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Histopathological Studies on the Effects of Chloroform and Methanolic Extracts of *Ilex kudingcha* in *Trypanosoma brucei* Infected Albino Wistar Rats

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Abstract
Histopathological studies of the effects of chloroform and methanolic leaf extracts of *Ilex kudingcha* in *Trypanosoma brucei* infected albino wistar rats were investigated. The toxicity and phytochemical study were also carried out using standard protocol. *T. brucei* infected animals were administered orally with 200 and 400 mg/kg b.w. of the extracts and 3.5 mg/kg b.w. of the standard drug (diminazene aceturate). Results on acute toxicity studies (LD_{50}) revealed no sign of lethality up to the dose of 5,000 mg/kg body weight but the liver and kidney histology of infected animals treated with 5,000 mg/kg b.w. of *I. kudingcha* extracts were observed to be hepatotoxic and nephrotoxic. The methanol extracts showed appreciably high *in vivo* anti-trypanosomal activities compared to the reference drug. Histological examination of the organs revealed serious pathological lesions in the liver of the infected animals without treatment (negative control). In the positive control animals (infected animals administered standard drug), mild multifocal aggregate of inflammatory leucocytes was observed. In the other experimental animals, no pathological lesion was observed in the liver, kidney, brain, and heart of infected animals treated with the methanolic extract and combined methanol and chloroform extracts. The effectiveness of the methanolic extract at reducing the lesions caused by the parasite is the same compared with the standard drug. Phytochemical analysis of the plant extracts showed that methanol extract contained appreciable high levels of alkaloids, saponin, tannins, phenol, and glycoside while flavonoid was not detected. Hence, the curative properties of methanolic extract of *I. kudingcha* as observed in the organs indicate its anti-trypanosomal properties but it should be consumed at minimal doses.

Keywords: *Ilex kudingcha*; *Trypanosoma brucei*; histopathology; *in vivo*.

1. INTRODUCTION

The use of plants as medicines predates written human history and some of the pharmaceuticals currently available to physicians are derived from plants that have a long history of use as herbal remedies [1, 2]. Ilex, whose common name is holly, is the only living genus of almost 600 species in the family Aquifoliaceae. Large-leaved Kudingcha (bitterspike leaf tea) is made from *Ilex kudingcha* and *Ilex latifolia* and has been consumed as a functional food in southern China for about 2,000 years [3]. Kudingcha is a particularly bitter-tasting tea that has been widely used in China throughout history. Over the last several years, Kudingcha has been considered as a dietetic beverage and is gaining popularity with names like "beauty-slimming tea," "longevity tea," "green-golden tea," and "clearing-heat tea." This plant has been reported for obvious antioxidant, anti-inflammatory, lipid metabolism, hepatoprotective, and antitumor activities [4]. This plant is also gaining popularity in Nigeria.

Trypanosomes are unicellular parasites transmitted by the tse-tse fly. Different species of this genus cause both human African trypanosomiasis (sleeping sickness) and African animal trypanosomiasis (AAT). The disease is also called "nagana" in cattle [5].

Trypanosomiasis in domestic animals, particularly in cattle, is a major obstacle to the economic development of affected rural areas. The disease results in acute, sub-acute, or chronic disease characterized by intermittent fever, anemia, occasional diarrhea, rapid loss of condition, and often death. Currently, chemotherapeutic agents constitute the principal method of control, as development of vaccines against AAT is still in progress. Trypanosome infections are known to cause immunosuppression responsible for the host's inability to eliminate the trypanosomes even after administration of trypanocidal drugs. Diminazene aceturate and isomethamidium chloride are the most currently used trypanocides, used both for prophylactics and curative purposes in the control of the disease in cattle. Unfortunately, the parasite has developed resistance to these drugs [6, 7].

There is no report on the anti-trypanosomal activities of *I. kudingcha* although few studies had reported on some biological activities of this medicinal plant. Moreover, in the light of the parasites' drug resistance and undesirable side effects of trypanosides, there is need for intensified efforts in search of potential medicinal plants that may contain nontoxic bioactive compounds. Hence, this study is aimed at evaluating the effect of *I. kudingcha* extracts on organs of albino wistar rats infected with *Trypanosoma brucei brucei*.

2. METHOD(S)

2.1. Plant Collection

Air-dried leaves of *I. kudingcha* were imported from China.

2.1.1. Plant preparation and extracts

The plant material was pulverized, finely sieved, and 60 g each separately soaked in 500 ml of 80% methanol and chloroform, respectively, for 24 h, after which they were filtered.

