BIOCHEMICAL AND CARCINOGENIC EFFECTS OF MONOSODIUM GLUTAMATE AND SOYA BEAN IN *WISTAR* RATS

BY

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CERTIFICATION

This is to certify that this work "Biochemical and carcinogenic effects of monosodiumglutamate and soya bean in Wistar rats" was carried out by I, Adaeze Bob Chile-Agada (REG.NO:20134877748) in partial fulfillment for the award of the degree of Doctor of Philosophy, (Ph.D) in Enzymology Biochemistry in the Department of Biochemistry of the Federal University of Technology, Owerri, ImoState.

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DEDICATION

This research work is dedicated to the Almighty God.

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ABSTRACT

This study was carried out to investigate the biochemical effects of short, medium and long term adminstartion of monosodium glutamate (MSG) and soya beans in both male and female *Wistar* rats, using standard methods. LD_{50} was carried out using rats which led to the choice of 1000, 2000 and 3000 mg/kg b.w for low, medium, and high doses of the samples. A total of 210 (105 males and 105 females) weanly Wistar rats weighing 70 – 78g were divided equally into three groups of 70 rats each representing the various experimental durations (2, 4, and 6 months). Each of these groups containing 70 rats was further divided equally into 14 subgroups, each containing 5 rats. Group 1 and 8 were controls for female and male rats fed normal raw chow and saline. Groups 2-7 (female rats) and 9-14 (male rats) were rats administered low dose (LD), medium dose (MD) and high dose (HD) MSG and soya beans respectively. 6-o-Malonylgenistin (98.47%) and Glycitin (98.11%) were the most abundant oestrogen-like compounds in the dehulled and oil of soya beans. The glucose and insulin levels of both male and female rats were significantly altered by HDs of both MSG and soya beans after 6 months while ALT, ALP and AST levels were significantly elevated after 4 and 6 months following administration of both MSG and soya beans and were more extensive in HD soya bean fed rats. No significant changes ($P > 0.05$) were observered in the urea levels after 2 and 4 months soya beans respective administration to female and male rats, while HD MSG significantly elevated the creatinine levels of male and female rats after 4 and 6 months administration. Administration of soya beans and MSG for 2 and 4 months had no significant effect ($P > 0.05$) on the bicarbonate and chloride levels. The HDL levels were significantly reduced while LDL, TC, and TG were significantly ($P < 0.05$) elevated after 6months HD administration of MSG and soya beans. Soya beans administration produced no significant change in the SOD levels for female rats and catalase levels in male rats after 2 months administration whereas the MD and HD administration of MSG significantly elevated MDA levels of both male and female rats after 4 and 6 months. Two and four months administration of soya beans and MSG produced no significant changes ($P > 0.05$) in the LH and FSH of female rats while MD and HD of MSG and soyabean significantly decreased the testosterone and LH of male rats. The colorectal, pancreartic, and ovarian cancer markers, and prostate specific antigen levels were significantly elevated after administration of MD and HD of MSG for 2, 4, and 6 months. This study has shown that the high dose adminstrtaion of monosodium glutamate and soya bean produced delecterious effects on the biochemical parameters and cancer markers on rats after six months extensive administration.

Keywords: biochemical indices, cancer markers, histopathology, monosodium glutamate, oestrogen-like substances, sex hormones, soyabean, *Wistar* rats

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Monosodium glutamate (MSG) has the chemical formula $C_5H_8NO_8NaH_2O$. It is chemically known as 2-aminopentane diotic or 2-amino glutaric acid; the sodium salt of the non-essential amino acid glutamic acid which is commonly used as a flavour enhancer in Chinese and Japanese foods (Inyang, Ojewumi and Ebuechi, 2012). It was first discovered by the Japanese chemist Kikonae Kedo in 1900 and is sold as a fine white crystalline substance similar in appearances to table salt (NaCl) and sugar. Also, it is used to intensify the natural flavour of certain foods (for example meat, vegetables and soups) without adding significant flavour of its own (Enemali and Danielson, 2014). Monosodium glutamate is composed of white colourless, odourless crystals that exist in two forms called enantiomers, although only the L forms are used as flavouring agents (Leung and Foster, 2003). The MSG contains 78% glutamic acid and 22% sodium and water (Okediran, Olurotimi, Rahman, Michael and Olukunle, 2015). It was initially synthesized from wheat gluten, but now produced in commercial quantities by bacterial fermentation (Onaolapo, Onaolapo, Mosaki, Akanji and Abiodun, 2013). It is found in unlimited amounts in a wide variety of packaged foods such as processed meat and poultry, semi preserved fish and fish products, food supplements, alcoholic beverages and seasoning (Erb, 2006). Monosodium glutamate is also found in a variety of vaccines, and it is now being sprayed on crops and can become airborne. However, the airborne effects of MSG sprays have not been extensively studied (Erb, 2006). Monosodium glutamate is found in some quantity in many natural food substances as either an additive or flavour enhancer in many commercially packaged food substances such as cheese, seafood, meat, broths, poultry and vegetables (Jinap and Hajeb, 2010; Onaolapo *et al.,* 2013).

The unique flavour and taste of this compound has been categorized and established as a separate taste sensation, “Umani” taste. It is a popular condiment in West African, districts and it is marketed in Nigeria with trade names like Ajinomoto, A-One or Vedan (Obaseiki-Ebor, MeGhee and Shankee, 2003).

The Food and Drug Administration (FDA) in USA categorized MSG as a safe substance in 1995. However, the FDA commissioned a report that an unknown percentage of the population might react to MSG and develop MSG symptom complex (Hassan, Arafa, Soliman, Atteia and Al-saeed, 2014). Its safety has generated much controversy locally and globally. In Nigeria, many people often use MSG as a bleaching agent for the removal of stains from clothes. As a result, there is a growing apprehension that its excellent bleaching properties could be harmful to the stomach mucosa, or worse still including terminal diseases in consumers when ingested as a flavour enhancer in food. Despite evidence of negative consumer response to MSG, reputable international and local organisations and nutritionists have continued to endorse MSG with the emphasis that it has no adverse reactions in humans (Eweka and Om’Iniabohs, 2008).

Plant derived substances, particularly soya bean foods and their isoflavones have received much attention as potential beneficial factors in diet, in Asian countries. Asian populations are known to have a lower incidence of cancers, cardiovascular morbidity and mortality and other chronic diseases, which epidemiologists hypothesize, may be attributable to their average daily intake of one serving of soya bean (Geeta, Khanna and Mahna, 2013). Soya bean (*Glycine max*) and its components are widely used in almost all sectors of agriculture, food industry, vetenary and human medicine (Geeta, Khanna and Mahna, 2013). Soya bean is the product with balanced content of essential amino acids, polyunsaturated fatty acids, vitamins, mineral elements and lecithin (Geeta, Khanna and Mahna, 2013). The health benefits associated with soya bean consumption has been linked to the content of isoflavones; and it is the main class of the phytooestrogens (Orgaard and Jensen, 2008). Soy proteins are derived from soya beans which have high protein content (35-40% of dry weight). Approximately, 90 % of the protein in soya beans exist as storage proteins, primarily β-conglycinin which is a glycoprotein composed of three subunits and glycinin which is a hexameric protein (Bayoumy, 2013). Soya bean is a widely used inexpensive nutritional source of dietary protein (McArthur, Walsh and Richardson, 1988). Its protein content (40 %) is higher and more economical than that of beef (18 %), chicken (20 %), fish (18 %) and groundnut (23 %) (IITA, 1990). It is also important as a vegetable protein source because of its cholesterol lowering abilities in patients with Type II hyperlipoproteinaemia (Alada, Akande and Ajayt, 2004). Apart from proteins, soya beans also contain carbohydrate (32 %), fats (20 %), minerals/ vitamins (5 %) and fiber (3 %) (Alada *et al.*, 2004).

Soya bean is a unique food because of its rich nutrient content, complex carbohydrate and dietary fiber content which contribute to their low glycaemic indices, which is beneficial to diabetic individuals and reduce the risk of developing diabetes (Amer, 2012).

1.2 Statement of the problem

The increasing use of MSG and soya bean in our homes has been observed, likewise the increasing knowledge of the toxic effects that comes with their usuage. Many researchers have looked at the iochemical effects of MSG and soya bean (Ibegbulem, Chikezie, Ukoha and Nwogu, 2016; Uyar, Ozdemir, Ilter, Akyildiz and Sivrikoz ,2016). But the carcinogenic property of MSG and soya bean has not been thoroughyly investigated. This work, however tried to look at both the biochemical, hormonal and carcinogemic effects of these two substances on both male and female weanly *Wistar* rats. The result will help us answer some of these assumptions such as: the safety of the usage of MSG as prolonged consumption has produced a myriad of toxic effects referred to as Chinese restaurant syndrome, characterized by sweating, nausea, headache, obesity and asthma to mention but a few. On the other hand the use of soya bean has been shown to be positive in its reduction of LDL-C, reduction of diabetes mellitus, as an anticancer agent, its buffering effect etc. So the functionality and integrity of the renal, hepatic organs and the activity of some antioxidant enzymes can also be affected by the prolonged use of MSG and soya bean. The level at which these enzyme activities and organs are affected is a question that needs to be answered.

1.3 Justification of the study

Histopathological studies are useful tools for identification and characterization of organ injury and recovery (Ali, Rahman, Islam, Mamun, Zannah *et al.,* 2014). Additionally, biochemical tests such as kidney function test (KFT), liver function test (LFT), lipid profile assessment are diagnostic parameters for ascertaining organ functionality and level of recovery from injuries during the course of therapeutic intervention. The present study is to investigate renal, and hepatic integrity and functionality and the activity of some antioxidant enzymes of *Wistar* rats in the presence of monosodium glutamate and soya bean using histological methods and blood diagnostic indices.

1.4 Objectives of the study

The aim of this work was to evaluate the effects of monosodium glutamate (MSG) and soya bean on some biochemical and carcinogenic markers parameters in *Wistar* rats.

Specific objectives

These include:

a. To determine the Oestrogen-like substances in soya bean (whole bean and oil).

b. To determine the LD_{50} of MSG and soya bean in mice.

c. To determine the effects of MSG and soya bean on sex and age.

d. To determine the effects of MSG and soya bean on serum hormone (insulin, testosterone, oestradiol, progesterone and luteinizing hormone) concentrations.

e. To measure the effects of MSG and soya bean on the liver and kidney functionalities, serum lipid profile and blood glucose concentrations.

f. To measure the effects of MSG and soya bean on antioxidant enzymes activities such as catalase, peroxidase, and superoxide dismutase.

g. To carry out histopathological studies on kidneys, heart, liver, uterus, testes, pancreas and the relative organs weights of the rats.

h. To determine the colorectal, pancreatic and ovarian cancer markers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of monosodium glutamate

Monosodium glutamate, also referred to as sodium glutamate, is a white odourless crystalline powder that occurs as the sodium salt of the naturally occurring non-essential amino acid, glutamate (Plate 2.1). Natural sources of monosodium glutamate include cheese and tomatoes. And just as glutamate improves the taste of meat, soups and stews, monosodium glutamate is primarily added to foods as a flavour enhancer (Wang, Zhang and Adhikari, 2019). As a flavour enhancer popularly referred to as the "umami" flavour, monosodium glutamate balances, intensifies and blends other taste perceptions (Wang *et al*., 2019). Different regulatory bodies provide unique safety profiles for the use of monosodium glutamate. And while the US Food and Drug Administration refers to monosodium glutamate as "generally recognized as safe" (RAS), the European Union refers to monosodium glutamate as a food additive restricted to a certain amount in some foods (Wang *et al*., 2019).

Monosodium glutamate is hydrophilic, but not hygroscopic, and hardly dissolves in organic solvents like ether. Monosodium glutamate is also thermally stable and is highly susceptible to the maillard reaction. It is generally produced via three methods: disruption of peptide bonds of vegetable proteins by hydrolysis, bacterial fermentation, chemical synthesis with acrylonitrile as the base material. Presently, the largest proportion of monosodium glutamate is produced by fermentation in a process similar to vinegar production, but including a neutralization step with the addition of sodium salts. The corynebacterium species is the fermenting organism most commonly used for the production of monosodium glutamate in a process that includes seeding of ammonia and incremental addition of sucrose sources which altogether react to generate amino acids from where l-glutamate can be separated. The processes involved in the industrial production of monosodium glutamate are highlighted in Figure 2.1.

Plate 2.1: Monosodium glutamate

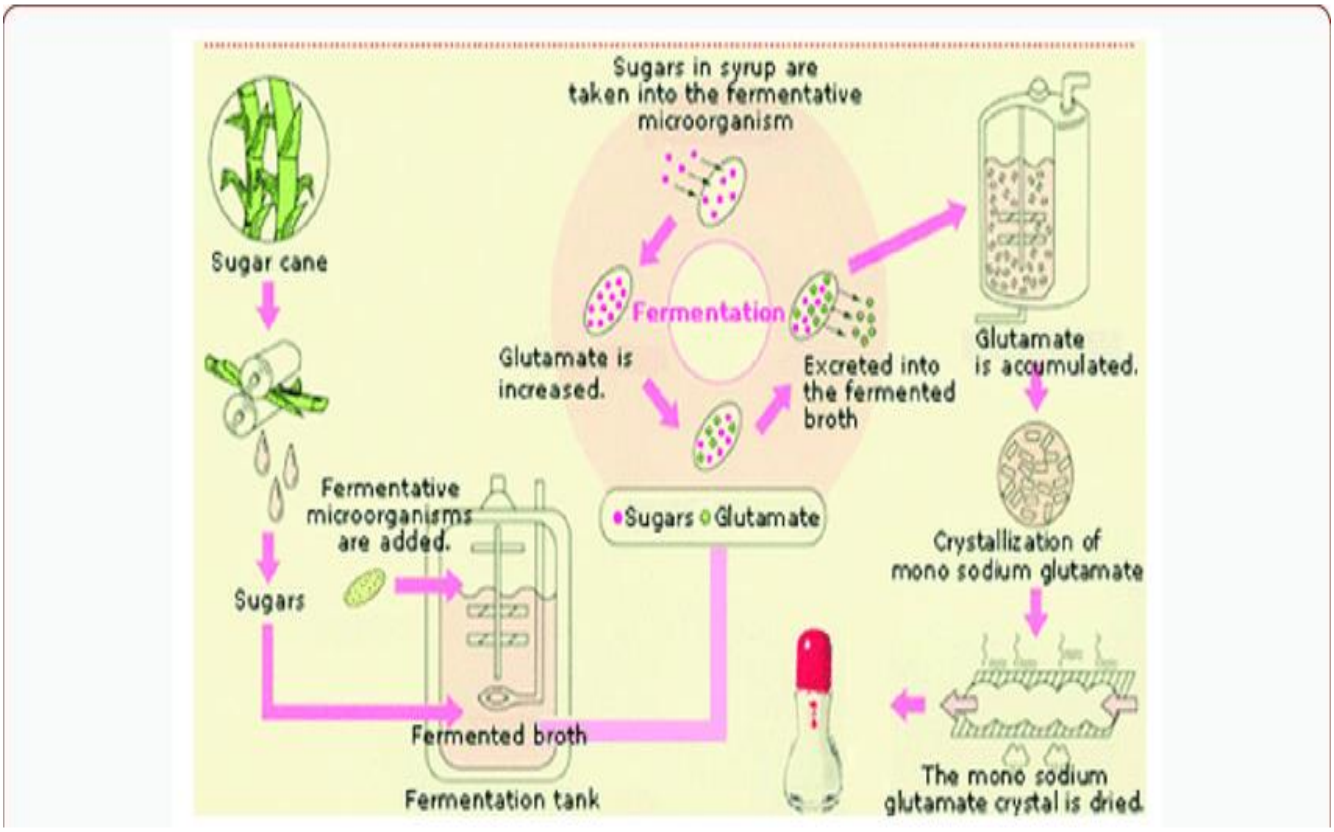

Figure 2.1: Industrial preparation of monosodium glutamate (Augustine *et al.,* 2019)

MSG has been described as a safe compound when intake is maintained at regulatory levels but could exacerbate undesirable effects when consumed by non-healthy individuals especially when consumed at levels $\geq$ 3g (Lidemann, 2001). Several chronic studies in clinical conditions have emerged with inconsistent reports regarding the chronic effects of MSG. Some studies have failed to relate any symptom complex of MSG to MSG intake (Freeman, 2006), while one study has reported an MSG-induced complication on fasting participants (Tarasoff and Kelly, 1993). Another study tested the effects of MSG on MSG-sensitive subjects and found inconclusive results even with extended administration (Geha *et al.*, 2000), and was later deemed irreproducible by later studies (Bakalar, 2008; Williams and Woessner, 2009; Shi *et al.*, 2010). Similarly, the suggested relationship between MSG and the pathophysiology of asthma has failed large scale scientific probity (Stevenson, 2000). Also, a product of the neurotransmitter, glutamate, their neuromodulatory effect in clinical trials has started since 2001 and is yet to provide a convincing relationship (Nicholas *et al.*, 2001).

2.2 Multicultural application of MSG

Monosodium is used in very small quantities by various consumers and institutions for food processing, feed pellets, and in commercial restaurant services. MSG also serves as a chelation agent, as the active constituent of N, N-diacetictetrasodium salt (GLDA). Asian countries account for up to 94% of the production of MSG worldwide because of a sustained workforce and high demand (Staples, 2016). China produces approximately 65% of worldwide MSG, consumes about 55% and exports about 44%, whereas Indonesia exports about 16% of worldwide MSG produced (Staples, 2016). Some reasons behind the various consumption capacities of continents were:

- Modernization, modified standards of living and dietary patterns, civilization and massive industrialization in Asian countries.
- Extensive usage in soup making, potatoes, stew and noodles in West Africa.
- Gender equality efforts, middle class proliferation and dependence on restaurant foods in South American countries.

However, in Northern American countries, due to increased concerns of MSG-induced obesity, the use of MSG was prohibited.

2.3 Chinese Restaurant Syndrome

Chinese restaurant syndrome is a complex symptom of MSG resulting from several systemic reactions that leads to severe illness and in some cases, death (Freeman, 2006). Till date, no causal factor or clear-cut elucidation of the associated pathophysiology has been substrantiated (Dutton, 2014). However, extremely high doses of exclusive intake of MSG may provoke Chinese Restaurant Syndrome in clinical settings (Dutton, 2014), though these effects neither persist nor escalate and become properly managed with counter dietary measures.

As early as 1997, it was established that doses as high as 5000 mg/kg body weight could induce complications (Yang *et al.*, 1997). Also the circulating levels of glutamate exceed permissible limits when MSG of > 5 g with or without foods is consumed, but normalizes spontaneously in few hours (Yang *et al.*, 1997). A later study eventually documented the manifestation of Chinese Restaurant Syndrome when individuals were subjected to $\geq$ 3 g single dose of MSG without food (Dutton, 2014). Also a study by Stevensson, (2000) established that asthmatic patients are more susceptible to Chinese Restaurant Syndrome than normal subjects.

2.4 Health effects of MSG

2.4.1 Effects of MSG on glucose levels

The hyperglycaemic effect of MSG especially in both kidney and serum samples have been established in earlier studies (Malik, V. and Ahluwalia, P. 1994). This established a direct relationship between MSG and blood sugar levels. This hyperglycaemic effect was attributable to stimulation of glyconeogenic enzymes with glutamine and glutamate as metabolic precursors (Malik, V. and Ahluwalia, P. 1994).

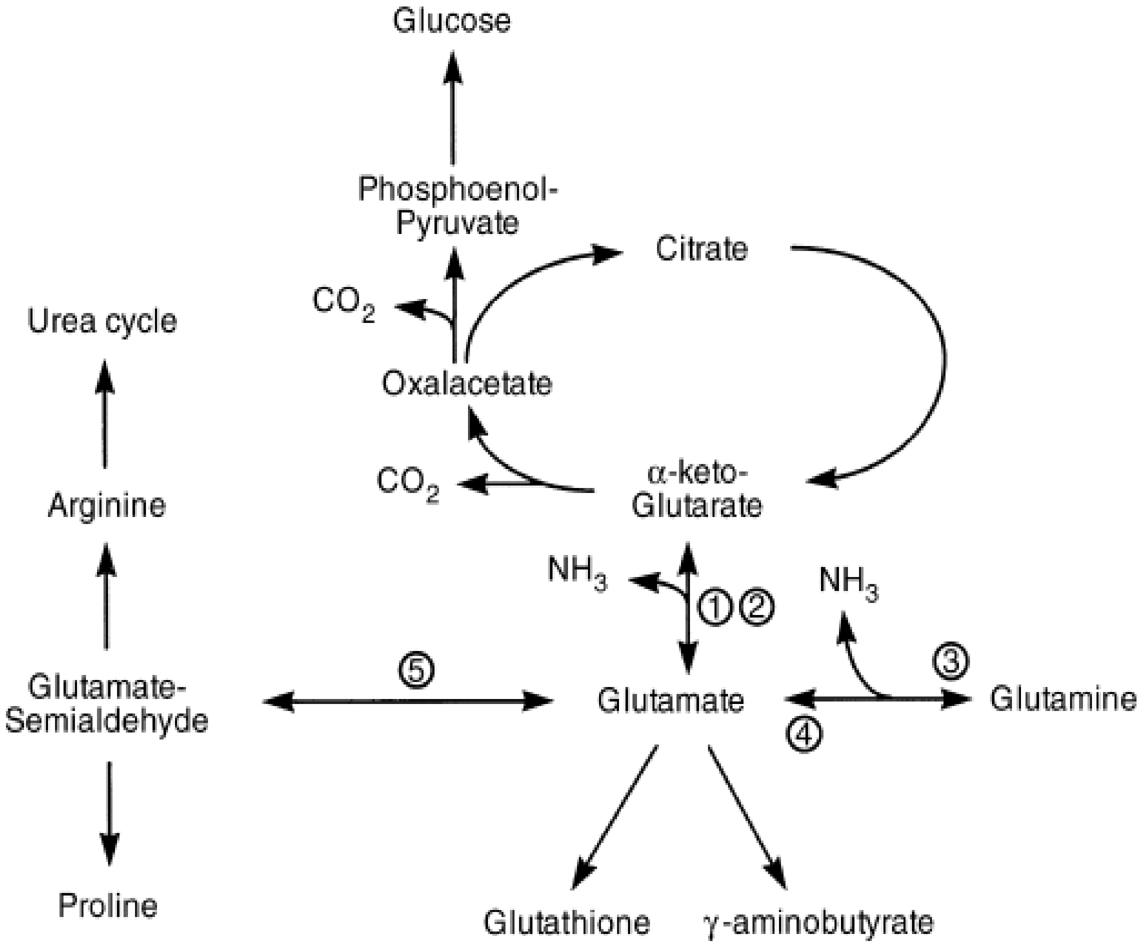

Figure 2.2: Glutamate metabolism and its link to gluconeogenesis (Stumvoll, Perriello, Meyer and Gerich, 1999).

Malik, V. and Ahluwalia, P. (1994) first described an MSG-induced stimulation of gluconeogenesis with a consequent suppression of glycolysis by upregulating and was later confirmed by Diniz *et al.* (2004). In addition, Diniz *et al* (2004) documented impaired glucose tolerance on account of MSG dietary supplementation, whereas Macho *et al.* (2000) reported that MSG caused insulin resistance in normoglycaemic experimental animals. Furthermore, MSG induced hyperglycaemic and hyperinsulinaemic conditions in rats; the hallmarks of impaired insulin sensitivity (Macho *et al.*, 2000). This hyperinsulinaemic condition was adjudged to have resulted from a compensatory switch to gluconeogenesis in response to excessive insulin concentration in the blood (Macho *et al.*, 2000). Some other studies have associated impaired β-cell response and elevated homeostatic model of assessment of insulin resistance (HOMA-IR) to extensive and excessive monosodium glutamate intake. Macho *et al* (2000) further established a link between impaired GLUT 4 transporters activities and MSG consumption, leading to hyperglycaemia. Similar observations have been documented for insulin receptors and insulin receptor substrates, and together with an impaired GLUT 4 transporter activities induced higher-than-normal glyconeogenesis which ultimately results to insulin resistance (Kakkar *et al.*, 1997). The MSG-induced impairment in glucose homeostasis also leads to tissue-level oxidative damage. The proposed mechanism behind this was related to the decreased influx of glucose-6-phosphate into the hexose monophosphate shunt due to a suppressed glucose-6-phosephate dehydrogenase, which thus decreases the synthesis of NADPH crucial for the biosynthesis of glutamate and other modulators of oxidative balance. In a nutshell, an MSG-induced impairment in glucose homeostasis consequently causes oxidative damage to tissues.

2.4.2 Effect of MSG on liver and kidney morphometrics

The consumption of MSG increases both the liver and kidney weights (Manal and Nawal, 2012). MSG elevate the circulating amounts of inflammatory agents which could have resulted in both liver and kidney weight gains. Potent antioxidants like vitamin E and ascorbic acid neutralizes the MSG-induced alterations in the liver and kidney weight, with vitamin E recommended as more potent (Onyema *et al.*, 2006). According to Onyema *et al.*, (2006), the reversal of the MSG-induced kidney and liver weight by vitamin E results from the inhibition of inflammatory agents.

2.4.3 Hepatic effect of MSG

MSG causes a significant increase in serum levels of alanine aminotransferase (ALT). With incremental doses, a 25.5 % and 45.2% ALT increase was recorded after MSG administration relative to the control animals (Eweka *et al.*, 2011). Since ALT is a reliable biomarker for hepatotoxicity, an elevated ALT shows a compromised liver integrity. The glutamate released from MSG dissociation yields ammonium ion as one of the degradation by-products. Except for immediate detoxification by the liver, ammonium ion causes serious hepatic dysfunction which releases ALT into the cytosol. The ammonium ion overload attacks the cell membranes of tissues forming complexes with surface polyunsaturated fats and further inhibits the mitochondria with subsequent leakage of these enzymes from their respective cellular compartments (Poli *et al.*, 1990). These observations agree with the reports of Farombi and Onyema (2006) that revealed extremely high amounts of ALT in the blood stream in rats fed with MSG-rich diets. They summarized that MSG produced hepatoxic radicals leading to ALT spillage into the blood stream, and should be avoided during the management of liver diseases. According to Vozarova *et al.* (2002), ALT spillage is indicative of impaired insulin sensitivity, type 2 diabetes mellitus and obesity, implying that MSG intake portends several other health risks. To counteract the hepatotoxic effects of MSG leading to elevated ALT, the exclusive or combinatorial administration of antioxidants has been suggested. Vitamin E particularly, and when combined with vitamin C attenuates the MSG-induced alterations in ALT levels (Manal and Nawal, 2012). This observation also corresponds with the findings of Gulec *et al.* (2006) that also reported a hepatoprotective effect of vitamin E.

2.4.4 Effect of MSG on other liver function indicators

The serum total protein levels remain unaffected by moderate intake of MSG while total bilirubin concentration was mildly suppressed (Manal and Nawal, 2012). Albumin on the other hand was significantly decreased in experimental animals subjected to MSG (Manal and Nawal, 2012). This indicates severe compromise of the functional integrity of the liver. Albumin and bilirubin estimation enables assessment of the functional states of the liver and suggests particularly the type of liver dysfunction that occurred. Alterations in albumin and bilirubin is suggestive of a chemical-induced hepatocellular injury and correlates with a reduction in the synthetic potentials of the liver arising from higher fluid retention in interstitial spaces of the liver (Giannini *et al.*, 2005). Furthermore, the γ-glutamyltransaminase were significantly elevated with MSG intake showing liver damage (Manal and Nawal, 2012). Onyema *et al.* (2006) posited that elevations in serum GGT levels only occurs during liver injury resulting from tissue oxidation, but however can be counteracted with the coadministration of potent antioxidants.

2.4.5 Effect of MSG on kidney functions

The consumption of MSG distorts the renal functionality (Vinodini *et al.*, 2010). The urea levels have been found to be suppressed by excessive MSG intake (Manal and Nawal, 2012). Alterations of the urea cycle are considered the major factor behind the decreased levels of serum urea after MSG rich diets. The serum urea nitrogen levels are diagnostic indicators of kidney function especially with extremely low levels, whereas high levels of serum urea nitrogen suggest possible heart failure, protein-rich diet or low fluid intake.

The status of the kidney can also be examined with the serum creatinine levels. Studies have shown distinct increase in creatine levels after intake of MSG (Farombi and Onyema, 2006; Vinodini *et al.*, 2010). The studies inferred that MSG comprises the functional capacity of the kidney by interfering with creatinine metabolism or its tubular excretion. Another suggestion is the evolution of free radicals that eventually targets and alters the renal tissue architecture (Phaniendra *et al.*, 2015). These alterations induced by MSG on the kidney are reversible with antioxidant supplementation (Vinodini *et al.*, 2010).

2.4.6 Effect of MSG on antioxidant defense system

The assessment of lipid peroxidation is an important indicator of oxidative damage because of the adverse effects reactive oxygen species have on the membranse (Selvakumar *et al.*, 2006). The administration of MSG causes the excessive generation of lipid peroxidation products (Okwudiri *et al.*, 2012) which was attributed to excessive production of reactive oxygen species. In earlier studies, homogenates from body tissues showed higher concentration of reactive oxygen species and lipid peroxidation products after MSG administration (Diniz *et al.*, 2005; Onyema *et al.*, 2006; Onyema and Farombi, 2006).

A compensatory action by the tissues to restore the antioxidant status after MSG administration also generates more lipid peroxidation products (Okwudiri *et al.*, 2012). Generally, the levels of non-protein thiols like glutathione in tissue homogenates reveals the degree of MSG-induced lipid peroxidadtion (Singh *et al.*, 2003; Onyema *et al.*, 2006). Glutathione plays a crucial role in scavenging of free radicals in the body and its alteration simply indicates high peroxidation of lipid bilayers especially in cells with higher metabolic functions (Onyema *et al.*, 2006). Glutathione maintains the antioxidant defense system in numerous ways involving direct chelation of free radicals and acyl peroxides which enables the maintenance of membrane integrity during lipid peroxidation (Price *et al.*, 1990).

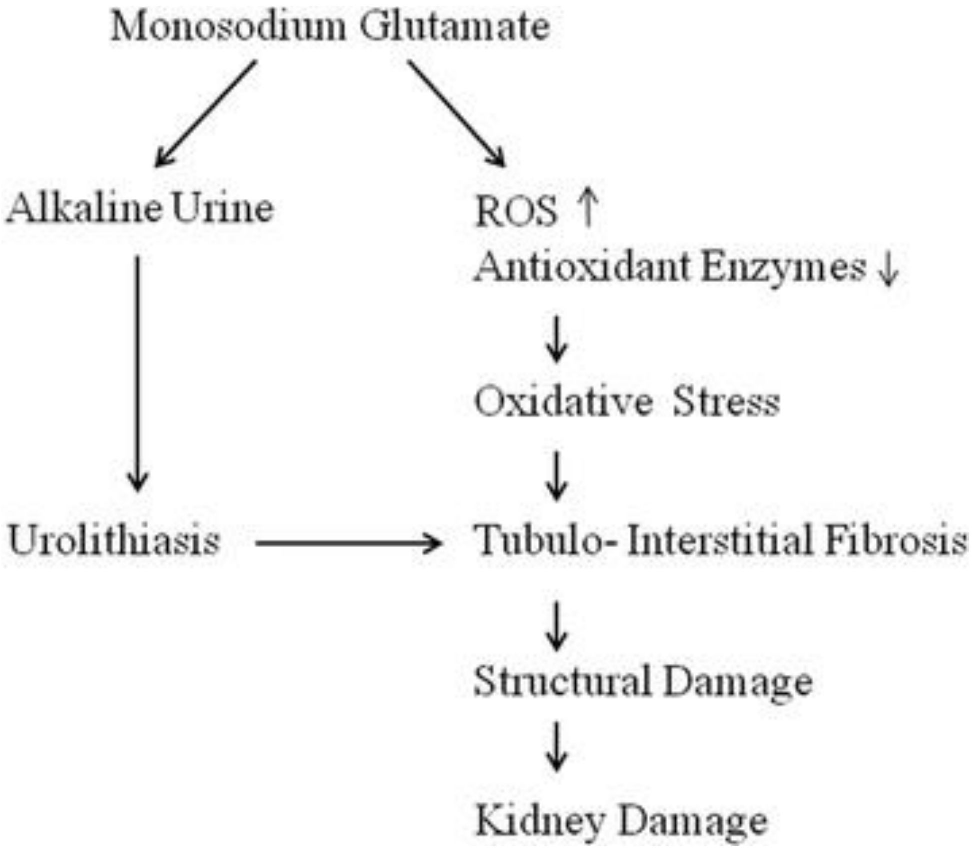

Figure 2.3: Effect of MSG-induced oxidative stress (Sharma, 2015)

The extent of depletion of glutathione reveals the extent of tissue degeneration (Anderson, Ambrose, and Garner.,1998). Likewise, the activities of glutathione-s-transferase directly correlates with the amount of tissue glutathione levels and integrity of the antioxidant defense system (Ketterer *et al.*, 1993). Glutathione peroxidase catalysis is the conjugation of glutathione to lipid peroxidation products thereby forming soluble innocuous conjugates (Neuefeind *et al.*, 1997). Furthermore, superoxide dismutase and catalase are extensively depleted with administration of MSG showing a compromised antioxidant defense system (Singh *et al.*, 2003). Okwudiri *et al.* (2012) explained that the low levels of these antioxidant enzymes after MSG intake resulted from glycation or inhibition by reactive oxygen species.

Also, as these enzymes become further depleted, lipid peroxidation products increase, thereby depleting the levels of glutathione as the only non-protein thiol capable of binding acyl radicals.

2.4.7 Effect of MSG on haematological parameters

Extensive intake of MSG significantly depletes the neutrophils (Ashaolu *et al.*, 2011). This reveals that MSG is toxic to either neutrophils or the progenitor cells in the bone marrow in both concentration and duration dependent manner. Neutrophils and monocytes are the immune cells charged with the first line defense against antigens (Hall, 2011). This thus is indicative of an MSG-induced immune compromise. Furthermore, MSG was found to elevate the circulating amount of lymphocytes, implying either a direct effect on the granulocytes or exacerbation of the alterations in macrophages (Ashaolu *et al.*, 2011). Macrophages show immunological effects by activating β-lymphocytes via the presentation of antigens and antigenic products to helper T-cells (Sembulingham, 2005). According to Barrett *et al.* (2010), the suppression of macrophages suppresses the secretion of inflammatory markers like cytokines thereby losing the capacity to activate and proliferate lymphocytes. This means that MSG indirectly causes an alteration in lymphocyte activation and proliferation (Ashaolu *et al.*, 2011). MSG also depletes the haematocrit and haemoglobin concentrations, indicating an induced reduction of functional duration of red blood cells circulation (Ashaolu *et al.*, 2011). This could also imply that MSG induced alterations of haematoporific stem cells. In addition, one of the effects of the autooxidation of blood cells induced by MSG, is the generation of micronucleated polychromatic erythrocytes (MNPCES) (Farombi *et al.*, 2006). Also, MSG intake affects the levels of mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) (Ashaolu *et al.*, 2011). This systematically points to a possible MSG-induced anaemia. Ashaolu *et al.* (2011) reported that the continuous and excessive intake of MSG causes pernicious anaemia (Macrocytic normochromic anaemia) inferred from elevated MCH levels. Another suggested mechanism behind this was that MS causes gastritis (gastric mucosa atrophy) due to the acidic component of MSG, which impairs the release of intrinsic factor and cobalamin absorption, the primary causes of macrocytic normochromic anaemia (Sembulingham, 2005). This summarily implies that MSG causes immunotoxicities (Eweka *et al*., 2011).

2.4.8 Effect of MSG on lipoprotein metabolism and cardiac morphology

MSG intake affects cholesterol and lipoprotein homeostasis (Egbonu and Osakwe, 2010), which portends cardiovascular risks. Also, MSG intake elevates triacylglycerol (TAG) levels in the blood due to the extensive degradation of lipid particles that heightens fatty acid mobilization from adipocytes (Collison *et al*., 2009). The circulating levels of fatty acids is found as the mechanistic link to the regulation of TAG metabolism (Schummer *et al.*, 2008). In a nutshell, as lipolysis increases, TAG biosynthesis or release consequently increases, and that eventually overburdens the capacity of very low density lipoproteins (VLDL) to mop up circulating TAG back to the adipocytes. On such conditions, hyperlipidaemia is inferred. MSG also induces metabolic syndromes like impaired insulin sensitivity and obesity (Schummer *et al.*, 2008), coronary diseases and atherogenesis (Palaniappan *et al.*, 2001), which are all resulting complications from an altered lipoprotein metabolism. This means that by up-regulating TAG and cholesterol metabolism, from extensive MSG consumption, animals are susceptible to various metabolic complications (Egbonu and Osakwe, 2010). When low doses of MSG were administered to animal models, no effects on either cholesterol or TAG metabolism was observed (Ahluwalia and Malik, 1998). The heart weight remains stable with low-moderate MSG intake while the extended intake of high doses of MSG increases the heart weight (Okon *et al.*, 2013). Increase in heart weight, otherwise referred to as cardiac enlargement, is mostly brought about by dilation or hypertrophy. Hypertrophy is the thickening of the left ventricular heart muscles, and in numerous cases is accompanied by other complications of the heart (Rogers and Blandell, 1990). Factors that increase the pump rate of the heart easily cause hypertrophy. Other predisposing factors include substance abuse, especially with tobacco and MSG, infection, genetics and metabolic syndrome. Kang and Izumo, (2000) likened the pump stress-induced hypertrophy to exercise-induced muscle mass increase. However, Okon *et al.* (2013) proposed that before hypertrophy sets in, fatigue, palpitations and angina occur.

2.4.9 Effect of MSG on reproductive health

In a recent clinical trial, histophathological examination of the fallopian tubes of subjects placed on MSG-rich diets, showed epithelial aberrations, and necrosis of the membrane between the myosalpinx and endosalpinx (Eweka *et al.*, 2011). According to Jovic *et al.* (2009), this distortion in the lumen of the fallopian tubes subsequently impairs the ciliary activities of the fallopian tubes. However, the report proposed that the MSG-induced cell death occurs from necrotic and/or apoptotic cell death (Jovic *et al.*, 2009).

2.4.10 Effect of MSG on pregnant and lactating women

Pregnant women and lactating women consumes the widest varieties of foods due to their peculiar metabolic needs. The foetus receives its nourishment via the placenta-mediated nutrient transfer. Notwithstanding, the transfer of nutrients from the mother to the foetus is a well regulated process, as studies have shown that even with high glutamate dietary intake, the foetal amounts remains fairly stable (Battaglia, 2000). Intravenously administered MSG, up to 220 mg/kg maternal weight, showed no effect on the foetus (Pitkin *et al.*, 1999). This led to the hypothesis that glutamate was unable to cross the placenta barrier (Pitkin *et al*., 1999). The teratogenic effects of MSG are yet to be established.

Also, the analysis of breast milk from lactating mothers subjected to glutamate-rich foods revealed no significant changes in glutamate levels of the breast milk (Bertrand *et al.*, 2002). Also, breastfed infants have shown preference of the naturally glutamate-rich breast milk to the cow milk (Baker *et al.*, 1999). Hence, MSG intake does not affect lactation or the breast-fed infants.

