EFFECT OF TEA INFUSION AS WEIGHT-LOSS AGENT IN WISTAR RATS

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CERTIFICATION

This is to certify that this thesis "**Effect of tea infusions as weight-loss agent in wistar rats**" was carried out by **Demason, Shirley James** with Reg. No: **20164991528** in partial fulfilment for the award of Master of Science (M.Sc) degree in Biochemistry in the Department of Biochemistry, Federal University of Technology, Owerri.

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DEDICATION

I dedicate this thesis to God Almighty and my father, HRH. Obol James Inah Okpokpo.

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ABSTRACT

The study evaluated the effect of tea infusion as weight loss agent in wistar rats. Parameters evaluated included the phytochemical content of tea infusion and their effects on body weight gain, relative organ weights, feed conversion ratio, lipid profile and proximate composition of faecal sample. Standard phytochemical methods were used to test for the presence of bioactive compounds in tea infusions. The infusions were obtained by measuring 150 ml of boiled water into a beaker, to which 1.5 g of tea sample was added and allowed to stand for 30 min before filtration. Eighteen male wistar rats were grouped into three of six rats each. Group A (normal control) were fed pelletized poultry feed and tap water ad libitum. Groups B and C were both fed pelletized poultry feed, green and black tea infusions, respectively for 28 days. The results of the phytochemical screening showed that tea infusion contained various metabolites; flavonoids, tannins, alkaloids, saponins, phytates, oxalates, and cyanogenic glycosides in varied quantities. The results demonstrated that the green tea infusion showed higher phytochemical content (tannins: 28.61±0.11, saponins: 8.17±0.02, flavonoids: 8.43±0.03, alkaloids: 9.78±0.01, phytates: 0.29±0.00 and cyanogenic glycosides: 559.50±0.50), compared to black tea (tannins: 4.90±0.04, saponins: 6.35±0.02, flavonoids: 5.15±0.04, alkaloids: 8.70±0.00, phytates: 0.22±0.00 and cyanogenic glycosides: 335.50±1.50). Black tea infused animals consumed the least feed and had lowest digestibility, had a significant drop in growth rate and body weight gain. Tea infusions caused a slight increase in the relative organ weight of treated animals. The infusions lowered plasma Low –density lipoprotein (LDL) and increased the high-density lipoprotein (HDL/LDL ratio). The effect of tea infusion on faecal proximate compositions indicated that excretion of minerals, lipid and fibre were enhanced in treated animals while reducing the excretion of water and carbohydrates. In conclusion, the weight-loss recorded by animals fed black tea was due to the excretion of minerals, fibre and lipid which was triggered by the action of polyphenols present in tea.

Keywords: faeces, lipid profile, phytochemicals, rats, tea infusions.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Weight loss, in the context of medicine, health, or physical fitness, refers to a reduction of the total body mass. The reduction could be due to a mean loss in fluid, body fat or adipose tissue or lean mass, namely bone mineral deposits, muscle, tendon, and other connective tissues. Weight loss can either occur unintentionally due to malnourishment or an underlying disease or arise from a conscious effort to improve an actual or perceived overweight or obese state. "Unexplained" weight loss that is not caused by reduction in calorific intake or exercise is called cachexia and may be a symptom of a serious medical condition. Intentional weight loss is referred to as slimming (Le Blanc, O'Connor & Whitlock, 2011).

Intentional weight loss refers to the total loss of body mass as a result of efforts to improve fitness and health, or to change appearance through slimming. Weight loss in individuals who are overweight or obese can reduce health risks, increase fitness, and may delay the onset of diabetes (Le Blanc *et al.,* 2011). It could reduce pain and increase movement in people with osteoarthritis of the knee. Weight loss can lead to a reduction in hypertension (high blood pressure), however whether this reduces hypertension-related diseases is unclear *(*Harsha & Bray, 2008).

It is generally regarded as a medical problem when at least 10% of a person's body weight has been lost in six months or 5% in the last one month (Huffman, 2002). Even lesser amounts of weight loss can be a cause for serious concern in a frail elderly person (Yaxley, Miller, Fraser & Cobiac, 2012).

Although, body mass index (BMI) is commonly used for determining obesity, where only height and weight is measured, it is not an accurate criterion for obesity evaluation (Peltz, Aguirre, Sanderson & Fadden, 2010).

Unintentional weight loss occurs as a result of inadequate nutrients in diet relative to a person's energy needs (generally called malnutrition). Disease processes, changes in metabolism, hormonal changes, medications or other treatments, disease- or treatment-related dietary changes, or reduced appetite associated with a disease or treatment can also cause unintentional weight loss (Huffman, 2002). Poor nutrient utilization can lead to weight loss, and can be caused by fistulae in the gastrointestinal tract, diarrhea, drug-nutrient interaction, enzyme depletion and muscle atrophy (Alibhai, Greenwood & Payette, 2005).

Weight loss occurs when the body is expending more energy in work and metabolism than it is absorbing from food or other nutrients. It will then use stored reserves from fat or muscle, gradually leading to weight loss. For athletes seeking to improve performance or to meet required weight classification for participation in a sport, it is not uncommon to seek additional weight loss even if they are already at their ideal body weight. Others may be driven to lose weight to achieve an appearance they consider more attractive. However, being underweight is associated with health risks such as difficulty in fighting off infection, osteoporosis, decreased muscle strength, trouble regulating body temperature and even increased risk of death (Sumithran, Priya & Joseph, 2013).

The least intrusive weight loss methods, and those most often recommended, are adjustments to eating patterns and increased physical activity, generally in the form of exercise. The World Health Organization recommended that people combine a reduction of processed foods high in saturated fats, sugar and salt and caloric content of the diet with an increase in physical activity (Harmon, 2010).

An increase in fiber intake is also recommended for regulating bowel movements. Other methods of weight loss include use of drugs and supplements that decrease appetite, block fat absorption, or reduce stomach volume. Bariatric surgery may be indicated in cases of severe obesity. Two common bariatric surgical procedures are gastric bypass and gastric banding (Albgomi, 2013). Both can be effective at limiting the intake of food energy by reducing the size of the stomach, but as with any surgical procedure both come with their own risks that should be considered in consultation with a physician. Dietary supplements, though widely used, are not considered a healthy option for weight loss (Neumark, Sherwood, French & Jeffery, 1999). Many are available, but only few are effective in the long term.

Virtual gastric band uses hypnosis to make the brain think the stomach is smaller than it really is and hence lower the amount of food ingested. This eventually result in weight reduction. This method is complemented with psychological treatment for anxiety management and with hypnopedia. Research has been conducted into the use of hypnosis as a weight management alternative. Acceptance and Commitment Therapy (ACT), a mindfulness approach to weight loss, has also in the last few years been demonstrating its usefulness (Neumark *et al.,* 1999).

For weight loss to be permanent, changes in diet and lifestyle must be permanent as well. Short-term dieting has not been shown to produce either long term weight loss or better health and it may be counterproductive (Allison, Faith & Myles, 1996). The healthiest weight loss regimen, therefore is one that consists of a balanced diet and moderate physical activity (Nedeltcheva, Kilkus, Imperial, Schoeller & Penev, 2010).

Continuing weight loss may deteriorate into wasting, a vaguely defined condition called cachexia. Cachexia differs from starvation in part because it involves a systemic inflammatory response. It is associated with poorer outcomes. In the advanced stages

of progressive disease, metabolism can change so that cachexia patients lose weight even when they are getting what is normally regarded as adequate nutrition and the body cannot compensate. This leads to a condition called anorexia cachexia syndrome (ACS) and additional nutrition or supplementation is unlikely to help. Symptoms of weight loss from ACS include severe weight loss from muscle rather than body fat, loss of appetite and feeling full after eating small amounts, nausea, anemia, weakness and fatigue (Payne, Wiffen & Martins, 2012).

Serious weight loss may reduce quality of life, impair treatment effectiveness or recovery, worsen disease processes and be a risk factor for high mortality rates. According to Mann *et al.* (2007), malnutrition can affect every function of the human body, from the cells to the most complex body functions such as, immune response, wound healing, muscle strength (including respiratory muscles), renal capacity and depletion leading to water and electrolyte disturbances, thermoregulation and menstruation.

Camellia sinensis (tea) is the most frequently consumed beverage worldwide besides water. It holds second position in consumption among beverages. The tea plant originated from Southeast Asia but is now being cultivated in 30 countries. About three billion kilograms of tea is produced and consumed yearly. Tea has been categorized into three main types on the basis of processing during manufacturing. The three types are manufactured from the leaves of *Camellia sinensis.* Of the tea produced worldwide, 78% is black tea (fully fermented), which is usually consumed in western countries; 20% is green tea (unfermented), which is commonly consumed in Asian countries, and 2% is oolong tea which is produced by partial fermentation and is mainly consumed in southern China. Tea possesses significant antioxidative, anti-inflammatory, antimicrobial, anticarcinogenic, antihypertensive, neuroprotective, cholesterol-lowering and thermogenic properties (Butt & Sultan, 2009).

1.2 Statement of the Problem

Many people desire to lose weight; their reasons include improving their appearance, increasing self-esteem, feeling better, reducing the risks of disease, and reducing the severity of medical problems and diseases that already exist. Depending on the choice of the weight-loss approach and the number of pounds one wants to lose, the effort can be appropriate or inappropriate and improve health or make it worse. To achieve this, people devise various methods such as nutritional support (the use of tea, dieting, etc.) and often times, the effect of this nutritional product (tea) is ignored.

1.3 Justification for the Study

Obesity is a significant public health concern worldwide, contributing to various chronic diseases such as cardiovascular disease, type 2 diabetes and certain cancers. Despite numerous interventions aimed at combating obesity, its rate continues to rise, emphasizing the need for more effective approaches for weight management. Green tea and black tea have gained attention for their potential role in promoting weight loss due to their rich content of bioactive compounds. While some studies suggest beneficial effects of tea consumption on weight management in both animals and humans, the evidence remains inconclusive and often conflicting.

1.4 Aim and objectives of the study

The present study is aimed at evaluating the effects of tea infusions as weight-loss agent in wistar rats.

The objectives of the study include;

i. Evaluation of the phytochemical components (tannins, phytates, oxalates, phenols, cyanogenic glycosides, saponins, flavonoids and alkaloids and phytosterols) of the tea infusions.

ii. Administration of infusions of the tea to rats and assessment of the lipid profile (HDL-c, LDL-c, VLDL, chylomicrons, total cholesterol and triacylglycerol), total feed consumed, weight gained/lost, growth rate, fluid consumed and feed conversion ratio.

iii. Determination of the proximate composition of faecal matter of rats-fed tea infusion to evaluate digestion patterns.

1.5 Significance of the study

The study will contribute to the existing body of scientific literature by providing insights into the potential of tea infusions in weight management.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical overview

Tea as a plant is grown in many countries of the world, mainly in China, Indonesia, India, Japan, and on the Ceylon Island (Fernandez *et al.,* 2001). According to ancient Chinese mythology, the tea plant was discovered thousands of years ago in south-east Asia. Aboriginal tea leaf infusions had been considered for medicine, and has become the most popular beverage all over the world today. The major tea producing countries are China, India, Sri Lanka, Japan, Taiwan, and Kenya (Wong, Sirisena, & Ng, 2022). The tea leaf market is differentiated and diverse. Basic tea division consist of green, oolong and black (Chen *et al.,* 2006; Hilal, 2017).

The tea plant is taxonomically classified as *Camellia sinensis* (L) of the Theaceae family. The genus *Camellia* incorporates more than 80 species in its taxonomy. The genus *Camellia* is classified into eight sections of which *Thea sp* comprises *C.sinensis, C. taliensis, C. irra wadiensis, C. grocilipes* and *C pubicosta.* There are two basic botanical varieties: Chinese tea shrub (*Camellia sinensis*) and the Indian tea *(Camellia assamica*) (Chu, 1997).

The tea plant can grow to a height of 30 feet and usually clipped to a height of 2.5 feet in cultivation. The shrub is heavily branched with dark-green, hairy, oblong, ovate leaves (Plate 2.1) cultivated and preferentially picked as young shoots. Its flowers are large, coloured white, pink or red and fruits are small and brown (Chu & Juneja, 1997). Older leaves are considered to be inferior in quality.

Plate 2.1: Fresh tea leaves of different sizes (Wikipedia contributors, 2024).