Thereafter, the filtrates were freeze dried, and the percentage yield was calculated. The yield was used to prepare the test extract administered (200 and 400 mg/kg body weight of rats). Moreover, 7% methanolic Tween 80 solution was served as the vehicle of plant extracts administration because chloroform extract yield was not soluble in water.

2.2. Phytochemical Test

The phytochemical analysis of the methanolic and chloroform extracts of *I. kudingcha* were conducted using procedures outlined by [8] for detection of alkaloids and flavonoids; [9] and [10] for detection of saponins, tannin, and glycosides.

2.3. Experimental Animals

A total of 54 mature albino male mice and 75 adult albino male rats were used for this study. The mice weighed between 24 and 26 g while the rats weighed between 100 and 120 g. The inbred experimental animals were obtained from the laboratory animal unit of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. They were housed in well-ventilated cages in the Animal House of Biology Research Unit, Science Laboratory Technology department, Akanu Ibiam Federal Polytechnic, Unwana and were fed with commercial feed (vital feeds, Gcom Nig. Ltd) and provided with clean water *ad libitum* and much water. The animals were screened for the presence of blood parasites using wet and Giemsa stained thin films prior to commencement of the experiment. The rats were allowed to acclimatize for 2 weeks before commencement of the study. The rules guiding the use of animals for scientific experiments were strictly obeyed.

2.4. Acute Toxicity and Lethality (LD_{50})

The acute toxicity and lethality of methanolic and chloroform extracts of *I. kudingcha* leaf were determined using the modified method of [11].

2.5. Test Organism and Determination of Parasitemia

T. brucei were obtained from the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The parasite was originally isolated from a slaughtered pig in Nsukka main abattoir early in 2006 and was maintained and multiplied in mice prior to use [12]. The parasites were maintained in the laboratory by continuous passage in mice intraperitoneally. Blood from the tail was used for the estimation of parasitemia in wet mount. The trypanosome count was determined by examination of the wet mount microscopically at 40× magnification using the "rapid matching" method of [13]. This method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2).

2.6. *In vivo* Anti-trypanosomal Activity of Plant Extracts

The 75 experimental animals were divided into 15 experimental groups A–O containing 5 animals per group. The groups consist of the following:

Group "A" was the uninfected and untreated control group administered with 0.2 ml of 7% methanolic Tween 80 solution and fed with food and water.

Group "B" was the *Trypanosoma* infected and untreated control (INT) group administered with 0.2 ml of 7% methanolic Tween 80 solution.

Group "C" was infected with *Trypanosoma* and administered with a single dose of diminazene aceturate (Diminal®) (445 mg diminazene diaceurate) prepared in 7% methanolic Tween 80 solution at a dose of 3.5 mg/kg intramuscularly.

Groups "D" and "E" were infected with *Trypanosoma* and treated orally with 200 and 400 mg/kg, respectively, of *I. kudingcha* leaf extracted with methanol.

Groups "F" and "G" were uninfected with *Trypanosoma* but administered orally with 200 and 400 mg/kg, respectively, of *I. kudingcha* leaf extracted with methanol.

Groups "H" and "I" were infected with *Trypanosoma* and treated orally with 200 and 400 mg/kg, respectively, of *I. kudingcha* leaf extracted with chloroform.

Groups "J" and "K" were uninfected with *Trypanosoma* but administered orally with 200 and 400 mg/kg, respectively, of *I. kudingcha* leaf extracted with chloroform.

Groups "L" and "M" were infected with *Trypanosoma* and treated orally with 200 and 400 mg/kg, respectively, of *I. kudingcha* leaf extracted with methanol and chloroform combined.

Groups "N" and "O" were uninfected with *Trypanosoma* but administered orally with 200 and 400 mg/kg, respectively, of *I. kudingcha* leaf extracted with methanol and chloroform combined.

Apart from the uninfected control (normal rat) group, each of these groups was given 1×10^4 parasites intraperitonially in 0.2 ml blood/PBS solution.

The animals were left for 5 days to develop parasitemia, and the level of parasitemia was determined by rapid matching method [13]. After 5 days post inoculation when the level of parasitemia had reached approximately 5×10^6 per microscope field, treatments commenced on the 5th day post infection and lasted for nine [9] days.