2.4.11 Effects on Fertility and Foetus

The effect of monosodium glutamate on foetal growth and fertility is a subject of crucial research in preclinical trials. In some studies, involving male experimental subjects, reversible changes in the testicular structural integrity as well as sperm morphology were found following MSG administration (Eweka and Om'iniabohs, 2008). Female rats exposed to both 0.08 and 0.04 mg/kg b.w. monosodium glutamate caused pathological changes in the fallopian tubes and oocytes (Eweka *et al.*, 2011; Eweka and Om'iniabohs, 2008). Studies have also shown that for these above mentioned studies, the doses administered were the basis of perceived experimental flaws. It is suggested that previous studies involving the administration of 1.46 g/kg b.w. and 2.92 g/kg produced effects irreconcilable with these previous studies (Eweka and Om'iniabohs, 2008).

In addition, wrong statistical analytical techniques and the omission of the exact number of pathological changes constituted further experimental flaw. In newborn mice, Eweka and Om'iniabohs, 2008, reported that the intake of monosodium glutamate causes the proliferation of primary spermatocytes and follicles. However, the small control of rats in the control group has offered less reliability of the study in terms of statistical accuracy. In the same study, pathological changes were reported after perinatal examination. Due to the duration, mode, and doses used in the aforementioned study, the translation of the result in clinical settings remain highly unlikely. Also, Yu *et al.* (2006) who investigated the gestational effect of monosodium glutamate, found that health of the pups was compromised, in addition to neurological impairments, growth hormones and insulin metabolism. Furthermore, dams subjected to monosodium glutamate administration produced offsprings showing functional and morphological cerebral distortions, as well as genotoxocity and apoptotic genes (Yu *et al.*, 2006). The use of extremely high doses and their route of administration, makes these studies non-trasmissible to human dietary conditions.

2.4.12 MSG-Induced Tumor Development and Obesity

A newly established model of obesity involves the administration of up to 4 mg/kg b.w. of monosodium glutamate for 10 days in newly born mice. In addition to this, reduction of the gene expressing adiponecton as well as increased secretion of inflammatory markers and chronic inflammation are obtainable (Hernandes-Bautista *et al.*, 2014; Tchkonla *et al.*, 2010). Hata *et al.* (2012) also demonstrated that mice subjected to MSG treatment showed elevated glucose, insulin and cholesterol levels. This indicates that alterations of both glucose and cholesterol metabolism are the notable mechanisms behind the MSG-induced obesity (Roman-Ramos *et al.*, 2011). Regarding the proinflammatory potentials of MSG, Roman-Ramos *et al.*, (2011) showed that MSG administration promoted elevated leptin and resistin, tumour necrosis factor and interleukin 6 over-expression. The induction of obesity using monosodium glutamate causes different metabolic alterations with profound gender differences in terms of bodyweight and ageing (Hernandez-Bautista *et al.*, 2014). Park *et al.* (2010) reported a correlation between liver damage and obesity induced inflammation and steatosis of the hepatocyte. Nakashini *et al.* (2008) suggested that MSG-induced hyperglycaemia and excess weight gain shows similar symptoms with nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. Hoang *et al.* (2007) later recorded increased cellular entropy, excitatory glutamate and non-neuronal receptors as well as sodium pumps, were crucial predisposing factors to cancer. Elevated free radical levels and lipogenesis of the liver that are both linked to insulin resistance and obesity, are observable during MSG-induced proliferation of the hepatocytes and hepatic carcinogenesis (Hoang *et al.* 2007). In addition, oxidoreductases and glucoronosyl transferases which are well known drug-metabolizing enzymes promoted the alterations of drug pharmokinetics after the administration of monosodium glutamate. Matouskova *et al.* (2015) found that MSG produced a compromised antioxidant system by suppressing the circulating levels of glutathione-s-transferases. The mechanism employed by these antioxidant enzymes is via the inactivation with these carcinogens or production of highly reactive species, more potent than the metabolized compounds. The activities of glutathione-s-transferase, cytochrome P_{450} and UDP glucoronosyl transferase has been correlated with the progression of colorectal cancer in both epidemiological and clinical studies (Beyerle *et al.*, 2015). Diethyl nitrosamine was used as a hepatotoxic agent before the administration of monosodium glutamate which enventually revealed hepatic carcinogenesis with symptoms of steatosis (Hata *et al.*, 2012). In another MSG-induced obesity model, the relationship between obesity and colorectal cancer was monitored after using intraperitoneal administration of 15 mg/kg b.w. azoxymethane to induce colorectal cancer. Elevated glucose, insulin and cholesterol were observed following the administration of monosodium glutamate (Hata *et al.*, 2012). The monosodium glutamate administration increased the expression of IGF-1 receptor mRNA. These findings simply imply that the administration of MSG enhances the susceptibility of azoxymethane-induced colorectal cancer. Furthermore, the treatment of pups with successive doses of monosodium glutamate brings about morphological and functional changes in alveolar macrophages (Liu *et al.*, 1989). In this aforementioned study, the researcher observed an increase in lipid profile of the cells as well as the presence of lamellar structures and lipid vacuoles. These changes suppress cellular senescence potentials and inhibition of the viability of tumour cells. Obesity also enhances the proliferation of tumours in MSG-treated animals. Later studies therefore proposed that an active insulin ERK-1 and 2 pathways, in addition to an anti-carcinogenic effect, could have influenced tumour development in obese animals (Liu *et al.*, 1989). It is also worthy of mention that while evidence has been synthesized for an MSG-induced tumour proliferation in animals, the experimental conditions are unsuitable for clinical settings and do not translate wholly to human tumourigenesis.

In vitro assessment of MSG was undertaken using immune cell cultures which resulted to the concentration dependent increase in the rate of aberration of chromosomes and crosstalk between sister chromatids of the lymphocytes. Indices of nuclear separations as well as DNA replication were unaffected. As indicated by the findings of Ataseven *et al.* (2016), monosodium glutamate at the cellular level may produce genetic abberations of the lymphocytes. B-cells also became more viable after a dose-dependent increase in MSG concentration.

Jovic *et al.* (2009) reported that the metabrotropic glutamate receptor plays a central role in MSG-induced apoptosis of nascent and memory β-cells. Though it was observed that both nascent and memory β-cells possess dissimilar receptors for glutamate. Thus, these differently expressed glutamate receptors of the β-cells are the ultimate basis for oxidative stress related proliferation of immune cells (Jovic *et al.*, 2009). In neonates, the intake of monosodium glutamate elevates the secretion of inflammatory cytokines like interleukins 12 and 1, β and suppresses tumour necrosis factor, and interleukins 10 and 4 (Jovic *et al.*, 2009).

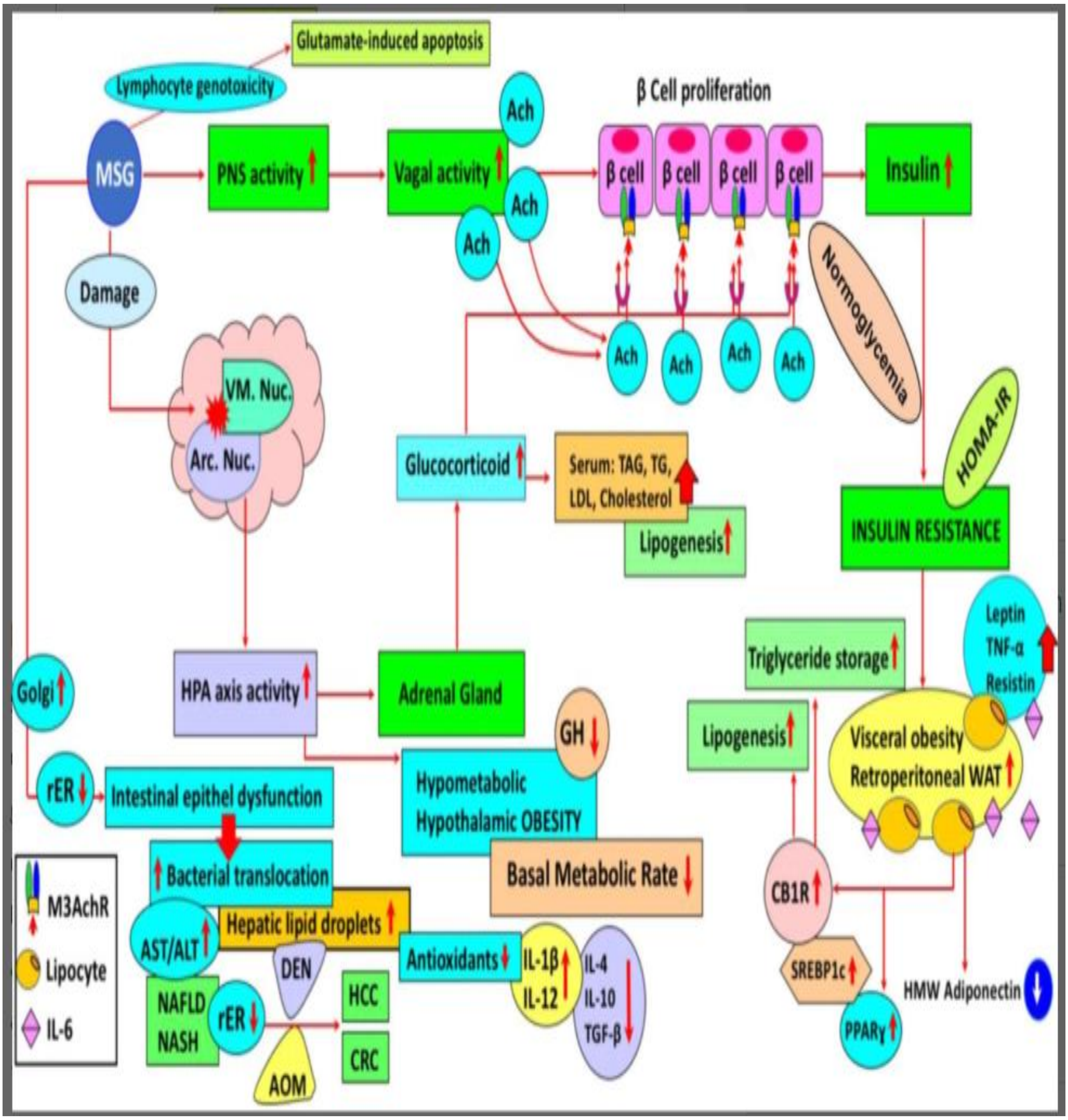

Figure 2.4: Synergistic action of the HPA axis and PNS hyperactivity in MSG induced obesity (Zanfirescu *et al*, 2019).

2.4.13 Effects of MSG on the Immune System

After an acute administration of MSG to neonates, no effect was observed on anti-inflammatory cytokine levels, whereas pro-inflammatory cytokines as well as insulin, leptin, and corticotropic hormones were significantly elevated (Castrogiovanni *et al.*, 2008). This simply means that both the neuroendocrine and metabolic immune activities were compromised in MSG-treated rats. Kohan *et al.* (2016) further posited that poor lipid absorption after MSG treatment corroborates to the damaging of membrane lining of the duodenum in response to inflammation. Post-natal MSG intake could impair the antioxidant defense system against pathogens (Nakadate *et al.*, 2016). This impairment brings about decrease in blood lymphocytes but has no effect on the phagocytic capacity of the neutrophils, whereas neonatal intake compromises the phagocytic activities of the macrophages.

Thermocyte apoptosis was also observed following the intraperitoneal administration of monosodium glutamate which eventually correlated with degree of oxidative stress (Pavlovic *et al.,* 2006; Farombi and Onyema, 2006). Pavlociv *et al.* (2007) also reported elevated xanthine oxidase and malondialdehyde activities with decreased catalase levels, after the administration of monosodium glutamate. These effects were ameliorated with the supplementation of notable antioxidants like ascorbic acid (Pavlovic *et al.*, 2005). Psychomobility examination of MSG-treated rats showed that the DTH effector T cells were dysfunctional during the migration inhibition assay (Pavlovic *et al.*, 2007). Earlier, Bellulardo *et al.*, (1990) showed a correlation between granular lymphocytes suppression and Nk cells cytotoxicity depression. Nevertheless, the designs of these studies make them unsuitable for translation to human dietary exposure.

2.4.14 Genotoxic effect of monosodium glutamate

The genotoxicities of MSG have been reported by several studies (Farombi and Onyema, 2006; Malik *et al.,* 1994). Malik *et al.,* (1994) reported that the generation of ROS is central to the genotoxic effect of MSG. In another study, Pavlovic *et al.* (2007) showed that MSG-generated ROS induced apoptotic lesions in rat thymus. Also, the study indicated that apoptosis induced by MSG was concentration dependent.

Elevate Malondialdehyde (MDA) and Xanthine oxidase were found preceding the MSG-induced apoptotic lesions primarily from DNA aberrations leading to apoptosis (Pavlovic, 2007).

2.4.15 Other effects of MSG

The inclusion of MSG in water improves the water intake of experimental animals, compared to plain water (Buzescu *et al.,* 2013). This improvement also became profound in human clinical trials, where both water and food intake significantly increased with the dietary inclusion of monosodium glutamate. Diniz *et al*. (2005) also included that despite the onset of overfeeding regarding the MSG-administered subjects, these experimental subjects also demonstrated profound elevated insulin and glucose levels as well as leptin and TAG levels in addition to profound insulin resistance and metabolic dysfunction. One of the gaps of the above mentioned study was equally the dose administered, as was considered impracticable in real life. Later on, Meyer-Gespach *et al*. (2016) hypothesized a connection between neurodevelopment and neural taste processing. Alterations in key players of memory and emotional learning were found after the administration of monosodium glutamate, as structures of the subcortex, and gustatory cortex were profound. The study equally reported that the performance of working memory remained unchanged. This later became a critical text for studies researching the relationship between the overconsumption of food and the alterations in memory and affective responses. Preclinical studies have inconsistently reported changes in bodyweight following large doses of MSG consumption, while a few studies have shown negative correlation, and others have a consistent direct proportionality between the amount of MSG consumed and weight gain (Kondoh and Torii, 2008).

The composition of constituent macronutrients in the preload used in the different research may have significantly amounted to these disparities of findings. MSG administration on several occasions have been found to alter glucose homeostasis and hepatic gene expression. During a glucose tolerance test, according to Bertrand *et al.* (1997), both diabetic and non-diabetic subjects showed elevated glucose tolerance and insulin secretion.

In another study involving feline experimental animals administered MSG directly into the uterus, caused the onset of β-cell dysfunction, steatosis, obesity, and alterations in both glucose and lipid metabolism (Tomankova *et al.*, 2015). Even glutamate levels were exponentially increased up to three folds in the rat's muscle (Cairns *et al.*, 2007). This implies that even minute increase in circulating amount of glutamate influences irritability of the muscles as well as some pain sensation in the muscle (Cairns *et al.*, 2007). Oral administration of monosodium glutamate also affects nociception (Zanfirescu *et al.*, 2017). Zainfirescu *et al.* (2017) found that high doses of MSG, up to 300 mg/kg b.w., altered the threshold of nociception, correlating with the elevated nitrates in the brain. Again the major flaws relate to unattainable actual doses consumed in real life could be administered over a very long period of time. In summary, the above mentioned studies have demonstrated that high doses of MSG affect both neurodevelopment, weight gain, and glucose metabolism.

2.5 Clinical Studies on MSG Administration

There has been conflicting reports emanating from clinical trials regarding the clinical effect of MSG on satiety, despite the enhancement of the palatability of food (Masic and Yeomans, 2017). Studies that eventually modified foods to achieve uniqueness and affinity for a particular taste sensation caused a reduction in overall palatability (Wilkinson and Brunstrom, 2016). Similarly, some reports have shown that both energy intake and hunger ratings diminish following MSG administration, whereas a study conducted on elderly subjects found no significant changes from either food containing MSG or in absence. Though Essed *et al.* (2009) pointed out that both the study group heterogeneity and age-related chemosensory diminishing perception could mask slight variations in the results. In contrary, Carter *et al.* (2011) improved the palatability of foods by the addition of small amounts of MSG which produced improved palatability and consumption without any alterations in energy intake. In similar design, the coadministration of chicken broth with monosodium glutamate, enhanced satiety without distorting energy intake levels in the control subjects (Luscombe-Marsh *et al.*, 2008). However, this study does not agree with the reports of Imada *et al.* (2014) that found a very significant rise in energy intake following MSG administration in comparison with normal broth. A comparable time stamp was recorded between an MSG-laddened meal and another test meal. These dissimilar reports however indicate that the effect of monosodium glutamate on the appetite correlates with the amount of constituent macromolecules in the food. Another study validated the above mentioned findings by comparing the satiety levels derived from MSG inclusion of either a protein-rich food or a carbohydrate rich food (Kochem and Breslin, 2017). The protein rich food when supplemented with monosodium glutamate, provoked increased satiety when compared to carbohudrate-rich food supplemented with monosodium glutamate. Masic and Yeomans (2017) found that the preference of higher doses of monosodium glutamate recorded decreased protein levels against the experimental groups who chose lower doses. This could mean that using palatability as a metric for choice of food indirectly shows a bearing on an individual’s dietary habits. Scientifically, there is sufficient evidence showing correlating satiety with protein contents of foods as leucine has been reported to increase satiety by suppressing food consumption (Laeger *et al.*, 2014). In another clinical study, the coadministration of chicken broth containing monosodium glutamate and fat-rich diets decreased both sugar and energy intake in healthy middle-aged women (Boutry *et al.*, 2010).

In comparison to normal cooking salt, supplementation of monosodium glutamate for 72 hours caused increased indigestion for 120 minutes thereby elevating circulating amounts of essential amino acids (Boultry *et al.,* 2010). Nevertheless, despite a sensory-specific related increase in satiety associated with MSG consumption, the consumption of other foods within this period drastically reduces. This implies that MSG may decrease appetite for other MSG-free foods, noting that the predominant macronutrient determines the effect of monosodium glutamate on energy intake and satiety. In addition, the nutritional status of an individual also determines the degree of cravings for MSG, as those with lower nutritional status prefer MSG-rich foods (Masic and Yeomans, 2017). Also, the neurological impact of the above mentioned findings have been investigated by Magerowski *et al.* (2018). They highlighted that inclusion of MSG in diets improved self-control in nutrition-related decisions among healthy participants, implying that glutamate may be responsible for the cognitive regulation food choices and eating attitudes. The disparities in the results could have emanated from methodological variations and discrepancies in food records. Also the lack of precision was another point of criticism. For example, in one of the studies (INTERMAP), one of the recruitment criteria was the ability to estimate the amount of MSG included in dishes by study applicants whereas in another study, participants were trailed to their preferred restaurants where food vendors were asked of estimates of MSG added in the foods. In another study (CHNS), regardless of the enormous sample size, the estimation of monosodium glutamate was achieved based on individual food consumption without any particular attention to frequency of consumption. One major issue of these studies was the wide variation of the mean values of the quantity of intake that at the end of the day no particular dose chosen gave similar findings among the studies. In another clinical trial, the Jiangsu Nutrition Study (JNS) showed a negative correlation between bodyweight and consumption of monosodium glutamate. In that study, family members were encouraged to report the quantity of MSG consumed per/meal (Shi *et al*., 2010). Given the imprecise method employed, reproducibility of the study has been a source of major concern. The Thai observational study employed a single dose of administration for 10 days, where the choice of dose occurred through the remainder of food after the first day of feeding. These highlights are essential to fully understand the effect of extensive and prolonged consumption.

2.6 Prevention of the toxic effect of MSG

The simultaneous administration of MSG with vitamin C protects against protein denaturation and associated toxicities (Hashen *et al.*, 2012). Farombi and Onyema (2006) found that quercetins, α-tocopherol and ascorbic acid reduce the elevated amounts of malondialdehyde, but the suppressed glutathione and glutathione-s-transferase. In addition, the antioxidants were effective in modulating superoxide dismutase, catalase and transferases in subjects treated with MSG. The liver enzymes ALT, aspartate amino transferase (AST) and γ-glutamyltransferase were significantly reduced when MSG intake was combined with either vitamin E or C (Onyema *et al.*, 2006).

Also, quercetin has been found to modulate cholesterol metabolism, insulin and leptin levels and other factors promoting glucose homeostasis (Seiva *et al.*, 2012). Superoxide dismutase and glutathione activities were restored by combining MSG intake with quercetin (Seiva *et al.*, 2012). Similarly, the reproductive health of MSG-treated subjects were reversed with diltiazem.

2.7 Description of soya beans

Two main sub genera form the major families of glycine namely, soja and glycine. *Glycine tomentella* and *Glycine Canescans* are the main subgenera for Glycine while *Glycine max, Glycine soja,* and *Glycine gracilis* are classified under the Soja subgenera.

Soya bean (*Glycine max*) (Plate 2.2) has hairy coverings all over its leaves and stems and bears its pod-like fruits annually. Up to 4 trifoliate-shaped leaflets in each leaf are obvious which shed before seed maturity. Before ripening, the greenish seeds are either curved or plainly shaped, not more than two seeds per pod and within 5 cm in length. Mature seeds are detectable with their light brown colour. Flowers have a combination of white to pink colour, though purple colour exists. Their pollens are shed into the stigma at maturity while the buds bear the mature anthers in a show of unique self-pollination.

2.7.1 Production of soya beans

The worldwide production of soybeans is over 200 million metric tonnes, and Africa accounts for more than one million metric tonnes (Shannon *et al*., 2005). Nigeria produces the largest ration of soya beans in Africa, with South Africa closely behind. Its by-products like haulms are used as animal feed, and generally, the growth in poultry farm increased the worldwide demand for soya bean as well as the need for increased importation in Nigeria. Zimbabwe and Zambia are also heave producers of soya beans in Africa. Other countries cultivate soya beans in small scale alongside cereals like maize, sorghum and cassava.

Plate 2.2: Soya bean seeds

2.7.2 Consumption of soya beans

More than half a million tons are consumed annually in Africa, where an additional 48000 tons are directly channeled as animal feeds (Zhang *et al.*, 2003). Nigeria and Uganda are the most Sub-Saharan African Consumers of soya beans. Soya bean is milled into milk and flour in Africa, and the fried curd serves as a breakfast meal. Soya bean is usually soaked and cooked for a long time to enhance its detoxification and enhance digestibility when consumed whole. Soya bean is presently used as a weaning food, supplemental diet and as a nutraceutical. More than half the produce of soya beans is consumed locally to enhance the nutritional status of malnourished infants and pregnant women. Soya beans are processed into soy cake, soy yoghurt and food seasoning. Dawadawa is one of the popular food seasonings produced from soya beans. Soya bean oil is presently considered as a healthier alternative to other cooking oils, and in some Nigerian restaurants, the soy-rice combination is one of the most demanded varieties by consumers.

2.7.3 Soya bean as a food ingredient

Domestic animals feed on soy products as major constituents of their feed. Amadi *et al.* (2018) posited that mixing soy products with regular cereals enhances the growth rate of poultry birds, serving as one of the most cost effective methods of improving the growth and vitality of farm animals. The major reason soya beans gained high dietary acceptance is due to its organoleptic properties as well as its nutrient density (Hammond and Jez, 2011). Many countries ferment soya beans powder into yoghurts, natto, miso and deserts as well as kinakoyuba, aburage, and tofu (Hammond and Jez, 2011). Soya bean is a widely adopted option for proteins for those with lactose intolerance and severe digestive complications (Businco *et al.*, 1992). The proximate analysis of soya beans is mineral (5%), carbohydrate (31%), protein (35%), moisture (12%) and fats (17%) (Messina, 2016). Soya bean contains a rich blend of essential amino acids which together with its rich phytochemical composition contributes to its health benefits (Kreijkamp-Kaspers *et al.*, 2004).

2.7.4 The protein content of soya bean products

The amino acid spread of soya beans is complementary to those for cereals which is why both types of foods are preferably used together. Soya beans lack most of the sulphur-containing amino acids but are rich in lysine sufficient enough to complement the poor lysine contents of cereals (Blomquist *et al*., 2005). A combination of rice and soya bean is a mainstay of most cultural diets due to a rich blend of lysine and methionine lacking in exclusive intakes. The report of Friedman and Brandon, (2001) showed that soya bean contains higher amount of proteins than other legumes and cereals. This sufficient protein composition and appreciable biological value represents a major reason why soya bean products are preferred over other oil seeds in animal nutrition. The dietary use of soya bean represents a compensation of both leguminous and cereal food proteins as chemical evaluation reveals a rich proportional mixture of constituents from leguminous and cereal food proteins. Certain practices that enhance the digestibility of soy proteins include soaking, extraction with boiling water, grinding and fermentation. Soya bean protein digestibility also varies according to the type of product, with milled soy flours showing lower digestibility than soy protein concentrate. Soya bean is mandatorily processed to mitiate the digestive complications from constituent chymotrypsin and trypsin inhibitors. Sulphur containing amino acids are specially included in diets to counteract the effects of these protein inhibitors. For infants that require methionine in very minute quantities, there is no justification of sulphur-containign amino acids when soya bean is used as a complementary diet. Medic *et al.* (2003) reported that dietary replacement of animal proteins with soya protein induces a hypocholesterolaemic effect.

Bressani *et al.* (2008) reported significant decrease in low and very low density lipoprotein cholesterol by up to 14.9%. Notwithstanding, some controversies trail the intake of soya beans especially the nutritional impairment seen with the excessive consumption of soya beans because of the presence of phytohaemagglutinins (Medic *et al.*, 2003). These glycoproteins participate in other reactions during the processing stage which could cause the synthesis of non-digestible compounds but have also been found to prevent gut atrophy by aiding the microbiota of the intestines (Otte *et al.*, 2001; Lajolo and Genevese, 2002).

2.7.5 Soya bean lipids and micronutrient contents

Soya bean oil contains significant proportion of essential fatty acids, fat soluble vitamins but contains extremely low amounts of vitamin D and K. Due to very low unsaturation, soya bean oil possesses high iodine values greater than those of well-known iodine containing oils like palm, sunflower, maize and peanut butter oils. The predominant fatty acids found in soya bean oil include linoleic, oleic, linolenic and palmitic acid. Soya bean has also been reported to contain fatty acids that enable cholesterol lowering effects. It is usually the considered source of essential fatty acids in poor resource settings. The intake of soya bean oil has been shown to enhance cholesterol lowering by > 300 mg/dl in hypercholesterolaemic individuals. Hydrogenation of soya bean oil occurs between 120^o to 230^oC (Nassiuma and Wafula, 2002) which is considered a disadvantage of the bleaching and hydrogenation of the oil. The oil contains both di and trienoic acids in very large quantities which predisposes the oil to trans-isomerism of linoleic and linolenic acids. Some texts have associated the hydrogenated soya bean oil to cardiovascular dysfunction as a result of the conversion of *cis* isomers to *trans* isomers (O’Brien, 2004).

2.7.6 Soya bean carbohydrates

Soya beans carbohydrates are mainly made up of polysaccharides and few oligosaccharides, with the major monomeric units as hexoses. These carbohydrates are non-structural in nature making up about 50% of the carbohydrates that include: little starch fragments, oligosaccharides and other low molecular weight sugars while the other 50% are mostly pectic polysaccharides.

Class 1 Allergy otherwise known as archaic food allergy. This type of soya allergy produces similar symptoms to pathogenic antigens. Anaphylactic shock, diarrhea, urticaria and vomiting are among the mainly experienced symptoms. In infants, poor immune response and cellular pervation of mesenteric tissues are evident. In addition, the manifestation of digestive and fever tolerance is probable, and in most likely cases, natural healing is usually expected.

Class 2 Allergy otherwise referred to as pollen or latex allergy. In this type of allergy, both transmucosal and percutaneous membranes are irritated, while healing is unlikely to be spontaneous.

Common symptoms include dyspora and other anaphylactic symptoms like facial edema, mouth and pharyngeal itching. Food proteins, mainly the hydrophilic low molecular weight proteins are the causative antigens. It is also worthwhile to note that digestive enzyme resistibility is usually a rare occurrence with this type of allergy because the assimilation of these allergens starts from the mouth rather than the gut.

Contact Urticaria: This is the irritation and swelling up of body parts, that affects the contact area of the body in contact with soybean products like tofu. Contact urticaria syndrome is divided into four stages: local urticaria and dermatitis – stage 1, generalized urticaria – stage 2, bronchial asthma, orolaryngeal defects, rhinitis and gut irritation – stage 3, and anaphylactic reactions – stage 4 (Jarmila *et al*., 2013).

Fig. 2.5: This figure showed Class 2; swelling of lips.

Fig. 2.6: This figure showed class 2; Allow indicated cellulites, scales with itching and dermatitis around lips

2.7.7 Soya consumption and phytoestrogen levels

Peculiar to soya bean, isoflavones strongly binds to very specific regions of protein molecules. Soya bean processing, culture, medical features, and varieties are the major factors that affects the variations of isoflavones. As reported by Bhathema and Belasquez (2002), isoflavones can easily be separated from the associated proteins by solubilization in alcohol, thereby weakening the binding between isoflavones and proteins. This provides further details regarding the disparities reported to dissimilar phytoestrogenic substances in different foods.

Furthermore, several studies have investigated the circulating levels of phytoestrogens in both preclinical and clinical trials in the presence or absence of soya products (Coward *et al.*, 1996). Usually, the concentration of isoflavones in the blood of humans that maintains a soya-free diet, is usually in $\leq$ 40 nm range whereas humans that maintain soy-based diet have isoflavones present in micromolar range (Watanabe *et al.*, 1998; Van Ere-Baart*et al.*, 2003). Different pharmacokinetic studies have found rapid absorption of isoflavones among health adults (Setchell, 2006). Both genistein, and diadein and their glycoside are usually metabolized to similar products, taking up to 420 minutes to attain peak plasma levels and up to 660 minutes for their glycoside products (Setchell, 2006). This implies that regulatory step as in the case of metabolism of their glycoside product, may be glycoside moiety cleavage. To support the hypothesis that isoflavones we excreted rapidly, Setchell, (2006) found that geneistein had a half-life of 420 minutes whereas diadzein had a half-life of 558 minutes. A couple of factors adjudged to influence the bioavailability of isoflavones include the isoflavones chemical nature, administered dose and food matrix, intestinal transit time and microflora (Setchell, 2006). The molecular structures of some of the phytoestrogens found in soya beans are represented below in Figure 2.7.

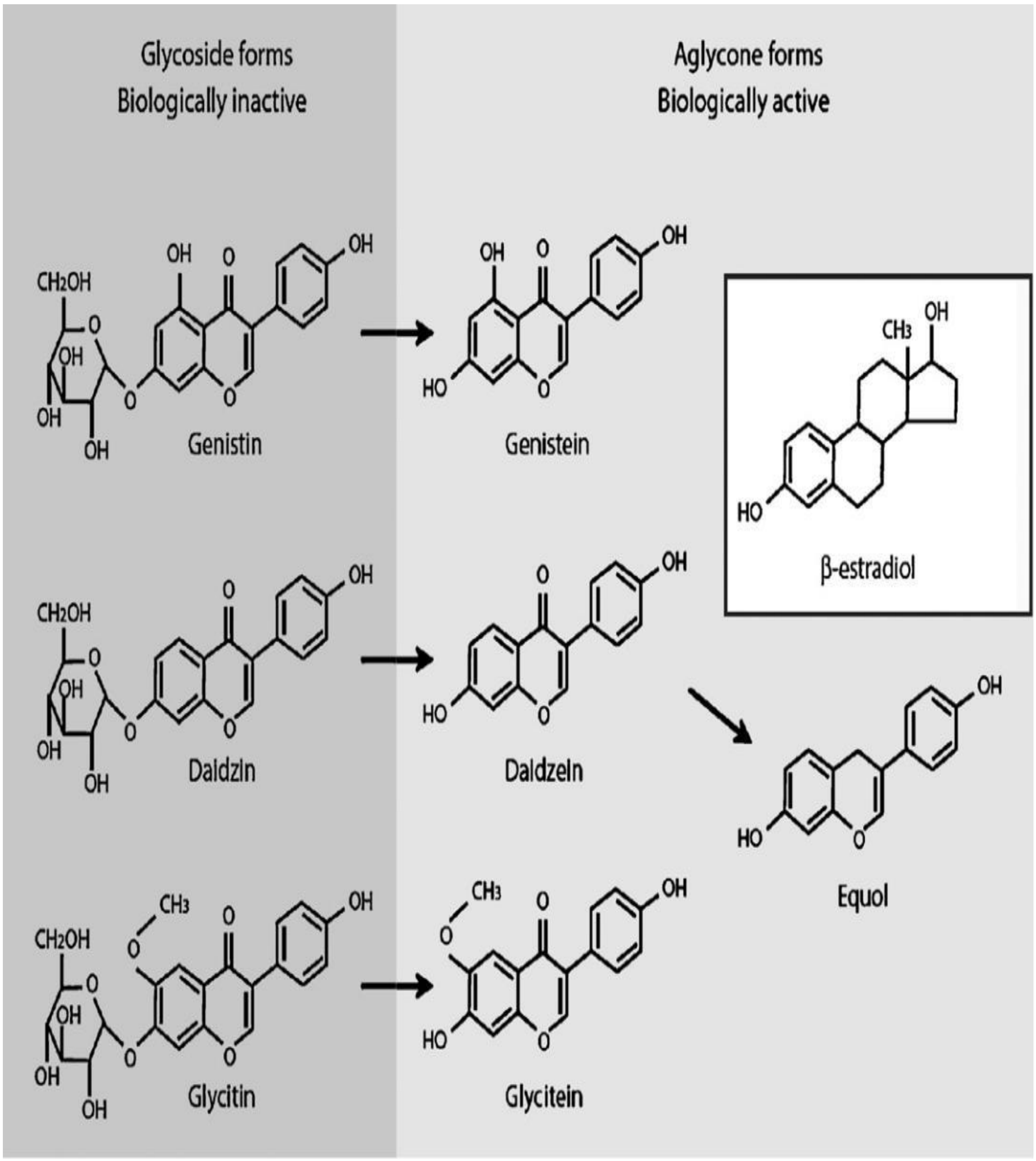

Fig. 2.7: The molecular structure of phytoestrogens (Cederroth, 2009)

2.7.8 Phytoestrogens: complex hormetic compounds

2.7.8a Phytoestrogens as hormetic complex compounds

In plants, the biosynthesis of phytoestrogens like soya isoflavones is affected by environmental challenges such sa nutrient drought or pest infection (Howitz and Sinclair, 2008). However, reports have shown that compounds synthesized during plant stress enhances stress resistance *in vivo*. Furthermore, a concept known as xenohormesis entails that chemicals from plants serve as reliable markers for environmental decay that allows animals build suitable defense under favourable conditions (Howitz and Sinclair, 2008). This phenomenon has become crucial in terms of the therapeutic value of stress-related bioactive compounds including polyphenols like vesveratrol, anthocyanidins, catechins, rutins, and kaempferols (Howrt and Sinclair, 2008). Dadzein and genistein, two widely research isoflavones have been widely studied for their roles in affectory metabolic pathways via estrogen-related or stress related pathways (Vedavanam *et al.*, 1999). Phytoestrogens affect a multitude of physiological changes such as cardiometabolic, neurological, nervous and reproductive systems, due to their strong binding to oestrogen receptors. Isoflavones elicit strong physiological response due to their capacity either strongly or preferentially bind to estrogen receptors. By this, isoflavones could promote either estrogenic or antiestrogenic activities conditioned by the tissue type and circulating levels of the isoflavone and estradiol (Cooke and Naaz, 2004). For instance, depending on the circulating levels of estradiol, geneistein could either provide antioestrogenic or become an additive agonist. According to Chen and DonoVan, (2004), genistein also shows biphasic properties in the intestines mediated by the estrogen receptors. This implies that those isoflavones which are usually produced under plant stress could avail medicinal effects as a result of selective estrogen receptor modulation (SERM).

2.7.8b Role of estrogens in metabolism

Both preclinical and clinical trials have shown the importance of estrogenic receptors during glucose and lipid homeostasis. As reported by Cook and Naaz (2004), estrogens alter adipocytes' activities by influencing synthesis or hydrolysis of lipids, adipogenesis or the mediation of energy or appetite balance. The hypothesis showing the effect of estrogens on metabolic processes emanated from the development of overweight and alteration in β-cell sensitivity in post-menopausal women due to suppressed oestrogen production. However, hormone replacement therapy reverses the above mentioned effect. Godsland (2005) showed that oestrogens are chief mediators of glucose homeostasis through a direct regulatory role on insulin signaling. As with the onset of menstrual cycle mediated by oestrogen, modulation of glucose metabolism during the luteal phase becomes more profound and almost non-responsive to antidiabetic drugs (Case and Reid, 2001). According to Takeda *et al.* (2003), evidence of synthesis from scientific studies have also shown that the impact of oestrogen activities on metabolic process came about after the onset of dyslipidemia, insulin resistance, and obesity following genetic mutations in aromatase that catalyses the synthesis of estrogens from androgens. Other findings have also supported the above mentioned findings showing the central role of estrogen receptors in both lipid and glucose homeostasis. Knockout mice for either estrogen receptors or the aromatase gene show accumulation of fats especially in the hepatic tissues (Takeda *et al.*, 2003). Nemoto *et al.* (2000), using knockout mice for aromatase enzyme demonstrated a relationship between β-oxidation associated genes and liver steatosis which was reversed following the administration of estrogen.

The role of estrogen receptor remains unclear as no particular phenotypical characteristics has been associated with mice without the estrogen receptor (Ohlsson *et al.*, 2000). Foryst-Ludwig *et al.* (2008) demonstrated that the administration of high-fat diet to knockout mice lacking estrogen receptors caused over-weight and accumulation of fats in the adipocytes compared to the wild-type mice. Lovet *et al.* (2004) utilized gender-specific studies to demonstrate the role of estrogen in glucose metabolism. In alloxan-induced diabetic and ovariectomized female rats, elevated blood glucose became more profound whereas perfusion of estrogen in male rats similar model normalizes the blood glucose levels (Lovet *et al.*, 2004). Heine *et al*. (2000) and Takeda *et al.* (2003) have associated glucose intolerance and poor β-cell response to the absence of aromatase and estrogen receptor.

In another study using knockout mice for both aromatase and estrogen receptors assays on muscle tissues showed a dysfunctional activity of glucose transporter 4 (GluT4) responsible for cellular importation of glucose (Barros *et al.*, 2006). Also varying estrogen levels especially during the estrous cycle disrupts endocrine pancreatic secretion of insulin. However, Aloso-Magdalena *et al.* (2008) showed that both endocrine disruptors and estrogens affect insulin secretion and provide cellular protection from oxidative damage, streptozotocin – induced organ damage and apoptosis (Le May *et al.,* 2006). Furthermore, many researchers have reported that estrogens mediate a specific case of glucose induced insulin release in special type of islets (Murtensson*et al.*, 2008). In addition to this, the hypothalamus and the central nervous system are indirect points of action of estrogens for the modulation of energy balance. In reponse to metabolic and endocrine crosstalk, the hypothalamus activates several metabolic pathways that ultimately produces metabolic, endocrine and behavioural changes leading to homeostatic balance. For this, both insulin and leptin are central to the hypothalamus-induced energy balance which ultimately enhances food consumption (Schwartz *et al.,* 2000).

As described by Meli*et al*. (2004), the importance of estrogen in the maintenance of energy balance cannot be overemphasized as deficiency of aromatase or alteration in the functional integrity of its receptors reduces energy expenditure and physical activity while estrogen supplementation improves locomotion (Ogawa *et al.*, 2003). The hypothalamic nuclei house the estrogen receptors and are the chief mediators of food intake and locomotory processes (Ogawa *et al.*, 2003). Meli*et al*. (2004), found that target specific silencing of the endocrine receptors in the hypothalamus of animals produced similar phenotypic features to those showing dysfunctional metabolic signals, energy balance, hyperphagia and obesity.