2.2 Tea classification and processing

The average per capita consumption of tea worldwide is approximately 120 ml/day (Gramza-Michałowska & Bajerska-Jarzębowska, 2007). Tea consumption preferences differ in various regions of the world. Tea consumption is specific in countries it is grown. Black tea and green tea are the most commonly consumed tea types, and globally, black tea accounts for over 70% of the annual tea production while green tea accounts for around 20% (Ho, Haufe, Ferruzi & Neilson, 2018). Following the high-volume production per year, black tea is the most consumed tea type and very preferred tea type in the West, including Europe, the United Kingdom, the United States, and India (Gramza-Michałowska & Bajerska-Jarzębowska, 2007). Green tea is mostly consumed in East Asia, especially in Japan, Korea, and Northern China; however, the popularity of green tea is rising elsewhere around the world, such as in Europe and the United States (Koch *et al.*, 2018). After black tea and green tea, oolong tea is the most recognized tea type, and it is widely enjoyed in Southern China and Taiwan (Ng *et al.*, 2018). Also, *Ilex paraguayensis* is a species of tea from South America. This plant is processed to obtain a final commercial product named yerba mate. The mate is a famous popular tea consumed in Argentina, Brazil, Uruguay, and Paraguay (Kujawska, 2018).

Tea can be harvested throughout the year while tea brewing quality is influenced by time. The most aromatic and delicate teas are collected in the spring. Top grade expensive teas however, are collected from young leaflets of top twigs and undeveloped leaves, showing uncommon suitable taste and aroma features (Chu & Juneja, 1997). Freshly harvested tea leaf is processed differently in different parts of the world, to give oolong (2%), green (20%) or black (78%) (Kuroda and Hara 1999). Tea leaf classifications is based on the different fermentation degree but there are three popular classes of tea: non-fermented (green), semi-fermented (oolong), and black (totally fermented). There are also other well-known teas like white (non- fermented), yellow (very lightly

fermented) and red (Pu-erh tea), which after the fermentation process undergo a long-term storage (Balentine *et al.*, 1997).

2.3 Components of tea

It is reported that tea contains nearly 4000 bioactive compounds while polyphenols contribute to 33% and catechins are the predominantly present polyphenol (KC, Parajuli, Khatri, & Shiwakoti, 2020). Other chemical constituents like alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds, and trace elements are also present (Namita, Mukesh & Vijay, 2012).

Tea is differentiated by its pigment. Green tea's main pigment is chlorophyll while that of black tea are orange *theaflavins* and brown, *thearubigens* (Higashi-Okai, Yamazaki, Nagamori & Okai, 2001), which appear during fermentation. Another important group of tea constituent are the alkaloids, which include caffeine (theine), theophylline and theobromine (Schulz, Engelhardt, Wegent, Drews & Lapczynski, 1999; Koch, Zagórska, Marzec & Kukula-Koch, 2019). Study has it that the fermentation process of tea leaves does not influence the caffeine level (Lin, Lin, Liang, Lin- Shian & Juan, 1998).

Tea leaves like other plant products could be a good source of polyphenolic substances, which have great impact on human health (Truong & Jeong, 2021; Klepacka, 2022). Fresh tea leaves consist 30% of flavonoids and phenolic acids, which make up 10% of the dry weight of black tea (Chaturvedula & Prakash, 2011). Flavonoid is believed to be the most important tea leaf polyphenol (Hollman, 2001), with the most important being epigallocatechin gallate (EGCG), which occurs only in tea leaves (Graham, 1992; Chu & Juneja, 1997). Tea contains several groups of polyphenols that include flavan-3-ols and their oligomers, flavonols and their glycosides, phenolic acids and hydrolysable tannins, theaflavins, and thearubigins (Zhang *et al.*, 2019). During

fermentation, large quantities of simple flavonoids in green tea are transformed into complex forms of theaflavins and thearubigenes (Graham, 1992; Dwyer & Peterson, 2013). Flavanols occur in plant tissues in monomeric forms of catechins and as polymers. Other compounds such as tannins are responsible for the specific astringent aroma and taste of tea (Riedl & Hagerman, 2001). Tea catechin is related with infusion quality, and young tea leaves are found to have the highest catechin content (Thanaraj & Seshardi, 1990). Catechins are a group of natural polyphenols of plants also found in green tea. There are eight major catechins (Fig. 2.2) present in green tea: catechin (C), catechin gallate (CG), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), gallocatechin gallate (GCG), and EGC gallate (EGCG) (Tao, Zhou, Zhao & Wei, 2016). Catechins account for 6-16% of natural polyphenols of dry green leaves.

Tea polysaccharides (TPS) is another main bioactive component of tea other than polyphenols (Xiao & Jiang, 2015). The content of polysaccharides in tea could be increased as the maturity of raw tea leaf increased, quite different from the pattern of tea polyphenols (Xiao & Jiang, 2015). In addition, TPS have diverse chemical characteristics, in terms of the monomer (mainly glucose, galactose, rhamnose, and arabinose, with little xylose and mannose), acidity (neutral or acidic), solubility (water-soluble or not), and conjugation with proteins, polyphenols, metal ions, selenium, strongly influencing the structure–function relationship (Wang, Li, Liu, Chen & Wei, 2015; Xiao & Jiang, 2015; Park *et al.*, 2017; Fan *et al.*, 2018; Sun, Warren & Gidley, 2018). For example, the complex of tea polysaccharides with lower content of polyphenols exert higher antioxidant activity than those with higher content of polyphenols, and conjugation with selenium could remarkably increase the antioxidant activity of tea polysaccharides (Wang *et al.,* 2015; Xiao & Jiang, 2015). Polysaccharides may contribute to the antioxidant, immuno-regulatory, anticancer, anti-diabetic, and anti-obesity effects of tea brewing and its extracts (Wang *et al.*, 2015; Xu *et al.*, 2015; Yuan *et al.,* 2015; Yang *et al.,* 2017).

Tea brewing and its extract also contain a considerable amount of amino acids (Zhu *et al.*, 2016). Aspartic acid, glutamic acid, arginine, alanine, tyrosine, and theanine have been reported as the major amino acids in tea, and the amino acid profile can be changed during fermentation (Bi *et al.*, 2016; Zhu *et al.*, 2016). Among them, theanine is a nonproteinic amino acid special to tea (Bi *et al.*, 2016). It has been summarized that L-theanine has positive effects on relaxation, cognitive performance, emotional status, sleep quality, cancer, cardiovascular diseases, obesity, and the common cold (Bi *et al.*, 2016; Türközü, & Şanlier, 2017).

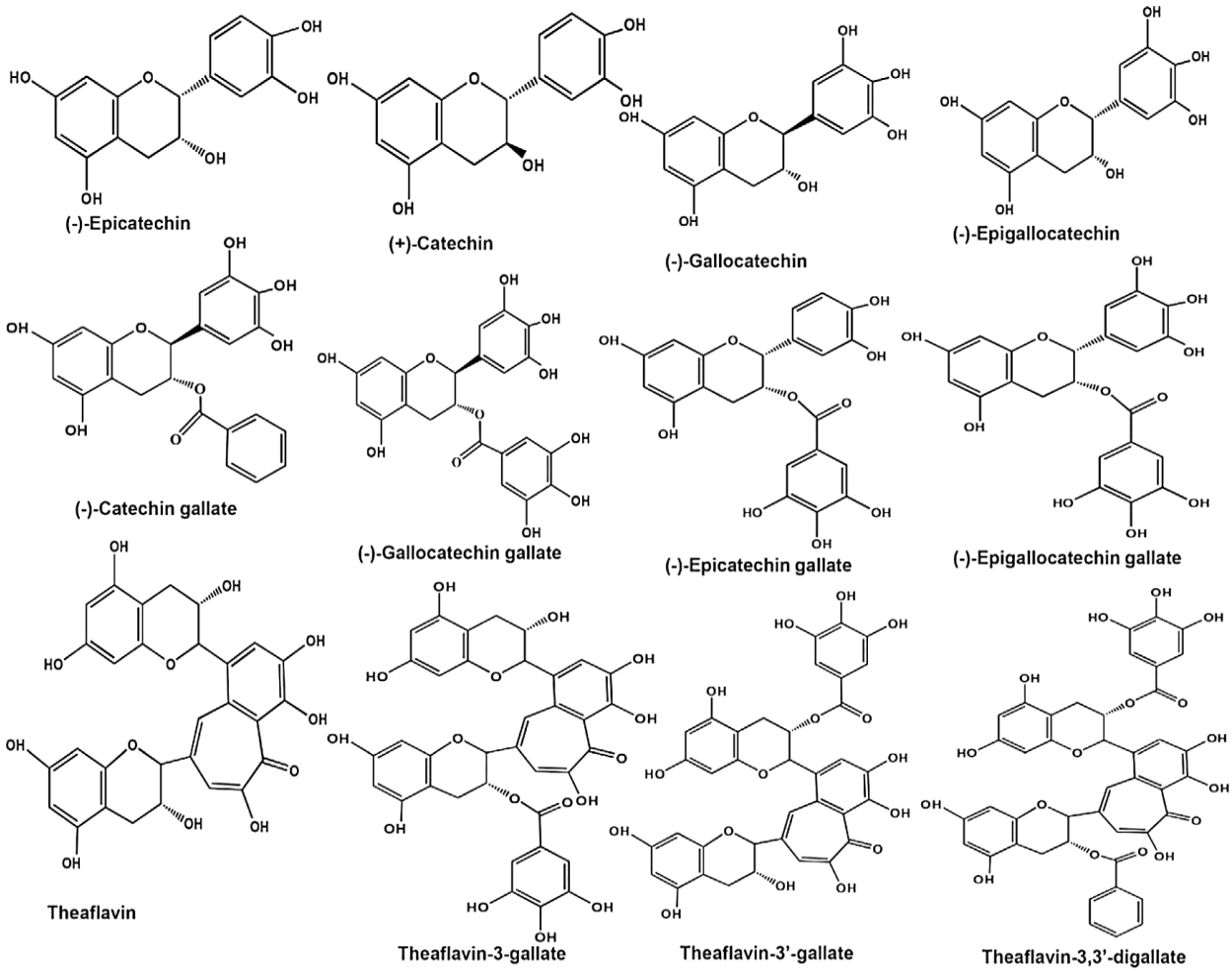

Fig 2.2: Eight major catechins and four theaflavins found in tea (Tao et al., 2016).

During fermentation, simple polyphenols are oxidized to more complex and condensed polyphenols which give the characteristic colours and flavours of black and oolong tea as shown in fig 2.3. (Ferruzzi, 2010). Another notable effect of fermentation is the gradual lowering of the flavanol content; however, the alkaloid content does not change significantly (Sava, Yang, Huang, Yang & Huang, 2001). Tea leaves with suitable quality are dried to inhibit further oxidation reactions, which serve as a basic requirement for product stability during storage (Temple, Temple, Van-Boxtel & Clifford, 2001).

Theaflavin (TF) and its derivatives, known collectively as theaflavins, are antioxidant polyphenols that are formed from the condensation of flavan-3-ols in tea leaves during the enzymatic oxidation of black tea. Theaflavin is formed from the polymerization of catechins at the fermentation or semi-fermentation stage during the manufacture of black or oolong tea. Theaflavin is generally considered to be the most effective component for the inhibition of carcinogenesis. Tea flavonoids are potent antioxidants in nature (Higdon & Frei, 2003; Lee *et al.*, 2016). Therefore, tea consumption leads to significant increase in the antioxidant capacity of the blood. Apart from being an antioxidant, theaflavins possess a broad spectrum of biological functions such as antibacterial, antitumor, antiviral, anti-inflammatory, and cardiovascular protection activities. The *TFs* could also strongly inhibit glucosyltransferase to protect tooth so as to prevent decayed tooth. Multiple epidemiological studies have reported an inverse association between black tea consumption and chronic incidence of mortality from congestive heart disease, stroke, atherosclerosis, pancreatic, bladder, and prostate cancers (Huang & Haung, 2006; Gardener, Rundek, Wright, Elkind, & Sacco, 2013).