2.7. Histological Study

Histological studies were conducted in the Pathology Laboratory, College of Veterinary Sciences, University of Nigeria, Nsukka. The animals were sacrificed by cervical dislocation 24 h after the last dose of the respective treatments and the organs were harvested. Primarily, pathological changes were observed in three main organs—liver, kidney, and heart—of the different groups of experimental mice. The method of [14] was used for processing of tissues.

The following scores were used to grade the degree of histopathological changes or lesions observed in the organs: not present (−), very mild (+), mild (++), moderate (+++), and severe (++++). The degrees of damages caused in the organs were scored or assigned as follows: not present (−), 1-4 foci/section examined (+), 5-8 foci/section examined (++), >9 foci/section examined (++++). Tubular necrosis was graded (++) when scattered cells were detected with pyknotic, karyorrhexis, karyolysis nuclei, or loss of polarity, (+++) when these changes were present in larger sections of a tubule, and (++++) when multiple tubules in an area were affected.

3. RESULTS

Yield of the methanol extract and chloroform extracts of *I. kudingcha* leaf: The percentage yield of the chloroform extract was 8.2% while methanol extract was 8.7% (Table 1).

3.1. Acute Toxicity and Lethality (LD50) Test

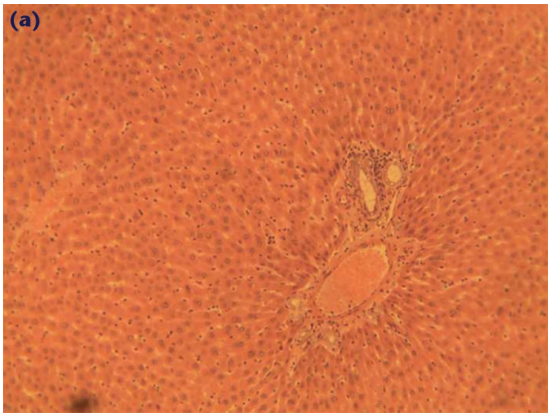
Studies on acute toxicity and LD_{50} of the individual extracts (methanol and chloroform separately) recorded no lethality in the mice at the different concentrations of 10, 100, 1,000, 1,900, 2,600, and 5,000 mg/kg. However, animals administered with 5,000 mg/kg body weight showed sign of weakness but no death was recorded within 24 h of administration.

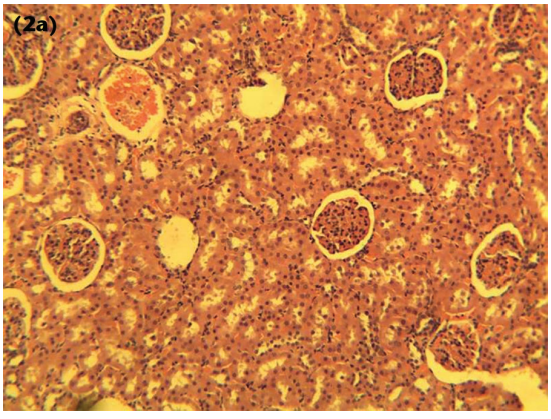
Table 1: Percentage yield of Ilex kudingcha extracts.

	Quantity of sample used (g)	Quantity of crude extract(g)	% Yield
Chloroform extract	60	4.9 g	8.2
Methanol extract	60	5.2 g	8.7

***Figure 1a*: A photomicrograph of the liver from animals administered 7% methanolic Tween 80 (normal control) showing normal hepatocytes arranged in chords around the central vein (V) radiating toward the portal triads (P). H&EX100.**

***Figure 2a*: A photomicrograph of the kidney from animals administered 7% methanolic Tween 80 (normal control) showing normal renal histomorphology. Normal glomeruli (G) in their Bowman's capsules, surrounded by numerous renal tubules (arrow), were observed. H&EX400.**





3.2. Histological Studies on Organs of Mice

Histopathological examination of the sampled organs from the two separate groups treated with methanolic and chloroform extracts showed varying degrees of changes from the normal histo-architectures (Figures 1a and 2a).

The liver showed varying degrees of degeneration and necrosis of the hepatocytes with consequent inflammation (hepatitis) characterized by infiltration of mononuclear leucocytes into the liver in varying severities (Figures 1b–1d).

The kidney showed degeneration and necrosis of the tubular epithelium involving mainly the proximal convoluted tubules. The sloughed necrotic epithelial cells thus appear as eosinophilic casts further down along the renal tubules (in the pars recta, distal convoluted tubules, and the collecting ducts). In addition, present on the kidneys are varying severities of interstitial nephritis, consequent to tubular necrosis with destruction of the tubular basement membranes (tubulorrhexis) (Figures 2b–2d).