Despite the uncertainties associated, alterations in neuropeptides in the hypothalamus, no relationship has been found with the phenotypic changes and the corresponding distortion of energy expenditure. Gao *et al.* (2007) supported a concept describing a direct interference of estrogens on regulation of accumulation of fats by the hypothalamus by modulation of synaptic activities via lectin-receptor mediated control on Stat 3 downstream signaling ultimately leading to weight loss. Thus, oestrogen in general are chief modulators of metabolic activities through direct and indirect influence on energy expenditure. Another high debuatable hypothesis is the influence on energy homoestasis by phytoestrogens through the mimicry of the metabolic signatures of estrogen.

2.7.8c Mechanisms of action of phytoestrogen

Phytoestrogens show structural similarities to estradiol, an oestrogen found in humans and attach to both α and βoestrogen receptors but with higher affinity and binding accuracy to the β-receptors (Paterni *et al.*, 2014). Yanagihara *et al.*, (2014) reported that the binding of estrogen to estrogen receptors, cellular localization occurs to the nucleus from the cytosol thereby modulating genetic expression via the activation/deactivation of transcriptional elements at the DNA promoter region. Another suggested mechanism of action of steroids is the attachment to cell surface receptors causing the synthesis of nucleotides in the cytosol thereby regulating the activities of specific genes through the binding or inhibition of transcription factors (Yanighara *et al.*, 2014). This means that phytoestrogens can mimic the activities of estrogens especially the aromatase inhibition and mediation of the secretion of globulin that specifically binds to sex hormones (Wang *et al.*, 2002). The location of estrogens in the human system cuts across several systems like the reproductive and nervous system, the lungs, placenta, gonads, and bones. This ultimately means that phytoestrogen possess the capacities to influence the activities of specific tissues (Rietjens *et al.*, 2013), in addition estrogen-receptor mediated control for instance α-estrogen receptors enhances cellular-proliferation while the β-receptors enhances mainly apoptosis of the cell (Rietjens *et al.,* 2013).

In addition to strong affinity for estrogen receptors, estrogen also induces other biological processes unrelated to receptor activities. Such biological processes include free radical scavenging potentials, serotonin and insulin growth factor receptors activation, acts as precursors for methylation of DNA, activation of several kinases like tyrosine, cyclic AMP-dependent protein kinase, myoinositol-activated protein kinase, phosphatidylinositol protein kinase, nuclear factor for kappa light chain enhancer of activated β-cells, histone protein and topoisomerase modulation (Rietjens *et al.* 2013). These activities of phytoestrogens cumulatively lead to antimutagenic, antioxidant, antiangiogenic and antiproliferative potentials (Ming *et al.*, 2013). However, it is difficult to substantiate whether phytoestrogens promote metabolic activities via the interplay of hormones or not, in particular metabolic processes mainly due to paucity of relevant information. This is because studies tend to pattern more to clinical applications rather than basic investigative studies centering on their mechanisms of actions.

2.7.8d Effects of soya protein and phytoestrogens on human metabolism

Surprisingly several conflicting results exist on the clinical outcomes of soya beans consumption. However, given that obesity and related metabolic complications are less prevalent in Asian populations, much attention has been given soya beans that comprise a greater percentage of Asian cuisines. Yang *et al.* (2004) has shown from epidemiological reports that in Japanese residents in Tokyo, type-2-diabetes is four times less prevalent than in Japanese residents in America. In that study, it was also reported that the consumption of soya bean correlated with lower risk of glycosuria, a well-documented indicator for diabetes. Furthermore, low risk of obesity and elevated high density lipoproteins were recorded in post-menopausal women placed on soya isoflavone-rich diet (Goodman- Gruen and Kritz-Silverstein, 2003).

Subsequent studies also found that the consumption of soya beans improves metabolic outcomes. A review of thirty-eight clinical trials showed that soya bean regular consumption enhanced the reduction of total cholesterol and low density lipoproteins (Greany*et al.*, 2004). Wu *et al.* (2006) also showed that the administration of soya isoflavones for twenty-four weeks produced lower fat mass in post-menopausal women. Also, twelve weeks of consumption of soya protein significantly reduces plasma levels of LDL with a corresponding decrease in body mass index (Allison *et al.*, 2003). Another six months' clinical study comparing the outcomes of administering soy isoflavones and estrogenic substances recorded significantly reduced fasting insulin and blood sugar levels by both test foods (Cheng *et al.*, 2004). Contrary to the outcome of the above mentioned studies, Anderson *et al.* (2007) found no significant effect of isoflavones on metabolic parameters like insulin levels, glucose, lipid profiles, and body weight. These conflicting reports pose problems in authenticating the clinical effect of soya beans and soya isoflavones. However, a critical comparison of these conflicting studies shows that differences in experimental design, duration, dose, and route of administration, as well as genetically influenced individual differences affects the studies in significant ways. Hence, more similarly designed studies are required to substantiate the metabolic effect of soya isoflavones.

2.7.8e Reproductive effect of phytoestrogens

Both positive and negative reproductive outcomes have been associated with the consumption of phytoestrogens. Genistein has been reported to induce the release of progesterone in the ovaries, as well as zygote implantation and maturation of the oocytes (Kim and Park, 2012). Other phytoestrogens have reversed cell proliferation, secretion of steroid hormones in the ovaries and enhanced apoptosis. Soya bean on the other hand has been found to impair sexual development by delaying puberty and oestrous cycle as well as altered the functional integrity of ovaries, pituitary glands and the hypothalamus. Though the reproductive toxicities associated with soya isoflavone consumption is not yet well documented, some studies have reported this possibility (Kim and Park, 2012).

In neonates, the implication of the consumption of soya bean rich foods has been carefully evaluated regardless of no physiological changes recorded (Cederroth *et al.*m 2010; Vandenplas *et al.*, 2011; Jefferson *et al.* 2012). Bedell *et al.*, (2012) demonstrated that the administration of soya beans phytoestrogens including coumestans, lignans, and isoflavones during any of the pre or post-menopausal conditions impairs symptoms of menopausal conditions, brought about by suppressed estrogen synthesis, disruption of vasomotor functions, atrophy of the vagina, and hot flashes, whereas the endometrium and breast remain unaffected. Several post-menopausal women often prefer phytoestrogens in soy rich foods like black cohosh (Kronenberg and Gugh-Berman, 2002) to hormone replacement therapy (Poluzzi *et al.*, 2014). In contrast to hormone-replacement therapy, lignans do not affect blood clotting during post-menopause and therefore are recommended as alternatives to hormone therapy (Bedell *et al.*, 2012).

In the study of Rosic *et al.* (2013), the psychological effect of soy isoflavones and phytoestrogens were studied showing a significant decrease in the somatic vegetative symptoms whereas the symptomatology of the urogenital glands were unaffected. Also no significant changes were recorded during the administration of red clower on density and thickness of the breast and lining of the endometrium, as well as the plasma gonadotropin levels (Powles *et al.*, 2008). Furthermore, the oil and aqueous extract of primrose, ginseng, dong quai, and red clower reduced the frequency of hot flushes during menopause (Eden, 2012). An inconclusive result was reached during the evaluation of the clinical effect of hop phytoestrogens on the above parameters (Keiler *et al.*, 2013). On male subjects, Hess *et al.* (2011) proposed that estrogens produced adverse outcomes on sperm quality and mobility as well as on the testosterone and follicle stimulating hormones. Despite some negative reports associated with the exposure to endocrine disruptors with structural similarities to estrogens, no particular report has implicated phytoestrogens (Cederroth *et al.*, 2010; GiwercMan, 2011). Recent review studies have shown that no significant connection exists between the consumption of soya isoflavones and the levels of androgen and estrogen (VanDie *et al.*, 2013), notwithstanding that both estrogen and androgen receptors are crucial to sustain prolonged fertility and the integrity of the epididymis and ducts (Hess *et al.*, 2011). Nevertheless, phytoestrogens have shown no positive outcome on male reproductive function compared to females.

2.8 Soya bean and health benefits

Soya bean is an important food source due to its perceived nutritional content and pharmaceutical relevance. Soya bean is rich in peptides and secondary metabolites such as phytosterols, isoflavones, trypsin inhibitors, phytates and saponins (Isanga and Zhang, 2008). Isoflavones such as genistein and diadzein as well as their metabolites; biochanin A and formononetin, and other glycosides, have been isolated from soya beans (Sabudak and Guler, 2009). These above mentioned flavonoids have been reported to produce anti-atherogenic effects and hypocholesterolaemic properties (Isanga and Zhang, 2008). In addition, two new flavonoids, Furowanin-A and Millewanin-F have been isolated from soya beans (Ito *et al.*, 2006). Isanga and Zhang (2008) found that phytochemical-rich extracts of soya beans produced cholesterol-lowering effects, cardiomodulatory, anticancer, and anti-diabetic effects. As a good source of calcium and structural proteins, soya beans are regarded as a dependable bone-building food as well as an anti-osteoporesis meal. The abundance of diadzein and genistein in soya also promotes its bone forming capacity as well as the enhancement of calcium storage.

Other flavonoids subclasses besides, isoflavones exists, for example anthocyanins, chalcones, flavonols and aurones. The breakdown of the isoflavones from soya beans has been described by Chen *et al.* (2003) and Setchell *et al.* (2005), as being subjected to enzymatic hydrolysis of the sugar moiety by β-glucosidases and brush border enzymes leaving the α-glycone products to further bowel metabolism.

The primary health benefit of the soya isoflavones is the potent antioxidant effect, whereas some studies have reported that soya isoflavone improves dysmenorrhea and mitigates obesity (Devi *et al.,* 2009; Dixit *et al.,* 2011). The clinical and epidemiological studies on soya isoflavones have shown that its consumption ameliorates breast cancer. Both yellow and black soybeans were reported to potentiate free radical scavenging effects in ovariectomized rats (Byun *et al.*, 2010), translating to a marked decrease in oxidative stress in post-menopausal women. Furthermore, Li *et al.* (2009) found that genistein suppresses telomerase expression via the involvement of both epigenetic and enetic processes. This finding established a new perspective to the mechanism of action of soya bean during the treatment of cancerous breast cells, i.e., by telomerase inhibition. However, the whole soya extract has been found more potent than pure genistein extract during carcinogenesis treatment (Kim *et al.*, 2008). Endothelial modulation has also been an attributed advantage of the consumption of soya bean via binding to the vascular endothelium and stimulation of inflammatory cascade (Nagarajan, 2010). Also, the anti-atherogenic effect of soya isoflavones is attributable to the modulation of monocyte activation (Nagarajan, 2010). As a very potent source of folic acid, soya bean plays a central role in amino acid metabolism and neurotransmission, cell division and gene expression (Djukic, 2007).

2.8.1 Soya isoflavones and heart disease

Dietary guidelines have recommended a preference of plant-based diet over animal-based foods for the regulation of blood lipid levels. According to the American Diabetes Association, plant-based foods are more nutritionally enriching and therapeutic, as compared to animal based diets (Ferdowsian and Bernard, 2009; Craig and Mangels, 2009). Several clinical and epidemiological findings have established that the consumption of isoflavone-rich diets potentiates antihypertensive, cholesterol-lowering and anti-diabetic effects (Ghosh and Scheepens, 2009). The studies of Sagara *et al.* (2004) found that soy proteins and isoflavones significantly reduces diastolic and systolic blood pressures as well as modulates cholesterol homeostasis in hypertensive conditions. Taku *et al.* (2010) found that the regular intake of soya isoflavones significantly decreased both blood pressures, heartbeat rate compared to control and prehypertensive subjects placed on placebo. Figure 2.8 shows an overview of health eefects of soya beans.

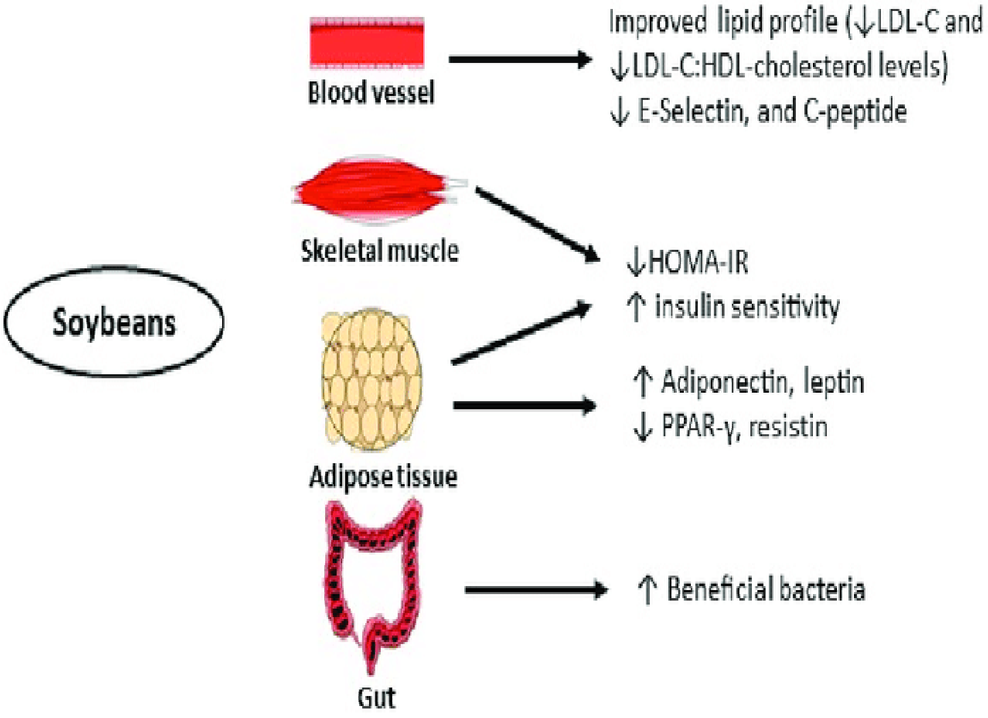

Fig. 2.8: Overview of health effects of soya beans

Nagarajan (2010) reported that the primary target of soy isoflavones is the amelioration of dyslipidaemia which is the major predisposing condition to cardiac involvements like myocardial infarction and atherosclerosis, and traditionally associated with metabolic syndromes and systemic inflammation. In addition, dyslipidaemia is as well a primary risk outcome for vascular diseases and stroke, thereby making soybeans a valuable nutraceutical (Sacks *et al.*, 2006).

2.8.2 Soya bean and biochemical parameters

Soya bean has been reported to significantly increase total plasma protein and albumin levels, which justifies its rich protein contents (Alada *et al.*, 2004). Further, in protein malnourished rats, soya bean significantly improved the plasma protein levels up to the recommended level (Medic *et al.*, 2003). Plasma electrolytes which are kidney function indicators have been shown to be significantly elevated, especially sodium, potassium and chloride, after the administration of soya bean rich meals in animal and human models (Alada *et al.*, 2004).

For the haematological parameters, soya bean mainly increases the packed cell volume and haemoglobin concentrations without any observable differences in effects among soya bean varieties (Alada *et al.*, 2004). Soya beans are recommended tropical diets for both Marasmus and Kwashiorkor patients because they are well known haematinics. Alada *et al.*, 2004).

2.8.3 Goitrogenic and estrogenic substances in soya beans

Glycitein-o-α-glucoside and glucosides genistein are the main goitrogenic compounds in soya beans. Other goitrogenic compounds found in minute amounts include coumesterol and 6,7,4-

trihydroxyflavone. Both diethylstilbesterol considered to be >100 times more active than genistein and diedzein more than 3 times active than genistein are among the oestrogenic compounds found in minute quantities in soya beans. In addition, notwithstanding that the iodine content of soya bean is insignificant, studies have shown that raw soya bean administered to rats caused thyroid hypertrophy. Also, both genistein and diadzein have both been reported to induce goiter and autoimmune dysfunction via the inhibition of thyroxine peroxidase. A clinical observation in Japan found that soya bean caused hyperthyroidism in healthy volunteers (Ishizuki *et al.*, 1991).

2.8.4 Soya beans and insulin resistance

Medicinal compounds from soya beans have been examined for their potentiation of insulin sensitivity, and the study found the stimulation of GLUT4 gene expression as the predominant mechanism of action (Kwon *et al.*, 2010). A study by Matsukawa *et al.* (2015) showed that the treatment of induced-insulin-resistant preadipocytes with soya beans resulted in improved GLUT4 expression in agreement with the findings of Wang *et al.* (2017). This led to improved glucose utilization in the preadipocytes. Inaguma *et al.* (2011) and Kwon *et al.* (2010) both confirmed that the high anthocyanin levels of soybeans were responsible for the insulin-sensitizing effects of soya beans.

Nizamutdinova *et al.* (2009) also demonstrated the improvement of insulin resistance achieved by soya beans using diabetic rat model.

2.8.5 Anticarcinogenic effects of soya beans

Soya bean has been widely reported to produce anticarcinogenic effects on the breast, colon, and prostate (Nagata *et al.*, 2002). A case controlled observational study demonstrated an inverse relationship between the consumption of soya bean and the risk of breast cancer in Singapore, whereas another study was unable to associate this outcome in China (Yuan *et al.*, 2003). In animal models of radiation-induced breast tumours, soya bean was found to slow down the progression of breast cancer (Constantinou *et al.*, 2008). Also, the potency of soya bean has been demonstrated against colon cancer and prostatic dysplacia in animal models (Makela *et al.*, 2001). Also, a variety of different cancer cell lines have been used both *in vivo* and *in-vivo* to describe the proliferative and apoptotic potentials of soya beans which in many cases have proven either mildly positive or inconclusive (Thiagarajan *et al.*, 2008). In patients with prostate cancer, the dietary supplementation with soya beans decreased the secretion of cancer markers like prostate specific antigen, testosterone and androgen receptor. This also was found in men at risk of prostate cancer development (Hamilton-Reeves *et al.*, 2007). However, in non-cancer subjects or patients not at risk of prostate cancer, the intake of soy produced no effect on levels of cancer markers indicating that the amelioration of reproductive cancer was specific to developed or developing cancerous cells (Jenkins *et al.*, 2003).

2.9 CANCER MARKERS

2.9.1 Prostate specific antigen (PSA)

PSA is a glycoprotein produced by cell either benign or malignant, of the prostate gland. Measurement of serum prostate-specific antigen (PSA) has become the most common event leading to the diagnosis of prostate carcinoma and may be the most commonly used cancer clinical test. The introduction of routine PSA-based screening over the past 20 years has led to a dramatic increase in the rate of disease detection and a subsequent stage shift at the time of diagnosis. For the past decade or more, a PSA value of 4.0 ng/mL has been considered to be the upper limit of normal (ULN). Challenging this long-held notion was a recent report from the Prostate Cancer Prevention Trial (PCPT) (Thompson *et al*., 2004). In an analysis of 2950 PCPT participants randomized to the study’s placebo group and who never had a PSA level 4.0 ng/mL or an abnormal digital rectal examination (DRE), we found that PSA levels of 0–4.0 ng/mL were associated with a positive predictive value of between 6.6–26.9%. Overall, 14.9% of men with prostate carcinoma were found to have high-grade disease, with this rate reaching 25% among men with a PSA level of 3.1– 4.0 ng/mL (Zhu *et al*., 2005).

2.9.1.1 PSA as a Tumour Marker

Prior to the introduction of PSA, prostatic acid phosphatase (PAP) measurements were used as a marker for prostate carcinoma. Although somewhat useful in monitoring patients with advanced disease, its low sensitivity in localized disease prevented any consideration for its use in the screening of prostate carcinoma (Antenor *et al*., 2005). The initial concept of PSA as a screening tool arose because of its improved sensitivity over PAP (Antenor *et al*., 2005). The widespread use of PSA in the clinical setting did not begin until the mid-1980s. The earliest observations had important clinical applications and included: 1) a decrease in the PSA level after hormonal therapy appeared to be correlated with response to treatment (Amling *et al*., 2001); 2) an increase in the PSA level after treatment appeared to precede and herald disease recurrence; and 3) after radical prostatectomy, PSA should be undetectable; if not, disease recurrence is the rule. Several of these early investigators rejected the possibility of using PSA for screening because of a substantial overlap in PSA values between patients with and those without carcinoma and the resulting poor test specificity (Amling *et al*., 2004). Recognizing the critical nature of specificity in a screening tool for prostate carcinoma, Freedland *et al*. (2004) established an ULN for PSA of 24 ng/mL in a case–control study. This value was selected because it was the mean plus two standard deviations (SDs) of a group of control men who had 1) a histologic diagnosis of benign prostatic hyperplasia (BPH) or 2) had no genitourinary symptoms. Of note, none of the patients had undergone a prostate biopsy. In a subsequent study by Meyer *et al*. (1999), investigators evaluated PSA in 2200 serum samples from 699 men, 378 of whom had prostate carcinoma. Controls in the study included 157 men ages 21–76 years who had a mean PSA concentration of 1.1 ng/mL, normal DRE findings, no prior history of any prostatic disease, no urinary symptoms, and no abnormal urinalysis results. By comparison, patients with prostate carcinoma had disease stages ranging from A1 (T1a) to D2 (M1) (TNM and Whitmore-Jewett staging systems). Using these definitions, the authors concluded that the normal value for PSA should be 2.5

ng/mL. They also expressed concern regarding the low test specificity as well as the degree of PSA variation within individuals over time (Fradet *et al*., 2009).

2.9.2 Tumour markers of ovarian cancer

The development of specific and sensitive biomarkers capable of diagnosing the onset of ovarian cancer remains a top priority. Studies have acknowledged this necessity to Early Detection Research Network (EDRN) have instituted guidelines to develop diagnostic markers (Bast *et al.*, 2005). However, those biomarkers used for the monitoring of ovarian cancer are also used to understand treatment and individual drug responses, and to differentiate benign from malignant tumours, as well as the early detection of diseases (Meyer and Rustin, 2000). Cancer Antigen 19-9 (CA19-9), Tumour-associated glycoprotein (TAG72) and Cancer Antigen 549 (CA549) are examples of tumor biomarkers targeted by various epitopes. In addition, Cytokeration 19 Fragment (CYFRA21-1) and the biomarker for proliferating cytokeratin are also documented for their uses during the diagnosis of ovarian cancer. However, CA125 represents the most documented marker for ovarian cancer (Meyer and Rustin, 2000).

2.9.3 Role of CA 125 in ovarian cancer

Cancer Antigen (CA 125) is referred to as the gold standard diagnostic biomarker for ovarian cancer (Hogdall, 2008). Studies have shown that CA 125, as a high molecular weight glycoprotein, is elevated in up to ninety percent of subjects with ovarian cancer (Hogdall, 2008). This glycoprotein is assayed using monoclonal antibodies that serves as agonists of ovarian cancer. CA 125 is localized in the epithelium of faetal tissues as well as epithelial cells of the coelom. It has two antigenic domains that strongly binds M11 and OC125 monoclonal antibodies. After achieving wide acceptance by regulatory bodies, CA 125 antigen is also used routinely to manage and monitor drug reponse during early or late stages of ovarian cancer (Markman, 2003). Also, high circulating concentration of CA 125 indicates serious and not mucinous cancers (Hogdall *et al.*, 2007). This biomarker is also used to case define disease recurrence, especially when cancer patients find the CA 125 levels normalized followed by a two-fold increase in the serum levels (Markman, 2003). Furthermore, recurrence can be diagnosed if the serum CA 125 levels fails to revert to normal levels following primary treatment. The relevance of CA 125 in the diagnosis and monitoring of ovarian cancer progress has been well documented (Verheijen *et al.*, 1999). Although some reports have shown that the levels of CA 125 increases three months before clinical detection (Yin and Lloyd, 2001). However, asymptomatic populations negates the adequacy of CA 125 sensitivity in screening for ovarian cancer (States *et al.*, 2003). Notwithstanding the widely recognized drawbacks in interpreting CA 125 activities, this biomarker enables the evaluation of the effectiveness of therapies for ovarian cancer (Duffy *et al.*, 2005).

2.9.4 CA 19-9

Carcer antigen 19-9, also known as sialyl-Lewis^{A}, is a tetrasaccharide which is usually attached to O-glycans on the surface of cells. It is known to play a vital role in cell-to-cell recognition processes. It is also a tumour marker used primarily in the management of pancreatic cancer.

2.9.4.1 CA19-9 as a diagnostic biomarker for pancreatic cancer

Serum levels of CA19-9 has been long established as a relevant diagnostic marker for both asymptomatic and symptomatic pancreatic cancer patients (Kim *et al.*, 2004). Kim *et al.* (2004) in an extensive cohort study involving 70940 subjects with asymptomatic conditions showed that only four of the subjects had higher than normal levels of CA19-9 and 1063 with mildly normal levels of CA 19-9 levels. In this study, the authors showed only a 0.9% positive predictive value despite a 98.5% specificity and 100% sensitivity. In the study of Satake *et al.* (1994), 12840 asymptomatic subjects were examined for their CA 19-9 levels as well as 8,706 subjects that satisfied the inclusion criteria of jaundice, epigastric pain and weight loss. Among the examined asymptomatic patients, 18 of these subjects showed elevated CA 19-9 levels were four actually had pancreatic tumour. Only 4.3% of the subjects that satisfied the case definition inclusion criteria, showed elevated CA 19-9 levels. After extensive diagnosis, 85 patients were diagnosed of pancreatic cancer with 28 resectable cases. Chang *et al.* (2006) recruited 5.343 asymptomatic subjects and found elevated CA19-9 levels in 385 of the patients. Of the group with elevated CA19-9 levels, only two patients were eventually diagnosed of pancreatic cancer. In this same study, the elevated serum CA19-9 showed a positive predictive value of only 0.5%, whereas the false positive elevation of the biomarker occurred in 325 subjects, also identifying the occurrence of more than fifty other kinds of cancers. This simply indicates that asymptomatic patients negates the use of CA19-9 as a screening tool for pancreatic cancer. This also applies to poorly defined cases of pancreatic cancer where the elevated CA 19-9 produced a predictive value of up to 0.9%. However, one significant outcome in the above mentioned studies was the presence of non-pancreatic neoplastic pathology in majority of the study participants that recorded high CA19-9 for pancreatic cancer in asymptomatic subjects. Symptoms like high pancreatic weight coupled with increased CA 19-9 improves the predictive value and pancreatic cancer diagnosis. A study involving 150 patients registered for pancreatic surgery without routine preoperative diagnosis showed an elevated CA 19-9 levels as well as elevated bilirubin and loss of weight (Tessler *et al.*, 2006). In this study, participants had a near 100% positive predictive value and specificity for pancreatic cancer was recorded despite challenges of the pancreas imaging (Tessler *et al.*, 2006). Older studies, of almost two decades back, that attempted to determine the diagnostic accuracy of CA19-9 for pancreatic cancer in a small sample size showed a 90% specificity and 81% median sensitivity (Steinberg *et al.*, 1990). In addition, a 95.7% false predictive value and 72.3% positive predictive value was recorded in the study. When the diagnostic threshold was increased to between 100 u/ml to 1000 u/ml, the sensitivity reduced to up to 41% with a concomitant increase in specificity of up to 99.8%. A more recent study that recruited 2,283 symptomatic subjects to test for the diagnostic accuracy of CA19-9 for pancreatic cancer, showed a false predictive value of 81%

and a 79% positive predictive value for sensitivity, whereas the specificity showed a 72% positive predictive value and 81% false predictive value (Goonetilleke and Siriwardena, 2007).

2.9.5 Classical Tumour Markers of Colorectal Cancer

Carcinoembryonic antigen is a widely used marker for epithelial tumours, especially for colorectal cancer. It is an oncofaetal glycoprotein antigen formed in the large bowel cells. The carcinoembryonic antigen test is an inexpensive test with up to 70% sensitivity rating for the monitoring and diagnosis of colorectal cancer (Queantmeier *et al.*, 1987). Despite being touted as a cancer marker, the carcinoembryonic antigen levels rise during inflammation of the hepatocytes and bowels, and pancreatitis. The normal circulating levels of the carcinoembryonic antigen is about 5 ng/ml. However, these values are raised up to 10 ng/ml in smokers showing symptoms of liver cirrhosis or ulcer colitis. In this study of Tan *et al.* (2009) that included 4285 participants monitored for recurrence of colorectal cancer using the carcinoembryonic antigen levels, the study recorded a 0.90% specificity, and a 0.64% sensitivity. Furthermore, Chen *et al.* (2010) monitored the use of carcinoembryonic antigen to detect relapses after operation. About 999 patients out of 4,841 total patients examined, showed increased carcinoembryonic antigen levels while 749 patients showed additional symptoms for recurrence. The study posited that the patients undergoing colorectal cancer treatment should be examined for the carcinoembryonic antigen levels trimonthly. However, it has been noted that the diagnostic accuracy of carcinoembryonic antigen levels is seldomly altered except in cases of adverse prognosis. CA 19-9 is another biomarker used for the diagnosis of gastric, colorectal and pancreatic cancers. Similar to the carcinoembryonic antigen, the specificity of this biomarker is low in terms of identifying the particular type of cancer and the specific organ affected. In comparison to carcinoembryonic antigen, CA 19-9 is less sensitive. However, when both biomarkers are used for diagnosis, the diagnostic accuracy for colorectal cancer increases. Moreover, the combined use of CA 19-9 and carcinoembryonic antigen is recommended after operation as a prognostic factor to determine the patient's chances of survival and the stage of the cancer. A study reported that elevated activities of Ca 19-9 and carcinoembryonic antigen were observed when colon cancer occurred in the sigma region (Nukatani*et al.,* 2012). The study further observed that the combined use of carcinoembryonic antigen and CA 19-9 for diagnosis had no effect on improving the sensitivity of the diagnosis. Rather, the diagnostic accuracy for carcionoembryonic antigen improves with advancing disease stage but showed no relationship with either number of the positive lymph nodes or the location of the tumour (Rupert *et al.*, 2009). Among these biomarkers, the tissue polypeptide specific antigen test, though very expensive, play a crucial role in the accurate diagnosis and monitoring of carcinomas affecting the gastrointestinal tract (Rupert *et al.*, 2009).

Secreted in various molecular cycle stages, this antigen formed as a linear polypeptide chain undergoes tissue migration after the splitting stage of mitosis. It is a moiety of cytokeratin after the hydrolysis of the carboxyl terminal (Rupert *et al.*, 2009). Rather than tumour mass, elevated tissue polypeptide specific antigen is indicative of tumour growth, and its serum levels in cancer proliferation as a reliable index of rate of division of the cell. The determination of this antigen may be useful in preoperative cancer stages. Acording to Mishaeli *et al.* (1998), using the tissue polypeptide specific antigen for colorectal cancer diagnosis attains a diagnostic accuracy between 60 and 80%. However, after early diagnosis of colorectal cancer using the tissue polypeptide specific antigen, the survival rate reduces with high concentration. The use of tissue polypepetide specific antigen has achieved clinical importance during the monitoring of non-response to treatments. This however controversially makes the tissue polypeptide specific antigen a more superior diagnostic marker for colorectal cancer compared to the carcinoembryonic antigen. Furthermore, physicians generally prefer the use of tissue polypeptide specific antigen for the determination of treatment duration due to its sensitivity.

Tumour associated glycoprotein-72 is another cancer diagnostic marker, formed in the cells of the renal pelvis, gastric epithelium and endomilium of the bile. As a mucin-like molecule, it is distributed across many tissue surfaces of the pancrease, colon, mammary glands and ovaries. It is recommended that the tumour associated glycoprotein-72 be examined alongside other tumour markers especially the carcinoembryonic antigen. Patients diagnosed of colorectal cancer showed elevated CA 19-9 in 27% of the recruited participants, while both carcinoembryonic antigen and the tumour associated glycoprotein-72 each were elevated in 43% of the recruited participants (Guadagni*et al.*, 1993). In that study, any of these markers were found elevated in 61% of the recruited participants.

The circulating tumour DNA is another predictive and monitoring biomarker for colorectal cancer. It is widely known as liquid biopsies capable of detecting colorectal cancer at early stages. Till date, the septin 9 cDNA hypermethylation analysis has been found to achieve the highest diagnostic accuracy for colorectal cancer. Bach *et al.* (2019) found up to 97% specificity and 100% sensitivity in the diagnosis of patients with colorectal cancer in both precancerous and post cancerous lessions of colorectal cancer. Luo *et al.* (2020) found a 86.8% specificity and 89.7% sensitivity using the circulating tumour DNA as a diagnostic tool for the assessment of colorectal cancer. Ma *et al.* (2019) conducted a septing hypermethylation assay and compared to the carcinoembryonic antigen assay, and found a 25% difference in sensitivity between both assays. Similarly, Toth *et al.* (2012) reported a 51.8% sensitivity for carcinoembryonic antigen assay and a 95.6% sensitivity for the septin 9 assay, with specificities of 85.2 for the carcinoembryonic antigen and 84.8 % for septin 9 assay. Notably, in the study of Toth *et al.*, (2012), septin 9 assay showed higher specificity. In contrary, Xie *et al.* (2018) reported that septin 9 assay produced higher sensitivity and specificity than the carcinoembryonic assay.

Another extracellular protein used for the diagnosis of colorectal cancer is the insulin-like growth factor binding protein 2 (IGFBP-2). This protein has been found to play a central role in the proliferation of cancer mediated by the heat shock protein-27. In three studies, the use of IGF BP-2 as a diagnostic marker for colon cancer was recently validated (Vocka *et al.*, 2019). Furthermore, since the host response to inflammation is a key prognosis to cancer proliferation, few inflammatory markers are believed to have reliable diagnostic accuracy for colorectal cancer. According to Dimitriu *et al.*, (2018), the neutrophyl lymphocyte ratio has been well documented as a diagnostic marker for colorectal cancer. However, when the levels exceed 4.7, the accuracy for disease free and 5-year survival becomes very doubtful, and especially when colorectal cancer progresses to stage II. In summary, IGFBP-2 can be reliably used, likewise carcinoembryonic antigen for the prognosis of overall survival of patients with colorectal cancer.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

Table 3.1: Equipment and sources

Equipment	Sources
Automatic Electrolyte Analyzer	Shimadzu UV-160A, Lakewood Carlifonia, USA
BT-3000 auto analyzer	Diamond Diagnostics Inc, Holliston, MA, USA
Centrifuge	Shimadzu UV-160A, Lakewood Carlifonia, USA
Colorimeter	Lovibond™ PFXi-995, Tintometer Limited, Amesbury, UK
Dessicator	East Biopharm, Hangzhou, Zhejiang, China
ELISA plate reader	Omega Bio-Tek Inc. - Norcross, Georgia USA
Gas Chromatography	Agilent Technologies 7890A, Santa Clara, Carlifonia, United States
Glucometer	Roche Diagnostics Indianapolis, IN, United States
Hematology Auto-Analyzer	Mindray,Boulevard, New Jersey, USA
Ichroma Machine	Shimadzu UV-160A, Lakewood Carlifonia, USA
Incubator	Shimadzu UV-160A, Lakewood Carlifonia, USA

Microscope	Shimadzu UV-160A, Lakewood Carlifonia, USA
Oven	Shimadzu UV-160A, Lakewood Carlifonia, USA
pH Meter	Uniscope , SM801A, England
Rotary Evaporator	SHB-520, Korea
Soxhlet Extractor	Uniscope , SM801A, England
Steam Bath	East Biopharm, Hangzhou, Zhejiang, China
Thermometer	East Biopharm, Hangzhou, Zhejiang, China
Water Bath	Biotechnics, Aberdeenshire, Scotland UK
Weighing Balance	South Cross Road Bradford

3.1.2 Chemicals/Reagents

Table 3.2: Chemicals/reagents and sources

Chemicals/reagents	Sources
4-dinitrophenyl hydrazine solution	British Drug House (BDH), England
Acetic acid	Sigma Aldrich St. Louis, MO, USA
Biochemical reagent kits	Randox lab Ltd, Antrim, UK.
Butanol	Sigma Aldrich St. Louis, MO, USA
CA 19-9 ELISA kit	Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA
CA-125 ELISA kit	Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA
Carbonate buffer	British Drug House (BDH), England
CEA ELISA kit	Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA
Dimethylether	Sigma Aldrich St. Louis, MO, USA
Ethanol	British Drug House (BDH), England
Ethylene diamine tetraacetic acid	British Drug House (BDH), England
Follicle Stimulating Hormone test kits	Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA
Glutathione reductase	Qualiken fine chemicals, New Delhi, India
Hexane	Sigma Aldrich St. Louis, MO, USA
Hydrogen peroxide	Sigma Aldrich St. Louis, MO, USA
Insulin kits	Syntron Bioresearch (USA).
Luteinizing Hormone Test kits	Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA
Potassium phosphate buffer	Qualiken fine chemicals, New Delhi, India
Propanol	British Drug House (BDH), England
Randox liver function test kits	140 London Wall, London, England

Randox renal function test kits	140 London Wall, London, England
Sodium azide	Omega Bio-Tek Inc. - Norcross, Georgia USA
Sodium bicarbonate	British Drug House (BDH), England
Sodium hydroxide	British Drug House (BDH), England
Sodium phosphate buffer	Qualiken fine chemicals, New Delhi, India
Sodium sulphate	Sigma Aldrich St. Louis, MO, USA
Sulphuric acid	Sigma Aldrich St. Louis, MO, USA
Testosterone Test kits	Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA
Thiobarbituric acid	Sigma Aldrich St. Louis, MO, USA
Trichloroacetic acid	British Drug House (BDH), England

3.2 Methods

3.2.1 Sample procurement and preparation

A brand of 99% monosodium glutamate (MSG) was obtained from Relief Market Owerri Imo State, Nigeria. Soya bean used for this study was equally obtained from Ekeonunwa Market Owerri Imo State, Nigeria. Aqueous extracts were obtained on weekly basis for the duration (181 days) of feeding adopted in this study. It was stored and kept away from direct sunlight.

3.2.2 Analysis of oestrogenic substances

The oestrogenous substances extraction was carried out following the modified method of Liggins (2008). The dried sample of soya beans was pulverized in a laboratory mortar and pestle. The pulverized samples were weighed and kept for analysis. Precisely, 0.250 g of the sample was dissolved in 20 ml of the 80% ethanol and homogenized. It was filtered and washed with 80% ethanol. The filtrate was evaporated at 45^0C. Five (5) ml of 0.1M acetate buffer, of pH 5.0, was added to hydrolyse the medium. The sample was later extracted three times with 3 ml of ethyl acetate. The extract was poured into a round bottom flask of the rotatory evaporator arrangement. It was separated by evaporating the solvent off the extract. Then the concentrated extract was dried of water by using the anhydrous sodium sulphate before gas chromatography analysis. The chromatographic conditions were; Gas Chromatogram HP 6890 synched with software (chemstation 09.01[1206]), injection type was split injection and split ratio was 20:1, the carrier gas was nitrogen, inlet temperature was 250^0C; capillary type AC-5, column dimensions was 30m x 0.25mm x 0.25µm, oven program initial temperature was at 5min for 1hr, 10^0C/min 1^{st} ramping for 20 minutes, 2^{nd} ramping at 15^0C/min for four minutes, detector was flame ionization detector with detector temperature of 320^0C, carrier gas was nitrogen and compressed air pressure 40psi. Regression was used for the verification of concentration response dependence linearity. The peak properties, mainly their retention times aided the identification when compared to the standards. The compounds were quantified from each calibration curves with methanol as internal standard for the phytochemicals.

3.2.3 Acute Toxicity (LD_{50}) determination

Acute toxicity study of the two samples were carried out according to the method of Lorke (1983) using 39 albino mice of both sexes of average weight between 13.2g – 19.2g. They were dosed orally with different gradual does (10 – 5000mg/kg body weight). The LD_{50} was calculated using the formular below:

$$\mathbf{LD_{50}=LD_{100} - \sum (a \times b)/n}$$

n = total number of animals in a group. a = the difference between two successive doses of administered extract. b = the average number of dead animals in two successive doses. LD_{100} = Lethal dose causing the 100% death of all test animals.