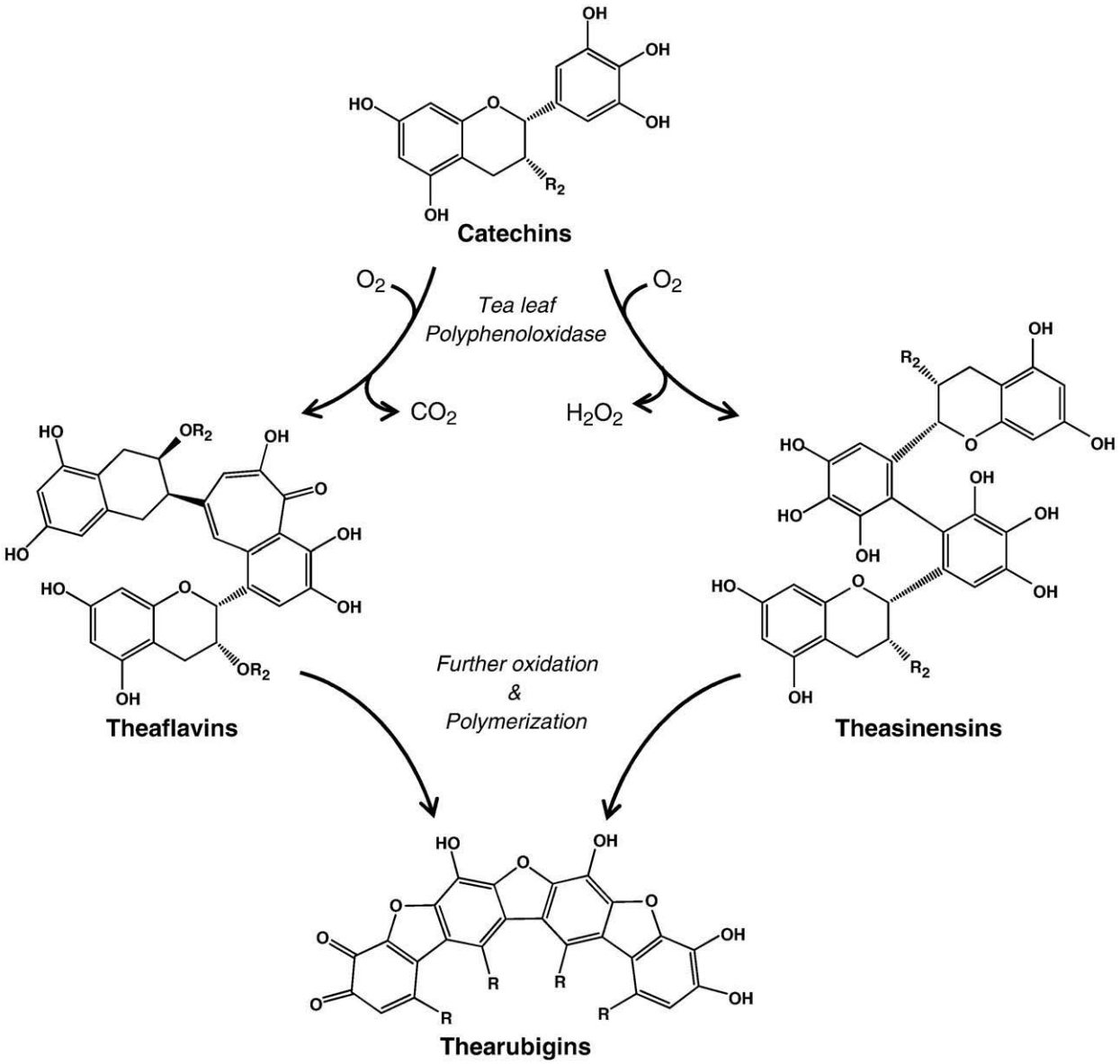

Fig 2.3: Polyphenol oxidation during fermentation to black tea (Ferruzzi, 2010).

2.4 Health benefits of tea

Tea is known to have beneficial effects on human health. These effects are largely due to its polyphenol content (Riemersma, Rice-Evans, Tyrrell, Clifford & Lean, 2001). Polyphenols are strong antioxidants found to act as anti-radical compounds, blocking free radicals (Gramza & Korczak, 2004). Studies found that according to the activity of tea polyphenols in scavenging superoxide radicals, tea could be helpful in preventing oxidative stress-related diseases, responsible for cellular disintegration and other degenerative diseases (Unno, Sugimoto & Kakuda, 2000).

Tea polyphenols are shown to have their effects on increasing the levels of enzymes detoxifying carcinogens in human cells (Yang, Lambert & Sang, 2009). The various components of tea are seen to impede carcinogen-induced DNA damage. Catechin is one of the most important constituents found in and demonstrates strong antioxidant activity by neutralizing free radicals (Trisha *et al.,* 2022). The catechins found in green tea include epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin-3-gallate and epicatechin, gallocatechins and gallocatechin gallate (Filippini *et al.,* 2020). EGCG is the predominant and most studied catechin in green tea (Peluso & Serafini, 2017; Yang & Zhang, 2019), as it is a powerful antioxidant believed to be an important determinant of the therapeutic qualities of green tea (Chen *et al.,* 2019; Gao, 2016). EGCG are found, in several studies, to bind to different target proteins in cells causing cell apoptosis (Yang & Wang, 2010). It is suggested that EGCG works by suppressing the formation of new blood vessels (angiogenesis) and regulating their permeability, thereby cutting off the blood supply to cancerous cells (Diniz, Suliburska & Ferreira, 2017, Rashidi, Malekzadeh, Goodarzi, Masoudifar & Mirzaei, 2017; Yang & Zhang, 2019).

In vitro studies and in vivo animal models have shown EGCG to be a potent chemo-preventative agent (Shirakami & Shimizu, 2018; Xu *et al.,* 2019; Filippini *et al.,* 2020). EGCG, has been shown

to reduce reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide and peroxynitrite (Sang, Lambert, Ho & Yang, 2011; Filippini *et al.,* 2020). Tea polyphenols are also strong chelators of metal ions, thus hampering the formation of reactive oxygen species. There is also some evidence that green tea polyphenols have a chemo preventive effect against cancers in smokers (Lee & Lee, 2006). The frequency of sister-chromatid exchange in lung cells was lower in smokers who consumed green tea. In a seven-year follow-up study of patients with breast cancer, it was found that increased consumption of green tea was associated with decreased numbers of axillary lymph node metastases especially among premenopausal patients with stage 1 and 2 breast cancers (Nakachi *et al.,* 1998). Early studies have linked tea drinking to both increased and decreased risks of esophageal cancer because of the high temperature at which the tea is consumed, but recent studies have also shown that the positive association between tea and esophageal cancer was because of the high temperature at which the tea is consumed (Gao *et al.,* 1994).

Butt and Sultan (2009) reviewed the anticarcinogenic and antimutagenic activities of green tea and suggested that it can reduce cancer prevalence and also provide protection. Yang and Landau (2000) supported that tea is not toxic and its consumption is suggested to prevent carcinogenesis. Both green and black teas have significant antimutagenic and anticlastogenic effects.

With respect to the effect of tea extracts on dental caries, oolong tea has been reported to contain substances, notably polyphenols, that have antibacterial properties against oral pathogens, such as streptococcus mutans, the bacteria closely associated with dental caries (Hamilton, 1995). Lingstrom, Wu and Wefel (2000) reported that frequent mouth rinsing with black tea infusion may contribute to oral health by inhibition of plaque, its acidity and its cariogenic microflora.

There is increasing evidence for a protective effect of tea consumption against cardiovascular health. The suggestion that regular tea intake, as part of a healthy dietary pattern, may play a role in maintaining cardiovascular health, reducing risk of cardiovascular diseases (CVDs) and decreasing all-cause mortality in adult populations has led to the development of research investigating potential health benefits of tea (Chung *et al.,* 2020). consumption of tea is thought to improve endothelial functions and to reduce blood pressure (Potenza *et al.*, 2007), and the findings of various studies support a role of tea in CVDs (Wang, Ouyang, Liu, & Zhao 2013; Lange, 2022).

2.5 Obesity and its health effects

Obesity is a complex, multifactorial, and largely preventable disease (Hruby & Hu, 2014), affecting, along with overweight, over a third of the world's population today (Ng *et al.,* 2014; Stevens *et al.,* 2012). Obesity is typically defined quite simply as excess body weight for height, but this simple definition belies an etiologically complex phenotype primarily associated with excess adiposity, or body fatness, that can manifest metabolically and not just in terms of body size (Hu, 2008). The aetiology of obesity is multifactorial with genetics, environmental factors, socioeconomic status, and behavioural factors all contributing to the development and persistence of obesity. Obesity results in a state of chronic inflammation, abnormal hormonal and immune system responses, and ultimately systemic metabolic dysregulation (Khanna, Welch & Rehman, 2022).

Obesity is a major risk factor for many of the most prevalent global chronic diseases including diabetes, hypertension, cardiovascular disease (CVD), kidney disease, chronic respiratory disease, and multiple types of cancers. It is estimated that obesity is directly responsible for at least 200,000 new cancer cases each year across Europe. Obesity increases the likelihood of various diseases and conditions which are linked to increased mortality. These include Type 2 diabetes mellitus

(T2DM), cardiovascular diseases (CVD), metabolic syndrome (MetS), chronic kidney disease (CKD), hyperlipidemia, hypertension, nonalcoholic fatty liver disease (NAFLD), certain types of cancer, obstructive sleep apnea, osteoarthritis, and depression (Swinburn *et al.*, 2011). Thus, while obesity is undoubtedly a condition, it also exacerbates pre-existing conditions and instigates new ones (Safaei, Sundararajan, Driss, Boulila & Shapi'I, 2021). More specifically, Bischoff et al. (2017), maintained that obesity can affect nearly every organ system, from the cardiovascular (CV) system to the endocrine system, central nervous system, and the gastrointestinal (GI) system. In addition, obesity is associated with the growing prevalence of several CV conditions, from hypertension and coronary heart disease (CHD) to atrial fibrillation (AF) and even total heart failure (Lavie, McAuley, Church, Milani, & Blair, 2014).

The development of certain cancers, including colorectal, pancreatic, kidney, endometrial, postmenopausal breast, and adenocarcinoma of the esophagus to name a few, have also been shown to be related to excess levels of fat and the metabolically active nature of this excess adipose tissue (Booth, Magnuson, Fouts, & Foster, 2015; Eheman *et al.*, 2012). Cancers have shown to be impacted by the complex interactions between obesity-related insulin resistance, hyperinsulinemia, sustained hyperglycemia, oxidative stress, inflammation, and the production of adipokines (Booth *et al.*, 2015).

Body mass index is regarded as the standard metric for determining underweight, normal-weight, overweight and obesity (Zierle-Ghosh & Jan, 2023). The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2 resulting from mass in kilograms and heights in meters.

An individual's BMI is important in the determination of potential future health issues ad has been widely used as a factor in the determination of various health policies (Zierle-Ghosh & Jan, 2023)..

Studies have shown that people with BMIs higher than 30 are at greater risk of dying from diabetes, cancer, heart diseases, etc. Although, several studies suggest that in some cases, a high BMI could protect a person from dying of heart failure, kidney failure and other chronic diseases (Poirier *et al.,* 2006).

2.5.2 Obesity and Cardiovascular diseases

Obesity directly and indirectly promotes CVD. Excess adiposity induces endothelial dysfunction, small vessel remodelling, and cardiomyocyte toxicity promoting atherosclerotic and vasospastic coronary heart disease, arrhythmias, cardiomyopathy, and congestive heart failure (Powell-Wiley, 2021). Additionally, obesity is a major risk factor for the development of known cardiovascular risk factors like diabetes, hypertension, dyslipidaemia, and chronic kidney disease (Khanna *et al.*, 2022). Prolonged excess calorie intake leads to excessive fat storage surpassing the limited storage capacity of adipose tissue for fatty acids. This leads to increased circulating free fatty acids and abnormal storage of fatty acids in organs that play a prominent role in overall metabolic regulation like the liver, pancreas, and skeletal muscle. The lipotoxicity of fatty acids in circulation and stored in organs key for metabolic regulations results in oxidative stress, inflammation, and metabolic dysregulation throughout the body (Khanna *et al.*, 2022). Adipose tissue is a complex secretory organ that plays several key roles in metabolism—modulating energy expenditure (EE), appetite, insulin sensitivity, bone metabolism, reproductive and endocrine functions, inflammation, and immunity. Adipocytes synthesize and secrete numerous proteins and hormones called adipokines which play important roles in endocrine regulation, immune function, and inflammation. Obese individuals have dysfunctional adipose tissue that secretes pro-inflammatory proteins like interleukin (IL)-6, tumor necrosis factor alpha, C-reactive protein (CRP), IL-18, while lean individuals' adipose tissue mostly secretes anti-inflammatory proteins like transforming growth factor beta, IL-4, IL-10, and IL-13 (Khanna *et al.*, 2022). The abnormal immune response and pro-

inflammatory state induced by obesity promotes insulin resistance, hypertension, renal disease, atherosclerosis and other chronic illnesses (Khanna *et al.*, 2022). Additionally, excessive adipose tissue in the epicardium surrounding the heart— frequently seen in overweight and obese individuals—promotes CVD. Epicardial adipose tissue leads to abnormal local adipokine expression, inflammatory cytokine production, and altered gene expression promoting coronary atherosclerosis, atrial fibrillation, and congestive heart failure (Konwerski, Gąsecka, Opolski, Grabowski, & Mazurek, 2022).