The heart and the brain did not show any change in their respective histo-architectures (Figures 3 and 4).

3.3. Phytochemical Test

Qualitative test result: In the methanol extract, alkaloids, tannin, saponin, and glycoside were detected. On the other hand, in the chloroform extract, only alkaloids and saponins were detected (Table 2).

Quantitative test result: In the methanol extract, the quantities of compounds detected in ascending order are tannin (1.03%), alkaloids (35.91%), and saponin (38.05%). In the chloroform extract, alkaloid was 25.3% while saponin was 25.5%. However, glycoside was not detected (Table 3).

***Figure 1b*: A photomicrograph of the liver from animals administered methanolic extract of *I. kudingcha* (1,900 mg/kg body weight) showing cytoplasmic vacuolations in the centrilobular hepatocytes (black arrow), inflammatory cells in the sinusoids (white arrow), and a hepatocellular megalocyte (blue arrow). Central vein (V). H&EX400.**

***Figure 1c–1d*: A photomicrograph of the liver from animals administered chloroform extract of *I. kudingcha* (1,900 mg/kg body weight) showing a mild periportal aggregation of inflammatory leucocytes (black arrow) as well as random aggregates of inflammatory aggregates in the sinusoids (white arrow). Portal area (P), Central vein (V). H&EX100, 400.**