3.2.4 Animal Husbandry

A total of two hundred and ten (210) weanly Wistar rats (70 – 78g) were acquired from Biochemistry Department, Federal University of Technology, Owerri, Imo State. The rats were acclimatized for 14 days, maintained *ad libitum* on water and growers mash bought from Owerri.

The rats were divided equally into three groups (70 rats each) representing the various experimental durations (2, 4, and 6 months). Each of these groups containing 70 rats were further divided equally into fourteen (14) subgroups each containing 5 rats, labelled, and orally administered according to the established LD_{50} as shown in the table below:

Table 3.3: Dosing schedule of rats with MSG and soybeans

Groups	Administration
1	Female rats administered daily 1000 mg/kg b.w (low dose) MSG
2	Female rats administered daily 2000 mg/kg b.w (medium dose) MSG
3	Female rats administered daily with 3000 mg/kg b.w (high dose) MSG
4	Female rats administered daily 1000 mg/kg b.w (low dose) soybean
5	Female rats administered daily 2000 mg/kg b.w (medium dose) soybean
6	Female rats administered daily with 3000 mg/kg b.w (high dose) soybean
7	Female rats fed normal rat chow and water
8	Male rats administered daily 1000 mg/kg b.w (low dose) MSG
9	Male rats administered daily 2000 mg/kg b.w (medium dose) MSG
10	Male rats administered daily with 3000 mg/kg b.w (high dose) MSG
11	Male rats administered daily 1000 mg/kg b.w (low dose) soybean
12	Male rats administered daily 2000 mg/kg b.w (medium dose) soybean
13	Male rats administered daily with 3000 mg/kg b.w (high dose) soybean
14	Male rats fed normal rat chow and water

After completion of the feeding duration, the animals were sacrificed by cervical decapitation under mild anaesthesia of ethyl ether. Both blood (collected by cardiac puncture) and sera was prepared for different analysis to be carried out.

The feed intake was calculated from the sum of daily feed intake obtained as the difference between the provided feed and leftover feeds. The weight gain was obtained from the difference in initial and final weights of the rats. The organ weights were obtained from the weights of each eviscerated organs in the electronic weighing balance.

3.2.5 Biochemical analyses

3.2.5.1 Blood sugar test

With a sterile lancet, blood samples were obtained from the tail of the rat. Fasting blood glucose was determined using glucose test strips. The strip was inserted into a glucometer for 5seconds, the process was repeated 3 times for all the animals and the values recorded.

3.2.5.2 Estimation of serum insulin level

The concentration of insulin in serum samples was estimated using Enzyme-Linked Immunoabsorbent Assay (ELISA) method with insulin kit from Syntron Bioresearch (USA). The sample used was non-haemolysed serum. Following a standard procedure, a sample of the standard curve was plotted and insulin concentrations in the samples were determined by interpolation from the standard curve (Elberry *et al*., 2015).

3.2.5.3 Determination of hepatic transaminases (Reitman and Frankel, 1957).

For alanine amino transaminase, fifty microlitres of the sample and five hundred microlitres of the ALT reagent (4-dinitrophenyl hydrazine solution) were mixed in a test tube, and the first absorbance at 340 nm was read after a minute. The timer was started at the same time and further readings of the absorbance were taken after one, two, and three minutes, and ALT activity was calculated as follows:

ALT activity (nm/min) = 1746 × Δ A 3 40 nm/min, ΔA 340nm/min = change in absorbance per minute for the sample, 1746 = Extinction coefficient.

For aspartate transaminase, fifty microlitres of the sample and five hundred microlitres of the AST reagent were mixed in a test tube, and the initial absorbance at 340 nm was read after a minute. The timer was started at once and further readings of the absorbance were observed after one, two, and three minutes. The activity of the enzyme was calculated as follows:

AST activity (nm/min) = 1746 × Δ A 3 40 nm/min, ΔA 340nm/min = change in absorbance per minute for the sample, 1746 = Extinction coefficient.

3.2.5.4 Determination of Serum Alkaline Phosphatase Activity (Belfield and Goldberg, 1971)

Ten microlitre of the sample was dispensed into a cuvette and mixed with five hundred microlitres of the reagent. The initial absorbance was read at 405nm and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation of the enzyme activity:

ALP activity (IU/l) = 2742 × ΔA 405 nm/min. Where: 2742 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the sample.

3.2.5.5 Determination of Total Bilirubin Concentration (Dufour, 2012)

Two hundred microlitre of reagent 1 was dispensed into test tube 1 (blank). One drop (Fifty microlitres) of reagent 2 was dispensed into test tube 2 (sample). One thousand microlitres of water was dispensed into the above two test tubes. Two hundred microlitres of test sample was also dispensed into the two test tubes. They were mixed and incubated for ten minutes at 25°C. One thousand microlitres of reagent 4 was dispensed into the two test tubes. They were mixed and incubated for a further five to thirty minutes at 25°C. The test tubes were inserted in a spectrophotometer and absorbance was read and recorded.

Total Bilirubin (mg/dl) concentration was calculated as

185 x Atb

185= a constant, Atb=Absorbance of Total Bilirubin, (578nm) = wavelength.

3.2.5.6 Determination of Serum Total Protein (Lubran, 1978)

Three test tubes were assembled, T_1 (blank), T_2 (standard) and T_3 (test sample). T_1 contained 3.0 millilitre distilled water and 5.0 millilitre biuret reagent, T_2 (standard) contained 3.0 millilitre standard protein solution and 3.0 millilitre biuret reagent. The contents were thoroughly mixed, incubated in a water bath (25˚C) for thirty minutes; absorbance was read at 560nm against the blank in a spectrophotometer. The total protein concentration was calculated as follows:

$$\frac{A}{As} \quad X \quad \text{Conc. of standard (g/dl)}$$

A = absorbance of sample, As = absorbance of standard

3.2.5.7 Determination of Serum Albumin (Doumas *et al,* 2005)

Three test tubes were assembled, T_1 (blank), T_2 (standard) and T_3 (test sample). The blank contained 3.0 millilitre bromocresol green reagent and 0.20 millilitre distilled water, the standard contained 5ml bromocresol green reagent and 0.20 millilitre standard protein solution. The test sample contained 5ml millilitre bromocresol green reagent and 0.20 millilitre plasma sample and their contents were mixed and allowed to stand for ten minutes. The absorbance was read against the blank at 630nm in a spectrophotometer. The albumin concentration was calculated as follows:

$$\frac{\Delta A_{standard} \text{ X } [Alb]_{standard}}{\Delta A_{unknown}}$$

3.2.5.8 Determination of Urea (Quraishi *et al*., 2013)

Ten microlitres (10µl) of sample was dispensed into test tube 1 (sample). Ten microlitres (10 µl) of standard (urea) was dispensed into test tube 2 (standard). Ten microlitres (10 µl) of distilled water was dispensed into test tube 3 (blank). Subsequently, to all the test tubes, fifty microlitres (50 µl) of the reagent labelled 1 was dispensed. Mixing and incubation was performed at 37°C for 10min. Precisely, 2.50 ml of standard reagent labelled 2 and 3 were dispensed into all the test tubes. Incubation at 37°C for 15min and thorough mixing was carried out. For at least 8 hrs, there was the observation of a blue colour which was stable.

The contents of the test tubes were transferred into a cuvette and read in a spectrophotometer and against the blank; the sample and standard's absorbance were read and recorded.

Concentration of Urea in sample was calculated as

$$\frac{\Delta\text{Asample x Standard conc.}}{\Delta\text{Astandard}} \qquad \text{(mg/dl)}$$

3.2.5.9 Determination of Creatinine (Quraishi *et al*., 2013)

One hundred microlitres (100 µl) of diluted water was dispensed into a test tube (reagent blank). One hundred microlitres (100 µl) of standard reagent was dispensed into a test tube (standard). Then one hundred microlitres of the specimen was dispensed into a test tube (sample) and one thousand microlitres (100 µl) of working reagent into all the test tubes. The working reagent and sample were mixed and after 30 seconds the absorbance was read. After 2 min, the standard's absorbance as well as that of the sample were read and recorded. Concentration of creatinine in sample was calculated as

$$\Delta\text{Asample} \quad \text{x} \quad \frac{\text{Standard conc. (mg/dl)}}{\Delta\text{Astandard}}$$

3.2.5.10 Determination of Serum Electrolytes (Quraishi *et al*., 2013)

The concentration of potassium ions, sodium ions and chloride ions in serum were assessed using the series electrolyte analyzers that apply ISE (Ion Selective Electrode) technology.

The activity of the specific ion in the sample at the electrode was converted to an electrical potential which is measured with a voltmeter. The voltage is theoretically proportional to the ionic activity. The voltage is finally converted to an electrical signal and displayed as a value on the screen. The ion concentrations in the serum was determined as follows:

3.2.5.11 Determination of Bicarbonate (HCO^-_3) (Quraishi *et al*., 2013)

One hundred and fifty microlitre of blood serum and reagent were added into the sealed reaction chamber, the $HC0^-_3$ ions in the serum precipitated into the reaction and released CO_2, leading to an increase in the gas pressure inside the chamber. The changes were detected by the pressure sensor and signals were sent to the microprocessor and the amount of $HC0^{-}_{3}$ ion in serum was determined.

3.2.5.12 Determination of Serun Triacylglycerol (TAG) Concentration (Tietz, 1990)

One thousand microlitres of triacylglycerol reagent was dispensed into test tubes containing ten microlitres of plasma, ten microlitres of standard and to an empty test tube (reagent blank). After vigorous mixing the test tubes were incubated for five minutes at 370 ºC. The absorbance was cautiously read within sixty minutes at 500 nm against blank reagent. The concentration of the triacylglycerol was calculated using the following:

$$\frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Standard}} \times \text{Conc. of standard}$$

3.2.5.13 Determination of Total Cholesterol Concentration (King and Wootton, 1959)

One thousand microlitres of the cholesterol reagent was dispensed into three separate test tubes containing ten microlitres of serum, ten microlitres of standard reagent and ten microlitres of distilled water respectively. The test tubes were incubated for five minutes at 370 ºC after they were vigorously mixed. The absorbance was cautiously read within sixty minutes at 500 nm against blank reagent, and the concentration deteremined as follows:

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 300$$

3.2.5.14 Determination of HDL-Cholesterol Concentration (Friedwald *et al,* 1972)

Two hundred microlitres of sample was mixed vigorously with five hundred microlitres of the reagent solution, the mixtures were set for five minutes at 37 ºC. The measurement was taken within sixty minutes against the blank and the absorbance was read at 546nm.

3.2.5.15 Determination of VLDL-cholesterol concentration (Friedwald *et al,* 1972)

Very low density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) were calculated as follows (Friedewald *et al*., 1972);

VLDL-c = $\frac{TG}{5}$, LDL-c = TC – HDL-c – VLDL-c

3.2.5.16 Determination of Malondialdehyde (MDA) (Ohkawa *et al*., 1979).

Normal saline (0.5ml) was pipetted into a test tube containing 0.5ml of the serum sample. About 2ml of thiobarituric acid (TBA)/trichloroacetic acid (TCA) mixture was added, allowed to boil for 1 hour, cooled to room temperature, and centrifuged at 4000rpm for 5min. The clear supernatant was read at 535nm. The concentration of MDA (nmol/ml) was calculated by using the following formula:

Concentration of the test = Abs(test) – Abs (blank) / 1.56 X 1000000

3.2.5.17 Determination of Catalase (CAT) activity (Aebi, 1984)

Distilled water (2.5ml) was pipetted into test tube containing 0.5ml H_2O_2, and about 40μl sample was added and mixed thoroughly**.** Rate of decomposition of hydrogen peroxide was read at 240nm at 30sec interval for 5 mins. CAT activity was determined by:

Measuring;

(Decrease in absorbance X 100/1) divided by protein amount in mg divided by time in min.

3.2.5.18 Superoxide Dismutase (SOD) activity (Marklund, 1980)

Sample extract (20ml) and 2.5 ml of 0.05 M carbonate buffer (pH 10.2) were mixed together and equilibrated in the spectrophotometer. In addition, 0.3 ml of 0.3 mM freshly prepared adrenaline was added and mixed by inversion. The increase in absorbance at 480 nm was monitored spectrophotometrically at 30 seconds intervals for 3mins.

3.2.5.19 Glutathione peroxidase (GSH-Px) activity

The GSH-Px activity was determined according to a modification of the method proposed by Paglia and Valentine (1967). The reaction medium was composed of potassium phosphate buffer 171 mM, sodium azide 4.28 mM, EDTA 2.14 mM, reduced glutathione 6 mM, NADPH 0.9 mM, and glutathione reductase 2 U.mL–1. The reaction took place at 22 °C (±1), starting with the addition of H_2O_2 0.72 mM. The absorbance of the samples was measured at 340 nm using a spectrophotometer. The measurements were taken every 15 seconds for 300 seconds. The GSH-Px enzymatic activity was expressed in enzymatic units per mL of sample ($U.mL^{-1}$).

3.2.5.20 Determination of Testeosterone

The serum testosterone levels were determined with a Testosterone Test System according to the manufacturer's instructions (Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA). Precisely 25µl of the serum reference calibrator was pippeted into an assigned well and 100 µl of the Testosterone Enzyme Reagent was added to each well. Afterwards the microplate was gently swirled for 30secs and covered. Next, the contents of the microplate was decanted by using an adsorbent paper to blot dry, then 350µl of the wash buffer was added and decanted by blot drying thrice. In a similar order of addition of the reagents, 100µl of the working substrate solution was added to all wells and incubated for fifteen (15) minutes at room temperature. Next, 50µl of stop solution was added to each well and gently mixed gently for 20 seconds and the absorbance in each well at 450nm was obtained using a reference wavelength of 620-630nm to minimize well imperfections in a microplate reader. By plotting a dose response curve with graph pad prism software, the concentration of testosterone in the test samples were determined and expressed as ng/ml.

3.2.5.21 Determination of Luteinizing hormone

The serum luteinizing hormone levels were determined with a Luteinizing Hormone Test System according to the manufacturer's instructions (Acculite Elisa Microwells, Monobind Inc. Lakeforest, USA). Precisely 25µl of the serum reference calibrator was pippeted into an assigned well and 100 µl of the L-H conjugate solution Reagent was added to each well.

Afterwards the microplate was gently swirled for 30secs and covered. Next, the contents of the microplate was decanted by using an adsorbent paper to blot dry, then 350µl of the wash buffer was added and decanted by blot drying thrice. In a similar order of addition of the reagents, 100µl of the working substrate solution was added to all wells and incubated for fifteen (15) minutes at room temperature. Next, 50µl of stop solution was added to each well and gently mixed gently for 20 seconds and the absorbance in each well at 450nm was obtained using a reference wavelength of 620-630nm to minimize well imperfections in a microplate reader. By plotting a dose response curve with graph pad prism software, the concentration of luteinizing hormone in the test samples were determined and expressed as mIU/ml.

3.2.5.22 Determination of Follicle Stimulating hormone

The serum follicle stimulating hormone levels were determined with a Follicle Stimulating Hormone Test System according to the manufacturer's instructions (Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA). Precisely 25µl of the serum reference calibrator was pippeted into an assigned well and 100 µl of the FSH Enzyme Reagent was added to each well. Afterwards the microplate was gently swirled for 30secs and covered. Next, the contents of the microplate was decanted by using an adsorbent paper to blot dry, then 350µl of the wash buffer was added and decanted by blot drying thrice. In a similar order of addition of the reagents, 100µl of the working substrate solution was added to all wells and incubated for fifteen (15) minutes at room temperature. Next, 50µl of stop solution was added to each well and gently mixed gently for 20 seconds and the absorbance in each well at 450nm was obtained using a reference wavelength of 620-630nm to minimize well imperfections in a microplate reader. By plotting a dose response curve with graph pad prism software, the concentration of FSH in the test samples were determined and expressed as mIU/ml.

3.2.5.23 Determination of Pancreatic Cancer Markers (CA 19-9)

The serum Pancreatic Cancer Markers (CA 19-9) were determined with a CA 19-9 ELISA kit according to the manufacturer's instructions (Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA). Precisely 25µl of the serum reference calibrator was pipetted into an assigned well and 100 µl of the biotinylated labeled antibody was added to each well. Afterwards the microplate was gently swirled for 30secs and incubated at room temperature for 30 mins. Next, the contents of the microplate was decanted by using an adsorbent paper to blot dry, then 350µl of the wash buffer was added and decanted by blot drying four more times. In a similar order of addition of the reagents, 100µl of the Ca 19-9 Tracer reagent was added to all wells, covered and incubated at room temperature for forty five (45) minutes. Next, the contents of the microplate was decanted again by using an adsorbent paper to blot dry, then 350µl of the wash buffer was added and decanted by blot drying five more times. In a similar order of addition of the reagents, 100µl of the working signal reagent was added to all wells, covered and incubated at room temperature for five (5) minutes. The Relative Light Units for each well was thus read, and a plot of the Relative Light Unit for the sample, against the corresponding reference was used to estimate the CA19-9 concentration expressed as ng/ml.

3.2.5.24 Determination of Colorectal Cancer Markers

The serum colorectal cancer markers (CEA) were determined with a CEA ELISA kit according to the manufacturer's instructions (Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA). Precisely 25µl of the serum reference calibrator was pippeted into an assigned well and 100 µl of the CEA Tracer Reagent was added to each well. Afterwards the microplate was gently swirled for 30secs and incubated at room temperature for 45mins. Next, the contents of the microplate was decanted by using an adsorbent paper to blot dry, then 350µl of the wash buffer was added and decanted by blot drying four more times. In a similar order of addition of the reagents, 100µl of the working signal reagent was added to all wells and incubated for fifteen (5) minutes at room temperature. The Relative Light Units for each well was thus read, and a plot of the Relative Light Unit for the sample, against the corresponding reference was used to estimate the CEA concentration expressed as ng/ml.

3.2.5.25 Determination of Ovarian Cancer Markers

The serum ovarian cancer markers (CA-125) were determined with a CA-125 ELISA kit according to the manufacturer's instructions (Accubind Elisa Microwells, Monobind Inc. Lakeforest, USA). Precisely 25µl of the serum reference calibrator was pippeted into an assigned well and 100 µl of the CA-125 Tracer Reagent was added to each well. Afterwards the microplate was gently swirled for 30secs and incubated at room temperature for 45mins. Next, the contents of the microplate was decanted by using an adsorbent paper to blot dry, then 350µl of the wash buffer was added and decanted by blot drying four more times. In a similar order of addition of the reagents, 100µl of the working signal reagent was added to all wells and incubated for fifteen (5) minutes at room temperature. The Relative Light Units for each well was thus read, and a plot of the Relative Light Unit for the sample, against the corresponding reference was used to estimate the CA-125 concentration expressed as ng/ml.

3.2.6 Histological Examination

Organ histological examination was carried out according to the methods of Banchroft *et al.* (1996). Tissues were taken from the kidney, heart, liver, uterus, testes, pancreas of the different animal groups, fixed in 10% formalin solution (pH = 7.2) for 24 hrs and washed with continuous flow of distilled water. The specimen were cleared in xylene, embedded in paraffin in hot-air oven set at 56^{o}C for 24h. Tissue block was prepared for sectioning at 4 mm thickness using a semi-automated rotatory microtome. The obtained tissue sections were collected on glass slides, dehydrated by immersing in serial dilutions of ethyl alcohol-water mixtures, cleaned in xylene and embedded in paraffin wax. Next, the specimens were deparaffinized and stained with hematoxylin-eosin (H&S) dye for histopathological examinations. Photomicrographs of the tissue sections were captured using charge-couple device (CCD) camera under light microscope at x400 magnification power.

3.2.7 Statistical Analysis

All data generated were subjected to statistical analysis. Values were reported as Mean ± Standard deviation (S.D) while one-way ANOVA was used to test for differences among treatment groups using Statistical Package for Social Sciences (SPSS) version 20 at 95% confidence interval ($p < 0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1: Oestrogen-like compounds

Table 4.1 shows the amounts of oestrogen-like compounds found in dehulled soybeans and soybeans oil. Eleven (11) compounds were isolated from the dehulled soybeans and soybean oil with 6-o-Malonylgenistin (98.47 %), Glycitin (98.11%), Genistin (96.69 %), coumestrol (88.80 %), and Genistein (84.64 %) being the most abundant oestrogen-like compounds.

Table 4.1: Oestrogen-like compounds in dehulled soya beans and soya bean oil

Name	**Dehulled Soybean (mg/100g)**	**Oil (mg/100g)**	**%CV**
Daidezein	42.63	7.96	68.53
Coumestrol	3.88	0.23	88.80

Genistein	28.49	2.40	84.46
Glycitein	1.98	0.28	75.22
Daidzein	0.54	0.11	66.15
Genistin	0.36	5.85 x 10^{-2}	96.69
Glycitin	0.26	2.47 x 10^{-2}	98.11
6-o-Acetyldaidzin	1.05 x 10^{-4}	1.71 x 10^{-5}	71.99
6-o-Acetylgenistin	6.74 x 10^{-5}	5.70 x 10^{-6}	84.40
6-o-Amalonyldaidzin	1.28 x 10^{-5}	7.78 x 10^{-6}	24.39
6-o-Malonylgenistin	5.01 x 10^{-5}	3.85 x 10^{-7}	98.47

4.1.2: Glucose and insulin levels

The glucose and insulin concentration of male and female rats administered varying doses of soybean and monosodium glutamate are represented in Table 4.2. In the first two months, no significant changes were observed in the glucose and insulin levels of the female rats following the administration of monosodium glutamate and soya beans. After 4 months the administration of high dose of MSG to the female rats significantly ($p<0.05$) elevated the glucose levels (126.75 mg/dl) when compared to the control (97.93 mg/dl) whereas the insulin levels of the female rats (42.96 mg/dl) significantly ($p<0.05$) reduced after 4 months administration of high dose MSG when compared to the control (42.96 ng/ml). After 6 months administration of both the medium dose (MD) and high dose (HD) MSG and only HD soya bean, significantly altered the blood glucose and insulin levels of the female rats. For the male rats, the blood glucose and insulin levels remained unchanged after two months administration of soya bean and MSG while after 4 months, the high dose of MSG significantly elevated the blood glucose levels (123.61 mg/dl) when compared to the control (102.85 mg/dl) and significantly lowered the insulin levels (52.01 ng/ml) when compared to the control (64.01 ng/ml). The administration of HD soya beans for 6 months to the male rats significantly elevated the blood glucose levels and decreased the insulin levels.

Table 4.2: Glucose (mg/dl) and insulin (ng/ml) levels of male and female rats administered soya beans and monosodium glutamate

DURATION	GROUPS	Glucose MSG	Glucose Soy	Insulin MSG	Insulin Soy
			FEMALES		
2 MONTHS					
	C	83.7±3.25^{a*}	83.71±3.25^{a*}	59.3±1.41^{a*}	59.31±1.41^{a*}
	LD	86.3±4.03^{a*}	79.64±9.47^{a**}	53.9±4.24^{a*}	54.08±3.95^{a*}
	MD	86.5±2.40^{a*}	84.65±6.57^{a*}	54.5±5.79^{a*}	56.35±5.58^{a*}
	HD	85.4±6.92^{a*}	90.45±2.61^{a*}	56.42±2.68^{a*}	58.75±5.26^{a*}
4 MONTHS					
	C	97.93±1.97^{b*}	97.93±1.97^{b*}	56.54±2.58^{a*}	56.54±2.58^{a*}
	LD	92.55±2.47^{ab*}	95.75±8.83^{b*}	55.74±2.08^{a*}	53.52±3.23^{a*}
	MD	95.8±3.11^{ab*}	93.65±3.60^{b*}	51.53±1.38^{a*}	55.35±2.23^{a*}
	HD	126.75±5.58^{cd*}	103.72±8.20^{b**}	42.96±0.89^{b*}	54.08±1.72^{a**}
6 MONTHS					
	C	97.7±9.33^{b*}	97.71±9.33^{b*}	60.95±4.03^{c*}	60.95±4.03^{b*}
	LD	90.6±2.96^{ab*}	104.42±10.04^{b*}	56.95±2.33^{a*}	59.65±2.19^{b*}
	MD	118.7±3.25^{c*}	112.35±5.58^{b*}	47.71±2.26^{b*}	61.65±2.75^{b**}
	HD	134.55±7.00^{d*}	125.27±6.22^{c**}	39.23±1.36^{d*}	44.65±2.61^{c**}
			MALES		
2 MONTHS					
	C	94.83±6.22^{a*}	94.84±6.22^{a*}	66.42±5.23^{a*}	66.43±5.23^{ac*}
	LD	94.05±1.76^{a*}	92.65±6.29^{a*}	65.85±4.77^{a*}	70.45±5.72^{a*}
	MD	91.77±6.50^{a*}	99.05±6.29^{a*}	69.85±2.05^{a*}	72.85±4.17^{a*}
	HD	98.71±5.79^{a*}	91.15±8.13^{a*}	70.41±6.82^{a*}	75.91±4.06^{a*}
4 MONTHS					
	C	102.85±8.13^{a*}	102.85±8.13^{a*}	64.01±3.23^{a*}	64.01±3.23^{c*}
	LD	97.30±3.39^{a*}	93.63±6.22^{a*}	62.70±2.24^{a*}	64.32±5.90^{c*}
	MD	105.44±10.04^{a*}	95.91±7.62^{a*}	65.31±2.56^{a*}	63.50±1.68^{c*}
	HD	123.61±6.50^{b*}	98.96±1.41^{a**}	52.01±1.96^{b*}	66.12±4.70^{ac**}
6 MONTHS					
	C	93.51±3.81^{a*}	93.51±3.81^{a*}	61.55±3.04^{a*}	61.55±3.04^{c*}
	LD	102.52±4.80^{a*}	91.55±2.37^{a**}	50.31±1.27^{b*}	65.35±2.47^{c**}
	MD	123.75±4.73^{b*}	105.45±5.58^{a**}	49.55±1.76^{b*}	54.73±3.11^{d*}
	HD	142.55±6.15^{c*}	120.95±8.13^{b**}	37.55±2.33^{c*}	45.15±3.88^{e**}

Values are means ± standard deviations, n=5. Values with different superscript letter(s) (a-e) down the column or symbols (* and **) across the row for each parameter are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean, LD – Low dose, MD – Medium dose, HD – High dose.

4.1.3: Liver enzyme concentrations

The hepatic dysfunction markers of rats administered MSG and soybeans are shown in Table 4.3. The ALT, ALP, and AST levels of the female rats were significantly ($p<0.05$) elevated after 2 months of administration of H.D MSG and soya beans. After 4 months administration of MD and HD MSG to the female rats, the ALT levels (120.86 and 123.32 U/L respectively), and ALP levels (311.73 and 320.99 U/L) were significantly ($p<0.05$) elevated when compared to the control. The result showed that 6 months administration of MD and HD soya bean to the female rats also significantly ($p<0.05$) elevated the ALT, ALP, and AST levels, when compared to the control. In the male rats, the low dose administration of MSG and soya beans for 2 months produced no significant ($p>0.05$) changes in the ALT, ALP and AST levels when compared to the control levels, whereas the ALT levels were significantly ($p<0.05$) increased by the MD and HD MSG, while the AST levels was significantly ($p<0.05$) increased by administration of MD and HD soya beans. After 4 months administration of MD and HD of soya bean, the ALT levels (139.20 and 135.08 U/L), the ALP (504.50 and 509.50 U/L), and the AST levels (274.00 and 280.50 U/L) were significantly ($p<0.05$) increased when compared to the control levels. Furthermore, all the doses of soya bean administered for 6 months significantly ($p<0.05$) elevated the ALT, ALP, and AST levels when compared to the control levels.

Table 4.3: Liver function parameters of rats administered monosodium glutamate and soya beans

DURATION	GROUPS	ALT (U/L)	ALT (U/L)	ALP (U/L)	ALP(U/L)	AST(U/L)	AST (U/L)
		MSG	**SOY**	**MSG**	**SOY**	**MSG**	**SOY**
2 MONTHS				**FEMALES**			
	C	87.00±7.07^{a*}	87.00±7.07^{a*}	261.00±18.38^{a*}	261.00±18.38^{a*}	149.00±29.69^{a*}	149.00±29.69^{a*}
	LD	82.50±9.19^{a*}	94.00±21.21^{b**}	277.00±31.11^{a*}	353.00±16.97^{b**}	163.00±16.97^{a*}	176.00±24.04^{b**}
	MD	83.00±4.24^{a*}	95.00±14.14^{b**}	421.50±19.09^{b*}	393.00±14.14^{c**}	153.50±20.50^{a*}	187.50±13.43^{c**}
	HD	96.00±7.07^{b*}	107.50±13.43^{c*}	456.50±16.26^{be*}	407.50±23.33^{c**}	179.50±14.84^{b*}	184.50±6.36^{c*}
4 MONTHS							
	C	104.86±3.31^{c*}	104.86±3.31^{c*}	293.14±6.63^{c*}	293.14±6.63^{ad*}	176.55±9.12^{b*}	176.55±9.12^{b*}
	LD	101.46±9.66^{c*}	115.51±5.81^{d**}	288.56±11.22^{c*}	283.63±14.47^{b*}	170.80±10.46^{b*}	180.45±14.07^{bc*}
	MD	120.86±7.02^{de*}	118.90±5.09^{d*}	311.73±3.72^{d*}	286.65±31.32^{b**}	179.00±13.43^{b*}	208.00±12.44^{d**}
	HD	123.32±5.40^{d*}	134.06±6.59^{e**}	320.99±10.74^{d*}	301.45±14.49^{d*}	192.00±12.12^{b*}	199.40±14.28^{d*}
6 MONTHS							
	C	116.16±5.17^{e*}	116.16±5.17^{d*}	471.90±12.86^{e*}	471.90±12.86^{c*}	209.40±12.72^{a*}	209.40±12.72^{d*}
	LD	122.85±9.26^{de*}	161.25±9.97^{fg**}	591.05±17.05^{f*}	599.05±33.58^{d*}	218.75±10.67^{a*}	259.25±9.82^{e**}
	MD	125.35±7.00^{d*}	155.30±6.78^{f**}	575.00±15.27^{f*}	635.85±20.15^{e**}	266.70±20.08^{c*}	297.70±20.08^{f**}
	HD	143.55±7.14^{f*}	170.75±4.87^{g**}	580.80±16.26^{f*}	671.70±13.01^{f**}	261.90±17.53^{c*}	305.90±20.50^{f**}
2 MONTHS				**MALES**			
	C	119.50±12.02^{g*}	119.50±12.02^{hc*}	430.00±18.38^{g*}	430.00±18.38^{g*}	184.50±14.84^{d*}	184.50±14.84^{gc*}
	LD	118.00±14.14^{g*}	119.00±24.04^{hc*}	425.50±30.40^{g*}	416.00±15.55^{g*}	187.50±9.19^{d*}	215.50±17.67^{hl*}
	MD	132.50±12.02^{hj*}	128.50±14.84^{h*}	445.00±38.18^{gh*}	501.00±21.21^{h**}	193.00±14.14^{d*}	266.00±9.89^{i**}
	HD	145.00±22.62^{h*}	143.00±7.07^{i*}	458.50±14.84^{gh*}	512.50±28.99^{hj**}	203.50±19.09^{d*}	288.50±10.60^{j**}
4 MONTHS							
	C	122.03±3.71^{i*}	122.03±3.71^{h*}	463.00±18.38^{h*}	463.00±18.38^{i*}	198.00±11.31^{d*}	198.00±11.31^{kld*}
	LD	116.81±5.04^{i*}	123.97±4.75^{h*}	453.50±13.43^{h*}	471.50±14.84^{i**}	188.90±12.58^{d*}	207.00±4.24^{ld**}
	MD	120.95±6.57^{i*}	139.20±5.26^{ie**}	462.50±9.19^{h*}	504.50±12.02^{h**}	208.65±13.64^{d*}	274.00±7.07^{m**}
	HD	133.80±7.60^{j*}	135.08±6.2^{ie*}	449.50±27.57^{h*}	509.50±23.33^{hj**}	217.90±18.24^{de*}	280.50±12.02^{mj**}
6 MONTHS							
	C	127.70±4.10^{i*}	127.70±4.10^{h*}	523.95±9.40^{i*}	523.95±9.40^{j*}	227.00±5.37^{e*}	227.00±5.37^{h*}
	LD	126.50±8.90^{i*}	141.40±8.76^{i**}	511.40±25.17^{i*}	657.20±43.84^{k**}	234.35±10.25^{f*}	252.70±9.05^{ne**}
	MD	140.20±13.29^{h*}	157.30±9.19^{jf**}	552.25±4.31^{j*}	663.05±16.61^{k**}	245.15±7.56^{g*}	260.70±13.85^{nie**}
	HD	152.00±5.23^{h*}	166.95±10.25^{j**}	582.50±11.73^{j*}	713.30±28.99^{l**}	257.35±16.89^{h*}	295.95±12.23^{of**}

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-n) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG-Monosodium glutamate, SOY-Soya bean, ALT-Alanine Transaminase, ALP – Alkaline Phosphatase, AST-Aspartate transaminase

4.1.4: Total bilirubin and conjugated bilirubin levels

The results for total bilirubin and conjugated bilirubin levels of the rats administered MSG and soya bean are presented in Table 4.4. The total bilirubin levels of the rats administered the LD, MD, and HD MSG (8.50, 11.50, and 10.50 µmol/l) for 2 months were significantly ($p<0.05$) elevated when compared to the control level (7.00 µmol/l), while administration of LD, MD, and HD soya beans significantly ($p<0.05$) reduced the conjugated bilirubin levels (4.50, 4.00, and 3.00 µmol/l) when compared to the control levels (5.00 µmol/l). The administration of HD MSG for 4 and 6 months significantly ($p<0.05$) increased the total bilirubin levels while no significant ($p>0.05$) changes were observed in the total bilirubin levels following LD, MD, and HD administration of soya beans; but the HD soya beans and MSG significantly ($p<0.05$) reduced the conjugated bilirubin levels. For the male rats, no significant ($p>0.05$) changes were observed on the total bilirubin levels after the administration of LD, MD, and HD soya beans throughout all experimental durations, whereas the administration of HD MSG for 2 months significantly ($p<0.05$) increased the total bilirubin levels (12.00 µmol/l) and decreased the conjugated bilirubin levels (3.00 µmol/l) when compared to the control (8.60 and 6.00 µmol/l respectively). The administration of HD MSG for 4 months significantly ($p<0.05$) increased the total bilirubin levels of the male rats and significantly ($p<0.05$) reduced the conjugated bilirubin, while all the doses of soya bean administered to the male rats significantly ($p<0.05$) decreased the conjugated bilirubin levels. All doses of MSG administered for 6 months to the male rats significantly ($p<0.05$) increased the total bilirubin levels and decreased the conjugated bilirubin levels in comparison to the control; while all the doses of soya bean administered for six months significantly ($p<0.05$) reduced the conjugated bilirubin levels.

Table 4.4: Total and conjugated bilirubin levels of rats administered MSG and soya beans

DURATION	GROUPS	TB (µmol/l)	TB (µmol/l)	CB (µmol/l)	CB (µmol/l)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	7.00±0.00^{a*}	9.00±0.00^{a**}	5.00±0.00^{a*}	5.00±0.00^{a*}
	LD	8.50±0.70^{b*}	9.50±0.70^{a*}	3.00±0.00^{b*}	4.50±0.30^{b**}
	MD	11.50±1.94^{c*}	9.00±0.70^{a*}	3.00±1.41^{b*}	4.00±0.00^{b**}
	HD	10.50±1.66^{cd*}	10.50±3.53^{a*}	3.00±0.00^{b*}	3.00±0.00^{ce*}
4 MONTHS					
	C	10.00±1.41^{d*}	10.00±1.41^{a*}	6.50±0.70^{c*}	6.50±0.70^{d*}
	LD	9.50±2.12^{d*}	8.50±0.70^{a*}	4.50±0.70^{a*}	5.50±0.70^{a**}
	MD	9.70±0.70^{d*}	9.50±0.70^{a*}	3.50±0.70^{b*}	4.50±0.70^{a**}
	HD	15.50±0.70^{e*}	8.00±1.41^{a**}	2.50±0.70^{b*}	3.00±1.41^{bce*}
6 MONTHS					
	C	9.90±2.49^{d*}	9.50±2.12^{a*}	4.50±0.70^{a*}	4.50±0.70^{a*}
	LD	14.50±0.70^{ei*}	10.20±1.41^{a**}	3.50±0.70^{b*}	4.50±0.70^{a**}
	MD	15.40±1.01^{e*}	9.00±0.00^{a**}	4.50±0.70^{a*}	5.00±1.41^{a*}
	HD	17.30±1.39^{f*}	10.50±0.70^{a**}	2.50±0.70^{b*}	3.50±0.70^{bc**}
2 MONTHS			**MALES**		
	C	8.60±1.41^{gb*}	8.00±1.41^{a*}	6.00±0.00^{d*}	6.00±0.00^{d*}
	LD	7.50±2.12^{ga*}	9.00±2.82^{a*}	4.00±0.00^{e*}	4.50±0.70^{a*}
	MD	9.00±1.41^{g*}	9.00±1.41^{a*}	3.00±0.00^{fb*}	4.00±0.00^{a**}
	HD	12.00±2.82^{h*}	9.50±2.12^{a**}	3.00±0.00^{fb*}	3.50±0.70^{bc**}
4 MONTHS					
	C	8.00±1.21^{g*}	8.00±1.41^{a*}	4.00±0.00^{ea*}	4.00±0.00^{b*}
	LD	7.00±1.11^{g*}	9.00±1.41^{a**}	3.50±0.70^{fb*}	4.50±0.70^{a**}
	MD	8.00±1.76^{g*}	9.50±2.12^{a*}	3.50±0.70^{fb*}	4.50±0.70^{a*}
	HD	13.00±2.82^{h*}	9.40±0.71^{a**}	2.00±0.00^{gb*}	3.00±0.00^{ce*}
6 MONTHS					
	C	7.50±0.70^{g*}	7.50±0.70^{a*}	6.00±1.41^{d*}	6.00±1.41^{d*}
	LD	13.00±1.41^{h*}	8.00±1.41^{a**}	5.00±0.00^{h*}	5.00±0.00^{a*}
	MD	14.50±2.12^{hi*}	8.00±1.41^{a**}	4.50±2.12^{eha*}	3.50±0.70^{bc**}
	HD	13.50±2.12^{hi*}	11.00±1.41^{a*}	3.50±0.70^{fb*}	2.50±0.70^{e**}

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-h) down the column or symbols (* and **) across the row for each parameter are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean, TB – Total Bilirubin, CB – Conjugated Bilirubin

4.1.5: Total protein and albumin levels

The total protein and albumin levels of male and female rats administered incremental doses of MSG and soya beans are presented in Table 4.5. The total protein levels of female rats administered LD, MD, and HD MSG for 2 months (66.50, 62.50, and 55.50 g/l respectively) were significantly ($p<0.05$) lower than the control (74.50 g/l) while the various soya bean doses produced no significant ($p>0.05$) changes on the total protein and albumin levels. Administration of HD MSG for 4 and 6 months significantly ($p<0.05$) decreased the total protein levels (58.25 and 50.75 g/l respectively) and albumin levels (35.26 and 29.77 g/l respectively). All the doses of MSG administered to the male rats for 2 months significantly ($p<0.05$) reduced the total protein levels, while only the HD soya bean caused a significant ($p<0.05$) decrease in their total protein levels. No significant ($p>0.05$) changes were observed in the albumin levels of the male rats administered MSG and soya beans for 2 months, while 4 months administration of HD MSG significantly ($p<0.05$) decreased the albumin and total protein levels. Administration of MD and HD MSG up to 6 months caused a significant ($p<0.05$) decrease in the total protein and albumin levels, while the HD soya beans significantly ($p<0.05$) decreased the albumin levels.