Obesity and obesity-induced cardiovascular risk factors have both been linked to the coronary atherosclerotic burden in autopsy studies of children and young adults (Powell-Wiley, 2021). In the absence of associated risk factors, obesity is thought to be directly associated with coronary atherosclerotic plaque formation primarily due to obesity-induced inflammation and increased oxidative stress resulting in apo-B lipoprotein oxidation and endothelial dysfunction (Powell-Wiley, 2021). Several large prospective analyses have indicated that the link between obesity and coronary artery disease is mediated largely by hypertension, dyslipidaemia, diabetes, and other comorbidities, whereas other prospective studies suggest a significant residual risk in obese individuals even after accounting for these risk factors (Ndumele *et al.*, 2016; Welsh, Hamad, Piña & Kulinski, 2024). Most likely both direct vascular dysfunction and injury from excess adiposity and indirect obesity-induced metabolic risk factors significantly contribute to coronary atherosclerosis and ischaemic heart disease.

Dyslipidaemia is the largest contributing factor to the development of atherosclerosis and subsequent atherosclerotic CVD in obese individuals (Wickramasinghe & Weaver, 2018). The entrance of apo-B lipoprotein particles within the arterial wall is the fundamental step that initiates and drives the atherosclerotic process from beginning to end (Sniderman *et al.,* 2019). Obesity and obesity-related metabolic diseases are strongly linked to dyslipidaemia that promotes atherosclerosis. Approximately 60–70% of patients with obesity have abnormal lipids including elevated serum triglycerides, very low-density lipoprotein, apolipoprotein B, non-HDL-C levels, and low serum HDL-C (Welsh *et al.,* 2024). LDL-C levels may or may not be significantly elevated, but there is an increase in small dense LDL particles which are pro-atherogenic because they are more easily oxidized and taken up by macrophages, enter the arterial wall more readily, and have a decreased affinity for the LDL receptor resulting in a prolonged time in the circulation (Klop, Elder & Cabezas, 2013). These abnormalities are driven by the combination of the greater delivery of free fatty acids and triglycerides to the liver from excess adipose tissue, insulin resistance, adipocyte dysfunction with reduced adiponectin and increased production of pro-inflammatory cytokines (Rosell, Appleby, Spencer & Key, 2006).

2.5.2 Obesity and osteoarthritis

There is a well-documented association between high BMI and the development of osteoarthritis in both men and women (Cicuttini & Spector, 1998; Park *et al.,* 2023). Initially, osteoarthritis has been considered to be a disease of articular cartilage, but recent research has indicated that the condition involves the entire joint (Dieppe, 2011; Man & Mologhianu, 2014). New bone is formed at the joint surface, probably in response to the cartilaginous damage (Hart *et al.,* 1993).

Obesity is typified by nutrient excess and insulin resistance, which are closely related to the excessive pro-inflammatory cytokine production seen in chronic inflammation (Hotamisligil,

2006; Messier, 2008; McArdle, Finucane, Connaughton, McMorrow & Roche, 2013). Nutrient excess produces reactive oxygen species, resulting in oxidative stress that damages cells and triggers an inflammatory response (Messier, 2008; Tan, Norhaizan & Liew, 2018). The increased inflammation blocks the protective action of insulin, which normally stimulates target cells to take up nutrients. Unfortunately, as excessive nutrients are consumed, neighboring cells and tissues that remain insulin-sensitive are placed at risk (Rehman & Akash, 2016). As insulin resistance progresses, inflammation is exacerbated, initiating a cycle of excessive nutrient intake/insulin resistance/ inflammation (Wisse, Kim, & Schwartz, 2007; Wu & Ballantyne, 2020). In some cells, nutrient excess impairs endoplasmic reticulum function and accelerates the accumulation of fatty acid derivatives that also promote inflammation (Hotamisligil, 2006; Park & Seo, 2020).

The broad inflammatory response characteristic of obesity was first demonstrated by Hotamisligil et al. (1993). They showed that the inflammatory cytokine tumor necrosis factor alpha (TNF-α) was overexpressed 5 to 10-fold in obese compared to lean mice. TNF-α activates signal transduction cascades that result in insulin resistance. The inflammatory response appears to be triggered and to reside predominantly in adipose tissue, which secretes a variety of hormones known collectively as adipokines. Deregulation of these proteins is associated with excessive weight gain, an inflammatory state, and a variety of chronic diseases, including knee OA (Messier, 2008; Taylor, 2021). Since obese individuals have higher concentrations of inflammatory markers, inflammation may contribute to functional limitation and disease progression in those with OA. Besides direct effects on the joint, inflammatory mediators can affect muscle function and lower the pain threshold (Messier, 2008).

Weight loss lowers serum leptin levels in OA subjects and is related to improved function (Miller *et al.*, 2004). Diet and physical exercise are the ideal methods for creating a negative energy balance and consequently causes weight loss. Medication, as a method of treating obesity, should be chosen only when the nutritional treatment has been shown to fail. Although medication helps in weight loss, and prevents relapse, it has many side effects and the patient still needs to follow a certain diet and exercise (Berghofer *et al.*, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and reagents

The following chemicals and reagents were used for analyses:

Petroleum ether (b.P 40-60^{o}C; M&B Ltd., England), HCl (BDH England), NaCl (M&B Ltd., England), concentrated sulphuric acid (MERCK, Germany), boric acid (BDH, England), acetone (MERCK, Germany), NaOH (BDH, England), HDL kit (Randox® Laboratories Ltd., United Kingdom), cholesterol kit (Randox® Laboratories Ltd., Antrim United Kingdom), Triglyceride kit (Teco Diagnostics, California, USA.) LDL kit (Biosystems diagnostics, S.A Costa Brava of Barcelona Spain), Chloroform (FIESER, Germany), ferrric chloride (Hamburg Chemical Co. Germany), lead acetate (FIESER, Germany), methanol (Guangdong Guanghua Sci. Ltd, China), ethanol (BDH, England), diethyl ether (Loba Chemie PVT Ltd., Mumbai), calcium chloride ($CaCl_2$) (Griffin and George, England), potassium permanganate ($KMnO_4$) (Guangdong Guanghua Sci. Ltd, China), ammonium chloride (NH_4Cl) (M&B Ltd., England), ammonium thiocyanate (Hamburg Chemical Co., Germany), Meyer's reagent (potassium mercuric-iodide solution), Folin-Denis reagent, bromocresol green and anthrone reagent (2g anthrone in 1L H_2SO) from Sigma Chemical Co.

3.1.2 Glassware and equipment

The following glassware and equipment were used for laboratory analyses; Electronic weighing balance (NAPCO, USA), dissecting set instruments, EDTA bottles (MERCK, Germany), water bath (Bio Techno Lab., India), Centrifuge (B. Bran Scientific and Instrument Company England), Refrigerator (LG Electronics Inc., South Korea), Oven (Bemco Inc., California, USA),

Spectrophotometer (Labomed, Inc., California, USA), Hot plate, (Lab. Soul, India). Electro thermal heater (Keison Company, UK), Muffle furnace (Bio Techno Lab., India), Crucible refluxing unit (Prime Scientific Industries, India).

3.1.3 Procurement of poultry feed, rats and tea

The pelletized poultry feed (a subsidiary product of UAC Nigeria Plc., Jos Nigeria) and tea used in the study were purchased from Douglas Market, Owerri, Nigeria. Eighteen albino rats (*Rattus norvegicus*) of the Wistar strain were purchased from the Animal Colony of the Department of Veterinary Medicine, University of Nigeria Nsukka, Nigeria.

The feed and tea used in the study were coded, described and their ingredients listed according to the manufacturers' descriptions (Table 1).

Table 3. 1: **Contents of feed and tea (manufacturer's description)**

Code/sample description	Ingredients
A (Poultry feed)	Cereals/grains, vegetable, protein, premix (vitamin/minerals), probiotic and enzymes, essential amino acids, antioxidant, antitoxins.
B (Green tea)	Polyphenols, amino acids, carbohydrate, protein, fluoride, aluminum, mineral and trace elements, vitamin, vitamin B, vitamin E and alkaloids.
C (Black tea)	White tea, green tea, hibiscus flower, rosehips, orange leaves, sweet blackberry leaves, lemon grass, roasted chicory cinnamon, peppermint, nutmeg, fennel seed, ginger, cloves, and licorice root.

Table 3.2: Nutritional information of *tea* and feed samples (manufacturer's description)

Sample description	Content/ Concentration
Camellia sinensis	
Energy	<10kg
Protein	<0.1kg
Fat	<0.1kg
Sodium	<5mg
Caffeine	NG
Vitamin B	NG
Vitamin C	NG
Vitamin E	NG
Polyphenol	NG
Fluoride	NG
Amino acids	NG
Feed	
Carbohydrate	43.62 %
Protein	12.69 %
Lipid	7.30 %
Fiber	19.99 %

NG = not given

3.2 METHODS

3.2.1 Preparation of tea infusion

One hundred and fifty milliliters (150 ml) of boiled water was measured into a 500 ml beaker. Then 1.5 g of each tea sample was added in separate beakers and allowed to stand for 30 min. The infusion was filtered off the leaves and its concentration determined by evaporating 50 ml to dryness in a pre-weighed 50 ml beaker.

3.2.2 Qualitative determination of phytochemical content of tea infusions

The qualitative determination of phytochemical content of tea infusions was carried out using the methods of Amadi, Agomuo & Ibegbulem (2004).

Saponins: An aliquot of the infusion was pipetted into test tubes, shaken vigorously for 1minute and the solution observed for stable froth.

Flavonoids: One milliliter of infusion was added to 1.0 ml of 10 % ferric chloride. The solution observed for formation of black precipitate.

Tannins: Three drops of lead acetate solution was added to 2.0 ml of infusion and observed for black precipitate formation.

Alkaloids: One milliliter of the infusion was treated with Meyer's Reagent (potassium, mercuric-iodide solution) and observed for cream coloured precipitate.

Phytosterol: One milliter of infusion was added to 2 ml of chloroform. Also, 2 ml of sulphuric acid was added to the solution and observed for coloured ring formation between infusion and chloroform layer.

3.2.3 Quantitative determination of phytochemical content of tea infusions

3.2.3.1 Determination of tannins

Tannins was determined using the Folin-Denis method as described by Pearson (1976). Two-point-five millilitres (2.5 ml) of sample was centrifuged and the infusion recovered. 1 ml of the supernatant was dispersed into 50 ml volumetric flask. Similarly, 1 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. Then, 1.0 ml Folin Denis reagent was measured into each flask followed by saturated Na_2CO_3 solution. The mixture was diluted to 50 ml in the flask and incubated for 90 min at room temperature. The absorbance of the samples were measured at 700 nm with reagent blank at zero. The % tannin was calculated.

% Tannin = An/As x C x100/w x Vf/Va

Where: An = absorbance of test sample

As = absorbance of standard solution

w = weight of sample used

Vf = total volume of extract

Va = volume analyzed

C = concentration of standard solution

3.2.3.2 Determination of flavonoids

Flavonoids was measured using the method described by Boham & Kocipai-Abyazan (1994). Ten milliliters of sample was determined with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered using Whatman filter paper. The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed to constant weight. The content of flavonoid was calculated and expressed in percentage.

$$\% \text{ flavonoid} = \frac{\text{Weight of flavonoid}}{\text{Weight of sample}} \times 100$$

3.2.3.3 Determination of saponins

Saponin was determined using the method described by Obadoni & Ochuko (2001). The saponin content was determined with 20 ml of each sample in 200 ml of 20 % ethanol. The suspension was placed over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol. The combined infusion was reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml diethyl ether was added and was shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

Sixty milliliters of n-butanol was added, the combined n-butanol infusion was washed twice with 10 ml of 5 % aqueous sodium chloride. The organic layer was separated using separating funnel and evaporated to dryness using a water bath. The infusion was dried to a constant weight and the saponin content was calculated and expressed in percentage.

$$\% \text{ Saponin} = \frac{\text{Weight of Saponin}}{\text{Weight of sample}} \times 100$$

3.2.3.4 Determination of cyanogenic glycosides

Analysis of cyanogenic glycosides was done using the AOAC (2000) method. Five millilitres (5 ml) of the sample was weighed and cyanide extraction was allowed to stay overnight and then filtered. Different concentration of KCN solution containing 20 to 100 mg/ml, cyanide was prepared for standard curve. Four millilitres (4 ml) of alkaline picrate was added to 1 ml of filtered sample and standard cyanide solution in different test tubes and incubated in a water bath for 15 min. After colour development, the absorbance was read at 490 nm against blank containing 1 ml

of distilled water and 4 ml picrate solution. The cyanide content was extrapolated from the cyanide standard curve. It was calculated using the formula:

$$\text{mg / kg cyanide} = \frac{\text{µg / mL of cyanide x final volume (L)}}{\text{Sample weight}}$$