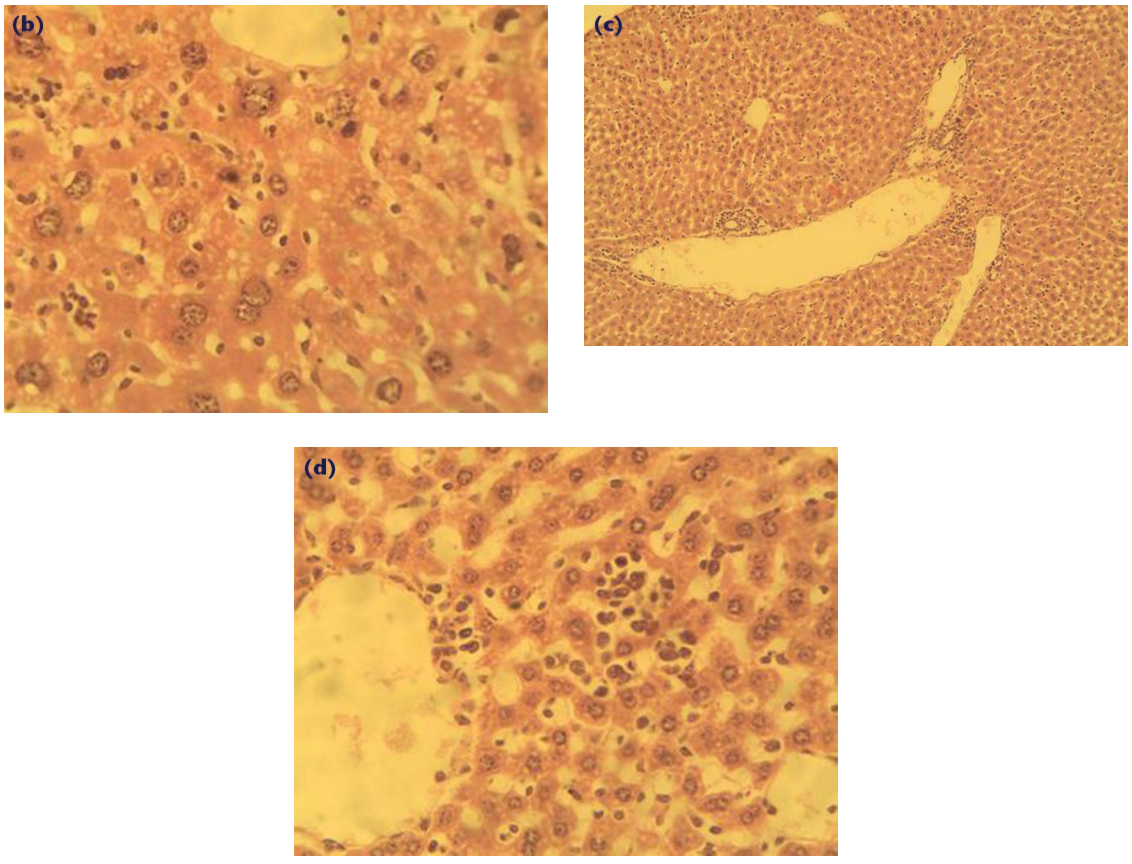


***Figure 2b–2c*: A photomicrograph of the kidney from animals administered methanolic extract of *I. kudingcha* (1,900 mg/kg body weight) showing sloughed necrotic tubular epithelial cells (appearing as eosinophilic casts) in the lumen of the tubules (white arrows) and a wide area of inflammatory cell infiltration (black arrow). Glomerulus (G). H&EX400, H&EX100.**
***Figure 2d*: A photomicrograph of the kidney from animals administered chloroform extract of *I. kudingcha* (1,900 mg/kg body weight) showing eosinophilic tubular casts in the lumens of some renal tubules and multifocal areas of inflammatory cellular infiltration of the renal interstitium (black arrow).H&EX100.**

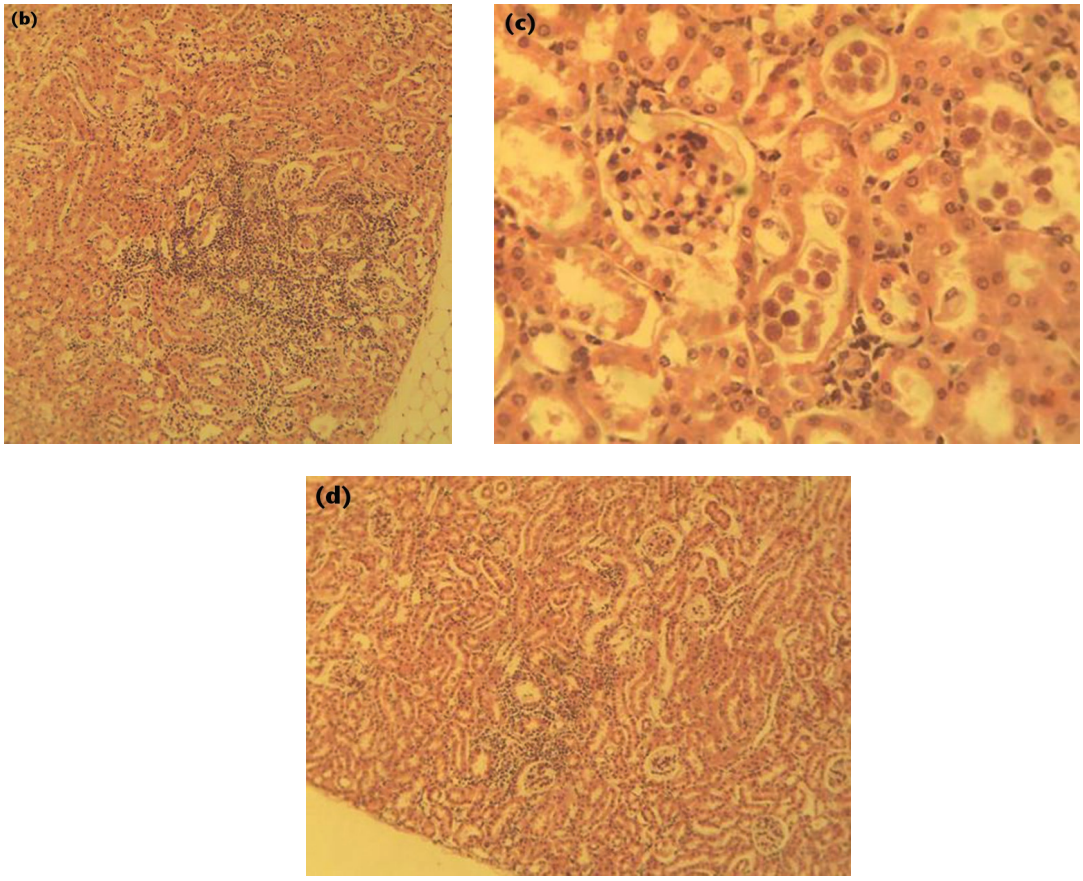


***Figure 3a*: A photomicrograph of the heart from animals administered methanolic extract of *I. kudingcha* (5,000 mg/kg body weight). Sections of these organs from this group did not show any change in their respective normal histo-architectures. H&EX400.**
***Figure 3b*: A photomicrograph of the heart from animals administered chloroform extract of *I. kudingcha* (5,000 mg/kg body weight). Sections of these organs from this group did not show any change in their respective normal histo-architectures. H&EX400.**