Table 4.5: Total protein and albumin levels of rats administered MSG and soya beans

DURATION	GROUPS	TP (g/l)	TP (g/l)	Albumin (g/l)	Albumin (g/l)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	$74.50\pm6.36^{a*}$	$74.50\pm6.36^{a*}$	$36.35\pm3.13^{a*}$	$36.35\pm3.13^{a*}$
	LD	$66.50\pm7.77^{b*}$	$73.50\pm6.36^{a**}$	$32.15\pm3.79^{a*}$	$34.71\pm3.92^{a*}$
	MD	$62.50\pm9.19^{b*}$	$76.50\pm0.70^{a**}$	$30.83\pm2.44^{a*}$	$37.25\pm2.86^{a*}$
	HD	$55.50\pm6.36^{c*}$	$73.00\pm1.41^{a**}$	$34.26\pm4.01^{a*}$	$35.18\pm2.55^{a*}$
4 MONTHS					
	C	$89.50\pm7.77^{d*}$	$89.50\pm7.77^{b*}$	$45.29\pm2.99^{b*}$	$45.29\pm2.99^{b*}$
	LD	$69.75\pm11.66^{bf*}$	$91.50\pm3.53^{b**}$	$43.19\pm2.85^{b*}$	$47.21\pm2.09^{b*}$
	MD	$65.47\pm6.39^{b*}$	$87.50\pm9.19^{b**}$	$40.88\pm2.16^{b*}$	$44.18\pm3.55^{b*}$
	HD	$58.25\pm8.13^{c*}$	$76.50\pm4.94^{a**}$	$35.26\pm3.42^{a*}$	$46.02\pm2.81^{b*}$
6 MONTHS					
	C	$85.30\pm5.65^{d*}$	$85.30\pm5.65^{b*}$	$47.81\pm3.22^{b*}$	$47.81\pm3.22^{b*}$
	LD	$67.50\pm2.12^{b*}$	$83.80\pm4.66^{b**}$	$40.50\pm3.87^{b*}$	$44.71\pm3.74^{b*}$
	MD	$53.80\pm4.66^{c*}$	$84.95\pm3.46^{b**}$	$34.06\pm3.17^{a*}$	$41.61\pm3.50^{b*}$
	HD	$50.75\pm3.46^{c*}$	$83.15\pm3.60^{b**}$	$29.77\pm3.62^{a*}$	$33.50\pm2.87^{a*}$
2 MONTHS			**MALES**		
	C	$81.00\pm2.82^{e*}$	$66.00\pm2.82^{c**}$	$39.31\pm3.45^{a*}$	$39.31\pm3.45^{ab*}$
	LD	$74.50\pm3.53^{fa*}$	$63.00\pm4.24^{c**}$	$32.35\pm2.80^{a*}$	$41.65\pm4.79^{b**}$
	MD	$75.00\pm5.65^{fa*}$	$68.50\pm2.12^{c**}$	$35.82\pm3.09^{a*}$	$39.02\pm2.61^{ab*}$
	HD	$78.00\pm8.48^{fa*}$	$73.50\pm7.77^{a*}$	$33.71\pm2.57^{a*}$	$37.63\pm2.90^{a*}$
4 MONTHS					
	C	$74.00\pm5.65^{f*}$	$74.00\pm5.65^{a*}$	$48.36\pm2.43^{b*}$	$47.36\pm2.43^{b*}$
	LD	$76.50\pm4.94^{f*}$	$75.50\pm2.12^{a*}$	$41.03\pm3.27^{b*}$	$45.90\pm2.83^{b*}$
	MD	$58.00\pm11.31^{gc*}$	$76.50\pm7.77^{a**}$	$44.69\pm2.05^{b*}$	$42.01\pm3.14^{b*}$
	HD	$49.70\pm5.23^{g*}$	$69.50\pm7.77^{a**}$	$32.26\pm2.47^{a*}$	$46.23\pm2.16^{b**}$
6 MONTHS					
	C	$90.45\pm4.73^{id*}$	$90.45\pm4.73^{b*}$	$46.42\pm2.92^{b*}$	$46.42\pm2.92^{b*}$
	LD	$85.35\pm11.66^{id*}$	$86.30\pm2.82^{b*}$	$44.10\pm3.94^{b*}$	$43.94\pm3.07^{b*}$
	MD	$66.90\pm5.65^{kb*}$	$85.70\pm6.64^{b**}$	$31.29\pm3.64^{a*}$	$45.18\pm2.71^{b**}$
	HD	$57.70\pm4.94^{lc*}$	$86.50\pm3.67^{b**}$	$29.37\pm2.80^{a*}$	$35.29\pm3.18^{a**}$

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-k) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean, TP – Total protein

4.1.6: Urea and creatinine levels

The urea and creatinine levels of rats administered MSG and soya beans are shown in Table 4.6. Soya bean administration for 2 months had no significant (p>0.05) effect on urea levels, whereas after 4 months, the high dose soya bean significantly (p<0.05) decreased the urea and creatinine levels. All doses of MSG significantly (p<0.05) decreased the urea levels at both 2 and 4 months administration while the creatinine levels were significantly (p<0.05) decreased by the MD and HD MSG administration. The administration of all doses of MSG to the female rats for 6 months significantly (p<0.05) decreased the urea and creatinine levels, while the low dose of soya bean administered for 6 months to the female rats produced comparable creatinine levels (7.00 mmol/l) to the control (7.35 mmol/l). The male rats administered LD, MD, and HD MSG for 2 months showed significantly (p<0.05) reduced urea levels (42.50, 43.50, and 43.00 mmol/l respectively) when compared to the control (58.70 mmol/l), while the creatinine level was significantly (p<0.05) increased by MD and HD MSG (7.45 and 7.95 mmol/l respectively) when compared to the control levels (6.30 mmol/l). No significant (>0.05) change was observed on the creatinine levels of rats administered soya beans, while the urea levels were significantly (p<0.05) decreased. Also, the 4 months administration of LD, MD, and HD MSG to male rats significantly (p<0.05) decreased the urea and creatinine levels, while LD and MD soya bean administration produced no significant (p>0.05) effects on the urea and creatinine levels. After 6 months, the urea and creatinine levels of the male rats were significantly (p<0.05) decreased by administration of LD, MD and HD MSG, while no significant (p>0.05) changes were observed in the creatinine levels of male rats administered LD and MD soya beans for 6 months.

Table 4.6: Urea and creatinine levels of rats administered monosodium glutamate and soya beans

DURATION	GROUPS	Urea (mmol/l)	Urea (mmol/l)	Creatinine (umol/l)	Creatinine (mmol/l)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	$58.50\pm3.53^{a*}$	$58.50\pm3.53^{a*}$	$5.15\pm0.49^{ad*}$	$5.15\pm0.49^{a*}$
	LD	$46.50\pm6.36^{b*}$	$27.95\pm3.18^{a**}$	$5.40\pm0.56^{a*}$	$5.75\pm0.49^{a*}$
	MD	$43.50\pm4.94^{b*}$	$29.80\pm3.22^{a**}$	$5.70\pm0.56^{a*}$	$5.60\pm0.56^{a*}$
	HD	$44.50\pm0.70^{b*}$	$29.40\pm3.37^{a**}$	$6.65\pm0.35^{b*}$	$6.65\pm0.49^{b*}$
4 MONTHS					
	C	$55.50\pm6.36^{a*}$	$55.50\pm6.36^{b*}$	$5.10\pm0.28^{ad*}$	$5.10\pm0.28^{a*}$
	LD	$44.00\pm5.65^{b*}$	$56.00\pm4.24^{b**}$	$5.45\pm0.35^{a*}$	$4.90\pm0.56^{a**}$
	MD	$43.50\pm2.12^{b*}$	$56.50\pm2.12^{b**}$	$7.15\pm0.35^{c*}$	$5.55\pm0.49^{a**}$
	HD	$38.50\pm2.12^{c*}$	$48.50\pm2.12^{c**}$	$7.55\pm0.77^{c*}$	$6.50\pm0.42^{b**}$
6 MONTHS					
	C	$62.70\pm4.94^{d*}$	$62.70\pm4.94^{d*}$	$4.90\pm0.14^{d*}$	$7.35\pm0.63^{c*}$
	LD	$42.05\pm3.18^{b*}$	$51.70\pm3.39^{b**}$	$5.50\pm0.56^{a*}$	$7.00\pm0.00^{c**}$
	MD	$38.05\pm4.87^{c*}$	$52.60\pm7.63^{b**}$	$5.70\pm0.28^{a*}$	$8.45\pm0.07^{d**}$
	HD	$34.55\pm2.47^{c*}$	$37.80\pm5.65^{a*}$	$7.35\pm0.63^{c*}$	$8.05\pm0.21^{d**}$
2 MONTHS			**MALE**		
	C	$58.70\pm9.19^{ega*}$	$58.50\pm9.19^{e*}$	$6.30\pm0.42^{eb*}$	$6.30\pm0.42^{eb*}$
	LD	$42.50\pm3.53^{fb*}$	$43.00\pm2.82^{f*}$	$6.35\pm0.77^{eb*}$	$6.65\pm0.49^{eb*}$
	MD	$43.50\pm3.53^{fb*}$	$44.50\pm3.53^{f*}$	$7.45\pm0.63^{f*}$	$6.00\pm1.13^{eb**}$
	HD	$43.00\pm1.41^{fb*}$	$42.00\pm2.82^{f*}$	$7.95\pm0.49^{f*}$	$5.95\pm0.35^{eb**}$
4 MONTHS					
	C	$61.00\pm8.48^{e*}$	$61.00\pm8.48^{e*}$	$6.50\pm0.42^{e*}$	$6.50\pm0.42^{eb*}$
	LD	$52.50\pm3.53^{ga*}$	$61.00\pm2.82^{e*}$	$7.25\pm0.21^{fc*}$	$6.85\pm0.21^{eb**}$
	MD	$43.00\pm1.41^{fb*}$	$58.00\pm7.07^{eb**}$	$7.45\pm0.63^{fc*}$	$6.95\pm0.49^{eb**}$
	HD	$41.50\pm4.94^{fb*}$	$63.50\pm6.36^{e**}$	$9.20\pm0.42^{g*}$	$7.85\pm0.49^{f**}$
6 MONTHS					
	C	$55.90\pm4.38^{g*}$	$55.90\pm4.38^{eb*}$	$9.00\pm1.27^{g*}$	$9.00\pm1.27^{g*}$
	LD	$38.80\pm2.40^{hc*}$	$54.35\pm5.30^{eb**}$	$11.10\pm1.55^{h*}$	$9.00\pm0.56^{g**}$
	MD	$37.55\pm5.30^{hc*}$	$47.20\pm4.80^{f**}$	$11.50\pm1.55^{h*}$	$10.30\pm1.69^{g*}$
	HD	$29.80\pm4.24^{i*}$	$38.90\pm2.12^{ga**}$	$13.05\pm1.48^{i*}$	$10.65\pm0.63^{h**}$

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-j) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean

4.1.7: Sodium and potassium levels

Table 4.7 presents the sodium and potassium levels of male and female rats administered varying doses of MSG and soya bean. The sodium and potassium levels after 2 months administration of MD and HD MSG to female rats were significantly ($p<0.05$) elevated while LD and MD soya bean produced no observable effect. After 4 months, no significant ($p>0.05$) change was observed in the potassium levels of rats administered the soya bean doses when compared to the control, while the sodium levels were significantly (p<0.050 increased by the HD soya bean. All the MSG doses administered showed a significantly ($p<0.05$) elevated the sodium levels and significantly l($p<0.05$) owered the potassium levels. At 6 months administration the sodium levels of the female rats administered the LD, MD, and HD MSG (170, 187, and 217 mmol/l) were significantly ($p<0.05$) higher than the control level (118.45 mmol/l). All doses of MSG significantly ($p<0.05$) decreased the potassium levels when administered for 6 months, while the MD and HD soya bean administration significantly altered the sodium and potassium levels of the female rats. For the male rats, the sodium levels significantly ($p<0.05$) increased after 2 months administration of MD and HD MSG and soya beans, while 2 months of LD MSG and soya bean administration produced no significant ($p>0.05$) effect on the potassium levels. After 4 months administration of LD soya bean, no significant ($p>0.05$) changes were observed in the sodium and potassium levels of the male rats, while MD and HD MSG administration significantly ($p<0.05$) increased the sodium levels and significantly ($p<0.05$) decreased the potassium levels. At 6 months administration, all MSG doses significantly ($p<0.05$) increased the sodium levels and as well significantly decreased the potassium levels when compared to the control levels, while no significant ($p>0.05$) differences were observed among the sodium levels of male control rats, and rats administered LD and MD soya beans.

Table 4.7: Sodium and potassium levels of rats administered MSG and soya beans

DURATION	GROUPS	Na(mmol/l)	Na (mmol/l)	K (mmol/l)	K (mmol/l)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	104.05±14.91^{a*}	104.05±14.91^{a*}	6.65±0.49^{a*}	6.65±0.49^{af**}
	LD	112.18±10.29^{a*}	110.10±5.51^{a*}	3.20±0.84^{b*}	5.70±0.28^{bf**}
	MD	129.60±6.37^{b*}	109.17±6.54^{a**}	3.30±0.14^{b*}	5.60±0.84^{bf**}
	HD	142.10±10.18^{c*}	120.43±11.83^{ac**}	3.45±0.63^{b*}	3.95±0.91^{c*}
4 MONTHS					
	C	130.05±3.88^{b*}	130.05±3.88^{bd*}	7.35±0.35^{c*}	7.35±0.35^{d*}
	LD	149.30±19.94^{c*}	126.45±8.55^{bc**}	6.30±0.28^{a*}	7.50±0.28^{d**}
	MD	161.55±16.75^{d*}	130.45±4.35^{b**}	4.70±0.42^{d*}	7.85±0.49^{d**}
	HD	183.50±10.18^{e*}	140.35±5.58^{d**}	4.10±0.14^{d*}	7.65±0.35^{d**}
6 MONTHS					
	C	118.45±12.65^{a*}	118.45±12.65^{a*}	8.52±0.81^{e*}	8.52±3.81^{e*}
	LD	170.00±5.37^{de*}	123.10±10.88^{a**}	7.16±0.60^{c*}	8.35±4.31^{e**}
	MD	187.65±9.26^{e*}	136.05±9.26^{d**}	6.15±0.95^{a*}	7.25±2.89^{d**}
	HD	217.65±5.44^{f*}	128.05±13.78^{b*}	4.98±0.05^{d*}	7.50±2.26^{d**}
2 MONTHS			**MALES**		
	C	104.24±10.68^{ga*}	104.24±10.68^{ea*}	5.75±0.21^{f*}	5.75±0.21^{fg*}
	LD	109.75±13.93^{ga*}	99.13±10.84^{ea*}	5.55±0.63^{f*}	5.25±0.77^{f*}
	MD	136.19±9.63^{h*}	115.01±8.60^{fga**}	4.60±0.42^{d*}	5.20±0.42^{f*}
	HD	149.65±12.65^{ic*}	130.15±10.95^{g**}	4.10±0.42^{d*}	4.20±0.28^{c*}
4 MONTHS					
	C	115.70±14.99^{g*}	115.70±14.99^{f*}	5.25±0.21^{f*}	5.25±0.21^{f*}
	LD	144.50±8.34^{ic*}	112.70±12.16^{f**}	5.35±0.21^{f*}	5.25±0.35^{f*}
	MD	147.90±3.81^{ic*}	120.25±7.70^{gfc**}	4.20±0.28^{d*}	6.00±0.28^{gh**}
	HD	205.90±17.96^{j*}	129.05±13.36^{gb**}	3.10±0.14^{b*}	6.30±0.70^{h**}
6 MONTHS					
	C	142.15±5.30^{i*}	142.15±5.30^{h*}	7.80±0.65^{c*}	7.80±0.65^{id*}
	LD	184.90±8.06^{je*}	139.50±12.30^{hi**}	6.05±0.68^{a*}	8.60±0.82^{je**}
	MD	188.40±8.34^{je*}	145.75±7.00^{h**}	6.06±0.20^{a*}	8.80±0.82^{je**}
	HD	198.75±20.85^{je*}	130.80±6.50^{i**}	5.65±0.38^{f*}	8.70±0.24^{je**}

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-j) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean.

4.1.8: Bicarbonate and chloride levels

The concentration of anions (bicarbonate and chloride) in male and female rats administered incremental doses of MSG and soya beans are represented in Table 4.8. Administration of all the doses of soya beans and MSG for 2 and 4 months produced no significant ($p>0.05$) changes in the bicarbonate and chloride levels of the male and female rats. Only the 6 months administration of MD and HD MSG significantly ($p<0.05$) elevated the bicarbonate and chloride levels of the female rats, while no significant differences were observed in these levels, in rats administered soya beans for 6 months. All doses of MSG administered for 6 months significantly ($p<0.05$) elevated the bicarbonate levels of the male rats, whereas the only the HD MSG significantly increased the chloride levels after 6 months administration.

Table 4.8: Bicarbonate and chloride levels of rats administered MSG and soya beans

DURATION	GROUPS	HCO_3 (mmol/l)	HCO_3 (mmol/l)	Cl (mmol/l)	Cl (mmol/l)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	28.50±4.94^{a*}	28.50±4.94^{a*}	91.50±2.12^{ad*}	91.50±2.12^{a*}
	LD	23.50±3.53^{a*}	25.50±3.53^{a*}	89.00±2.82^{ad*}	92.50±3.53^{a*}
	MD	25.00±5.65^{a*}	24.00±2.82^{a*}	90.50±3.53^{a*}	95.50±4.94^{a*}
	HD	25.50±2.12^{a*}	26.00±4.24^{a*}	93.50±2.12^{a*}	92.50±4.94^{a*}
4 MONTHS					
	C	24.00±5.65^{a*}	24.00±5.65^{a*}	98.00±2.82^{a*}	98.00±2.82^{a*}
	LD	26.50±7.77^{a*}	27.50±3.53^{a*}	94.00±5.65^{a*}	104.50±12.02^{a*}
	MD	27.00±2.82^{a*}	28.50±4.94^{a*}	98.00±8.48^{a*}	94.50±6.36^{a*}
	HD	24.50±2.12^{a*}	27.00±2.82^{a*}	96.50±3.53^{a*}	98.00±4.24^{a*}
6 MONTHS					
	C	29.50±2.12^{a*}	29.50±2.12^{a*}	74.50±6.36^{b*}	74.50±6.36^{b*}
	LD	27.00±1.41^{a*}	23.00±4.24^{a*}	79.00±2.82^{bc*}	72.00±9.89^{b*}
	MD	33.00±4.24^{b*}	29.50±3.53^{a*}	82.00±9.89^{cd*}	78.50±4.94^{bc*}
	HD	36.00±4.24^{b*}	24.50±3.53^{a**}	88.50±7.77^{ad*}	79.50±3.53^{bc*}
			MALES		
2 MONTHS					
	C	25.00±1.41^{ca*}	25.00±1.41^{a*}	92.00±2.82^{ea*}	92.00±2.82^{a*}
	LD	24.00±1.41^{ca*}	23.50±0.70^{a*}	92.00±0.00^{ea*}	90.00±5.65^{a*}
	MD	23.00±4.24^{ca*}	26.00±2.82^{a*}	96.00±0.00^{ea*}	94.00±2.82^{a*}
	HD	24.50±0.70^{ca*}	21.50±2.12^{a*}	94.00±2.82^{ea*}	93.00±7.07^{a*}
4 MONTHS					
	C	26.50±2.12^{ca*}	26.50±2.12^{a*}	101.00±5.65^{ea*}	103.00±5.65^{a*}
	LD	26.00±2.82^{ca*}	26.50±4.94^{a*}	92.50±2.12^{ea*}	96.50±6.36^{a*}
	MD	24.50±3.53^{ca*}	29.00±2.82^{a*}	97.00±1.41^{ea*}	96.50±2.12^{a*}
	HD	28.00±1.41^{ca*}	25.50±4.94^{a*}	96.00±8.48^{ea*}	100.00±15.55^{a*}
6 MONTHS					
	C	29.50±2.12^{ca*}	29.50±2.12^{a*}	84.00±2.82^{gd*}	84.00±2.82^{c*}
	LD	32.50±3.53^{db*}	29.50±3.53^{a*}	84.50±4.94^{gd*}	83.00±4.24^{c*}
	MD	32.50±4.94^{db*}	31.00±4.24^{a*}	87.50±3.53^{gd*}	85.50±6.36^{c*}
	HD	34.00±1.41^{db*}	29.50±4.94^{a*}	93.00±2.82^{e*}	80.00±0.00^{c*}

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-j) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean.

4.1.9: Lipoprotein concentration

The concentration of lipoproteins of rats administered MSG and soya beans for 2, 4, and 6 months, are shown in Table 4.9. LD administration of soya beans and MSG for 2 months produced no significant ($p>0.05$) changes in the HDL levels, the LDL levels of the female rats were significantly decreased by the LD, MD and HD administration of soya beans. The administration of MD and HD MSG for 2 months significantly ($p<0.05$) decreased the HDL levels (0/60 and 0.65 mmol/l respectively) of the female rats, when compared to the control level (1.05 mmol/l).The administration of LD, MD, and HD MSG for 6 months significantly ($p<0.05$) decreased the HDL level and increased the LDL levels, while the MD and HD soya beans significantly ($p<0.05$) decreased the concentration of HDL and elevated the LDL levels. In the male rats, the administration of MD and HD of both MSG and soya beans for 2 months significantly ($p<0.05$) increased the LDL levels and decreased the HDL levels when compared to the control levels. After 4 months administration of the MSG doses, the HDL levels (1.55, 1.45, and 1.45 mmol/l) were significantly ($p<0.05$) decreased in comparison to the control (2.35 mmol/l), while the soya bean doses produced no significant ($p>0.05$) changes in the HDL levels of the male rats. The results showed that the MD and HD doses of MSG and soya bean significantly ($p<0.05$) increased the LDL levels after 4 months administration. After 6 months administration of the MSG and soya bean, all doses significantly ($p<0.05$) increased the LDL levels and decreased the HDL levels when compared to the control.

Table 4.9: Lipoproteins concentration rats administered MSG and soya beans

DURATION	GROUPS	HDL (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	LDL (mmol/l)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	$1.05\pm0.07^{a*}$	$1.05\pm0.07^{a*}$	$3.03\pm0.28^{a*}$	$3.03\pm0.38^{a*}$
	LD	$0.80\pm0.28^{a*}$	$1.67\pm0.24^{a**}$	$2.34\pm0.14^{b*}$	$2.67\pm0.31^{b*}$
	MD	$0.60\pm0.28^{b*}$	$1.52\pm0.10^{a**}$	$3.27\pm0.50^{c*}$	$1.51\pm0.16^{c**}$
	HD	$0.65\pm0.35^{b*}$	$1.05\pm0.07^{b**}$	$3.51\pm0.70^{c*}$	$1.99\pm0.27^{d**}$
4 MONTHS					
	C	$2.25\pm0.21^{c*}$	$2.25\pm0.41^{c*}$	$2.97\pm0.66^{d*}$	$2.97\pm0.66^{a*}$
	LD	$1.80\pm0.14^{d*}$	$2.20\pm0.14^{c**}$	$3.33\pm0.41^{c*}$	$2.59\pm0.29^{b*}$
	MD	$1.85\pm0.07^{d*}$	$2.55\pm0.07^{d**}$	$3.80\pm0.76^{c*}$	$2.42\pm0.16^{b**}$
	HD	$1.35\pm0.21^{af*}$	$2.25\pm0.21^{c**}$	$4.90\pm0.59^{e*}$	$2.83\pm0.07^{a**}$
6 MONTHS					
	C	$3.05\pm0.21^{e*}$	$3.05\pm0.21^{e*}$	2.23 ± 0.09^{b}	2.23 ± 0.09^{d}
	LD	$1.55\pm0.21^{f*}$	$2.60\pm0.14^{c**}$	4.69 ± 0.38^{e}	2.15 ± 0.09^{d}
	MD	$1.50\pm0.14^{f*}$	$1.80\pm0.14^{a**}$	4.83 ± 0.04^{e}	3.39 ± 0.07^{f}
	HD	$1.25\pm0.07^{a*}$	$1.75\pm0.21^{a**}$	6.54 ± 0.19^{f}	4.10 ± 0.53^{g}
2 MONTHS			**MALES**		
	C	$0.90\pm0.00^{ga*}$	$0.90\pm0.00^{e*}$	$2.06\pm0.08^{ga*}$	$2.06\pm0.08^{d*}$
	LD	$0.90\pm0.00^{ga*}$	$0.80\pm0.00^{f**}$	$2.32\pm0.07^{hb*}$	$2.25\pm0.22^{d*}$
	MD	$0.75\pm0.21^{hb*}$	$0.75\pm0.3^{g*}$	$2.47\pm0.41^{hb*}$	$2.64\pm0.50^{b*}$
	HD	$0.55\pm0.21^{hb*}$	$0.65\pm0.12^{h*}$	$2.86\pm0.70^{h*}$	$2.89\pm0.28^{ab*}$
4 MONTHS					
	C	$2.35\pm0.21^{jc*}$	$2.35\pm0.21^{c*}$	$1.45\pm0.04^{i*}$	$1.45\pm0.04^{c*}$
	LD	$1.55\pm0.21^{kf*}$	$2.25\pm0.35^{c**}$	$4.85\pm0.52^{jke*}$	$1.60\pm0.45^{c**}$
	MD	$1.45\pm0.35^{kf*}$	$2.05\pm0.21^{c**}$	$4.73\pm0.52^{je*}$	$1.97\pm0.66^{d**}$
	HD	$1.45\pm0.07^{kf*}$	$2.20\pm0.28^{c**}$	$4.81\pm0.29^{jke*}$	$2.15\pm0.63^{d**}$
6 MONTHS					
	C	$2.75\pm0.07^{j*}$	$2.75\pm0.07^{d*}$	$2.01\pm0.38^{gb*}$	$2.01\pm0.38^{d*}$
	LD	$1.30\pm0.14^{kf*}$	$2.25\pm0.21^{c**}$	$5.03\pm0.24^{k*}$	$2.89\pm0.32^{a**}$
	MD	$1.35\pm0.21^{kf*}$	$1.55\pm0.07^{a**}$	$5.75\pm0.07^{l*}$	$3.91\pm0.21^{g**}$
	HD	$1.25\pm0.21^{ka*}$	$1.60\pm0.28^{a**}$	$6.39\pm0.21^{mf*}$	$4.81\pm0.69^{h**}$

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-h) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean, HDL – High Density Lipoproteins, LDL – Low Density Lipoproteins

4.1.10: Total cholesterol and triacylglycerols

Table 4.10 shows the concentration of total cholesterol (TC) and triacylglycerols (TAG) following LD, MD and HD administration of MSG and soya beans for 2, 4, and 6 months to male and female rats. No significant ($p>0.05$) changes on the TC and TAG were observed after administration of soya beans for 2 and 4 months on the female rats while for the 2 months administration, the MD and HD MSG significantly ($p<0.05$) increased the TC and TAG levels. The LD and MD MSG produced no significant ($p>0.05$) changes in the TC levels when administered for 4 months to the female rats when compared to the control rats, while all the MSG doses significantly ($p<0.05$) elevated the triacylglycerol levels (5.10, 5.50, and 5.75 mmol/l) when compared to the control level (4.40 mmol/l). The administration of the MSG doses for 6 months to female rats significantly ($p<0.05$) increased their TC and TAG levels while only the MD and HD administration of soya beans significantly increased the TC and TAG levels when compared to the control. For the 2 months duration, the male rats administered LD, MD, and HD MSG had comparable TC levels to the control, whereas the soya bean doses produced no significant ($p>0.05$) changes to the TAG levels when compared to the control. Both the MD and HD MSG and soya bean significantly ($p<0.05$) increased the TC levels after 4 months administration to the male rats, while the administration of HD MSG significantly increased the TAG levels (5.70 mmol/l) when compared to the control level (4.25 mmol/l). The 6 months administration of the MSG and soya beans to the male rats significantly ($p<0.05$) elevated the TC and TAG levels when compared to their control levels.

Table 4.10: Total cholesterol and triacylglycerol levels of rats administered monosodium glutamate and soyabeans

DURATION	GROUPS	TC (mmol/l)	TC (mmol/l)	TG (mmol/l)	TG (mmol/l)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	$3.85\pm0.23^{a*}$	$3.85\pm0.23^{a*}$	$2.60\pm0.29^{a*}$	$2.60\pm0.29^{a*}$
	LD	$3.92\pm0.16^{a*}$	$3.65\pm0.07^{a*}$	$3.87\pm0.17^{b*}$	$2.65\pm0.27^{a**}$
	MD	$4.75\pm0.16^{b*}$	$3.80\pm0.14^{a**}$	$4.40\pm0.28^{c*}$	$2.24\pm0.07^{a**}$
	HD	$5.08\pm0.40^{c*}$	$3.56\pm0.23^{a**}$	$4.60\pm0.28^{c*}$	$2.60\pm0.14^{a**}$
4 MONTHS					
	C	$6.05\pm0.35^{d*}$	$6.05\pm0.35^{b*}$	$4.40\pm0.56^{c*}$	$4.40\pm0.56^{b*}$
	LD	$6.15\pm0.35^{d*}$	$5.65\pm0.49^{b**}$	$5.10\pm0.42^{d*}$	$4.30\pm0.28^{b**}$
	MD	$6.75\pm0.77^{d*}$	$5.85\pm0.21^{b**}$	$5.50\pm0.42^{d*}$	$4.40\pm0.14^{b**}$
	HD	$7.40\pm0.42^{e*}$	$5.95\pm0.21^{bd**}$	$5.75\pm0.21^{d*}$	$4.35\pm0.35^{b**}$
6 MONTHS					
	C	$6.10\pm0.28^{d*}$	$6.10\pm0.28^{de*}$	$4.10\pm0.14^{c*}$	$4.10\pm0.14^{b*}$
	LD	$7.45\pm0.63^{e*}$	$6.20\pm0.28^{e**}$	$6.05\pm0.21^{e*}$	$4.25\pm0.21^{b**}$
	MD	$7.50\pm0.14^{e*}$	$6.85\pm0.35^{f**}$	$5.85\pm0.21^{e*}$	$5.05\pm0.35^{c**}$
	HD	$9.05\pm0.35^{f*}$	$5.60\pm0.15^{c**}$	$6.30\pm0.42^{e*}$	$5.00\pm0.14^{c**}$
			MALES		
2 MONTHS					
	C	$3.43\pm0.04^{ga*}$	$3.43\pm0.04^{a*}$	$2.37\pm0.17^{fa*}$	$2.37\pm0.17^{a*}$
	LD	$3.87\pm0.08^{ga*}$	$3.54\pm0.17^{a**}$	$3.25\pm0.07^{g*}$	$2.47\pm0.24^{a**}$
	MD	$3.86\pm0.24^{ga*}$	$3.92\pm0.09^{g*}$	$3.22\pm0.24^{g*}$	$2.62\pm0.24^{a**}$
	HD	$4.10\pm0.53^{ha*}$	$4.05\pm0.05^{g*}$	$3.42\pm0.24^{gj*}$	$2.52\pm0.10^{a**}$
4 MONTHS					
	C	$4.65\pm0.21^{h*}$	$4.65\pm0.21^{h*}$	$4.25\pm0.21^{hc*}$	$4.25\pm0.21^{b*}$
	LD	$7.35\pm0.35^{ie*}$	$4.75\pm0.07^{hi**}$	$4.75\pm0.21^{h*}$	$4.50\pm0.14^{b*}$
	MD	$7.10\pm0.28^{ie*}$	$4.85\pm0.49^{ij**}$	$4.60\pm0.56^{kh*}$	$4.15\pm0.21^{b*}$
	HD	$7.40\pm0.28^{ie*}$	$5.20\pm0.28^{j**}$	$5.70\pm0.28^{ike*}$	$4.25\pm0.35^{b**}$
6 MONTHS					
	C	$5.50\pm0.42^{j*}$	$5.50\pm0.42^{b*}$	$3.70\pm0.14^{j*}$	$3.70\pm0.14^{d*}$
	LD	$7.40\pm0.42^{ie*}$	$6.00\pm0.14^{de**}$	$5.35\pm0.21^{i*}$	$4.30\pm0.14^{b**}$
	MD	$8.15\pm0.35^{k*}$	$6.45\pm0.21^{e**}$	$5.25\pm0.35^{i*}$	$4.95\pm0.35^{c**}$
	HD	$8.85\pm0.49^{kf*}$	$7.45\pm0.49^{f**}$	$6.05\pm0.35^{ke*}$	$5.20\pm0.42^{c**}$

Values are means ± standard deviations n=5 Values with different superscript letter(s) (a-m) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean, TC – Total Cholesterol, TAG – triacylglycerol

4.1.11: Superoxide dismutase and catalase levels

Table 4.11 shows the superoxide dismutase and catalase levels of rats administered monosodium glutamate and soya beans for 2, 4, and 6 months. The result showed no significant ($p>0.05$) changes occurred in the SOD and CAT levels following 2 and 4 months administration of LD, MD, and HD soya beans, while all the doses of MSG significantly ($p<0.05$) decreased the SOD and MSG after 4 months administration. The soya bean administration significantly ($p<0.05$) decreased the SOD and CAT levels when administered for 6 months, while the MD and HD administration of MSG significantly ($p<0.05$) decreased the SOD levels (29948.50 and 27647.00 U/gHb respectively) when compared to the control (38049.50 U/gHb) and CAT levels (25.85 and 25.40 U/gHb) when compared to the control (45.85 U/gHb). For the male rats, the administration of LD, MD, and HD MSG for 2 months significantly ($p<0.05$) increased the SOD levels, while the administration LD and MD soya bean for 2 months produced SOD levels comparable to the control. The CAT levels of male rats administered LD and MD of MSG and soya beans were comparable to the control levels. The SOD levels of male rats administered MD and HD MSG and soya beans were significantly ($p<0.05$) lower than their control levels, while no significant changes were observed in the CAT levels of the male rats administered the soya bean doses for 4 months. The 6 months administration of MD and HD MSG significantly ($p<0.05$) decreased the SOD levels of the male rats when compared to the control, while the administration of MD and HD soya bean for 6 months significantly ($p<0.05$) decreased the CAT levels of the male rats. The SOD levels of the male rats administered LD, MD, and HD soya beans (24277.50, 27166.50, and 26967.50 U/gHb respectively) were significantly ($p<0.05$) lower than the control rats (47060 U/gHb).

Table 4.11: Superoxide dismutase and catalase levels of rats administered monosodium glutamate and soya beans

DURATION	GROUPS	SOD (U/gHb)	SOD (U/gHb)	CAT(U/gHb)	CAT(U/gHb)
		MSG	**SOY**	**MSG**	**SOY**
2 MONTHS			**FEMALES**		
	C	$20320.00\pm1583.91^{a*}$	$20320.00\pm1583.91^{a*}$	$45.85\pm1.34^{a*}$	$45.85\pm1.34^{a*}$
	LD	$16645.50\pm1378.15^{b*}$	$21245.00\pm544.47^{a**}$	$35.15\pm2.61^{b*}$	$49.00\pm1.41^{a**}$
	MD	$16402.00\pm562.85^{b*}$	$22530.00\pm3436.53^{a**}$	$25.85\pm2.47^{c*}$	$47.50\pm1.26^{a**}$
	HD	$8880.50\pm1074.09^{c*}$	$19625.00\pm1308.14^{a**}$	$25.40\pm5.09^{c*}$	$46.50\pm3.53^{a**}$
4 MONTHS					
	C	$20835.00\pm739.63^{ai*}$	$20835.00\pm739.63^{a*}$	$47.50\pm2.12^{a*}$	$47.50\pm2.12^{ad*}$
	LD	$18103.00\pm506.28^{b*}$	$22315.00\pm302.64^{a**}$	$32.50\pm3.53^{b*}$	$43.50\pm4.94^{ad**}$
	MD	$16818.50\pm1144.80^{b*}$	$19348.50\pm101.11^{a**}$	$29.00\pm4.24^{c*}$	$44.50\pm2.12^{ad**}$
	HD	$11248.50\pm1646.85^{c*}$	$18978.50\pm936.91^{a**}$	$27.50\pm0.70^{c*}$	$33.50\pm3.53^{b**}$
6 MONTHS					
	C	$38049.50\pm2358.20^{d*}$	$38049.50\pm2358.20^{b*}$	$45.85\pm1.34^{a*}$	$45.85\pm1.34^{a*}$
	LD	$34350.00\pm5389.56^{dl*}$	$23163.00\pm2172.23^{a**}$	$35.15\pm2.61^{b*}$	$39.70\pm3.67^{b*}$
	MD	$29948.50\pm2137.58^{el*}$	$18746.50\pm4489.42^{a**}$	$25.85\pm2.47^{c*}$	$38.25\pm2.47^{bc**}$
	HD	$27647.00\pm1927.57^{e*}$	$13825.00\pm2432.44^{c**}$	$25.40\pm5.09^{c*}$	$33.20\pm2.82^{bc**}$
			MALES		
2 MONTHS					
	C	$19014.50\pm303.34^{fa*}$	$19014.50\pm303.34^{da*}$	$28.00\pm4.24^{dc*}$	$38.00\pm4.24^{ce**}$
	LD	$12820.50\pm2093.74^{g*}$	$17805.00\pm289.91^{d**}$	$29.00\pm4.24^{df*}$	$36.50\pm3.53^{c**}$
	MD	$12798.00\pm704.27^{g*}$	$17668.00\pm243.24^{d**}$	$30.50\pm4.94^{df*}$	$32.00\pm2.82^{c*}$
	HD	$7973.50\pm504.16^{hc*}$	$13844.00\pm670.33^{e**}$	$23.00\pm4.24^{c*}$	$34.50\pm2.12^{c**}$
4 MONTHS					
	C	$23165.00\pm2787.41^{i*}$	$23165.00\pm2787.41^{fa*}$	$44.00\pm4.24^{eg*}$	$44.00\pm4.24^{dfa*}$
	LD	$22791.00\pm2647.40^{i*}$	$15482.50\pm1802.41^{g**}$	$33.50\pm3.53^{f*}$	$40.50\pm4.94^{de**}$
	MD	$19061.00\pm311.12^{fa*}$	$12442.50\pm2137.58^{g**}$	$29.50\pm6.36^{df*}$	$41.50\pm2.12^{de**}$
	HD	$15197.00\pm688.72^{j*}$	$13504.00\pm336.58^{g**}$	$24.00\pm5.65^{dc*}$	$43.00\pm7.07^{da**}$
6 MONTHS					
	C	$47060.50\pm1694.93^{k*}$	$47060.50\pm1694.93^{hb*}$	$48.65\pm2.61^{e*}$	$48.65\pm2.61^{fa*}$
	LD	$46037.00\pm4821.05^{k*}$	$24277.50\pm1820.79^{fa**}$	$42.00\pm1.55^{g*}$	$48.15\pm1.90^{fa**}$
	MD	$32480.00\pm2539.92^{l*}$	$27166.50\pm1347.03^{fa*}$	$33.20\pm2.54^{f*}$	$38.55\pm3.88^{ce**}$
	HD	$33392.00\pm2527.19^{l*}$	$26967.50\pm3260.46^{fa*}$	$26.15\pm2.89^{c*}$	$36.00\pm6.78^{c**}$

Values are means ± standard deviations, n=5. Values with different superscript letter(s) (a-l) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean, SOD – Superoxide dismutase, CAT – Catalase

4.1.12: Glutathione peroxidise and malondialdehyde levels

Table 4.12 shows the concentration of glutathione peroxidise and malondialdehyde of rats administered MSG and soya beans. The glutathione peroxidase levels of the female rats were significantly ($p<0.05$) decreased by the administration of MSG and soya beans for 2 months while no significant changes were observed on the malondialdehyde levels after the 2 months administration of MSG and soya beans. The 4 months administration of LD, MD, and HD MSG significantly ($p<0.05$) decreased the glutathione levels (66.95, 54.40, and 50.85 U/L) and increased the MDA levels (55.00, 51.00, and 81.00 nmol/ml respectively) when compared to their respective controls (76.05 U/L and 35.00 nmol/ml respectively). The 6 months administration of MSG significantly reduced the GPx levels and significantly ($p<0.05$) elevated the MDA levels. No significant difference was observed in the GPx and MDA levels of rats administerd LD soya bean for 6 months relative to the control levels. For the male rats the LD administration of MSG and soya bean for 2 months has no significant ($p>0.05$) effect on the GPx and MDA levels. After 4 months administration, the LD, MD and HD MSG significantly ($p<0.05$) decreased the GPx levels and elevated the MDA levels when compared to the control. No significant effect was observed on the GPx levels of the rats administered the various soya bean doses, for 4 months, while the LD and MD administration of soya bean to the male rats for 4 months significantly ($p<0.05$) increased the MDA levels (37.50 and 41.50 nmol/ml respectively) The administration of MD and HD MSG and soya bean, significantly ($p<0.05$) decreased the GPx levels and significantly ($p<0.05$) increased the MDA levels.