3.2.2.5 Determination of oxalates

Oxalates was determined by the titration method described by Baker (1952). Two millilitres (2 ml) of the infusion was placed in 250 ml volumetric flask suspended in 190 ml distilled water. Then, 10 ml of 6N HCl solution was added to the sample solution and the suspension digested at 100°C for 1hr. The sample was then cooled and made up to 250 ml mark of the flask with distilled water. The sample solution was filtered and duplicate portion of 125 ml of the filtrate was measured into the beaker and four drops of methyl red indicator was added, followed by the addition of conc. ammonia solution in drops until the solution changed from pink to yellow. The solution was then heated to 90°C, cooled and filtered to obtain the precipitate containing ferrous ion. The filtrate was again heated at 90°C and 10 ml of $CaCl_2$ solution was added with consistent stirring. The solution was allowed to stand overnight and then centrifuged and filtered. The precipitate was completely dissolved in 10 ml of 20 % H_2SO_4 and diluted to 200 ml with distilled water. An aliquot of 125 ml of the filtrate was heated to near boiling and titrated against 0.05 ml $KMnO_4$ solution to a pink colour which persisted for 30 sec. The oxalate content of each sample was calculated as:

$$\frac{T \times (Vme)\,(Df) \times 10^5}{(ME) \times Mf} \quad \text{(mg/100g)}$$

Where: T = titre of $KMnO_4$ (ml)

Vme = volume-mass equivalent

Df = dilution factor

ME = molar equivalent of $KMnO_4$

Mf = mass of flour used

3.2.2.6 Determination of phytates

The determination of phytate was done using the method of Maga (1982). 2 ml of the infusion was measured into 250 ml conical flask and 100 ml of 20 % HCl was added and allowed to stand for 3 hrs. The solution was then filtered with Whatman No.1 filter paper and 50 ml of the filtrate was introduced into a 250 ml beaker with 10 ml of HCl added to improve the acidity. Then, 10 ml of 0.3 % ammonium thiocyanate solution was added to each sample solution as indicator and titrated against 0.1 M $FeCl_3$ until a brownish yellow colour that persisted for 5 mins was obtained. The phytate content was calculated from the titer value.

$$\text{Phytic acid} = \frac{\text{Titre value x 0.001 95 x 119 x 100}}{2}$$

3.2.2.7 Determination of alkaloids

Alkaloids was determined using the method described by Harbone (1984). One-point-five millilitres (1.5 ml) of infusion was pipetted and evaporated. The residue was dissolved in 3 ml of phosphate buffer solution of pH 4.5. The solution was then transferred to a separation funnel and was thoroughly mixed with 3 ml of bromocresol green solution (0.03 %). Chloroform (5 ml) was added after 30 min and shaken for 2 min. The lower layer was then separated and measured in percentage.

$$\% \text{ Alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100$$

3.2.4 GC-MS analysis of tea

The GC-MS analysis was performed on an Agilent 7890A using the method described by Sulaiman et al. (2020). Firstly, the two concentrates of green tea and black tea infusions were reconstituted

in methanol. The sample was injected into the GC-MS and vaporized at 250 ◦C. The compounds were subsequently separated using an HP5MS column fused with phenylmethyl siloxane. The carrier gas, helium, operated at a flow rate of 1 mL per min. The compounds, which had been separated, were detected using a GC-FID detector and mass spectrometry for compound identification; each signal was fragmented.

3.2.5 Determination of concentration of tea infusion

Two empty beakers were weighed and 50 ml of infusion added, respectively. The samples were placed on hot plate and allowed to evaporate to dryness. The beaker was then allowed to cool in a desiccator, re-weighed to constant weight and the concentration calculated as:

Final weight of beaker – initial weight of beaker = weight of solute / 50 ml

3.2.6 Grouping and treatment of the experimental rats

The rat feeding study was conducted in accordance with the protocols approved by the Departmental Experimental Animal Ethics Committee and in accordance with international standard for laboratory animal use and care as captured in NIH (1985). Eighteen (18) male Wistar rats were randomly allotted according to their weight into 3 groups of 6 rats each. The weights ranged from 87-120 kg. The rats were housed in groups in metabolic cages with provision for food and fluid troughs. The rats were maintained under the same light and dark condition and an ambient room temperature of 25.00 ± 2.0°C. They were acclimatized and fed for 14 days with supply of pelletized poultry feed and water *ad libitum.* After the period of acclimatization, tap water was provided as the only source of fluid for the control group, while groups B and C had tea infusions as their only source of fluid.

Group A served as the normal control with 6 rats. They were fed pelletized poultry feed and tap water for 28 days. Groups B and C, with 6 rats each served as the first and second test groups.

They were both fed pelletized poultry feed, and 0.20 g/50 ml of green and 0.28 g/50 ml of black tea infusions respectively for 28 days. The remnant feed and fluid for each group were collected, measured and recorded per day and total daily amount of feed and fluid consumed per group were calculated as shown in Appendix 1.

Body weight gained was calculated as: Final weight of each rat (g) – initial weight (g).

$$\text{Growth rate} = \frac{\text{Weight gain}}{\text{Number of days}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

3.2.7 Collection of blood and faecal samples

At the end of the feeding study, the rats were re-weighed and anaesthetized by exposure to mild dichloromethane vapour soaked in cotton wool. Incisions were made into the thoracic region and blood collected by cardiac puncture using 5 ml hypodermic syringe and needles while the heart was still beating. The blood was immediately transferred into ethylene diamine tetra acetic acid (EDTA) sample bottles and mixed gently but thoroughly. The uncoagulated blood was centrifuged at 5,000 rpm for 5 min and the plasma separated with the aid of sterile Pasteur. Faecal samples were collected from the three groups, weighed, dried and blended into powder form and labeled before analysis.

3.2.8 Determination of relative organ weight

The relative organ weights of animals was carried out using the method of Duchen et al. (1984). After blood sample collection, the animals were dissected to remove the respective organs. The heart, kidneys, spleen and liver were each excised, blotted dry with absorbent paper and weighed

using an analytical balance. The percentage relative organ weights of the rats were estimated as weight of each organ divided by the final body weight of the rat multiplied by 100.

3.2.9 Determination of lipid profile

3.2.9.1 Total cholesterol determination

The determination of total cholesterol was done using the method of Allain, Poonm & Chan (1974)

Principle

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{cholesterol + fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-Amino antipyrine} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 4H_2O$$

Procedure

About 1.0 ml of the working reagent was pipetted into the test tubes labelled blank (B), standard (S) and test (T). Thereafter, 0.01 ml of distilled water was added to the blank (B), then 0.01 ml of cholesterol standard was also added to standard (S), and 0.01 ml of sample was added to test (T). The solutions were thoroughly mixed respectively and incubated at 37º C for 5 min. or at room temperature (25º C) for 15 min. it was determined by measuring absorbance of the standard (Abs.S), and test sample (Abs.T) against the blank within 60 min.

$$\text{Cholesterol in mg/dl} = \frac{\text{Absorbance sample x concentration of standard}}{\text{Absorbance of standard}}$$

3.2.9.2 High density lipoprotein determination

Determination of High-Density Lipoprotein was done using the methods of Grove (1979).

Principle

Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) contained in the serum precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The cholesterol is quantitatively monitored through the actions of cholesterol esterase, cholesterol oxidase and chromophore (quinoneimine) formed from dichlorophenolsulphonate (DCPS) by the action of peroxidase.

$$\text{Cholesterol esters} + H_2O \xrightarrow{Cholesterol\ esterase} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + 1/2O_2 + H_2O \xrightarrow{Cholesterol\ oxidase} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{DCPS} \xrightarrow{Peroxidase} \text{Quinoneimine} + 4\ H_2O$$

Procedure

An aliquot of sample (0.2 ml) of the serum and 0.5ml of reagent was pipetted into centrifuge tubes, mixed thoroughly, allowed to stand for 10 minutes at room temperature and centrifuged at 4,000 rpm. Thereafter, pH 7.0 was pipetted into the tubes and incubated at 37ºC for 5 minutes, the absorbance of the sample solutions were measured at 500 nm against the blank. The colour was stable for 2 hours.

3.2.9.3 Determination of triacylglycerol

The determination of triacylglycerol was carried out using the method of Searcy (1969).

Principle

The enzymatic reaction sequence employed in the assay of triacylglycerol is as follows:

Triglycerides $\xrightarrow{Lipase}$ glycerol + fatty acids

Glycerol +ATP $\xrightarrow{Glycerol\ kinase}$ glycerol-1-phosphate + ADP

Glycerol-1- phosphate O_2 $\xrightarrow{GTP}$ DHAP + H_2O_2

Procedure

An aliquot (0.1 ml) of reagent was pipetted into tubes, labeled according to manufacturer's instruction and placed in a 37°C heating block for 4 minutes. Then 0.01 (10 µl) of sample was added to the respective tubes. The tubes were mixed by shaking and incubated for 5minutes at 37°C. The absorbance of sample was measured against the blank reagent within 60 min at 520 nm wavelength. It was calculated using the formula below:

$$\text{conc. of triacylglycerol in sample} = \frac{\text{Absorbance sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

3.2.9.4 Determination of low-density lipoprotein

The determination of low-density lipoprotein was carried out using the method of Assman, Jabs, Kohnert, Nolte, & Schriewer (1984).

Principle

Low-density lipoprotein (LDL) in the sample, with polyvinylsulphate. Their concentration was calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by means of

Cholesteryl esters + H_2O $\xrightarrow{Cholesterol\ esterase}$ Cholesterol + fatty acids

Cholesterol + $1/20_2$ + H_2O $\xrightarrow{Cholesterol\ oxidase}$ Cholestenone + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + DCPS $\xrightarrow{Peroxidase}$ Quinoneimine + 4 H_2O

Procedure

Zero-point-four millilitres (0.4 ml) of serum and 0.2 ml of reagent were pipetted into centrifuge tubes and thoroughly mixed. The solution was allowed to stand for 15 minutes and centrifuged at 4000 rpm for 15 minutes. About 20 µl of distilled water, cholesterol standard and sample supernatant was pipetted into the tubes labelled reagent blank, standard and sample respectively. The content of the test tubes were thoroughly mixed and incubated at 37°C for 15 minutes. It was determined by measuring the absorbance of the standard and sample at 500 nm against blank. The colour was stable for at least 3 min and calculated as:

$$\frac{\text{Abs. sample}}{\text{Absorbance of standard}} \times \text{conc. of standard x sample dilution factor} = \text{conc. of supernatant}$$

Total cholesterol – cholesterol in supernatant = LDL. chol.

3.2.9.5 Determination of very low- density lipoprotein

This was estimated by dividing the value of triglyceride by a factor of 5 if the value is mg/dl or 2.2 in mmol/ L. During fasting, the VLDL to total plasma triglyceride ratio is relatively fixed at 1:5 (Driver *et al.*, 2016).

3.2.9.6 Determination of chylomicrons

Chylomicron was determined using the method of Lindgren, Jensen & Hatch (1972).

Principle

The presence of chylomicrons is tested by the qualitative "standing plasma" test also known as the refrigeration test. Specimen is allowed to stand undisturbed for 12-24 hours between 2- 8 $^{\circ}$C. In refrigerated serum, chylomicrons are the largest lipoproteins with the least density which rises to form a creamy top layer, very low-density lipoprotein remain dispersed and serum becomes turbid. The determination of chylomicrons was visually inspected.

Procedure

The samples were collected in tubes containing EDTA and centrifuged at 2800 rpm for 15 min. at 4°C and plasma extracted. About 3 ml of plasma was covered with 2.5 ml of a NaCl-NaBr solution of 1.006 kg/L in ultracentrifugation tubes and centrifuged for 23 min. at 30000 rpm. The tubes were sliced 45 mm from bottom and the top fraction which contained primarily of chylomicrons was measured. It was called "chylomicron positive" if an ultracentrifuge supernatant had a triacylglycerol concentration of more than 60 mg/L.

3.2.10 Analysis of faecal content

3.2.10.1 Determination of protein content

A small amount of processed faecal sample (0.1 g) was weighed into a clean conical flask of 250 ml capacity. A digestion catalyst (3 g) was added into the flask and concentrated sulphuric acid (20 min) was also added and the sample heated to digest according to Kjeldahl method (Kjeldahl 1883) until the content changed from black to sky –blue coloration. The digest was cooled to room temperature. The content was then diluted to 100 ml with distilled water. A dilute digest (20 ml) was measured into a distillation flask and placed on an electro thermal heater or hot plate and the distillation flask was connected to a receiver that contains 10 ml of 2 % boric acid indicator. Then 40 ml of 40 % sodium hydroxide was also injected into the digest through a syringe that was attached to the mono-arm steel head until the digest became strongly alkaline. The mixture was heated to boiling and ammonia gas distilled via the condenser. The color of the boric acid changed from purple to green after ammonia distillate was introduced. Then 0.1 N of hydrochloric acid was used to titrate the distillate, until the solution changed back to purple from green after hydrochloric acid was added. The percentage of nitrogen in the sample was calculated using the formula:

$$\% \text{ organic nitrogen} = \text{titre value} \times \frac{1.4 \times 100 \times 100}{1000 \times 20 \times 0.1}$$

Where titre value = the volume of HCl used in titrating the ammonium distillate.