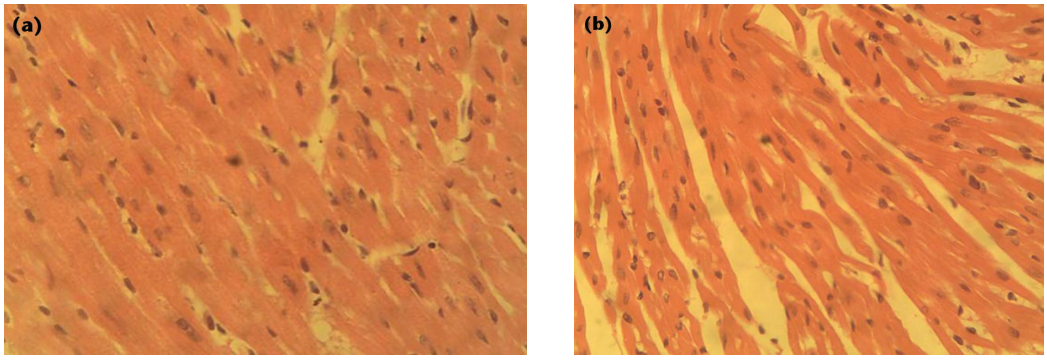


***Figure 4a*: A photomicrograph of the brain from animals administered methanolic extract of *I. kudingcha* (5,000 mg/kg body weight). Sections of these organs from this group did not show any change in their respective normal histo-architectures. H&EX400.**
***Figure 4b*: A photomicrograph of the brain from animals administered chloroform extract of *I. kudingcha* (5,000 mg/kg body weight). Sections of these organs from this group did not show any change in their respective normal histo-architectures. H&EX400.**

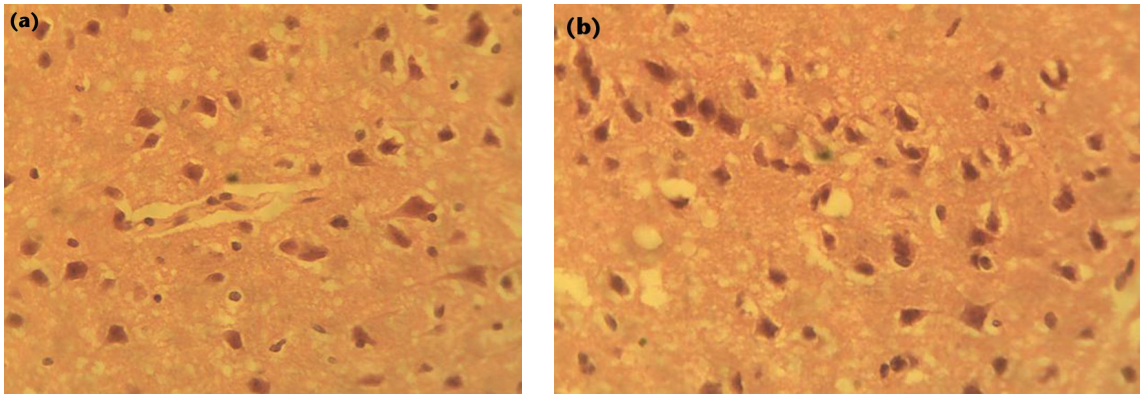


Table 2: Qualitative phytochemical screening of *I. kudingcha*.

Phytochemicals	Methanol	Chloroform
Alkaloid	++	++
Flavonoid	–	–
Tannin	+++	–
Saponin	+++	++
Glycoside	++	–

Table 3: Quantitative phytochemical screening of *I. kudingcha*.

Phytochemicals	Methanol (%)	Chloroform (%)
Alkaloids	35.91	25.3
Flavonoid	–	–
Tannin	1.03	–
Saponin	38.05	25.5
Glycoside	ND	ND

ND = not detected.

3.4. In vivo Effects of Methanol Extract of I. kudingcha on Parasitemia Count of *T. b. brucei* Infected Rats

On day 0 before infection, parasitemia count in the experimental animals were 4.33 ± 1.20 (negative control), 5.66 ± 0.33 (positive control), 4.00 ± 0.00 (200 mg/kg plant extract group), and 4.33 ± 0.33 (400 mg/kg plant extract group). On days 3 to day 6 post infection, the infected groups treated with the varying doses of methanol extract of *I. kudingcha* did not show any significant ($p < 0.05$) decrease in parasitemia count compared with the *Trypanosoma* infected and untreated control group but the positive control group (standard drug—diminazen aceturate) reduced parasitemia significantly on day 6 post infection. On day 9 post infection, the standard drug had cleared all parasites from the blood stream of the rats. However, the effect of the plant extract on parasitemia was dose-dependent on day 9. The methanol extract at 200 mg/kg reduced parasitemia significantly compared to the negative control. At day 15 post infection, parasitemia was not observed in the positive control group treated with the standard drug. On the other hand, parasitemia increased in the groups treated with the plant extracts. Percentage suppression of parasitemia in the treatment groups (standard drug, methanol extract at 200 mg/kg b.w. and 400 mg/kg b.w.) were 100, −20.02, and −0.03%, respectively (Table 4).