Table 4.12: Glutathione peroxidise and malondialdehyde concentrations of rats administered MSG and soya beans

DURATION	GROUPS	GPx (U/L)	GPx (U/L)	MDA (nmol/ml)	MDA (nmol/ml)
		MSG	**SOY**	**MSG**	**SOY**
			FEMALES		
2 MONTHS					
	C	$76.70\pm2.26^{a*}$	$76.70\pm2.26^{ad*}$	$22.00\pm5.65^{a*}$	$22.00\pm5.65^{a*}$
	LD	$63.45\pm2.19^{b*}$	$42.40\pm4.24^{b**}$	$23.00\pm4.24^{a*}$	$18.50\pm7.77^{a*}$
	MD	$55.70\pm0.98^{c*}$	$56.90\pm5.23^{c*}$	$25.50\pm6.36^{a*}$	$20.00\pm9.89^{a*}$
	HD	$44.10\pm5.79^{d*}$	$44.15\pm8.98^{b*}$	$20.00\pm4.24^{a*}$	$19.50\pm9.19^{a*}$
4 MONTHS					
	C	$76.05\pm5.30^{a*}$	$76.05\pm5.30^{c*}$	$35.00\pm2.82^{b*}$	$35.00\pm2.82^{b**}$
	LD	$66.95\pm4.45^{b*}$	$77.75\pm3.88^{c**}$	$55.00\pm4.24^{c*}$	$25.50\pm2.12^{a**}$
	MD	$54.40\pm5.51^{c*}$	$68.60\pm8.90^{d**}$	$51.00\pm1.41^{c*}$	$20.00\pm2.82^{a**}$
	HD	$50.85\pm2.05^{c*}$	$62.85\pm4.59^{d**}$	$81.00\pm4.24^{d}*$	$24.50\pm2.12^{a**}$
6 MONTHS					
	C	$70.70\pm2.26^{a*}$	$70.70\pm2.26^{c*}$	$31.41\pm3.53^{a*}$	$31.50\pm3.53^{b*}$
	LD	$54.45\pm4.15^{b*}$	$73.65\pm3.32^{c**}$	$40.00\pm4.24^{b*}$	$35.00\pm4.24^{b*}$
	MD	$47.62\pm3.04^{d*}$	$68.05\pm2.75^{d**}$	$52.50\pm6.36^{c*}$	$36.00\pm2.82^{b**}$
	HD	$40.31\pm2.11^{d*}$	$59.40\pm3.39^{a**}$	$93.00\pm4.24^{e*}$	$41.00\pm1.41^{c**}$
			MALES		
2 MONTHS					
	C	$84.75\pm3.74^{e*}$	$84.75\pm3.74^{e*}$	$19.00\pm4.24^{a*}$	$19.00\pm4.24^{da*}$
	LD	$79.35\pm3.60^{ea*}$	$88.50\pm3.25^{e**}$	$18.00\pm1.41^{a*}$	$18.50\pm3.53^{da*}$
	MD	$67.30\pm4.52^{fgb*}$	$86.95\pm3.74^{e**}$	$24.50\pm6.36^{a*}$	$31.00\pm4.24^{e*}$
	HD	$62.30\pm5.65^{fb*}$	$81.85\pm2.33^{e**}$	$36.50\pm4.94^{b*}$	$36.00\pm1.55^{e*}$
4 MONTHS					
	C	$92.65\pm5.02^{g*}$	$92.65\pm5.02^{f*}$	$31.50\pm3.53^{d*}$	$31.50\pm3.53^{eb*}$
	LD	$82.95\pm2.33^{e*}$	$93.75\pm2.05^{f**}$	$35.00\pm1.41^{b*}$	$33.00\pm2.82^{ef*}$
	MD	$67.50\pm4.66^{fb*}$	$90.80\pm6.74^{f**}$	$40.00\pm2.82^{b*}$	$37.50\pm2.12^{fg*}$
	HD	$70.15\pm3.32^{fb*}$	$91.80\pm2.96^{f**}$	$48.00\pm2.82^{c*}$	$41.50\pm2.12^{g**}$
6 MONTHS					
	C	$81.85\pm1.76^{e*}$	$81.85\pm1.76^{e*}$	$36.50\pm2.12^{b*}$	$36.50\pm2.12^{fb*}$
	LD	$71.95\pm3.88^{ga*}$	$82.75\pm3.46^{e**}$	$40.50\pm2.12^{b*}$	$35.50\pm1.94^{fb**}$
	MD	$61.95\pm2.61^{f*}$	$72.50\pm2.26^{hc**}$	$52.50\pm3.53^{c*}$	$42.00\pm1.41^{gc**}$
	HD	$52.45\pm2.19^{hb*}$	$65.90\pm3.53^{gd**}$	$61.00\pm4.24^{e*}$	$47.00\pm2.82^{hc**}$

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-h) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate, SOY – Soya bean, GPx – Glutathione peroxidise, MDA – Malondialdehyde

4.1.13: Female reproductive hormone levels

The results for the female reproductive hormones of rats administered MSG and soya beans for 2, 4, and 6 months were shown in Table 4.13. No significant changes were observed in the luteinizing hormone (LH) levels of rats administered MSG for 2 and 4 months when compared to the control levels while only the MD and HD administration for 6 months significantly ($p<0.05$) decreased the LH levels. Administration of soya beans produced no significant ($p>0.05$) effect on the LH levels for the 2, 4, and 6 months feeding period. The result for the progesterone (PRG) levels of the rats showed that the MSG and soya bean doses administered respectively for 2 and 4 months caused no significant ($p>0.05$) changes. The 4 months administration of MD and HD MSG significantly ($p<0.05$) decreased the PRG levels (13.55 and 14.10 ng/ml respectively) when compared to the control level (15.05 ng/ml) while all the soya bean doses significantly ($p<0.05$) decreased the PRG levels. The results for the 6 months administration of MSG and soya beans showed significant ($p<0.05$) decrease in the PRG levels when compared to their respective control levels. No significant change was observed in the oestrogen levels after the 2 months administration of LD, MD, and HD MSG while the LD, MD, and HD soya beans significantly ($p<0.05$) decreased the oestrogen levels (68.50, 76.00, and 56.50 pg/ml respectively) when compared to the control level (62.65 pg/ml). The administration of the extracts respectively for 4 and 6 months significantly ($p<0.05$) increased the oestrogen levels when compared to the control. The result for the FSH levels showed that the administration all the doses of MSG and soya beans for 2 and 4 months produced no significant ($p>0.05$) effect. The LD, MD, and HD administration of MSG significantly ($p<0.05$) decreased the FSH levels (79.50, 74.00, and 66.50 pg/ml respectively) relative to the control level (87.50 pg/ml) while only the 6 months HD soya bean administration, significantly ($p<0.05$) decreased the FSH levels (0.35 pg/ml) relative to the control (0.53 pg/ml).

Table 4.13: Female reproductive hormones of rats administered monosodium glutamate and soya beans

DURATION	GROUPS	LH (mIU/ml)	LH (mIU/ml)	PRG (ng/ml)	PRG (ng/ml)	E_2 (pg/ml)	E_2 (pg/ml)	FSH (mIU/ml)	FSH (mIU/ml)
		MSG	**SOY**	**MSG**	**SOY**	**MSG**	**SOY**	**MSG**	**SOY**
2 MONTHS									
	C	$0.54\pm0.12^{a*}$	$0.54\pm0.12^{a*}$	$11.80\pm1.16^{a*}$	$11.80\pm1.16^{a*}$	$62.65\pm3.19^{a*}$	$62.65\pm3.19^{a*}$	$0.25\pm0.04^{a*}$	$0.25\pm0.04^{a*}$
	LD	$0.57\pm0.01^{a*}$	$0.57\pm0.31^{a*}$	$10.10\pm2.54^{a*}$	$12.60\pm2.40^{b*}$	$69.50\pm1.60^{a*}$	$68.50\pm6.36^{b*}$	$0.23\pm0.05^{a*}$	$0.22\pm0.01^{a*}$
	MD	$0.49\pm0.17^{a*}$	$0.51\pm0.16^{a*}$	$13.05\pm0.91^{a*}$	$14.85\pm3.74^{b*}$	$70.00\pm1.14^{a*}$	$76.00\pm2.82^{c**}$	$0.20\pm0.03^{a*}$	$0.25\pm0.04^{a*}$
	HD	$0.51\pm0.15^{ba*}$	$0.63\pm0.38^{a*}$	$11.60\pm2.12^{a*}$	$15.15\pm6.57^{bc*}$	$66.50\pm9.19^{a*}$	$56.50\pm6.36^{d**}$	$0.21\pm0.06^{a*}$	$0.21\pm0.05^{a*}$
4 MONTHS									
	C	$0.65\pm0.03^{b*}$	$0.65\pm0.03^{a*}$	$15.05\pm0.21^{b*}$	$15.05\pm0.21^{c*}$	$86.00\pm2.82^{b*}$	$86.00\pm2.82^{e*}$	$0.38\pm0.04^{b*}$	$0.38\pm0.04^{b*}$
	LD	$0.67\pm0.06^{b*}$	$0.67\pm0.04^{a*}$	$14.40\pm1.13^{b*}$	$15.85\pm0.77^{c*}$	$78.00\pm1.41^{c*}$	$75.00\pm7.07^{ac*}$	$0.35\pm0.06^{b*}$	$0.39\pm0.05^{b*}$
	MD	$0.65\pm0.05^{b*}$	$0.71\pm0.01^{a**}$	$11.30\pm1.41^{a*}$	$13.55\pm2.05^{b*}$	$65.50\pm3.53^{a*}$	$70.5\pm2.12^{ab**}$	$0.31\pm0.02^{b*}$	$0.34\pm0.05^{b*}$
	HD	$0.65\pm0.03^{b*}$	$0.80\pm0.02^{a**}$	$10.80\pm1.41^{a*}$	$14.10\pm0.70^{b**}$	$63.00\pm4.24^{a*}$	$73.5\pm3.53^{ac**}$	$0.32\pm0.06^{b*}$	$0.36\pm0.03^{b*}$
6 MONTHS									
	C	$0.78\pm0.06^{c*}$	$0.78\pm0.06^{a*}$	$15.15\pm0.49^{b*}$	$15.15\pm0.49^{c*}$	$87.50\pm3.53^{b*}$	$87.50\pm3.53^{d*}$	$0.53\pm0.02^{c*}$	$0.53\pm0.02^{c*}$
	LD	$0.83\pm0.11^{c*}$	$0.77\pm0.02^{a**}$	$12.35\pm0.77^{c*}$	$14.55\pm0.7^{bc**}$	$75.50\pm4.94^{c*}$	$79.50\pm0.70^{c*}$	$0.48\pm0.04^{d*}$	$0.51\pm0.02^{c*}$
	MD	$0.60\pm0.12^{b*}$	$0.79\pm0.01^{a**}$	$9.45\pm0.63^{d*}$	$15.35\pm0.77^{c**}$	$76.00\pm2.82^{c*}$	$74.00\pm4.24^{ac*}$	$0.43\pm0.06^{e*}$	$0.48\pm0.03^{c*}$
	HD	$0.51\pm0.11^{ba*}$	$0.82\pm0.02^{a**}$	$9.55\pm1.20^{d*}$	$15.75\pm0.49^{c**}$	$56.00\pm5.65^{d*}$	$66.50\pm3.53^{b*}$	$0.35\pm0.02^{b*}$	$0.40\pm0.04^{d*}$

Values are means ± standard deviations n=5. Values with different superscript letter (s (a-f)) $p < 0.05$) down the column or symbols (* and **) across the row for each parameter, are significantly different. MSG – Monosodium glutamate, SOY – Soya bean, LH – Luteinizing hormone, PRG – Progesterone, E_2 – Oestrogen, FSH – Follicle stimulating hormone

4.1.14: Male reproductive hormone levels

The male reproductive hormones of rats administered monosodium glutamate and soya beans were presented in Table 4.14. The 2, 4, and 6 months administration of LD MSG and soya bean produced no significant ($p>0.05$) effect on the testosterone levels while the MD and HD MSG and soya bean significantly ($p<0.05$) decreased the testosterone levels of the rats. The LD, MD and HD MSG and soya bean significantly ($p<0.05$) decreased the luteinizing hormone of the male rats when administered for 2 months while no significant ($p>0.05$) effect was observed with 4 months administration. For the 6 months administration, the MSG doses significantly ($p<0.05$) decreased the luteinizing hormone levels of the male rats (0.73, 0.54, and 0.52 mIU/ml respectively) relative to the control level (0.82 mIU/ml) while only the MD and HD soya bean significantly ($p<0.05$) increased the luteinizing hormone level (0.70 and 0.60 mIU/ml respectively). The result for the FSH level showed no significant ($p>0.05$) change observed for 2 months administration of LD, MD, and HD MSG and soya bean. At 4 and 6 months, only the administration of MD and HD MSG significantly ($p<0.05$) increased the FSH level while only the HD soya bean significantly ($p<0.05$) increased the FSH level.

Table 4.14: Male reproductive hormones of rats administered monosodium glutamate and soya beans.

DURATION	GROUPS	Ttos (ng/ml)	Ttos (ng/ml)	LH (mIU/ml)	LH (mIU/ml)	FSH (mIU/ml)	FSH (mIU/ml)
		MSG	**SOY**	**MSG**	**SOY**	**MSG**	**SOY**
2 MONTHS							
	C	$4.48\pm0.00^{a*}$	$4.48\pm0.00^{a*}$	$0.82\pm0.04^{a*}$	$0.82\pm0.04^{a*}$	$0.32\pm0.05^{a*}$	$0.32\pm0.05^{a*}$
	LD	$4.25\pm0.19^{a*}$	$4.86\pm0.09^{a**}$	$0.56\pm0.09^{b*}$	$0.73\pm0.23^{b**}$	$0.28\pm0.05^{a*}$	$0.30\pm0.04^{a*}$
	MD	$2.59\pm0.15^{b*}$	$3.61\pm0.26^{b**}$	$0.45\pm0.05^{c*}$	$0.71\pm0.11^{b**}$	$0.30\pm0.03^{a*}$	$0.31\pm0.01^{a*}$
	HD	$0.92\pm0.09^{c*}$	$3.25\pm0.19^{b**}$	$0.42\pm0.06^{c*}$	$0.70\pm0.07^{b**}$	$0.33\pm0.04^{a*}$	$0.29\pm0.03^{a*}$
4 MONTHS							
	C	$4.40\pm0.36^{a*}$	$4.40\pm0.36^{a*}$	$0.74\pm0.05^{d*}$	$0.80\pm0.05^{a*}$	$0.45\pm0.02^{b*}$	$0.45\pm0.02^{be*}$
	LD	$4.20\pm0.26^{a*}$	$4.66\pm0.11^{a**}$	$0.71\pm0.04^{d*}$	$0.82\pm0.02^{a**}$	$0.40\pm0.04^{b*}$	$0.40\pm0.04^{bc*}$
	MD	$3.12\pm0.08^{d*}$	$3.61\pm0.28^{b**}$	$0.71\pm0.02^{d*}$	$0.85\pm0.04^{a**}$	$0.36\pm0.03^{a*}$	$0.42\pm0.02^{b**}$
	HD	$3.14\pm0.14^{d*}$	$3.30\pm0.28^{b*}$	$0.70\pm0.01^{d*}$	$0.82\pm0.01^{a**}$	$0.31\pm0.05^{a*}$	$0.38\pm0.03^{b**}$
6 MONTHS							
	C	$6.78\pm0.35^{e*}$	$6.78\pm0.35^{c*}$	$0.82\pm0.02^{a*}$	$0.82\pm0.02^{a*}$	$0.69\pm0.04^{c*}$	$0.69\pm0.04^{d*}$
	LD	$6.50\pm0.27^{e*}$	$6.82\pm0.12^{c*}$	$0.73\pm0.03^{d*}$	$0.80\pm0.02^{a**}$	$0.62\pm0.06^{c*}$	$0.64\pm0.02^{d*}$
	MD	$5.72\pm0.28^{f*}$	$5.68\pm0.37^{d*}$	$0.54\pm0.04^{b*}$	$0.70\pm0.02^{b**}$	$0.53\pm0.05^{d*}$	$0.67\pm0.03^{d**}$
	HD	$4.41\pm0.12^{a*}$	$5.12\pm0.17^{d**}$	$0.52\pm0.03^{b*}$	$0.60\pm0.02^{c**}$	$0.44\pm0.06^{b*}$	$0.50\pm0.06^{e**}$

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-f) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate., SOY – Soya bean, Ttos – Testosterone, LH – Luteinizing hormone, FSH – Follicle stimulating hormones

4.1.15: Activities of cancer markers

Table 4.15 shows the cancer markers of rats administered monosodium glutamate and soya bean. No significant ($p>0.05$) change was observed in the colorectal cancer markers of the female rats administered MSG and soya beans. No significant ($p>0.05$) difference was observed in the colorectal markers of female rats administered soya beans for 4 and 6 months while the MD and HD MSG significantly elevated the CEA levels when administered respectively for 4 and 6 months. No significant ($p<0.05$) change was observed on the levels of pancreatic cancer markers for 2 months soya bean administration while all doses of MSG administered for 2 months significantly ($p<0.05$) increased the CA-19-9 levels (1.10, 1.85, and 1.50 U/ml respectively) relative to the control (0.30 U/ml). Only the administration of MD and HD soya bean for 4 and 6 months, significantly ($p<0.05$) increased the CA-19-9 levels of the female rats, when compared to the control, while all the doses of MSG administered for 4 and 6 months significantly ($p<0.05$) increased the CA-19-9 levels. The result for the ovarian cancer marker (CA-125) showed that only the HD soya bean administered for 2, 4, and 6 months respectively, significantly ($p<0.05$) elevated the CA-125 levels while the MD and HD MSG administered for 4 months and LD, MD and HD MSG administered for 6 months significantly ($p<0.05$) increased the CA-125 levels. For the male rats the administered soya bean doses for 2, 4, and 6 months produced no significant ($p>0.05$) effect on the CEA levels while administration of MD and HD MSG significantly ($p<0.05$) increased the CEA levels. The administration of MD and HD MSG for 2 and 4 months significantly ($p<0.05$) increased the PSA levels while after 6 months, all the doses significantly ($p<0.05$) elevated the PSA concentration when compared to the control level. The PSA levels of rats administered LD and MD soya beans were comparable to the control level while rats administered MD and HD soya bean for 4 and 6 months recorded significantly ($p<0.05$) higher PSA levels when compared to the control.

Table 4.15: Cancer markers of rats administered monosodium glutamate and soya bean.

DURATION	GROUPS	CEA (U/ml)	CEA (U/ml)	CA-19-9 (U/ml)	CA-19-9 (U/ml)	CA-125 (U/ml)	CA-125 (U/ml)
		MSG	**SOY**	**MSG**	**SOY**	**MSG**	**SOY**
2 MONTHS				**FEMALES**			
	C	$0.75\pm0.10^{a*}$	$0.75\pm0.10^{a*}$	$0.30\pm0.02^{a*}$	$0.30\pm0.08^{a*}$	$0.80\pm0.14^{a*}$	$0.80\pm0.14^{a*}$
	LD	$0.70\pm0.17^{a*}$	$0.50\pm0.14^{a**}$	$1.10\pm0.49^{b*}$	$0.25\pm0.07^{a**}$	$0.85\pm0.21^{a*}$	$1.20\pm0.20^{a**}$
	MD	$0.70\pm0.14^{a*}$	$0.45\pm0.17^{a**}$	$1.85\pm0.23^{c*}$	$0.30\pm0.00^{a**}$	$0.75\pm0.21^{ae*}$	$1.10\pm0.14^{a**}$
	HD	$0.75\pm0.11^{a*}$	$0.60\pm0.14^{a*}$	$1.50\pm0.25^{c*}$	$0.25\pm0.07^{a**}$	$0.95\pm0.49^{a*}$	$1.70\pm0.14^{b**}$
4 MONTHS							
	C	$0.85\pm0.07^{a*}$	$0.85\pm0.07^{b*}$	$0.65\pm0.05^{d*}$	$0.65\pm0.35^{b*}$	$0.90\pm0.28^{a*}$	$0.90\pm0.28^{c*}$
	LD	$0.85\pm0.21^{a*}$	$0.85\pm0.07^{b*}$	$1.95\pm0.07^{c*}$	$0.65\pm0.21^{b**}$	$1.05\pm0.21^{a*}$	$0.85\pm0.07^{c**}$
	MD	$1.15\pm0.07^{c*}$	$0.90\pm0.14^{b*}$	$1.85\pm0.20^{c*}$	$1.00\pm0.42^{c**}$	$1.55\pm0.21^{b*}$	$0.85\pm0.07^{c**}$
	HD	$1.60\pm0.14^{de*}$	$0.75\pm0.07^{b**}$	$1.85\pm0.07^{c*}$	$0.90\pm0.14^{c**}$	$1.85\pm0.07^{bc*}$	$1.35\pm0.07^{d**}$
6 MONTHS							
	C	$1.45\pm0.21^{d*}$	$1.45\pm0.21^{c*}$	$0.85\pm0.07^{e*}$	$0.85\pm0.07^{bc*}$	$2.10\pm0.28^{c*}$	$2.10\pm0.28^{e*}$
	LD	$1.95\pm0.21^{ef*}$	$1.30\pm0.42^{c**}$	$1.60\pm0.01^{c*}$	$1.05\pm0.21^{c**}$	$3.80\pm0.14^{d*}$	$2.30\pm0.28^{e**}$
	MD	$2.15\pm0.21^{f*}$	$1.70\pm0.28^{c*}$	$2.45\pm0.03^{f*}$	$1.80\pm0.28^{d**}$	$3.75\pm0.21^{d*}$	$2.50\pm0.28^{e**}$
	HD	$2.60\pm0.28^{g*}$	$1.55\pm0.35^{c**}$	$2.50\pm0.01^{f*}$	$1.90\pm0.28^{d**}$	$4.00\pm0.28^{d*}$	$3.20\pm0.28^{f**}$
				MALES			
2 MONTHS						**PSA (ng/ml)**	**PSA (ng/ml)**
	C	$0.75\pm0.07^{ha*}$	$0.75\pm0.07^{d*}$	$0.25\pm0.02^{ga*}$	$0.25\pm0.02^{a*}$	$0.31\pm0.00^{a*}$	$0.31\pm0.00^{ad**}$
	LD	$0.75\pm0.07^{ha*}$	$0.60\pm0.14^{d*}$	$1.55\pm0.03^{hc*}$	$0.28\pm0.05^{a**}$	$0.36\pm0.18^{a*}$	$0.24\pm0.00^{a**}$
	MD	$0.75\pm0.07^{ha*}$	$0.75\pm0.07^{d*}$	$1.75\pm0.07^{hc*}$	$0.30\pm0.07^{a**}$	$0.48\pm0.14^{bd*}$	$0.27\pm0.02^{ac**}$
	HD	$0.70\pm0.14^{ha*}$	$0.75\pm0.21^{d*}$	$1.45\pm0.21^{hjc*}$	$0.25\pm0.04^{a**}$	$0.52\pm0.12^{b*}$	$0.37\pm0.01^{b**}$
4 MONTHS							
	C	$0.95\pm0.21^{iac*}$	$0.95\pm0.21^{e*}$	$0.30\pm0.11^{id*}$	$0.30\pm0.11^{a*}$	$0.23\pm0.01^{c*}$	$0.23\pm0.01^{ad*}$
	LD	$0.80\pm0.14^{ha*}$	$0.90\pm0.00^{e**}$	$1.05\pm0.49^{j*}$	$0.40\pm0.12^{a**}$	$0.20\pm0.01^{c*}$	$0.24\pm0.01^{a*}$
	MD	$1.30\pm0.42^{jcd*}$	$0.85\pm0.21^{e**}$	$1.35\pm0.37^{hj*}$	$0.65\pm0.11^{b**}$	$0.33\pm0.02^{a*}$	$0.30\pm0.02^{ce*}$
	HD	$1.45\pm0.35^{jcd*}$	$1.10\pm0.28^{e**}$	$1.40\pm0.23^{hj*}$	$1.55\pm0.39^{d*}$	$0.44\pm0.03^{d*}$	$0.41\pm0.03^{b*}$
6 MONTHS							
	C	$1.35\pm0.49^{jd*}$	$1.35\pm0.49^{f*}$	0.70±0.01ie*	$0.70\pm0.21^{b*}$	$0.20\pm0.01^{c*}$	$0.20\pm0.01^{d*}$
	LD	$1.55\pm0.07^{jd*}$	$1.25\pm0.35^{f*}$	$1.65\pm0.21^{hc*}$	$0.80\pm0.22^{bc**}$	$0.34\pm0.00^{a*}$	$0.34\pm0.04^{e*}$
	MD	$2.10\pm0.28^{kf*}$	$1.45\pm0.35^{f**}$	$1.70\pm0.14^{hc*}$	$1.00\pm0.21^{c**}$	$0.36\pm0.00^{a*}$	$0.38\pm0.05^{b*}$
	HD	$2.40\pm0.42^{kgf*}$	$1.75\pm0.21^{f**}$	$1.85\pm0.07^{hc*}$	$1.20\pm0.21^{c**}$	$0.49\pm0.02^{b*}$	$0.50\pm0.02^{f*}$

Values are means ± standard deviations n=5. Values with different superscript letter(s) (a-k) down the column or symbols (* and **) across the row for each parameter, are significantly different ($p < 0.05$). MSG - Monosodium glutamate. SOY – Soya bean, CEA – Colorectal cancer marker, CA – 19-9 – Pancreatic cancer marker, PSA – Prostate specific antigen

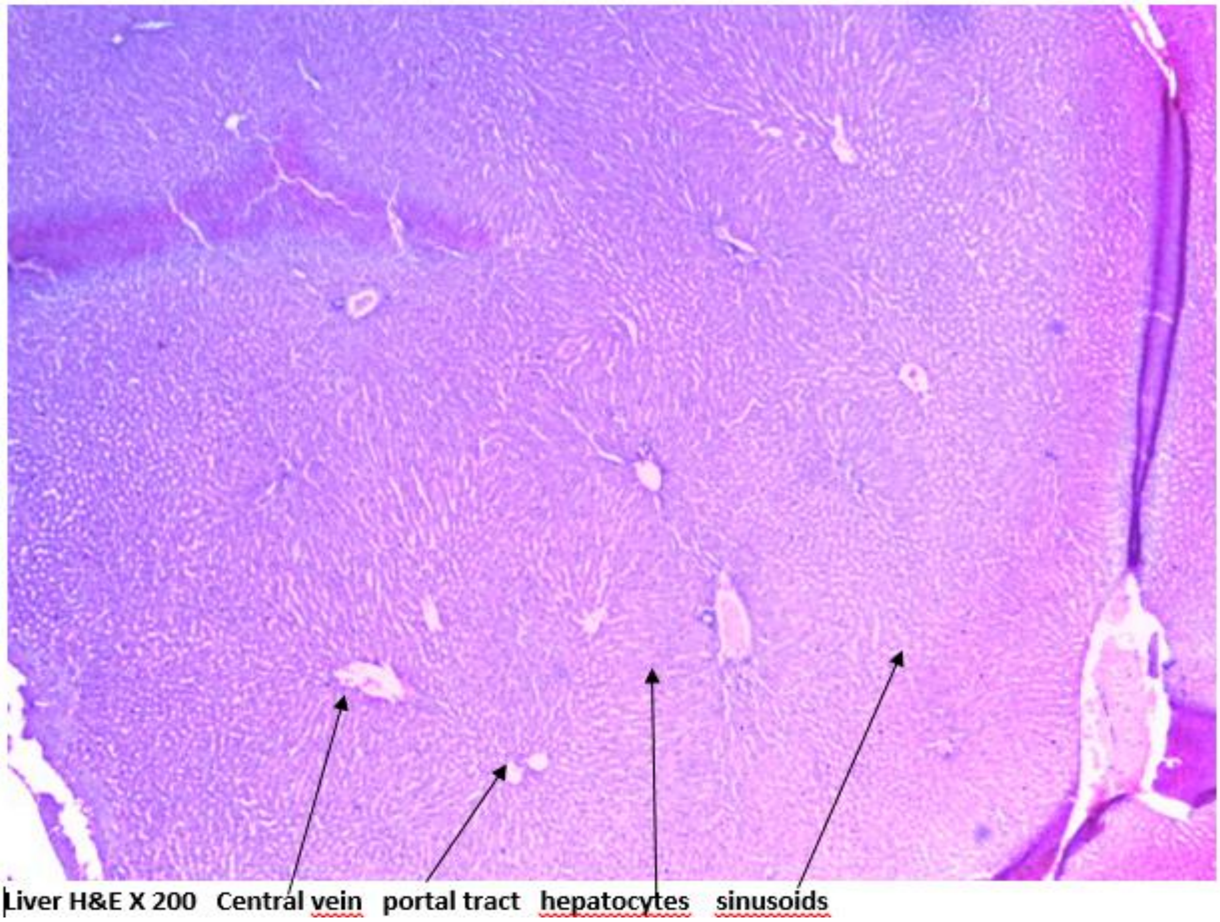

Plate 1: Photomicrograph of the liver of the control female rats showing normal histologic features (x 400)

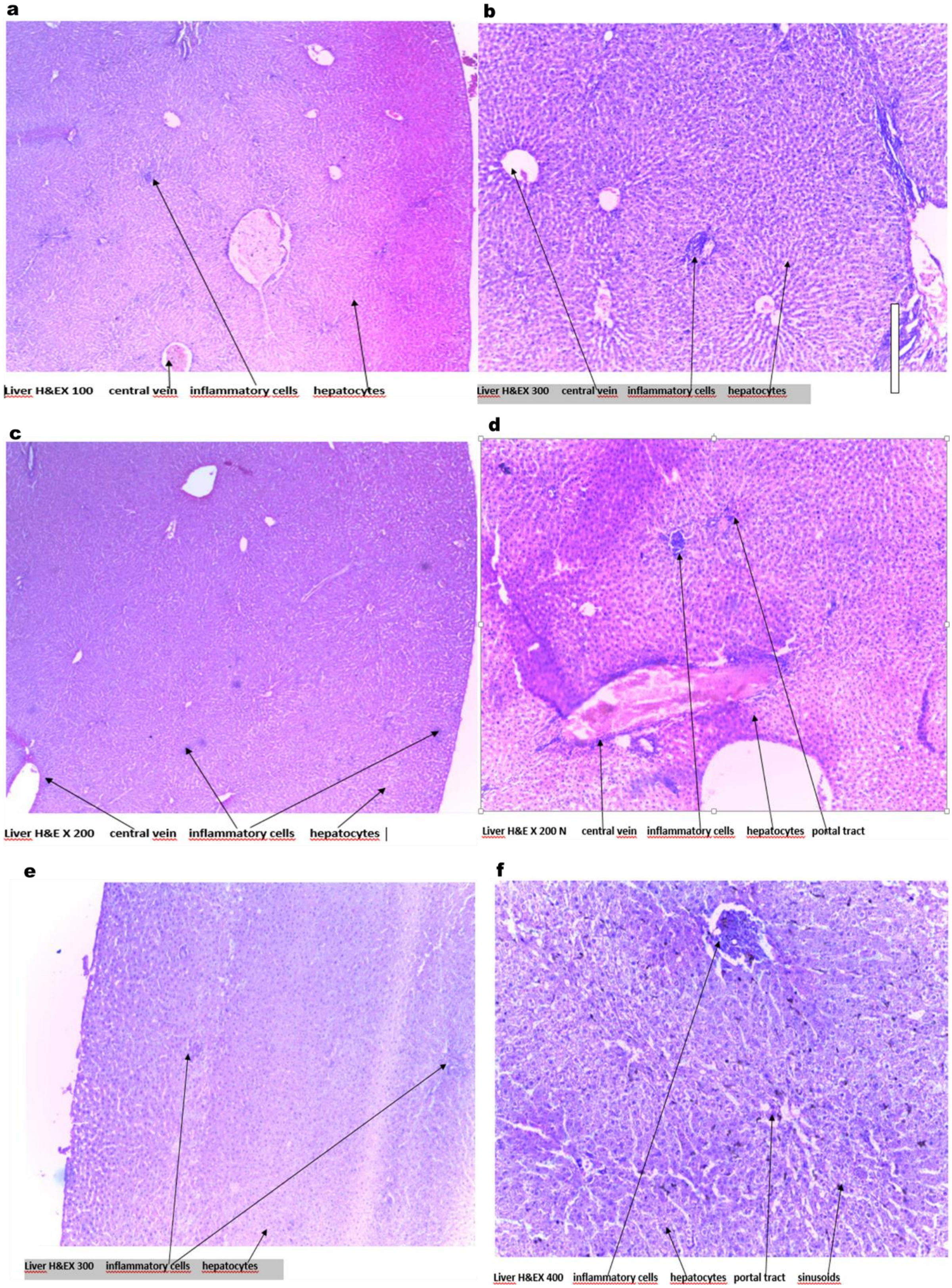

Plate 2 a – f: Photomicrograph of the liver cell of the representative female rats administered soya bean and MSG respectively showing inflammation of the hepatocytes (x400)

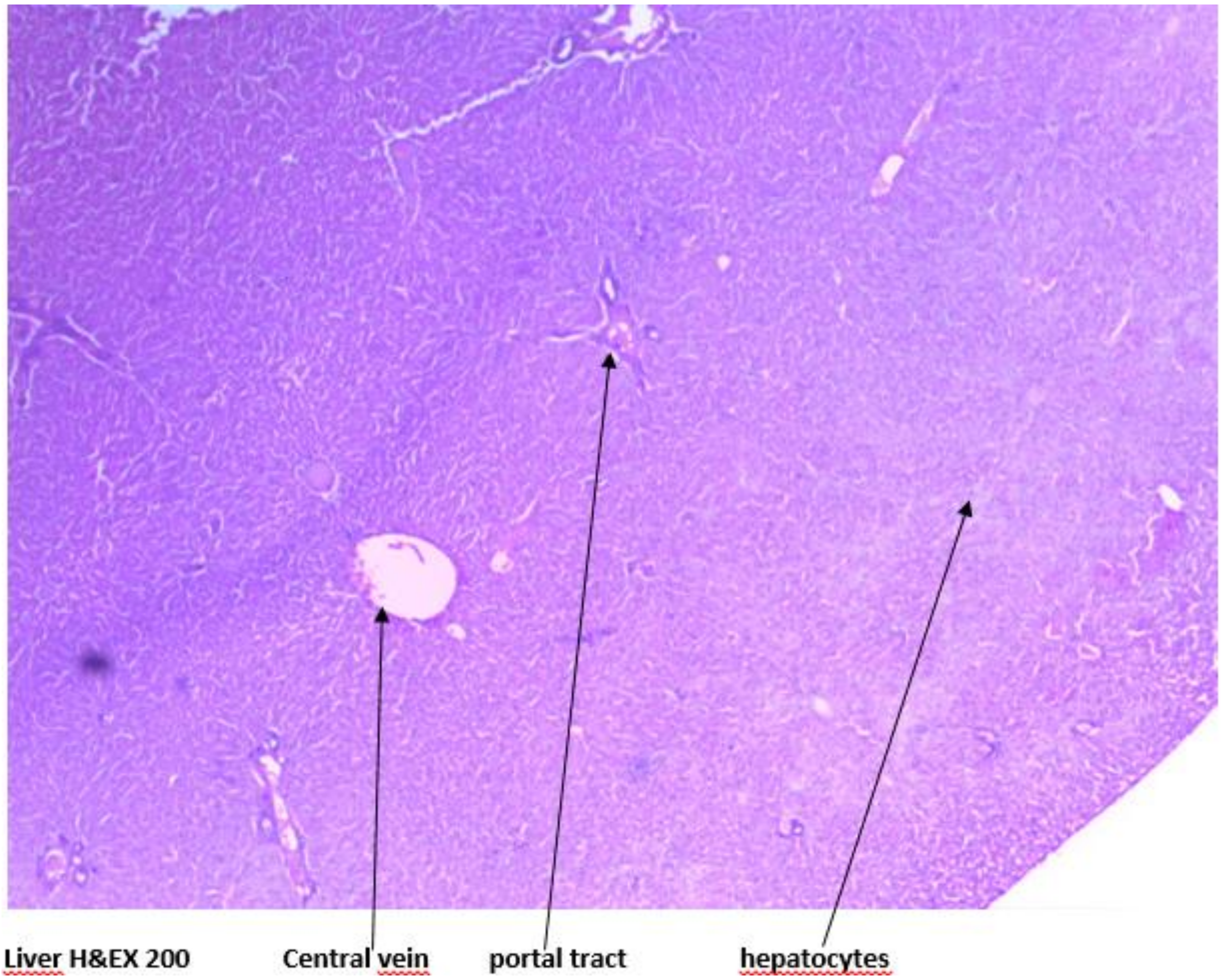

Plate 3: Photomicrograph of the section of the liver of the control male rats showing normal histologic features (x400)