1.4 = Nitrogen equivalent to the normality of HCl used in titration of 0.1N

100 = the total volume of digest dilution

100 = percentage factor

1000 = conversion factor from gram to milligram

20 = integral volume of digit analyzed or distilled

0.1= the weight of sample in gram digested.

3.2.10.2 Determination of carbohydrate

This was carried out using the method of Hedge & Hofreiter (1962). A small amount (0.1 g) of faecal sample was weighed into a volumetric flask, distilled water (1 ml) and 1.3 ml of 62 % perchloric acid was added and shaken for 20 min for complete blending. The solution formed was filtered with a glass filter paper. The filtrate measuring 1ml was collected and transferred into a 10 ml test tube and diluted to volume with distilled water. Then 5 ml of anthrone reagent was added to 1 ml of working solution and mixed with 1 ml of distilled water and the solution read at 630 nm wavelength using a mixture of distilled water (1 ml) and anthrone reagent (5 ml) as blank solution. Thereafter, 0.1 ml glucose was also prepared and treated as the sample with anthrone reagent and absorbance was read and the value of carbohydrate (glucose) was evaluated using the formula below:

$$\%\ \text{CHO as glucose} = \frac{25 \times \text{absorbance of sample}}{\text{absorbance of standard glucose} \times 1}$$

3.2.10.3 Determination of moisture

This was performed using the evaporation method of deMan, Finley, Hurst, & Lee (2018). One gram of sample was weighed into a porcelain evaporating dish and placed in an oven with temperature of 10^{o}C for six hours. The evaporating dish was cooled afterwards in a desiccator at room temperature. It was reweighed and recorded. The formulae for calculating moisture is:

$$\%\ \text{moisture} = \frac{\text{weight of fresh sample - weight of dried sample}}{\text{Weight of fresh sample}} \times \frac{100}{1}$$

3.2.10.4 Lipid determination

Lipid determination was done using the AOAC (2000) method. The sample measuring 2 grammes was wrapped in filter paper and put on a soxhlet extraction and the extractor placed on a pre-weighed dried flask. Acetone was then introduced into the soxhlet extractor and held by a retort stand clamp. Cold water was allowed into the condenser and the heated solvent refluxed as a result. The lipid in the solvent was extracted in the process of continuous refluxing. When the lipid was completely extracted, the condenser and extractor was disconnected while the solvent evaporated to concentrate the lipid. The flask was dried in the air oven to constant weight and re weighed to obtain the weight of lipid. The calculation was done using the formulae below:

$$\% \text{ lipid} = \frac{\text{Weight of flask and extract-weight of empty flask}}{\text{Weight of sample extracted}} \times \frac{100}{1}$$

3.2.10.5 Determination of ash

Ash was determined using the AOAC (2000) method. A gram of dried sample was weighed into a preheated weighed porcelain crucible. The crucible was then put into a muffle furnace and regulated to temperature of 63^{o}C for 3hr and allowed to cool to room temperature and reweighed. Percentage weight of ash calculated using the formulae below:

$$\% \text{ Ash} = \frac{\text{weight of crucible + ash sample – weight of crucible}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.2.10.6 Determination of fibre

Fibre was determined using the AOAC (2000) method. A small amount of sample (0.1g) was weighed, gelatinized and partially digested with alpha- amylase and enzymatically digested with protease and amyloglucosidase to remove starch and protein, followed by the addition of 95% of ethanol to precipitate all fibre. The solution was then filtered and the fibre collected, dried and weighed. Percentage weight of fibre was calculated using the formula below:

% Fibre = weight of residue – weight of protein and ash

3.2.11 Statistical Analysis

Data was analyzed relative to the control using student's t-test and one- way- analysis of variance (ANOVA) at 95 % confidence interval ($P \leq 0.05$) and percentage coefficient of variation (CV %) where appropriate.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1: Phytochemical contents

Table 4.1 presents the qualitative phytochemical screening of the tea infusions. The presence of phytochemical is shown with a + sign while a – sign would mean absence. Phytochemical screening revealed the presence of presence of flavonoids, tannin, alkaloids, saponin and phytosterols in the tea infusions.

Table 4.1: Qualitative phytochemical content of green and black tea

Parameter	Green tea	Black tea
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	+
Saponins	+	+
Phytosterols	+	+

Key: + = Presence of phytochemicals

4.1.2: Phytochemical Contents of the tea infusions

Quantitative phytochemical screening of the tea infusions is presented in Table 4.2. The table revealed that the mean concentration of tannin, alkaloid, flavonoid, saponins, phytate, oxalates, cyanogenic glycoside was higher in green tea than observed in black tea. Tannin presented the highest concentration (28.61%), followed by alkaloids (9.78%) in green tea while alkaloids in black tea was the most concentrated phytochemical (8.7%), followed by saponins (6.35%). The following antinutrients were also present; phytate, oxalate and cyanogenic glycoside.

Table 4.2: Phytochemical content (%) of the tea infusions

Parameter	Concentration (%)	
	Green tea	**Black tea**
Tannins	28.61 ± 0.11^{a}	4.90 ± 0.04^{b}
Phytates	0.29 ± 0.00^{a}	0.22 ± 0.00^{b}
Oxalates	1.55 ± 0.00^{a}	1.76 ± 0.00^{b}
Cyanogenic glycosides	559.50 ± 0.50^{a}	335.50 ± 1.50^{b}
Saponins	8.17 ± 0.02^{a}	6.35 ± 0.02^{b}
Flavonoids	8.43 ± 0.03^{a}	5.15 ± 0.04^{b}
Alkaloids	9.78 ± 0.01^{a}	8.70 ± 0.00^{b}

Values are mean ± Standard deviation of duplicate determinations. Values on the same column bearing the same superscript letter are not significantly different ($p < 0.05$).

4.1.3 GC-MS results of green tea

The GC-MS analysis of green tea infusion is presented in Table 4.3. In this analysis, 17 phytochemical constituents were detected. Butylated hydroxytoluene, 9-octadecenoic acid, 2-8-pentadecen-1-ol acetate, 9, 17-octadecadiena and 5-eicosene predominated the chemical composition of the green tea infusion in decreasing order, while tricosane, .gamma.-sitosterol and 6-octadecenoic acid were the lowest.

Table 4.3: GC-MS results of chemical constituents of green tea

S/N	Retention Time	Area %	Name of compound
1	10.133	39.42	Butylated hydroxytoluene
2	10.798	6.50	Butylated hydroxytoluene
3	21.382	2.48	Oleic acid
4	25.762	3.69	9,12-octadecadienoic acid
5	25.980	1.05	Tricosane
6	26.358	2.29	9,12-octadecadienoic acid
7	28.420	2.62	9,17-octadecadienal
8	28.006	7.53	Tricosane
9	29.734	6.52	Hentriacontane
10	29.961	1.99	9-octadecenoic acid
11	31.190	8.79	5-eicosene
12	31.623	0.58	. gamma. -sitosterol
13	31.230	2.81	2-8-pentadecen-1ol acetate
14	31.871	8.74	2-8-pentadecen-1ol acetate
15	34.549	3.65	2-8-pentadecen-1ol acetate
16	36.804	3.27	13-octadecenoic acid
17	38.125	-1.49	6-octadecenoic acid

4.1.4 GC-MS results of black tea

The GC-MS analysis of black tea infusion is shown in Table 4.4. In this analysis, 30 phytochemical constituents were detected. Black tea infusion's chemical composition was dominated by 11-octadecenoic acid, 9,12-octadecadienoic acid, hexadecenoic acid and 1-octadecene in decreasing order, with heptafluorobutyric acid, disparlure, heptadecyl and acetic acid having the lowest concentration.

Table 4.4: GC-MS results of chemical constituents of black tea

S/N	Retention Time	Area %	Name of compound
1	8.000	0.18	Cyclopentane
2	8.732	0.43	Cyclopentane
3	11.859	0.08	Acetic acid
4	13.397	1.02	5-tetradecane
5	16.273	0.33	Heptadecyl
6	16.715	0.33	7-hexadecenal
7	17.016	0.16	Disparlure
8	17.641	0.15	Heptadecyl
9	17.782	1.52	1-octadecene
10	18.276	0.45	Trichloroacetic acid
11	18.858	0.42	9-eicosene
12	19.071	0.31	Cyclohexane
13	19.324	0.83	Oxirane
14	19.835	0.44	Pentadecafluorooctanoic acid
15	19.974	0.31	Heptadecanoic acid
16	20.367	14.41	Hexadecenoic acid
17	21.108	0.68	Heptafluorobutyric acid
18	21.253	0.93	Cis-vaccenic acid
19	21.386	1.28	2-bromopropionoic acid
20	21.809	6.41	1-octadecene
21	22.292	2.20	Cyclohexane
22	22.554	1.76	Acetic acid
23	22.964	2.76	1-cyclohexylnonene
24	23.194	2.31	Tetradec-11-en-1-yl
25	23.427	21.45	9,12-octadecadienoic acid
26	23.565	33.38	11-octadecenoic acid
27	24.165	3.70	Methyl stearate
28	25.486	0.22	Heptafluorobutyric acid
29	34.202	1.03	9,17-octadecadienal
30	35.083	0.33	Squalene

4.1.5: Performance characteristics

The effect of tea infusions on performance characteristics like body weight gain, growth rate, feed and fluid consumption and feed conversion ratio of rats are shown in Table 4.3. From the table, animals fed green tea gained more body weight, had higher growth rate and consumed more feed compared to those fed with water and black tea. Animals fed black tea recorded a drop in growth rate and an increase in feed conversion ratio compared to the control and those fed green tea. There was a reduction in fluid consumption in animals fed tea infusions compared to the control group.

Table 4.5: Effects of tea infusions on performance characteristics of rats

Group		Performance characteristics				
		Body weight gain (g)	**Growth rate (g/day)**	**feed consumed (g)**	**Fluid consumed (ml)**	**Feed conversion ratio**
Water (control)		169.50	6.05	118.25	133.03	2.80
Green tea		183.10	6.53	131.86	21.28	2.90
Black tea		129.20	4.61	68.42	100.00	3.50
	Mean	160.60	5.73	84.77	106.17	3.10
	SD	28.03	0.90	33.40	57.40	0.37
	% CV	17.45	15.70	31.45	67.71	11.90

4.1.6: Relative organ weights

The effect of tea infusion on relative organ weights of liver, kidneys, spleen and heart is presented on Table 4.4. Organ to body weight ratios was not significantly affected in groups treated with tea infusions for liver, kidneys and spleen when compared with the control group. However, there was a significant reduction in heart to body weight in green tea treated animals compared to other groups. The relative organ weight in green treated groups were; liver (4.37±0.96), kidneys (0.55±0.13), spleen (0.40±0.16), and heart (0.26±0.05), while black tea treated groups recorded; 4.96±1.02, 0.71±0.17, 0.45±0.16, and 0.38±0.05 in liver, kidneys, spleen and heart respectively.

Table 4.6: The effect of infusion on relative organ weights of rats

Group	Liver	Kidneys	Spleen	Heart
Water	4.26 ± 0.79^{b}	0.56 ± 0.12^{b}	0.40 ± 0.16^{b}	0.39 ± 0.04^{b}
Green tea	4.35 ± 0.96^{b}	0.55 ± 0.13^{b}	0.40 ± 0.23^{b}	0.26 ± 0.05^{a}
Black tea	4.96 ± 1.02^{b}	0.71 ± 0.17^{b}	0.45 ± 0.16^{b}	0.38 ± 0.05^{b}

Values are mean ± SD of determinations. Values on the same column bearing the same superscript letter are not significantly different ($p< 0.05$).

4.1.7: Lipid profile

The effect of tea infusions on lipid profile parameters is presented in Table 4.5. Total cholesterol concentration was not significantly different among the experimental groups (contr: 199.17 ±6.01 mg/dL, green tea: 173. 33±3.01 mg/dL, and black tea: 204.00±3.22 mg/dL). Animals fed green tea had the lowest total cholesterol compared with the control group and those fed black tea. There was an increase in HDL concentration in treated groups compared to the control, however, this increase was only significant in green tea treated animals. LDL concentration in treated groups presented no significant reduction when compared to the control groups (contr: 108.5 ±1.94 mg/dL, green tea: 94.17±2.48 mg/ dL, and black tea: 74.50 ±2.34 mg/dL). There was difference in triglyceride concentration in green tea treated animals compared to the control and animals fed black tea. Chylomicron concentration in black treated animals showed significant increase when compared with the control group and green tea treated groups. No significant difference was observed for VLDL concentration and HDL/LDL ratio among the experimental groups.