3.5. In vivo Effects of Chloroform Extract of I. kudingcha on Parasitemia Count of *T. b. brucei* Infected Rats

The chloroform extract had no significant effect on the parasitemia count in the rats treated with this extract. Although, parasitemia count reduced on days 6 and 9 post infection (days 1 and 4 after treatment) in the group treated with 200 mg/kg b.w.

Table 4: *In vivo* effect of methanol extract of *I. kudingcha* on parasitemia count of *T. b. brucei* infected rats.

Day (PI)/day after treatment	Parasitemia count (log number/ml)			
	INT	TBD 3.5 mg/kg b.w.	TBIK 200 mg/kg b.w.	TBIK 400 mg/kg b.w.
0/0	4.33 ± 1.20	5.66 ± 0.33	4.00 ± 0.00	4.33 ± 0.33
3/0	27.00 ± 8.96	26.66 ± 3.52	31.67 ± 1.67	28.33 ± 1.67
6/1	31.66 ± 24.49	1.00 ± 1.00*	27.00 ± 8.96	26.67 ± 3.54
9/4	30.00 ± 2.88	0.00 ± 0.00*	3.00 ± 2.08*	0.0 ± 0.01*
15/10	36.66 ± 3.33	0.00 ± 0.00*	44.00 ± 16.00	36.67 ± 5.41
Percentage Suppression (%)	0	100.00	−20.02	−0.03

Values represented in mean ± SEM.
*shows significant difference ($p < 0.05$) compared with TBDW group; INT: *T. b. brucei* infected not treated rats administered 0.2 ml of 7% methanolic Tween 80 solution; TBD: *T. b. brucei* infected rats administered 3.5 mg/kg b.w. of diminazen aceturate; TBIK 200 mg/kg: *T. b. brucei* infected rats administered 200 mg/kg b.w. of *I. kudingcha;* TBIK 400 mg/kg: *T. b. brucei* infected rats administered 400 mg/kg b.w. of *I. kudingcha.*
PI = post infection.

Table 5: *In vivo* effect of chloroform extract of *I. kudingcha* on parasitemia count of *T. b. brucei* infected rats.

Day (PI)/day after treatment	Parasitemia count (log number/ml)			
	INT	TBD 3.5 mg/kg b.w.	TBIK 200 mg/kg b.w.	TBIK 400 mg/kg b.w.
0	4.33 ± 1.20	5.66 ± 0.33	4.66 ± 0.33	5.33 ± 0.66
3	27.00 ± 8.96	26.66 ± 3.52	24.66 ± 5.48	20.66 ± 0.88
6	31.66 ± 24.49	1.00 ± 1.00*	19.33 ± 5.36	31.66 ± 4.40
9	30.00 ± 2.88	0.00 ± 0.00*	16.33 ± 6.83*	23.66 ± 8.56
15	36.66 ± 3.33	0.00 ± 0.00*	38.66 ± 13.33	45.00 ± 6.50
Percentage suppression (%)	0	100.00	−5.46	−22.75

Values represented in mean ± SEM.
*shows significant difference ($p < 0.05$) compared with TBDW group; INT: *T. b. brucei* infected not treated rats administered 0.2 ml of 7% methanolic Tween 80 solution; TBD: *T. b. brucei* infected rats administered 3.5 mg/kg b.w. of diminazen aceturate; TBIK 200 mg/kg: *T. b. brucei* infected rats administered 200 mg/kg b.w. of *I. kudingcha;* TBIK 400 mg/kg: *T. b. brucei* infected rats administered 400 mg/kg b.w. of *I. kudingcha.*
PI = post infection.

of rat, this were not significantly ($p > 0.05$) different compared to the Trypanosoma infected but untreated control group. On day 15 post infection, parasitemia increased drastically leading to −5.6% suppression of parasitemia. In the group treated with 400 mg/kg b.w. of rat, parasitemia reduced on day 9 post infection (day 4 after treatment) but not statistically significant. On day 15 post infection, parasitemia increased drastically leading to −22.75% suppression of parasitemia (Table 5).