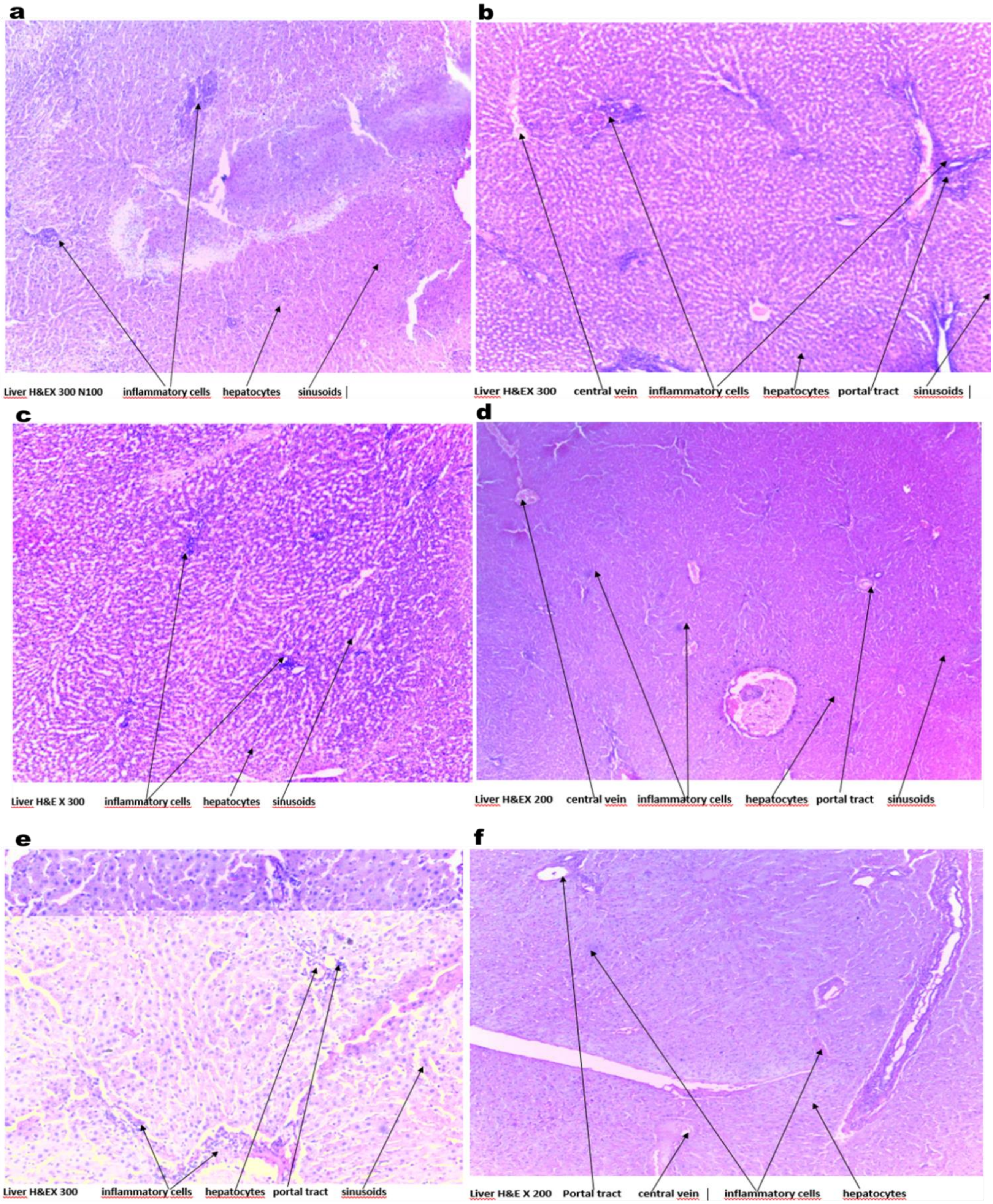

Plate 4 a – f: Photomicrograph of the section of the liver cell of the representative male rats administered soya bean and MSG respectively showing inflammation of the hepatocytes (x400)

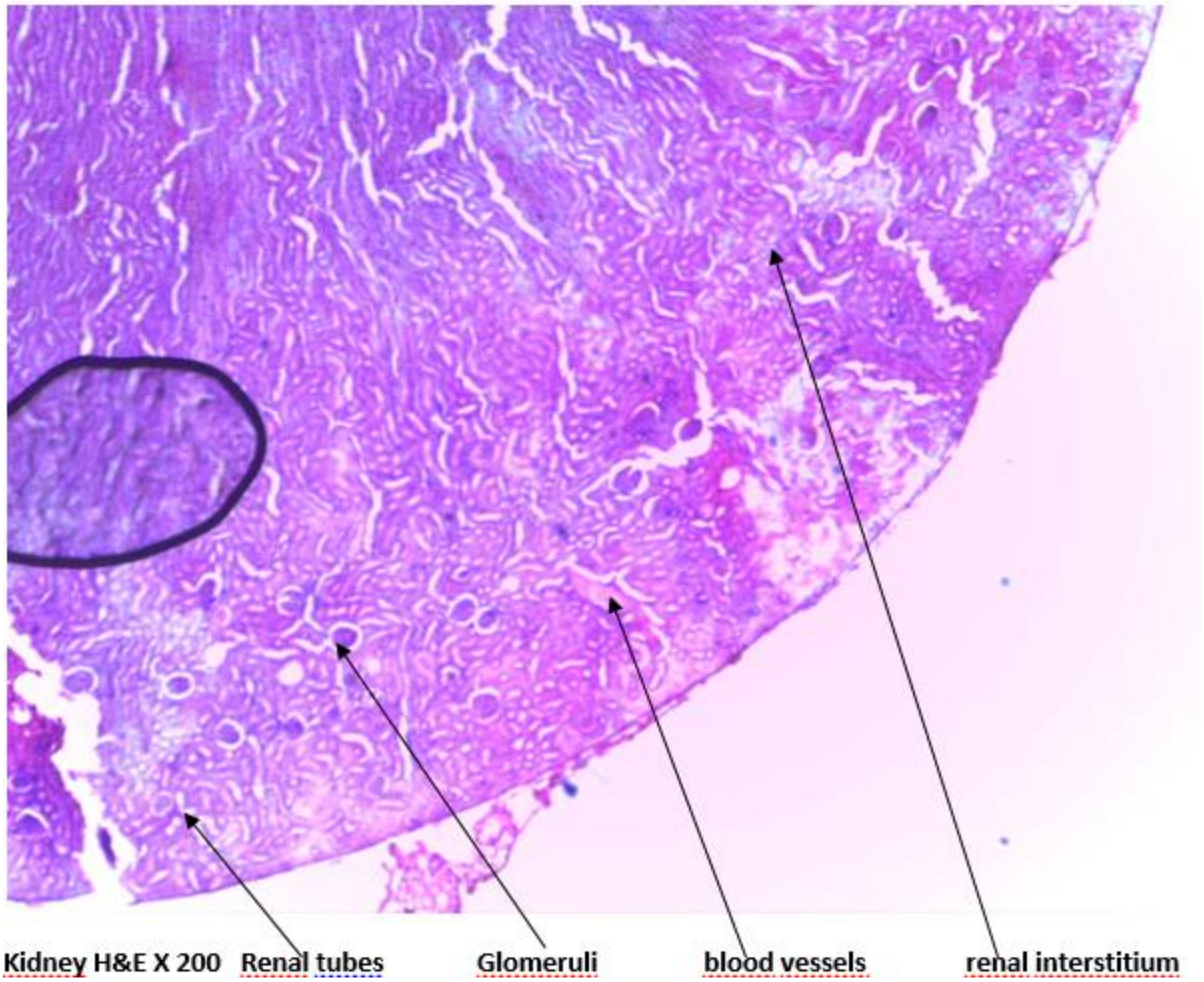

Plate 5: Photomicrograph of the renal cortex of the control female rat, showing the cytoarchitecture of the kidney. The glomeruli were intact with the normal endothelium. This urinary space and bowmam’s capsule were also intact and normal. The juxtaglomerular apparatus also has the normal appearance. The urinary tubules, linings and lumen were normal, connective tissue were adequate.

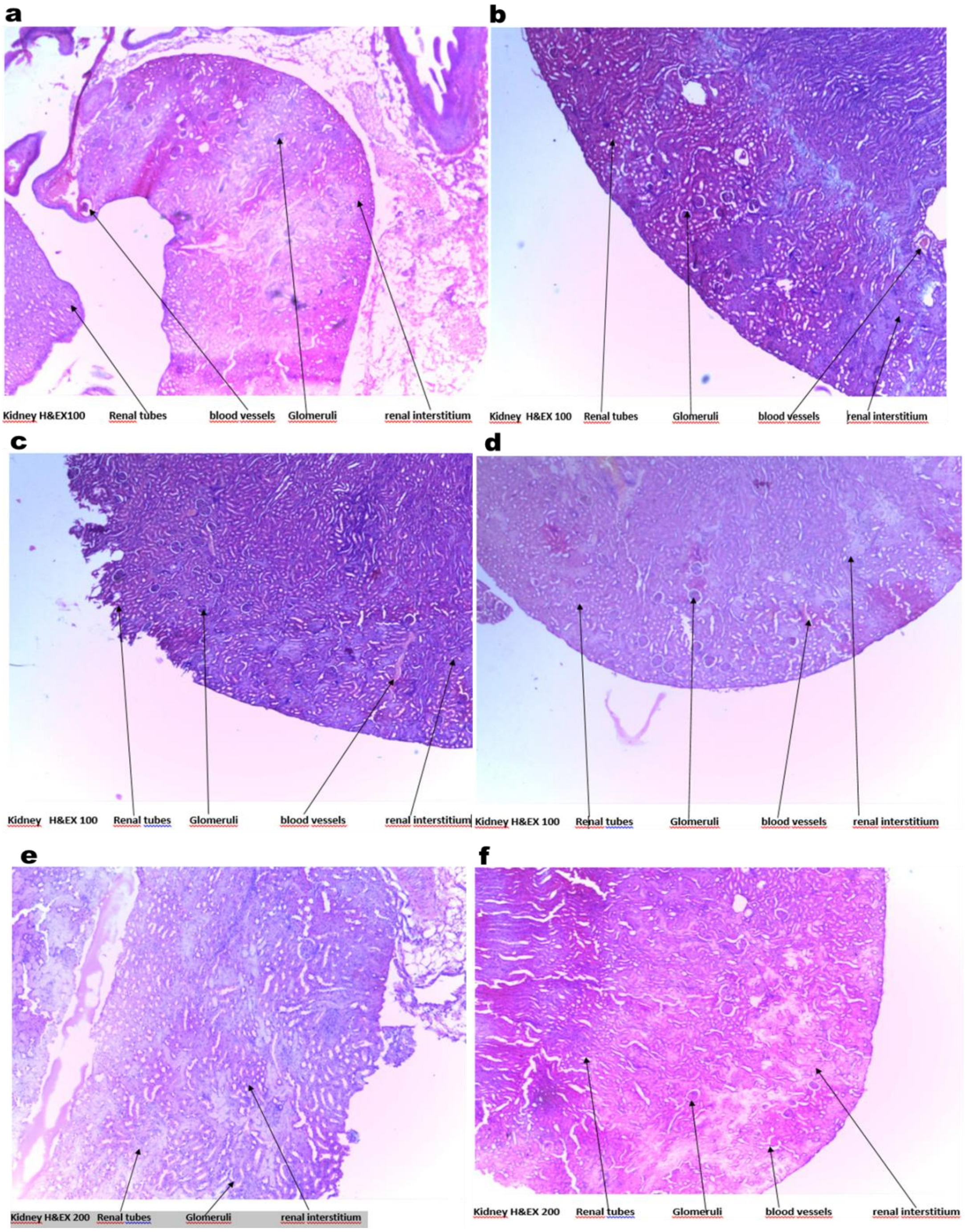

Plate 6 a – f: Photomicrograph of the section of the renal cortex of the female rats administered soya bean and MSG respectively, showing the glomeruli with interact endothelial cells. The glomerulus is bifocated. The glomeruli presented with more dilated urinary space, whose urinary capsule lining were not distinct, or the juxtaglomerula apparatus not well placed. The lumen tubules were dilated. The tubular linings were merged together as the nuclei were enlarged (hyperplasia)

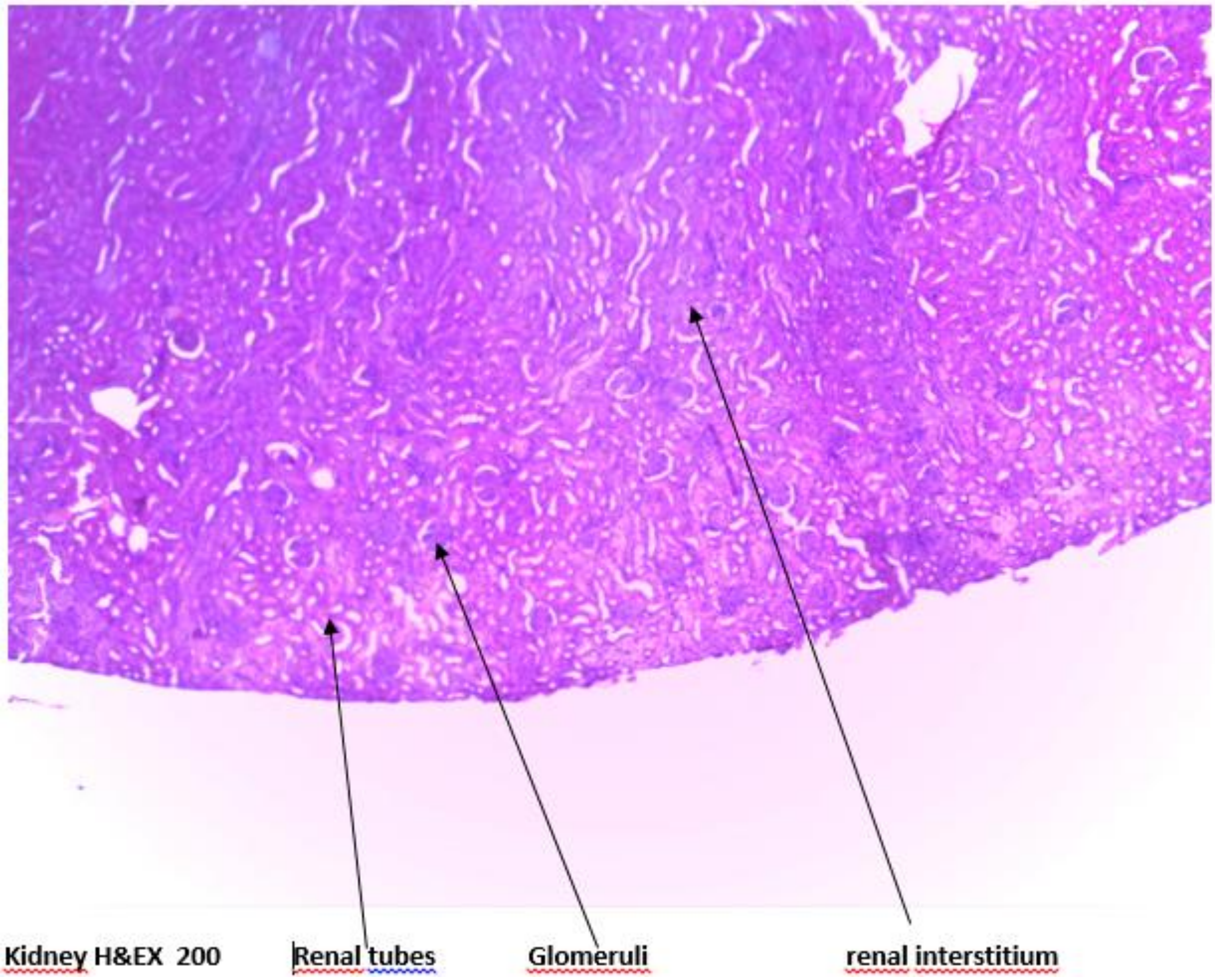

Plate 7: Photomicrograph of the renal cortex of the representativr male rat in the control group, showing the cyto-architecture of the kidney. The glomeruli were intact with the normal endothelium. The urinary space as bowman's capsule were also intact as normal. The juxteglomerular apparatus also had normal appearance. The urinary tubules, lining and lumen were normal, connective tissue were adequate.

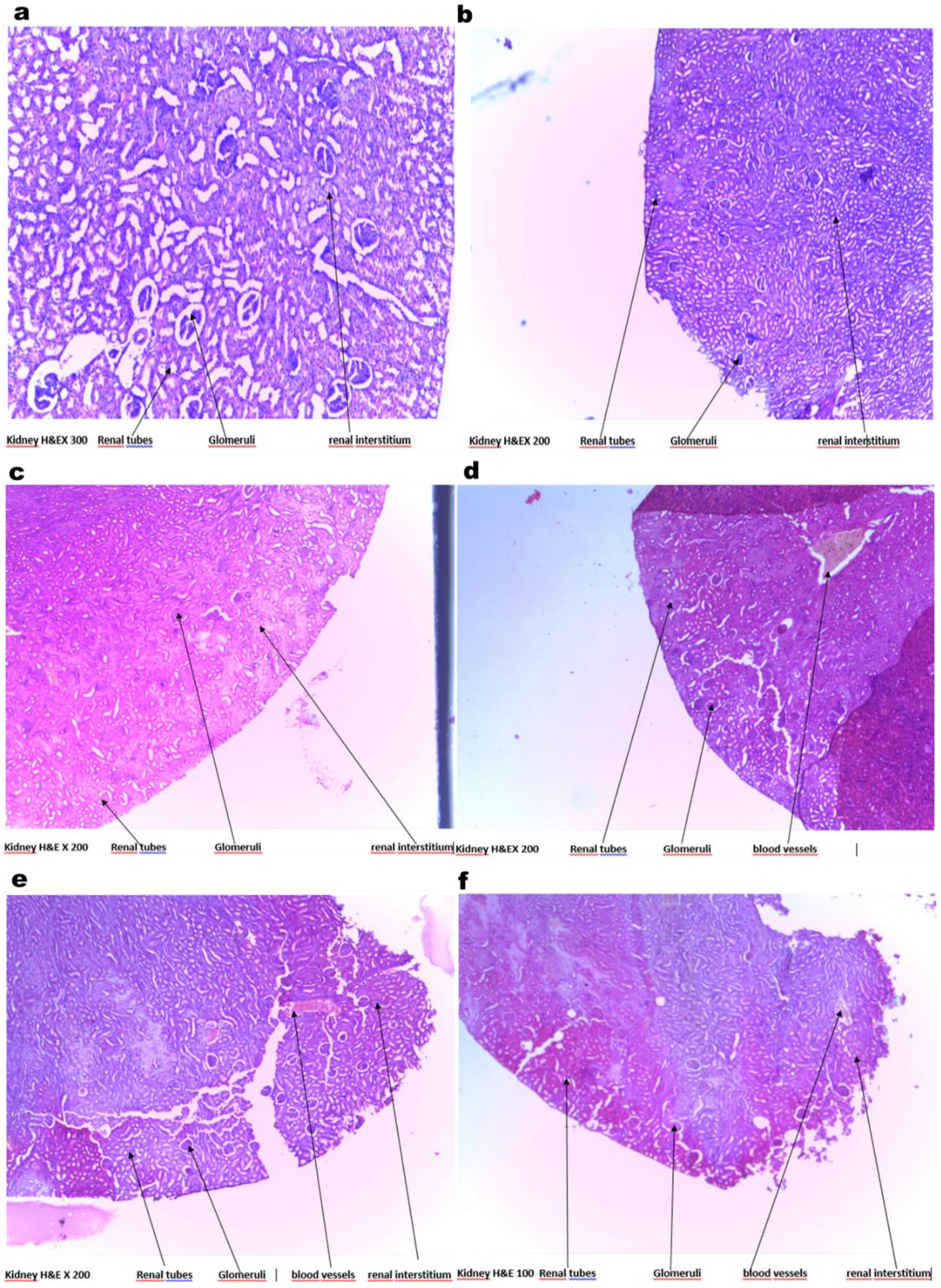

Plate 8 a – f: Photomicrograph of the renal cortex of the representative male rats administered soya bean and MSG, showing a glomerulus with normal endothelium but the urinary space was deleted (wider). The bowman’s capsule were not well outlined. There were losses of the cells of the connective tissue as tubules. The organ were slightly affected

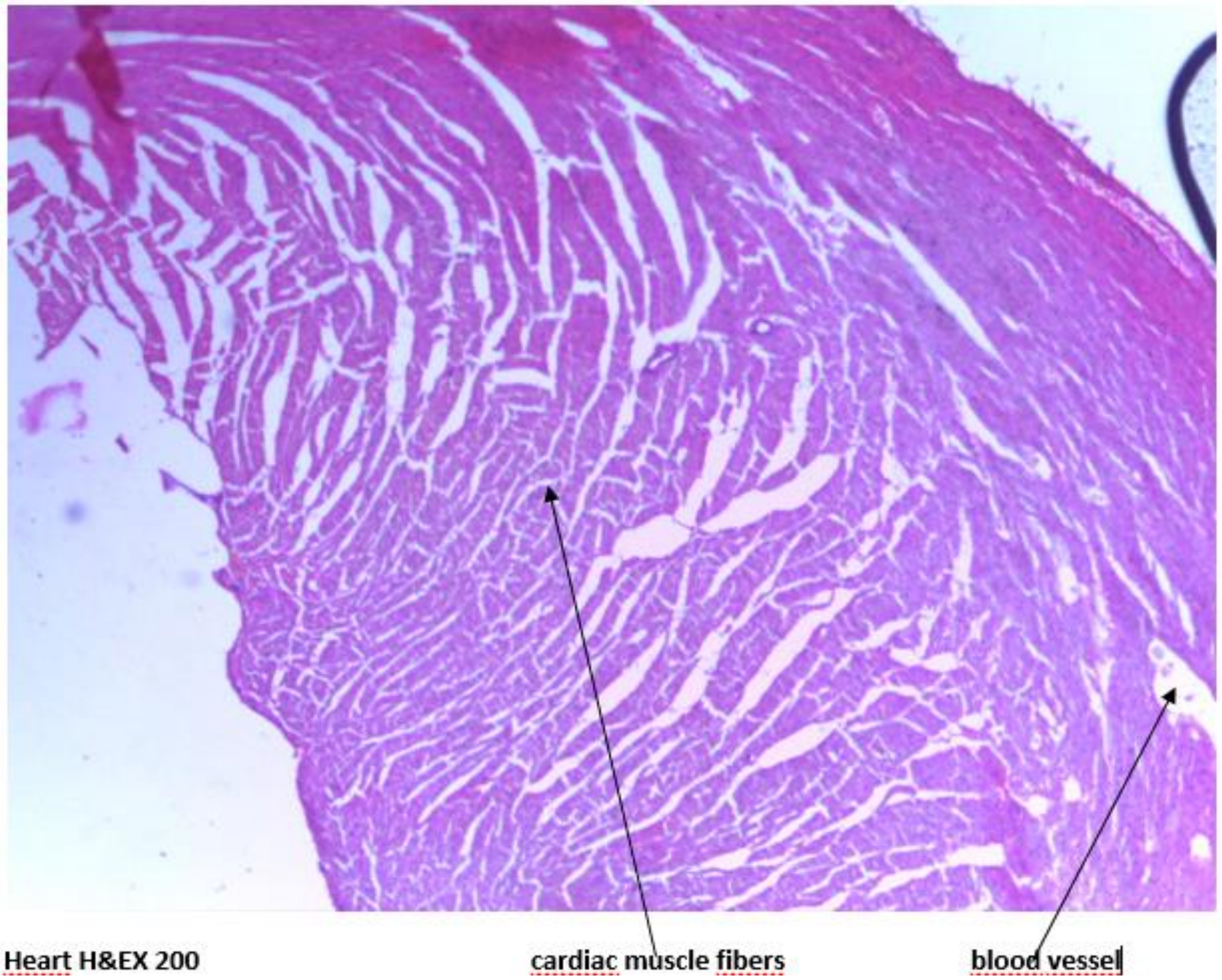

Plate 9: Photomicrograph of the section of the cardiac muscle of the representative female rat in control group, showing the bundles of the muscle appearing scanty, also the connective tissue appeared scanty. The intercalated disc appeared long as less in number than any section.

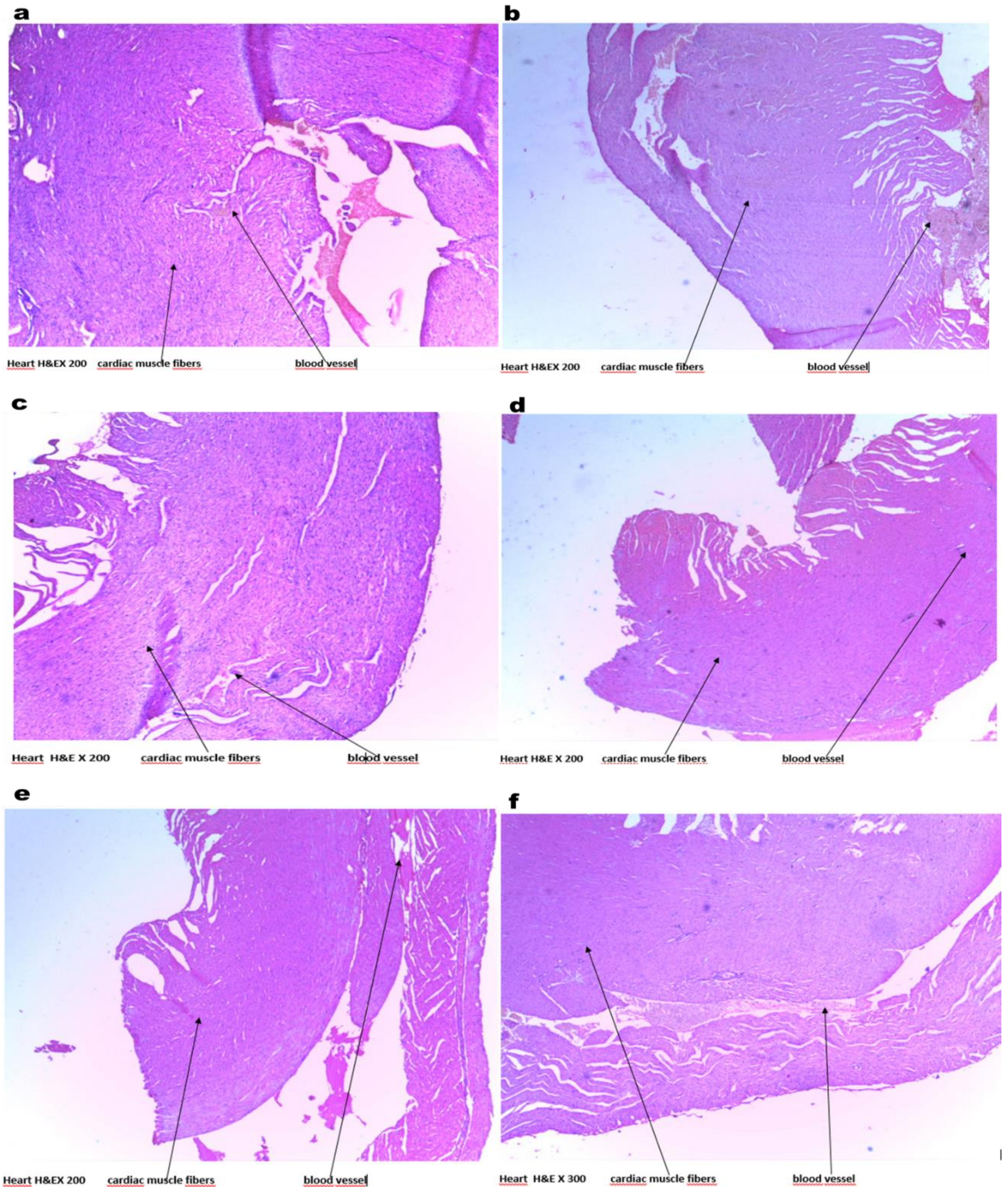

Plate 10 a – f: Photomicrograph of the section of the cardiac muscle of the representative female rats administered soya beans and MSG, showing thickened bundles of muscles unit, the bundles meshed together so that the branding pattern of the cardiac muscle are easily distinguished out. The intercalated discs appeared mostly at the periphery, some nuclei are seen at the center of the bundles.

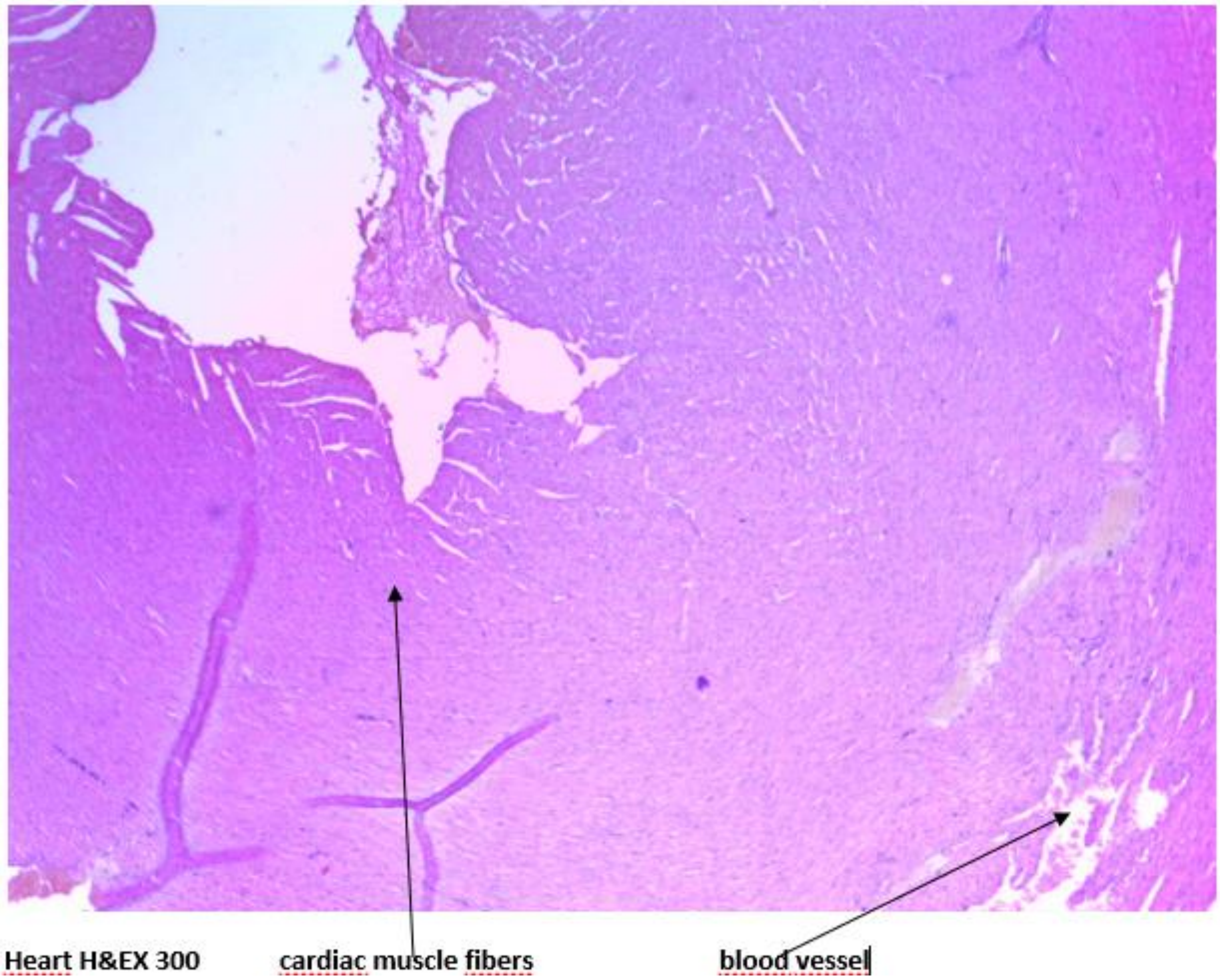

Plate 11: Photomicrograph of the section of the cardiac muscle of the male rats in the control group showing the muscle bundles of the heart appearing scanty or depletion, also the connective tissue appeared scanty. The intercalated disc appeared long are less in number than any section.

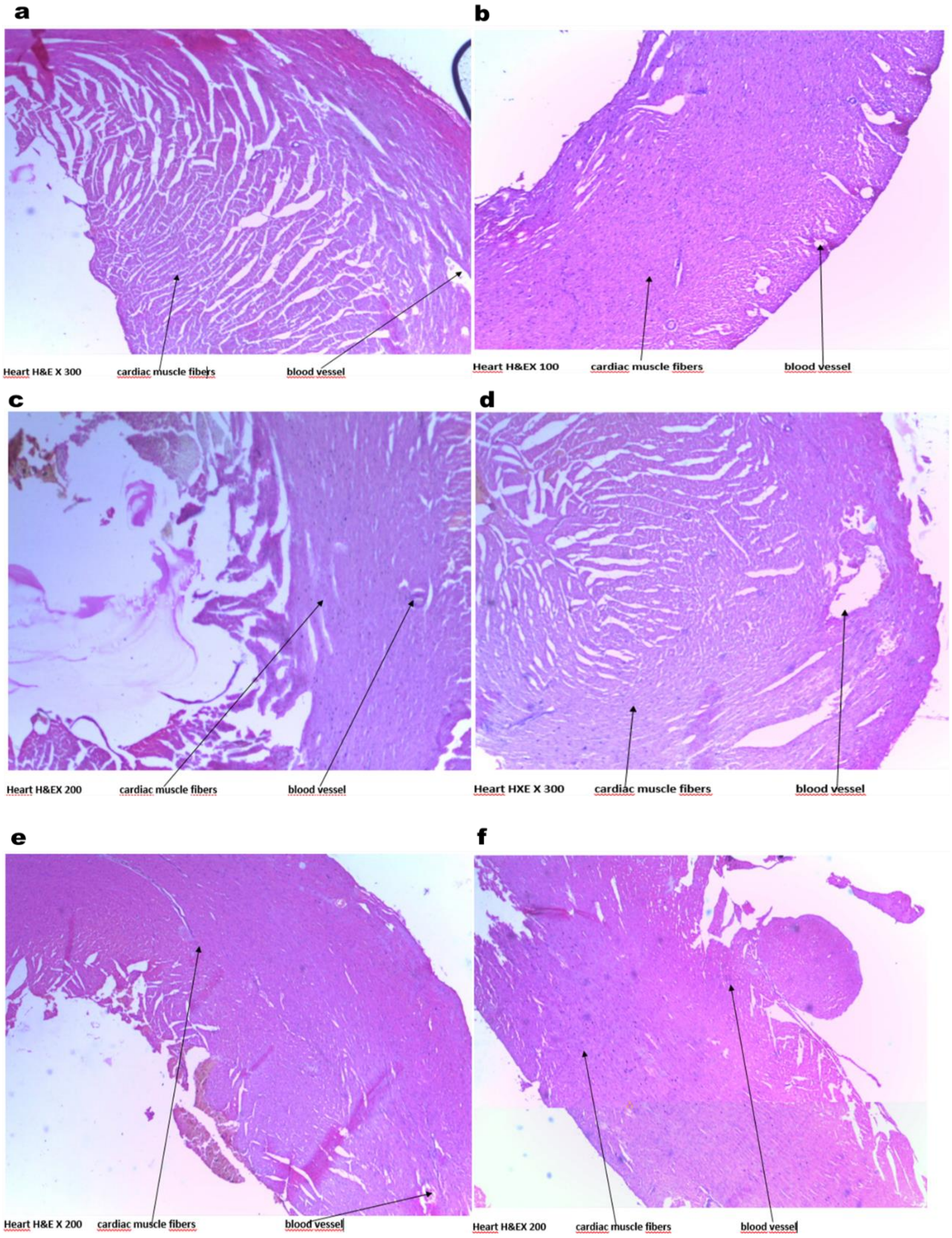

Plate 12 a – f: Photomicrograph of the section of the cardiac muscle of male rats admistered soya bean and MSG respectively, showing the cardiac muscle bundles that are slender and branched but the branching is not as in the normal cardiac network. The intercalated disc appeared thickened and longer, also is seen all over the section (metaplasia of the cells). The tissue is high vascularized.

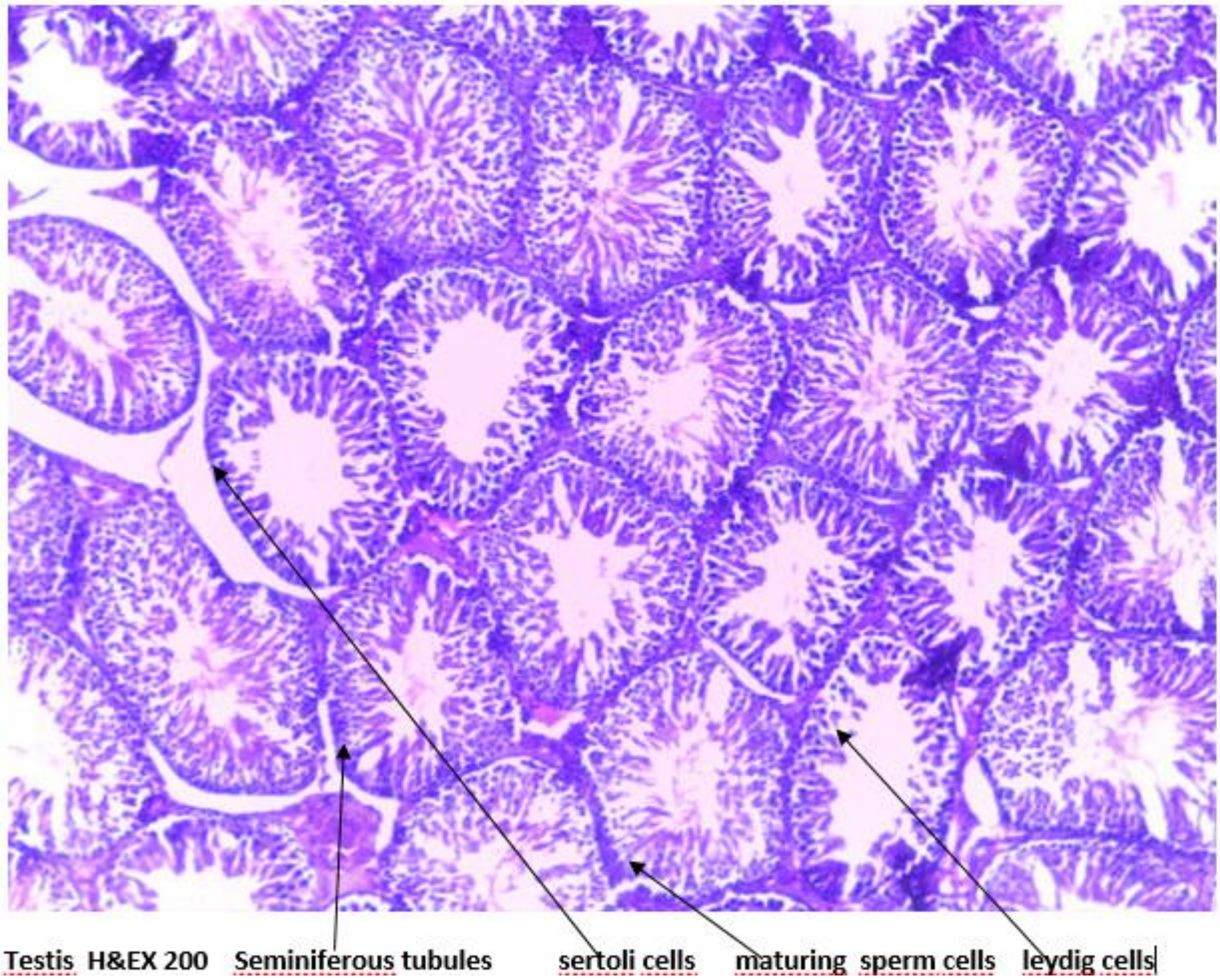

Plate 13: Photomicrograph of the section of the control testes showing the seminiferous tubules.. The section showed the tubules been filled with the conspicuous spermatogonia cell lined one cell on top of the other. The lumen of the tubules is filled with thread like substances called the spermatozoa, representing the different stages of spermatogenesis.