Table 4.7: Effect of tea infusions on lipid profile of albino rats

Group	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Chylomicron (mg/dL)	HDL/LDL ratio
Water (control)	199.17±6.01^{b}	157.67±2.80^{b}	56.16±1.94^{b}	108.5±1.94^{b}	3.88±0.07^{b}	98.33±3.26^{b}	0.51±0.02^{b}
Green tea	175.33±3.01^{b}	109.33±2.58^{b}	59.66±2.33^{a}	94.17±2.48^{b}	2.70±0.08^{b}	76.33±2.94^{b}	0.62±0.02^{b}
Black tea	204.00±3.22^{b}	138.00±3.71^{b}	57.33±1.75^{b}	74.50±2.34^{b}	3.47±0.10^{b}	102.17±2.92^{a}	0.76±0.02^{b}

Values are mean ± SD. Values on the same column bearing the same superscript letter are not significantly different ($p < 0.05$).

4.1.8: Faecal samples

The effects of tea infusions on faecal proximate compositions is presented in Table 4.6. In this analysis, moisture and carbohydrate concentration were higher in the control group when compared with treated groups, with the lowest observed in animals treated with black tea. Animals treated with black tea recorded higher ash and fibre content than observed in the control and green tea treated groups, while protein and lipid content were increased in animals treated green tea compared to the control and black tea treated groups.

Table 4.8: Effect of tea infusion on faecal content of albino rats

Group		Parameter (%)					
		Ash	**Moisture**	**Carbohydrate**	**Protein**	**Lipid**	**Fibre**
Water(control)		5.00	12.70	8.16	10.50	2.40	61.24
Green tea		5.35	10.48	4.63	11.81	3.95	63.78
Black tea		9.70	4.21	2.32	10.06	2.70	71.01
	Mean	6.68	9.13	5.18	10.79	3.01	65.34
	SD	2.6	4.40	3.17	0.91	0.82	5.06
	CV%	38	48	61	8	27	7

SD = standard deviation; CV = coefficient of variation.

4.2 DISCUSSION

Phytochemical screening revealed that both tea infusions contained all the phytochemical assayed (alkaloid, tannin, saponin, flavonoid, phytates, oxalates, phytosterol and cyanogenic glycoside) (Table 4.1-2). The phytochemicals were present at varied quantities. Alkaloids have been shown to exhibit antiproliferation, antibacterial, antiviral, insecticidal, and anti-metastatic activities (Dey *et al.,* 2020). Therapeutically alkaloids are known anesthetics, cardioprotective and anti-inflammatory agents (Henrich, Mah, & Amirkia, 2021). Alkaloids play vital pharmacological activities, acting as human therapeutic arsenal, such as antioxidant compounds, antitumoral drugs, analgesics, anti-inflammatories and stimulants (Bhambhani, Kondhare, & Giri, 2021). Flavonoids and phenolic compounds in various plants have been reported to have multiple biological effects like antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic properties.

Tannins are active antioxidant, antitumor, and antimicrobial agents (Abeysinghe, Kumara, Kaushalya, Chandrika & Alwis, 2021). Tannins may accelerate blood clotting in certain conditions, reduce blood pressure, decrease serum lipid level, anti-microbial defence and modulate immunoresponse (Marcińczyk, Gromotowicz-Poplawska, Tomczyk & Chabielska, 2022). Polyphenols protects against development of certain cancers, cardiovascular diseases, diabetes, and neurodegenerative diseases (Cory, Passarelli, Szeto, Tamez & Mattei, 2018; García-Aguilar, Palomino, Benito, & Gullen, 2021). These results indicate that the extracts are potential sources of useful drugs in medicines.

Furthermore, tea infusions recorded some antinutrient compounds such as phytate, Cyanogenic glycosides and Oxalate. These antinutrient are compounds that interfere with intake, absorption and utilization of nutrients. Antinutrients may further elicit very harmful biological responses while some are used as pharmacologically active agents. Appreciable amount of phytate was

recorded in green tea infusion and it is known that phytate has a strong affinity for calcium, magnesium, iron, copper, and zinc preventing absorption. Oxalic acid are presents in many plants, they bind calcium and prevents its absorption. Glucosinolates interfere with the uptake of iodine, chelate metals and flavonoids thus reducing absorption (Lee, Kim, & Woyengo, 2020).

The green tea infusion showed significantly higher composition of most chemicals assayed (alkaloid, flavonoid, tannin, and saponin) than the black tea infusion and therefore could serve as better source of these compounds for medicinal purposes. These results corroborate with those of Mahammad et al. (2023), who reported higher phenols and flavonoid content in green tea infusion than observed in black tea infusion in a study on the simultaneous consumption of green and black tea infusions from *Cnidoscolus aconitifolius* leaves with metformin treatment on type II diabetic rats.

Tea infusions can alter appetite (Brett, 2012). There was an increase in appetite for food in the animals fed green tea and a decreased appetite for fluid. The enhanced feed intake may have been due to sudden withdrawal from the fluid (green tea infusion) and a biochemical need to compensate for body water need by producing metabolic water. Metabolic water is naturally produced in the body as a byproduct of metabolism; when oxygen serves as the final recipient of protons in oxidative phosphorylation. The observed increase in body weight gain in animals fed green tea might be attributed to the animals sudden withdrawal from fluid compared to those treated with black tea. Animals treated with black tea recorded a decrease in appetite for food and increased appetite for fluid, which caused a reduction in body weight gained and growth rate with a lower feed conversion ratio. Although, polyphenols was more abundant in green tea than in black tea, the weight gain observed in green tea treated animals could be as a result of the bioavailability of these compounds. The absorption rate of oolong and black tea polyphenols is higher than that of green tea polyphenols (Sun *et al.,* 2018). Many factors, regarding absorption, metabolism,

distribution and excretion in the body, can influence the bioavailability of tea polyphenols (Peng *et al.,* 2018; Tang *et al.,* 2019). Generally, the bioavailability of tea polyphenols is relatively low, mainly due to the low rate of absorption through gastrointestinal tract (Chen, Lee, Li & Yang, 1997; Tang *et al.,* 2019). For example, it was reported that < 2% of the EGCG dose given orally was available in the systemic blood in rats (Chen, Lee, Li & Yang, 1997; Tang *et al.,* 2019). Also, the reduction in weight of black tea treated animals might be due to the role of polyphenols present in tea. Polyphenols are known to hinder the activity of digestive enzymes (amylase, glycosidase and lipase) thereby helping to reduce the digestion of carbohydrates and fats thus lowering energy uptake. Plant based phenolic compounds suppress appetite either by slowing down secretion of appetite-stimulating hormones, modulating Melanin-concentrating hormone receptors, inactivation of appetite sensors (Godfrey *et al.,* 2017).

There was a slight increase in the organ weight of treated animal especially those fed black tea, except for the heart. The heart weight of treated animals did not increase, this might be due to the phytochemical components in the infusion, which are known to act as cardio protective agents. (Table 6). The increase in organ weight might be due to the adaptation of cells or their organelles to new steady states. Weight changes of these tissues are useful measures of their pathological conditions (Ibegbulem, 2012).

Phytochemicals in tea infusions may have played a role in the reduction of these "bad cholesterol" while maintaining HDL levels (good cholesterol) thereby reducing heart disease risks. Several studies have reported that green tea may affect cardiovascular function through LDL-cholesterol oxidation (Ishikawa *et al.,* 1997; Mckay *et al.,* 2010). The inhibition of LDL-cholesterol, associated with risks of atherosclerosis and heart disease by green tea has been attributed to the presence of epicatechin and epigallocatechin gallate antioxidant activity (Avri, *et al* 2018). Raederstoff *et al*. (2003) reported that catechins reduced plasma cholesterol level and the rate of

cholesterol absorption, plasma triglyceride and HDL-cholesterol did not change significantly. Yokozawa *et al.* (2002) reported that green tea effectively inhibited LDL-cholesterol oxidation and elevated antioxidant activity. Furthermore, green tea polyphenols may exert an anti-atherosclerotic action by virtue of its antioxidant properties by increasing HDL-cholesterol levels.

Studies have shown that drinking black tea on a regular basis helps to reduce the onset of cardiovascular diseases (Gardner, Ruxton, & Leeds, 2007). Davies *et al.,* (2003) also reported that the addition of black tea to diets reduced LDL in hypercholesterolemic humans. Hodgson *et al.,* (2012) reported that long term drinking of black tea improved blood pressure either from lower or higher sides. Gregling *et al.,* (2014), reported the same benefit of black tea on blood pressure among normal adults. From their findings, black tea played a role in reducing the risk of complications associated with hypertension.

The dietary fibre in faecal samples of treated animals was higher in the black tea group. Energy from protein, carbohydrates and lipid was wasted via excretion. The energy/nutrient loss of protein was 43.44 kcal and lipid 29.42 kcal which may have contributed to weight loss of the treated animals. Pan *et al.,* (2016) reported that the polyphenol present in black tea inhibited obesity by suppressing the digestion and absorption of lipid and complex sugars. There was increased excretion of minerals through faeces upon treatment with the teas as seen in the ash content. Minerals serve as cofactors in metabolism and their loss may have affected cellular metabolism in the treated rats.

CHAPTER FIVE

CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 CONCLUSION

There is growing interest on whether tea infusions could contribute to weight loss and the effects of these infusions. From the results of the various parameters, green and black tea infusions were found to quantitatively and qualitatively contain phytochemicals. The infusions had an effect on the body weight gain and growth rate of those treated especially animals that consumed the infusion in large quantity. There was slight increase in relative weight of some organs, however, the increase in relative organ weight of treated animals did not have any adverse effect on animals. Tea infusion caused a reduction in LDL of treated animals. The effect of tea infusion promoted the excretion of protein and lipid in treated animals. From the results, black tea can be said to be a weight loss agent. The weight loss recorded by animals fed black tea infusion may be attributed to the excretion of minerals which may serve as cofactors and lipids which may serve as energy sources and was triggered by the action of polyphenols present in the tea. The effect of tea infusion on treated animals were more beneficial than injurious. Thus, moderate use of tea can thus be recommended for weight- loss or weight management.

5.2 RECOMMENDATIONS FOR FURTHER STUDY

Study should be done on the effect of tea infusions on liver function and kidney function parameters.

5.3 CONTRIBUTION TO KNOWLEDGE

The present study revealed that although higher phytochemical content was detected in green tea than was seen in black tea, black tea proved to be a better alternative in weight management due to the fact that black tea polyphenols are more readily absorbed than green tea polyphenols.

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APPENDICES

Appendix 1: Table showing initial weight, final weight, weight gain and growth rate of each rat.

Group	Sample Identity	Initial weight (g)	Final weight (g)	Weight gain	Growth rate
CODE A (poultry feed) **CONTROL**	A1	119.4	176.0	56.6	2.02
	A2	104.0	157.6	53.6	1.91
	A3	102.7	154.0	51.3	1.83
	A4	98.6	151.2	52.6	1.87
	A5	95.9	149.5	53.6	1.91
	A6	87.2	139.5	51.8	1.85
		$\bar{X}$=101.3	**$\bar{X}$ =154.63**	**$\bar{X}$=44.61**	**$\bar{X}$=1.89**
		SD =10.69	**SD=12.10**	**SD=1.88**	**SD=0.06**
Group B (green tea)	B1	120.5	171.5	51	1.82
	B2	105.2	157.8	52.6	1.87
	B3	98.9	152.4	53.5	1.91
	B4	96.1	141.3	45.2	1.61
	B5	92.0	136.4	44.4	1.58
	B6	98.2	141.1	42.9	1.53
		$\bar{X}$=101.81	**$\bar{X}$=121.5**	**$\bar{X}$=39.76**	**$\bar{X}$=1.72**
		SD=10.11	**SD=13.18**	**SD=4.61**	**SD=0.16**
Group C (Black tea)	C1	117.5	151.2	33.7	1.20
	C 2	108.1	132.4	24.3	0.86
	C3	110.6	145.8	35.2	1.25
	C4	86.3	124.6	38.3	1.36
	C5	95.9	134.5	38.6	1.37
	C6	88.1	127.3	39.2	1.4
		$\bar{X}$=101.08	**$\bar{X}$=135.96**	**$\bar{X}$=34.88**	**$\bar{X}$=1.24**
		SD=12.83	**SD=10.46**	**SD=5.61**	**SD=0.20**

Table showing body weight gained, growth rate, feed consumed, fluid consumed and feed conversion ratio of each group.