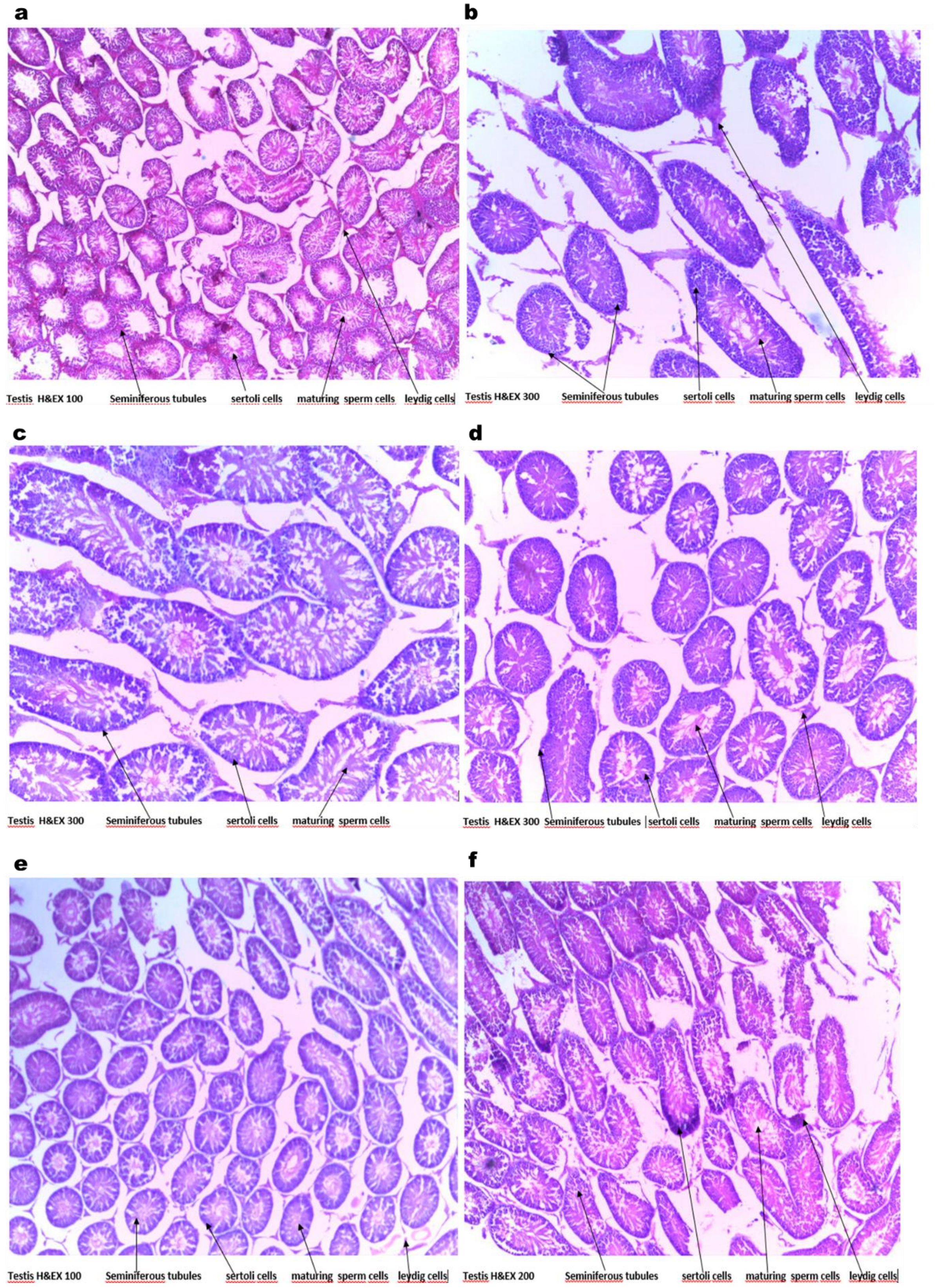

Plate 14 a – f: Photomicrograph of the section of the testes showing the seminiferous tubule. In these tubules few spermatogonia cells were slightly depleted. The lumen of the tubule were empty, no spermatozoa seen. Also there were slight loss of the scertoli cells.

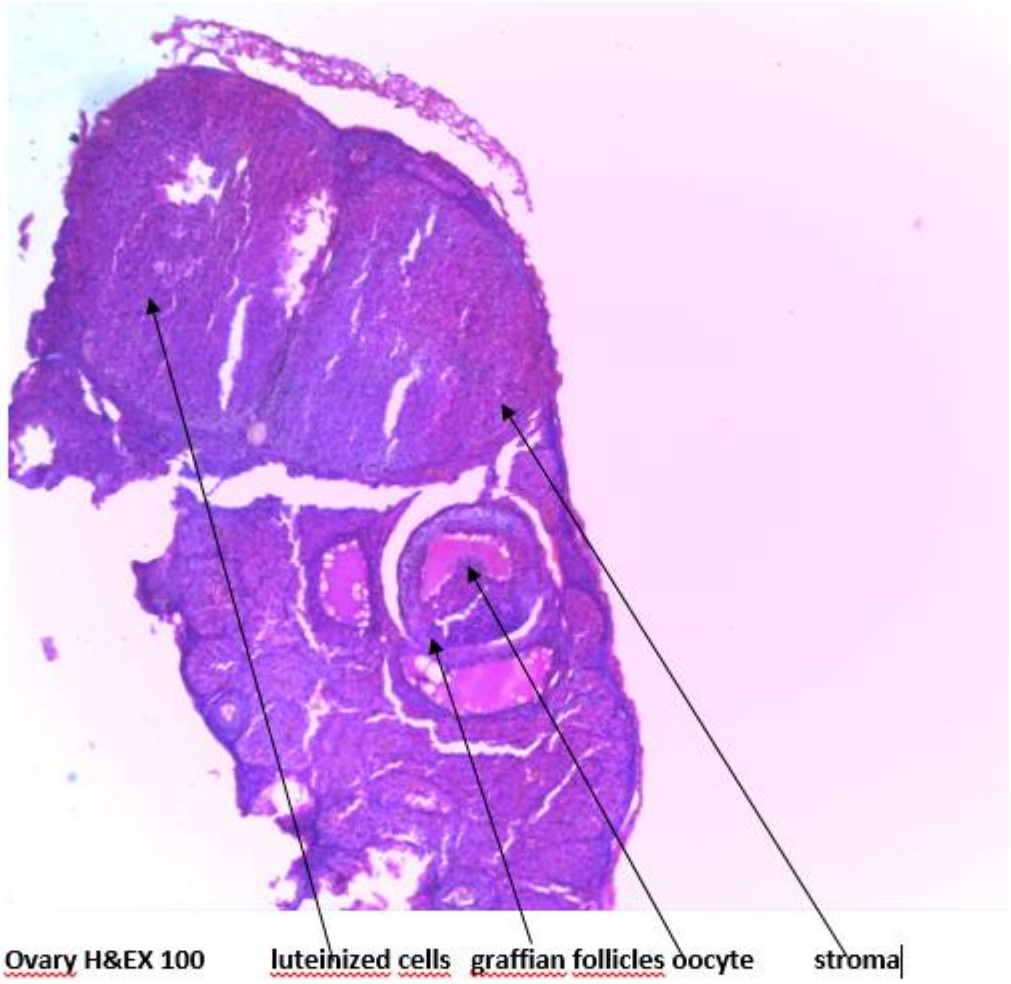

Plate 15: Photomicrograph of the section of the ovary of the representative female rat in control group showing the cortex. The section showed ovarian graffian follicle that are mentioned within which is a corpeus leutum and other growing a ovarian are vacuolated to form a ovarian cavity. The connective tissue is intact and thick or adequate. The nuclei are normal. Other follicle seen appeared normal.

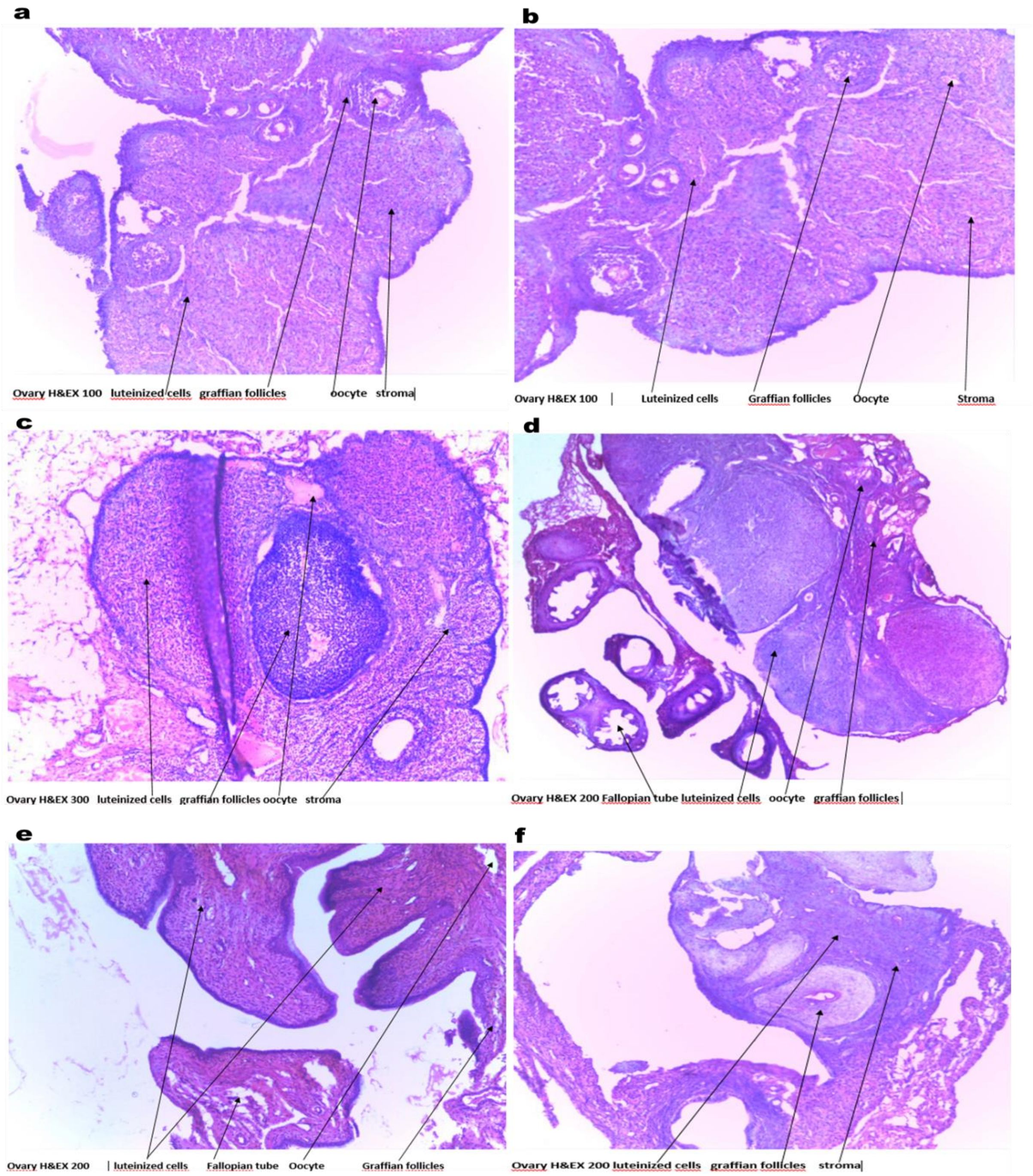

Plate 16 a – f: Photomicrograph of the ovary section of the representative female rats admiminstered soya bean and MSG showing part of the cortex and medulla. The cortex showed a graffian follicle with a developing oocyte. The medulary ray runs in cords that cross each other, within which a tiny graffian follicle are not mentioned. The nuclei also undergo metaplasia. Connective tissue cells are intact.

4.2 Discussion

The dietary inclusion of phytoestrogens is currently regulated in many developed countries due to its endocrine disrupting potentials. Exposure to phytoestrogens principally occurs through soya beans and its products. Among the reported phytoestrogens in the dehulled soya beans and its oil, diadzein, genistein, coumestrol and the remaining nine compunds isolated were found in therapeutic doses. Daidezein and genistein; two of the most abundant phytoestrogens in both the dehulled soya bean and soya bean oil, are isoflavones, while coumestrol is a coumestan. Compared to fruits and nuts (Liggins *et al*., 2000a), vegetables (Liggins *et al*., 2000b), and cereals (Liggins *et al*., 2000c), the soya bean contained therapeutically higher doses of genistein, daidzein, and coumestrol. A study that investigated the biochemical effect of diadzein on Wistar rats found hormone-related influence on energy metabolism and food intake. In humans, recent studies revealed the therapeutic benefits of daidzein in medicine for treating osteoporosis, menopause, cardiometabolic dysfunction, and some hormone-related cancers. Genistein is profound for its strong antioxidant properties. It produces atheroproteic effect via the inhibition of the leukocyte-endothelium interaction (Si and Liu, 2007) while its inhibitory effect on hexose transporter, methyl transferases, tyrosine kinases, and topoisomerase has been reported (Fang *et al*., 2007; Gossner *et al*., 2007; Nakamura *et al*., 2009). This may provide the scientific backing behind the reported regulatory effect of soya bean on cell proliferation and apoptosis (Shu *et al*., 2001; Nagata *et al*., 2007).

The structural formula of coumestrol mimics estradiol enabling the inhibition of 3α-hydroxysteroid dehydrogenase and aromatase (Blomquist *et al*., 2005), which are enzymes that modulate the biogenesis of steroid hormones (Amin and Buratovich, 2007). As an estrogen mimic, coumestrol is a potent endocrine disruptor capable of affecting all organ systems that are regulated hormonally via estrogens such as the nervous, skeletal, and reproductive system (Rajesh, 2005; Jefferson *et al.*, 2012). Also, some studies have associated the suppression of glycogen synthesis and hypocholesterolemic effect of soya bean to the coumestrol (Preedy, 2013).

Numerous studies have flagged the flagrant consumption of soybeans in various forms (Shahbazian *et al.*, 2007). In this study low, medium, and high doses of soya beans were administered to examine the effects on blood glucose and insulin levels. The results showed that both the male and female rats showed adverse biochemical responses in glucose metabolism as the administration of both MSG and soya bean persisted beyond 2 months. However, the male rats were more sensitive to incremental doses of the samples than the female rats. This hyperglycaemia found in both male and female rats especially with high doses of soya bean might be attributed to potent gastric emptying delays resulting from high viscos arabinogalactans, galactomannans, and pectins that inadvertently delays glucose uptake from the blood stream. This study was in agreement with the reports of Ascencio *et al*., (2004) who observed significant elevations in blood glucose levels on administration of gradient doses of soya beans. On the contrary, Chang *et al.*(2008) found that administration of soya beans longer than 40 days had no effect on blood glucose levels. The insulin levels after administration of low and medium doses of soya beans for 2 and 4 months remained comparable to the control rats. However, all high doses administered for 6 months significantly lowered the insulin levels. In agreement with the findings of this study, Wagner *et al*., (2008) reported that extremely high doses of soya beans could be hypoinsulinemic. In support of this finding, Noriega-López *et al*., (2007) presented a significantly lowered insulin level after excessive intake of soybeans.

Hyperinsulinemia has also been shown to be produced in mice due to insulin resistance following soya beans treatment (Kavanagh *et al*., 2008). It is known that insulin plays an important role in increasing glucose intake by cells through inducing the translocation of glucose transporter GLUT4 from intracellular sites to the cell surface. Therefore, hyperglycemia might be attributed to impaired glucose intake by cells due to decreased GLUT4 expression despite hyperinsulinemia. Wagner *et al*., (2008) reported that when 8 mg/day of soya bean is injected subcutaneously in rats, it elevates serum insulin levels and impairs glucose tolerance. This suggests that soya beans may influence insulin release, as the efferent pancreatic branch of vagus nerve could stimulate insulin secretion during the cephalic phase following feeding of additive in rats. MSG has been implicated as a causative agent for several ailments including leptin and insulin resistance (Brice *et al*., 2002), possibly influencing energy balance, leading to overweight. Over dose of MSG can increase both insulin secretion and blood glucose level suggesting presence of insulin resistance (Hugues *et al*., 2002). In this present study, the effects of gradient doses of MSG administration on glucose and insulin levels were identified. The results indicated that low or medium levels of MSG intake produces minor alterations in blood glucose and insulin levels, however, high dose of MSG significantly elevated the blood glucose levels with a corresponding suppression of insulin secretion. Some reports have shown that intake of L-glutamate causes obesity and diabetic like conditions in rat and mice (Iwase *et al*., 2000). This is majorly caused by excessive and prolonged glucose retention in free circulation with decrease insulin levels. Some researchers also proposed that a significant increase in blood glucose after MSG administration was probably because of its conversion glutamic acid moiety of MSG to oxalacetate by D-amino acid oxidase thereby producing glucogenic precursor (Niaz *et al*., 2018). Also, in support of the findings of this study, Araujo *et al.*, (2019) suggested that the hyperglycaemic effect of MSG could arise as a result of poor ability to oxidize glutamic acid in the pancreatic cells and that MSG acts via phosphoenolpyruvate carboxykinase in decreasing blood glucose. The study of Dayer and Dayer, (2010) validates the findings of this present study, showing no alterations in plasma insulin concentration in short term administration of low and medium doses of MSG which was attributed to the low stimulation of glutamate receptors in β pancreatic cell (Brice *et al*., 2002). Another theory suggested by Tanizawa *et al.* (2002) was that glutamate alone does not stimulate insulin secretion. The activation of glutamate dehydrogenase enzyme (GDH), stimulates the conversion of glutamate to α- ketoglutarate and play more important role in insulin secretion, thus, GDH stimulation may have remained normal consequently causing no changes in insulin levels. The GDH enhances glutamate oxidation and increases ATP production by providing the TCA cycle with substrate (α-ketoglutarate) and therefore stimulates insulin secretion. Some other studies have also provided conflicting findings on the effect of prolonged MSG intake on insulin levels. Hugues *et al*. (2002) reported a hyperinsulinemic and hypoglycaemic effect of high dose administration of MSG. They found that glutamate lowered blood glucose level during rise in insulin secretion. Macho *et al*. (2000) further explained that insulin resistance in MSG treated group could also be due to changes in insulin binding or post-receptor insulin effects in target tissues.

The evaluation of the hepatic effects of soya beans remains paramount and in this study, low, medium, and high doses of soybean were administered to experimental animals, and their effects on the liver were evaluated. The result showed that changes in concentration of hepatic enzymes mostly occurred with medium and high doses administration from >4 months. Similar findings have been reported by Leng *et al*., (2011) arguing that the soya bean when consumed in moderated amounts contains little antinutrients that could cause hepatotoxicity. Furthermore, Wiwanitkit *et al*., (2001) have shown that excessive intake of soya bean caused the elevation of liver function markers as well as increase in inflammatory markers from liver homogenates. The ALT is regarded as being a more specific indicator of liver inflammation, since AST may be elevated in diseases of other organs such as heart disease or muscle disease (Hall and Cash, 2012). Although ALT and AST are synthesised in the liver, they are also present in serum and in various tissues. In particular, ALT serum levels become elevated during liver diseases, and therefore, it is considered a more specific marker for liver injury than AST (Kunutsor *et al*., 2013). Mild or moderate elevations of ALP or AST are nonspecific and may be caused by a wide range of liver diseases (Hall and Cash, 2012). This means that the prolonged intake of high dose of soya beans could have induced the onset of liver diseases. From the results of this study, the serum levels of ALT and AST were affected by administration of medium and high doses of MSG when compared with the control group. The normal level of ALT in the rats fed MSG is an indication that liver synthetic function was not challenged by low dose administration of MSG. The results of this present study is in agreement with the report of other investigators who have reported no changes of AST and ALT consequent to low and medium dose MSG administration for 28days (Onyema *et al*., 2006). Eweka *et al.* (2011) however observed a gradual increase in AST level after 4 months MSG administration, with a non-significant change in ALT. The mean serum activity of alkaline phosphatase was significantly higher in all the groups of rats that received medium and high doses of MSG for > 2months when compared with the control group. This aligns with the study of Zhelyazkov and Stratev, (2019). Alkaline Phosphatase (ALP) is a biomarker enzyme for assessing the integrity of plasma membrane (Brichacek and Brown, 2019). Increase in the activities of alkaline phosphatase is an indication that there could be damage due to cytotoxic effect of MSG thereby resulting to leakage of this enzyme from the liver into the serum (Zhelyazkov and Stratev, 2019). Such increase in alkaline phosphatase activities can constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital processes since there may be indiscriminate hydrolysis of phosphate esters in the tissue. The increased ALP activity may also be due to the increased synthesis in the presence of increasing biliary pressure. Again, Rocek *et al.* (2001)

demonstrated that MSG administration could alter the intestinal function thereby releasing intestinal ALP.

Both total and conjugated bilirubin are assessments applied to determine both the functional integrity of the liver, and to differentiate between types of liver damage (Agomuo and Amadi, 2020). The elevated total bilirubin levels and consequent decrease in conjugated bilirubin, associated with the MSG administration shows that MSG compromises bilirubin clearance via glucoronic acid conjugation. This is the main predisposing factor to jaundice.

The functional status of the liver is reflected by total protein and albumin levels because the liver is enriched with machineries for the synthesis of serum proteins excluding γ- globulins, hence, liver damage is characterized by hypoproteinemia and hypoalbuminemia which can affect the whole physiological status of animals (Pachathundikandi and Varghese, 2006). In this present study, a reduction in total protein and albumin observed in the subjects fed MSG and soya bean for 6 months in relation to control group indicated hypoproteinemia and hypoalbuminemia, and by extension a progressive liver damage.

Investigation of the overall homeostatic and hemodynamic effect of both MSG and soybean, due to associated health concerns became highly necessary. From the findings, by implication, the soya bean intake during gestation led to renal damage. The measurement of the urea and creatinine in the dams administered with the soybeans provides further backing to possible renal damage or congestive heart failure. Other studies have also shown the relevance of the urea/creatinine ratio in ascertaining renal failure, proposing that elevated decreased urea levels with concomitant increase in creatinine levels is diagnostic of acute renal. Hence, this study clearly proposes that the common practice of excessive soybean consumption interferes with renal hemodynamics and organ integrity.

Furthermore. Anderson *et al*, (2007) reported that substitution (albeit medium doses) of soy protein for animal protein results in hyperfiltration and glomerular hypertension with resulting protection from diabetic nephropathy." This observation therefore highlights the renal protective effects of soya bean when used in minimal amounts. However, in this study the decreased urea levels with short term administration of soya bean as compared to the 4 months and 6 months administration, remains inexplicable. No current data in literature has investigated this outcome.

For the unaltered plasma sodium, and potassium concentrations in the rat fed soya bean low and medium doses, compared to the control rats in this study, may indicate that soybean contains moderate levels of these electrolytes. For instance, soya bean contains 1.6% potassium, 21% sodium and 0.8 % chloride, which was regarded as a balanced mix of electrolytes (IITA, 1990). Thus, it is inferable that with the increasing level of soya bean in the soybean diet preparations in the present study, the potassium content also becomes higher due to a synergistic action with sodium in the sodium/potassium pump. All MSG administration schedule on the other hand, altered the sodium and potassium levels The results of the present study therefore support the observation of Syzdek *et al.* (2007) who reported that as the serum sodium levels increases, the potassium levels concomitantly decreases. Many processes in the body, especially in the brain, nervous system, and muscles, require electrical signals for communication (Syzdek *et al.*, 2007). The movement of sodium is critical in generation of these electrical signals. Too much or too little sodium therefore can cause cells to malfunction, and extremes in the blood sodium levels (too much or too little) can be fatal (Syzdek *et al.*, 2009). A high serum Na level above the clinically accepted range is indicative of dehydration and shock, which means that both except for low dose of MSG administered for 2 months, all other dosing schedule of MSG could lead to severe dehydration. In addition, the decrease in circulating potassium levels as were the cases for rats administered medium and high dose of MSG and soybeans for 4-6 months, is indicative of chronic kidney disease. Furthermore, the results showed that no differences existed in the bicarbonate and chloride levels between the control and test samples. This implied that that plasma anions were unaffected by low, high and medium doses of soya beans and MSG administered for a short and medium term duration. This result agrees with the reports of Okon *et al.* (2013) who reported that no changes in plasma electrolytes were recorded when *Glycine max* was administered. Although anion gap is mostly used for examination of acid-base disorders (Kraut and Madias, 2007), such increase in both anions after high dose administration of MSG was suggestive of acute nutrient deprivation (Patel *et al*., 2011), preeclampsia (Kashyap *et al*., 2006), hypertension (Taylor *et al*., 2007) and acute kidney diseases (Kraut and Madias, 2007).

The beneficial effects of soya bean protein on serum lipid and lipoprotein concentrations and thus on cardiovascular diseases have been well-documented. Many studies have reported a correlation between the oral intake of soya bean protein and serum lipid profile (Mizushige *et al*., 2007). The increased number of studies performed over the past 10 to 12 years has provided evidence that soya bean consumption has a positive effect on serum lipid profile and that it might protect against the accumulation of cholesterol on the vascular walls and thus improve cardiovascular health. Consequently, it may also inhibit the early progression of coronary artery atherosclerosis (Lovati *et al*., 2000; Gianazza *et al*., 2003). These studies showed that soy protein intake was positively associated with HDL-C and negatively associated with total cholesterol, non-HDL-C, and TG (Fassini *et al*., 2011). This aligns with the findings of this present study. The result further showed that both HDL and LDL levels were not adversely affected by short periods of administrating low and medium doses of soya bean, but showed adverse effects with prolonged administration of high doses of soya beans.

Among the soya bean dosing schedules, only the medium dose (MD) and high dose (HD) administration for six months altered the TC levels. This was consistent with the report of Sirtori and Lovati (2001). In other words, no low or moderate dosage group displayed a lipidaltering effect. The results of this present investigation collaborates the findings by Retelny *et al*. (2008) and Matthan *et al*. (2007) but conflicted with the study by Wangen *et al*. (2001). The components of the soya bean diet that are responsible for lipid lowering effects and the mechanisms involved remain uncertain but maybe connected to the saponin and fibre contents. Recent research has focused primarily on efforts to identify the components of soya bean protein that are responsible for its beneficial effects on the cardiovascular system. However, some experimental studies have shown that the isoflavones contained in soya beans and many soy-based products are responsible for these effects (Vidyavati *et al*., 2010). Conversely, various studies reported that isoflavone-free soy protein preparations reduce serum cholesterol (Fukui *et al*., 2002). How do soya beans exert these effects on the aortic wall and blood lipid profile? The pathways of the effects remain unclear. Adams *et al*. (2004) concluded that the consumption of peptides from purified soya bean beta-conglycinin has an inhibitory effect on the development of atherosclerosis, which greatly exceeds the effect of whole-isoflavone soy protein isolate and does not depend on low density lipoprotein cholesterol (LDL-C) receptors or effects on serum lipoproteins. On the other hand, the findings of this study on the effect of MSG on TC and TG indicated that the MSG was potentially cardiotoxic after prolonged consumption. It has been established that MSG levels greater than 149 mg/dl caused hypertriglyceridemia, and severity of triglyceride is further classified by serum values falling within classification value ranges. The findings of this study agree with the reports of Singh *et al*. (2012) who reported that MSG induced transient, but more prolonged hypertriglyceridemia depending on duration and dosage of administration. This implied that for the safety of the cardiovascular system, MSG consumption should be limited to short periods in very low quantities.

The MSG-induced suppression of the antioxidant enzymes was profound. The findings of this study showed that even minimal mounts of MSG for two months compromised the antioxidant defence system. Soya bean on the other hand produced no effect on SOD until prolonged administration for upto 6 months. This implied that persistent use of soya bean may caused oxidative stress while for MSG use, co-administration with well known antioxidant sources appeared critical. Huang *et al*., (2006) reported that prolonged administration of *Glycine max* seed based formulated feeds showed slight increase in the levels of SOD in liver homogenates. This finding disagrees with the results of this present study. Superoxide dismutase catalyzes the dismutation of superoxide anion to hydrogen peroxide, for further detoxification to water by catalase (Mukherjee *et al*., 2003). The decrease in activity of SOD after treatment with MSG in this present study implied that the synthesized antioxidant enzyme had been suppressed. This was in line with the findings of Liao *et al.* (2020) and Ibegbulem *et al.* (2016). No alterations were found for the CAT levels after 4 months administration of low dose (LD) and MD soya beans. The report of Kim *et al.* (2004) corresponds to the findings of this study for the CAT levels. From the results, the GPx activities were affected after administration of low and medium dose of soya bean even at short periods, whereas the MDA levels were distorted with administration beyond 2 months. This simply indicated that cellular components were at risk with prolonged administration of both MSG and soya beans. GPx is regarded as one of the key enzymes for the detoxification of reactive oxygen species. Fuentealba *et al.* (1994) posited that glutathione levels become altered in advanced exposure to toxicants. This implies that the toxicity of phytoestrogens in soya bean was implicated by the reduction in the intracellular levels of glutathione. On the other hand, malondialdehyde (MDA), increased in the groups fed medium and high dose of soya bean provides clues as to the degree of lipid peroxidation caused. This also implied that even prolonged low level consumption of MSG, predisposes the body to cellular damage and excessive lipid peroxidation.

Among the female hormones, only luteinizing hormone was not affected by both soya bean and MSG intake. Soya bean consumption even at minimal doses for short periods distorted progesterone and oestrogen levels, whereas a prolonged and excessive intake of soya bean disrupted FSH levels. These observations agrees with the study of Kurzer (2002). The effects of soya bean on both estrogen and progesterone were mainly due to the presence of therapeutic doses of oestrogen-like compounds in soya beans.

The MSG when administered for short periods produced no effect on the female reproductive hormones. Mondal *et al*., (2018) posited that MSG impairs the functions of the ovary probably by augmenting the release of FSH, LH and estradiol; promoting the follicular maturation and improving the biochemical mechanism for antioxidant defense. This however, contradicted the findings of this study. Both medium and high dose MSG produced similar effects on the progesterone levels. These findings were similar to the findings of Zia *et al*, (2014) who initially reported decreased levels of progesterone in the plasma and interstitial tissue in MSG treated animals, which were significantly lower than those found in control animals. The reason for decrease in progesterone levels was possibly due to decreased levels of luteinising hormone due to MSG treatment. Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species (King and Brucker, 2010). Hence, this study has shown that administration of MSG could elicit hormonal imbalance. Similarly, the estrogen levels were unaffected by low dose of MSG whereas medium and high doses of MSG suppressed the synthesis of estrogen. Estrogen, or oestrogen, is the primary female sex hormone. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. The decrease in the estrogen levels found in this study, and as well by Soltysik and Czekaj (2013), was possibly because MSG causes inactivation of enzyme aromatase which catalyzes the conversion of testosterone to estradiol, therefore resulting in decreased estradiol synthesis. Thus, the intake of medium and high doses of MSG requires serious caution over the impending reproductive toxicities caused.

For the male reproductive hormones, low doses of both MSG and soya bean were not toxic to testosterone activities. However, medium and high doses suppressed testosterone production. The lowering of testosterone concentrations suggests marked tubular degeneration, meiotic interruption, depletion of sperm concentration and degradation of germinative epithelium (Richburg and Boekelheide, 1996). This suggested the development of male infertility. The LH was extensively suppressed by both soya bean and MSG. The sensitivity of luteinizing hormone to either MSG or soya bean is yet to be elucidated in literature. However, as indicated by Creasy (2008) a low LH indicates suppressed potency, hormonal imbalance and sexual dysfunctions in males. Hence, the intake of excessive amounts of soya beans is toxic to the testes. However, the results showed no effect on the FSH levels up to 4 months administration of any of the low, medium or high dose of soya bean. The MSG decreased FSH levels only after prolonged intake of either medium or high dose MSG. The successful and complete male germ cell development is dependent on the balanced endocrine interplay of hypothalamus, pituitary and testis. In this case, the reproductive toxicities of the soya bean were evident at prolonged and excessive administration.

The result showed that soya bean had no effect on colorectal cancer markers of the experimental animals regardless of amount and duration of administration. Thus, soya bean was not carcinogenic to the colon or rectum. The MSG on the other hand elevated the colorectal cancer markers, on excessive administration under prolonged administration. This means that moderate amounts when administered in short duration was not carcinogenic. Carcinoembryonic antigen (CEA) is one of the most widely used tumor markers worldwide. Its main application is mostly in gastrointestinal cancers, especially in colorectal malignancy. Although in use for almost 30 years, the clinical value of CEA in colorectal cancer is still underutilized. Patients with tumors in the colon generally have a higher incidence of increased CEA concentrations than those with malignancies on the other organs (Li *et al.*, 2009). This means that the CEA serves as an accurate indicator of colorectal cancer. Several studies have attempted to elucidate the predominant mechanism behind the carcinogencity of potassium MSG. A widely accepted theory is the generation of highly reactive oxygen species that promote the proliferation of sensitive organs. Hence it was possible that the administration of MSG induced the biosynthesis of colorectal cancer markers through the generation of reactive oxygen species.

The utility of serum CA 19-9 levels to provide meaningful prognostic information about the effect of soya beans on pancreatic cancer markers, was shown in this study. The result showed the susceptibility to pancreatic cancer with soya beans intake; increasing over time. From the CA-19-9 levels low amounts of soya bean was not carcinogenic to pancreatic cells. This also meant that the low intake of soya beans portended no damage to the pancreas. It could mean that, contrary to high levels of phytoestrogens obtainable from excessive intake of soya beans, the low doses provided safe levels of phytoestrogens. Sequel to this, multiple studies have demonstrated that a treatment related increment in CA 19-9 serum levels is associated with prolonged survival and is an independent predictor of overall survival of ulcer patients (Ballehaninna and Chamberlain, 2012). From the findings of this study, MSG increased the concentrations of the pancreatic cancer markers irrespective of the dose, duration, and sex. This meant that MSG consumption is a major predisposing factor to pancreatitis. Similar observations were made by Boonnate *et al.* (2015) who posited that daily MSG dietary consumption was associated with enhanced β-cell hemorrhages and fibrosis. Similarly MSG-induced increases of markers of ovarian cancer were both dose and duration dependent. From the results the levels of CA-125 increased as the feeding duration stretched to 6 months especially with the medium and high doses. This observation agreed with the reports of Ali *et al*. (2014) who showed that MSG-treated rats showed degenerative changes of the ovary with many atretic follicles and vacuolated stroma. For soya bean, only the high dose increased the circulating levels of ovarian cancer marker. This implied that extensive and excessive consumption of soybean could lead to ovarian cancer.

Furthermore, this study investigated the potentials of MSG, to cause any alterations in the PSA levels of Wistar rats. In this study, both medium and high dose MSG elevated the PSA levels when compared to the control rats. This also meant that the high dose of MSG increased PSA levels when compared to the control, low and high dose group. In recent years MSG has been a mainstay of most delicacies around the globe. The increasingly popular use of MSG for most cuisines may be related to reports of its cytotoxic activity (Olowofolahan *et al*., 2020). This seemed to imply that the more the usage of MSG the more endangered the prostate health becomes.

For soya bean, the more the amount and duration of administration the more the circulating levels of PSA. Prostate-specific antigen (PSA), also known as gamma-seminoprotein or kallikrein-3 (KLK3), is a glycoprotein enzyme encoded in humans by the KLK3 gene. The PSA is a member of the kallikrein-related peptidase family and is secreted by the epithelial cells of the prostate gland. The PSA is produced for the ejaculate, where it liquefies semen in the seminal coagulum and allows sperm to swim freely (Balk *et al*., 2003). It is normally present in the blood at very low levels. Increased levels of PSA may suggest the presence of prostate cancer. However, prostate cancer can also be present in the complete absence of an elevated PSA level, in which case the test result would be a false negative. The PSA levels can also be increased by prostatitis, irritation, benign prostatic hyperplasia (BPH), and recent ejaculation, producing a false positive result (Bernal-Soriano). This means that additional tests are required to affirm that the elevations in PSA levels following high dose soya bean administration, was truly caused by the disruption of the prostate. Elsewhere, it has been reported that similar plants, containing equivalent phytoestrogens like soya beans, enhances the proliferation and growth of tumor cells through disruption of cell cycle progression. For example, substances that inhibits growth of cervical cancer cells, can also show proliferation-inhibitory effects in prostate cancer cells (Hong *et al*., 2019). Interestingly, since androgen-refractory prostate cancer cells are more resistant to apoptosis and lead to prostate cancer recurrence, excessive consumption of soya beans should be avoided or taken alongside phytase rich foods which could provide therapeutic benefits to patients with recurrent prostate cancer.

The adverse effects of MSG and soya bean administrations noticed at medium anmd high doses seem to suggest that a 70kg human would need to consume only diets containing 140,000 mg (140) or 210,000 mg (210g) per dose per day of both supplementsfor the periods under study.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Soya bean contains therapeutic doses of diadzein, and genistein, known for numerous medicinal effects while the reproductive toxicity of soya bean is underscored by high contents of coumestrol. Minimum intake of monosodium glutamate and soya bean, irrespective of the gender produced minimal toxic effect to the system. However, glucose metabolism as well as insulin secretion were compromised with prolonged and excessive use of MSG and soya bean. Similarly, organs like the liver, kidney, and heart were also susceptible to harm when monosodium glutamate and soya bean were persistently consumed at high doses. Also, the systemic potentials to withstand oxidative stress whittled as both amount and consumption duration of both MSG and soya bean increased. Soya bean showed deleterious effects on female reproductive hormones. On the other hand, MSG only showed adverse effect on reproductive health with excessive and prolonged intake thereby requiring very minimal intake. Monosodium glutamate was observed to be potentially carcinogenic given the increases of ovarian, colorectal and pancreatic cancer markers even at minimal short term intake. In general, the consumption of both monosodium glutamate and soya bean should be less frequent and limited to very small amounts.

5.2 RECOMMENDATIONS FOR FURTHER STUDIES

- **a.** Given the toxic effects of both MSG and soya beans at certain doses, the vascular and hematological effect should be investigated.
- **b.** The effect of MSG on various cancer cell lines as well as the effect of soya beans on rapidly proliferating gametocytes should be undertaken.
- **c.** Further studies are recommended to attempt to separate coumestrol from soya bean and determine if the toxicities associated with soybean consumption will be mitigated.
- **d.** Since the consumption of soya bean is most frequent among pregnant women and children, further studies should verify its maternal, postpartum, and developmental effects (on the child).

5.3 CONTRIBUTION TO KNOWLEDGE

- **a.** Development of pancreatic cancer by soya bean at high doses or prolonged usage.
- **b.** MSG could cause pancreatitis irrespective of dose, duration and sex.
- **c.** We found out that MSG could cause colorectal cancer when used for a prolonged period.
- d. Increases in ovarian cancer marker by MSG were dose and duration dependent.
- e. Medium and high doses of MSG and soya bean suppressed oestrogen and testeoterone production which could lead to male amd female infertility.

5.4 LIST OF PUBLISHED JOURNAL

- **a.** **Bob Chile-Agada, A.**, Nwachukwu, N., Ibegbulem, C.O. and Ene, A.C (2021): Biochemical effects of short-long Term extensive Administration of Monosodium Gluatamate and Soybean on Wistar Rats. *Asian Journal of Biochemistry, Genetics and Molecular Biology* **8(3):** 14-27

b. **Bob Chile-Agada, A.,** Nwachukwu, N., Ibegbulem, C.O. and Ene, A.C. (2021): Renal and Cardiovascular Effects of prolonged intake of Monosodium Glutamate and Soybean on Wistar Rats. *South Asian Research Journal of Natural Products.* **5(1):** 8 - 20

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