Group	Body weight gained (g)	Growth rate (g/day)	Feed consumed (g)	Fluid consumed (ml)	Feed conversion ratio
A	169.5	6.05	3311.2	3725	2.8
B	183.1	6.53	3564.3	596	2.9
C	129.2	4.61	1908.6	2708	3.5
Mean	160.6	5.73	2928.03	2343	3.1
Standard deviation	28.03	0.9	891.88	1596.11	0.37
Coefficient value%	17.45	15.70	30.00	68.00	11.9

Table showing test result of phytochemical Analysis of two samples

Parameter	Green tea	Duplicate	Black	Duplicate
Tannin%	28.72	28.50	4.857	4.950
Phytate%	0.301	0.298	0.222	0.220
Oxalate %	1.549	1.551	1.760	1.761
Phenol %	1.334	1.338	0.811	0.805
HCN (mg/kg)	559	560	337	334
Saponin %	8.15	8.20	6.35	6.30
Flavonoid %	8.46	8.40	5.20	5.11
Alkaloid %	9.80	9.77	8.70	8.70
	$\bar{X}$=77.16	**$\bar{X}$=77.25**	**$\bar{X}$=45.61**	**$\bar{X}$ =45.23**
	SD=182.31	**SD=182.65**	**SD=110.16**	**S D=109.17**

Table showing organ weight measurement for each rat

Group	Identity	Liver	Kidney	Spleen	heart
Control A	back	7.7	0.9	0.4	0.7
	tail	7.0	1.2	0.8	0.7
	hand	7.7	1.0	1.1	0.7
	head	7.1	0.7	0.8	0.5
	leg	5.9	0.7	0.4	0.6
	plain	4.8	0.8	0.4	0.5
		$\bar{X}$=6.7	$\bar{X}$=0.88	$\bar{X}$=0.65	$\bar{X}$=0.61
		SD=1.14	SD=0.21	SD=0.29	SD=0.09
SAMPLE B	hand	7.0	1.0	0.9	0.5
	plain	7.0	0.9	0.7	0.3
	back	7.4	0.9	0.4	0.4
	tail	6.7	0.7	0.6	0.4
	head	4.8	0.7	0.2	0.4
	leg	5.8	0.7	0.6	0.4
		$\bar{X}$=5.61	$\bar{X}$=0.81	$\bar{X}$=0.56	$\bar{X}$=0.4
		SD=0.97	SD=0.13	SD=0.24	SD=0.06
SAMPLE C	back	7.0	0.9	0.8	0.6
	plain	5.9	0.9	0.6	0.5
	Leg	6.2	0.9	0.6	0.5
	Tail	6.1	0.9	0.7	0.5
	Head	8.4	1.3	0.8	0.6
	hand	6.6	0.9	0.7	0.4
		$\bar{X}$=6.7	$\bar{X}$=0.96	$\bar{X}$=0.67	$\bar{X}$=0.57
		SD=0.92	SD=0.16	SD=0.15	SD=0.13

Table showing relative organ weight of Wistar rats

Group	Identity	Liver	Kidney	Spleen	Heart
	Back	4.8	0.57	0.25	0.44
CONTROL	Tail	4.54	0.77	0.51	0.45
	Hand	4.3	0.56	0.62	0.39
	Head	4.74	0.46	0.53	0.33
	Leg	3.76	0.46	0.26	0.39
	Plain	3.44	0.57	0.28	0.35
		$\bar{X}$=4.26	$\bar{X}$=0.56	$\bar{X}$ =0.40	$\bar{X}$=0.39
		SD=0.79	SD=0.12	SD=0.16	SD=0.04
SAMPLE A	Hand	5.13	0.73	0.65	0.36
	Plain	4.96	0.70	0.63	0.21
	Back	4.85	0.59	0.26	0.26
	Tail	4.74	0.49	0.42	0.28
	Head	2.79	0.40	0.11	0.23
	Leg	3.67	0.44	0.38	0.25
		$\bar{X}$=4.35	$\bar{X}$=0.55	$\bar{X}$=0.42	$\bar{X}$=0.26
		SD=0.96	SD=0.13	SD=0.23	SD=0.05
SAMPLE B	Back	5.27	0.67	0.60	0.45
	Plain	4.04	0.61	0.41	0.34
	Leg	4.87	0.70	0.31	0.39
	Tail	4.53	0.66	0.29	0.37
	Head	6.74	1.04	0.64	0.48
	Hand	4.36	0.59	0.46	0.26
		$\bar{X}$ =4.96	$\bar{X}$ =0.71	$\bar{X}$=0.45	$\bar{X}$=0.38
		SD=1.02	SD=0.17	SD=0.16	SD=0.05

Table showing test result for lipid profile analysis of samples

Group A	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**RANGE (mg/dl)**
CHOL.	197	195	201	193	199	210	200-240
TRIG.	155	159	161	154	157	160	36-165
HDL	57	55	58	53	56	58	40-59
LDL	108	111	103	106	110	113	100-159
VLDL	3.9	3.9	3.8	3.8	3.9	4	2-30
CHYLO	99	101	95	95	97	103	75-1000nm

Group B (green tea and feed)

Group	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**RANGE (mg/dl)**
CHOL.	175	178	173	171	176	179	200-240
TRIG.	109	106	110	107	111	113	36-165
HDL	56	59	60	59	61	63	40-59
LDL	94	96	93	90	95	97	100-159
VLDL	2.7	2.6	2.7	2.6	2.8	2.8	2-30
CHYLO.	75	79	77	72	75	80	751000nm

Group C (black tea and feed)

Group	C1	C2	C3	C4	C5	C6	RANGE (mg/dl)
CHOL.	203	208	201	207	200	205	200-240
TRIG.	133	141	136	139	141	143	36-165
HDL.	55	57	56	58	58	60	40-59
LDL	74	78	72	76	72	75	100-159
VLDL	3.3	3.5	3.4	3.5	3.5	3.6	2-30
CHYLO.	102	107	100	104	99	101	75-1000nm

Table showing test result for faecal sample analysis for rats.

Group	Ash %	Moisture %	Carbohydrate %	Protein %	Lipid %	Fibre %
A	5.00	12.70	8.16	10.50	2.40	61.24
B	5.35	10.48	4.63	11.81	3.95	63.78
C	9.70	4.21	2.32	10.06	2.70	71.01

Table showing wasted feed for each group for 28 days

Control	Green tea	Black tea
7.00	3.20	2.10
3.50	4.80	5.30
10.00	7.00	7.20
4.90	9.10	8.00
5.00	5.30	3.30
3.50	5.30	5.00
4.20	9.10	9.00
11.00	2.80	6.20
9.70	7.40	4.10
2.10	15.00	3.10
3.00	3.10	6.80
15.20	10.00	10.00
20.30	10.00	8.20
25.00	4.00	5.20
19.40	5.50	4.00
25.10	3.10	4.20
10.50	3.10	6.40
28.30	3.10	3.50
11.20	2.80	2.10
16.70	7.20	2.10
12.20	4.50	10.00
14.00	3.50	10.00
10.60	2.91	5.50
5.00	5.00	2.20
3.10	3.70	2.50
6.00	3.70	2.50
6.00	2.20	2.30
8.00	4.10	2.10
$\bar{X}$ =10.74	**$\bar{X}$ = 5.37**	**$\bar{X}$ = 5.10**
SD = 7.35	**SD = 2.99**	**SD = 2.79**

Table showing remaining feed for each group for 28 days

Control	Green tea	Black tea
0.00	0.00	0.00
7.00	16.10	60.00
0.00	32.00	50.00
12.00	0.00	10.00
7.00	0.00	108.00
8.00	0.00	108.20
6.00	0.00	40.10
6.10	0.00	22.40
8.00	20.00	20.00
0.00	35.00	37.00
0.00	0.00	33.00
19.80	35.20	70.20
13.00	35.00	117.70
17.00	46.00	93.60
32.00	0.00	77.80
35.00	50.20	90.00
9.00	0.00	98.33
40.10	0.00	11.00
10.00	0.00	100.00
36.10	35.00	107.00
54.00	0.00	79.50
45.00	10.03	102.00
30.00	10.00	96.63
10.00	25.10	107.21
10.00	0.00	115.00
72.20	0.00	89.20
52.10	0.00	147.50
49.52	18.00	120.00
$\bar{X}$ = 21.03	**$\bar{X}$ = 13.12**	**$\bar{X}$ = 75.40**
SD = 19.87	**SD = 16.61**	**SD = 39. 97**

Table showing remaining fluid for each group for 28 days

Control	Green tea	Black tea
5.00	90.00	100.00
5.00	140.00	140.00
7.00	150.00	110.00
28.00	79.00	130.00
10.00	150.00	132.00
8.00	150.00	128.00
4.00	122.00	110.00
6.00	115.00	130.00
27.00	125.00	100.00
33.00	100.00	104.00
30.00	110.00	101.00
19.00	103.00	0.00
25.00	112.00	0.00
46.00	119.00	31.00
20.00	120.00	43.00
20.00	115.00	0.00
20.00	123.00	0.00
13.00	110.00	0.00
21.00	121.00	10.00
13.00	150.00	0.00
5.00	150.00	0.00
9.00	150.00	0.00
18.00	150.00	16.00
8.00	150.00	15.00
5.00	150.00	42.00
31.00	150.00	0.00
28.00	150.00	0.00
11.00	150.00	58.00
$\bar{X}$ =16.96	**$\bar{X}$ = 128.71**	**$\bar{X}$ = 53.57**
SD =10.92	**SD = 21.68**	**SD = 54. 63**

Table showing total feed consumed by each group for 28 days.

Control	Green tea	Black tea
143.00	146.80	147.90
139.20	129.10	84.70
140.00	111.00	92.80
134.00	140.90	132.00
138.00	144.70	38.50
138.50	144.70	105.00
139.80	140.90	118.60
132.91	147.20	123.80
132.60	122.60	108.90
147.90	100.00	113.90
147.00	146.90	73.00
115.20	104.80	48.20
116.73	105.00	67.00
108.00	100.00	57.00
99.00	144.50	47.50
89.90	96.90	132.60
130.50	146.90	46.50
81.60	146.90	40.90
128.80	147.20	68.40
97.20	107.80	38.00
83.80	145.50	43.40
91.00	136.50	37.30
109.40	147.10	32.80
135.00	120.00	58.30
136.00	146.30	0.00
71.80	146.30	27.70
91.90	147.80	23.90
92.5	127.90	7.40
$\bar{X}$ = 118.25	**$\bar{X}$ = 131.86**	**$\bar{X}$ = 68.42**
SD = 23.27	**SD = 18.36**	**SD = 40.84**

Table showing fluid consumed by each group for 28 days.

Control	Green tea	Black tea
145.00	60.00	50.00
145.00	10.00	110.00
143.00	0.00	40.00
122.00	71.00	20.00
140.00	0.00	18.00
142.00	0.00	22.00
146.00	28.00	40.00
144.00	35.00	20.00
123.00	25.00	50.00
117.00	50.00	46.00
120.00	40.00	49.00
131.00	47.00	150.00
125.00	38.00	150.00
104.00	31.00	119.00
130.00	30.00	107.00
130.00	35.00	150.00
130.00	27.00	150.00
137.00	40.00	150.00
129.00	29.00	140.00
137.00	0.00	150.00
145.00	0.00	150.00
141.00	0.00	150.00
132.00	0.00	134.00
142.00	0.00	135.00
145.00	0.00	108.00
119.00	0.00	150.00
122.00	0.00	150.00
139.00	0.00	92.00
$\bar{X}$ = 133.03	**$\bar{X}$ = 21.28**	**$\bar{X}$ = 100**
SD = 10.92	**SD = 21.68**	**SD = 51.97**

Appendix 2: Showing formulae for calculating the initial weight, final weight, gain and growth rate.

Number of days of experimental feeding studies =28 days

$$\text{Growth rate} = \frac{\text{weight gain}}{\text{No. of days}}$$

Where, weight gain (g) = final weight (g) – initial weight (g)

- Weight gained = final weight of each albino rats – initial weight of rat

Appendix 2b: Showing formulae for calculating body weight gain, growth rate, feed consumed, fluid consumed and feed conversion ratio.

Feed consumed = (wasted food (g) + Remaining feed – Total feed given each group)

- Where, weight gain (g) = final weight (g) – Initial weight (g)
- $\text{Feed conversion ratio (FCR)} = \frac{\text{feed consumed (g)}}{\text{Weight gain (g)}}$

Appendix 2c: Showing formula for calculating relative organ weight

Relative organ weight = organ weight divided by final body weight of rats multiply by hundred

Appendix 3: picture showing different brands of tea used for infusion

Black tea

Green tea