3.6. In vivo Effects of Chloroform Extract Combined with Methanol Extract of *I. kudingcha* on Parasitemia Count of *T. b. brucei* Infected Rats

At days 6 to day 9 post infection (days 1-4 after treatment), the infected groups treated with the standard drug (diminazen aceturate) and varying doses of methanol extract of *I. kudingcha* showed significant ($p < 0.05$) decrease in parasitemia count compared with the *Trypanosoma* infected and untreated control group. On day 9 post infection (day 3 after treatment), the standard drug had cleared all parasites from the blood stream of the rats. At day 15 post infection (day 9 after treatment), further significant reduction in parasitemia was observed in the group treated with 400 mg/kg b.w. of rat but at 200 mg/kg b.w. of parasitemia did not reduce significantly when compared to the infected but untreated group. The infected rats treated with methanol extract at 200 and 400 mg/kg body weight of rat showed nonsignificant ($p > 0.05$) decrease in parasitemia count compared with the infected rats, treated with diminazen aceturate. On days 6 and 15 (post infection), the plant extract at 400 mg/kg b.w. of rat significantly ($p < 0.05$) reduced parasitemia compared to 200 mg/kg b.w. of rat. On day 15 post infection, percentage suppression of parasitemia in the treatment groups (standard drug, methanol extract at 200 and 400 mg/kg b.w.) were 100, 25.45, and 62.74%, respectively (Table 6).

Table 6: *In vivo* effect of combined methanol–chloroform extract of *I. kudingcha* on parasitemia count of *T. b. brucei* infected rats.

Day (PI)/day after treatment	Parasitemia count (log number/ml)			
	INT	TBD 3.5 mg/kg b.w.	TBIK 200 mg/kg b.w.	TBIK 400 mg/kg b.w.
0	4.33 ± 1.20	5.66 ± 0.33	4.66 ± 0.88	3.33 ± 0.66
3	27.00 ± 8.96	26.66 ± 3.52	25.66 ± 3.52	33.66 ± 6.33
6	31.66 ± 24.49	1.00 ± 1.00*	13.33 ± 1.66*	3.66 ± 0.88*
9	30.00 ± 2.88	0.00 ± 0.00*	12.00 ± 1.52*	9.00 ± 3.46*
15	36.66 ± 3.33	0.00 ± 0.00*	27.33 ± 10.26	13.66 ± 4.91*
Percentage suppression (%)	0	100.00	25.45	62.74

Values represented in mean ± SEM.
*shows significant difference ($p < 0.05$) compared with TBDW group; INT: *T. b. brucei* infected not treated rats administered 0.2 ml of 7% methanolic Tween 80 solution; TBD: *T. b. brucei* infected rats administered 3.5 mg/kg b.w. of diminazen aceturate; TBIK 200 mg/kg: *T. b. brucei* infected rats administered 200 mg/kg b.w. of *I. kudingcha;* TBIK 400 mg/kg: *T. b. brucei* infected rats administered 400 mg/kg b.w. of *I. kudingcha.*
PI = post infection.

3.7. Histological Studies on Organs of Rats

Observations on the effect of the individual extracts (methanol and chloroform separately) and combined extracts (methanolic/chlororform) at 200 and 400 mg/kg body weight, respectively, on the organs of animals infected with trypanomiasis is presented in Tables 7-9.

The histopathological section of liver, kidney, brain, and heart of rats infected and treated with 200 and 400 mg/kg b.w. of *I. kudingcha* methanolic extract did not show any deviation from the normal histo-architecture (Table 7). Figures 5a-5c and 6a-6c show the photomicrograph of sections from the liver and brain.

The histopathological section of liver, kidney, brain, and heart of rats infected and treated with 200 and 400 mg/kg b.w. of chloroform leaf extract of *I. kudingcha* did show some deviations from the normal histo-architecture in the liver and kidney but not dose dependent (Table 8). Figures 5d and 6c show the photomicrograph of sections from the liver and brain.

The histopathological section of liver, kidney, brain, and heart of rats infected and treated with 200 and 400 mg/kg b.w. of combined methanolic and chloroform leaf extract of *I. kudingcha* did not show any deviation from the normal histo-architecture (Table 9). Figures 5c and 6c show the photomicrograph of sections from the liver and brain.

Table 7: Histopathological observations in organs of animals infected with *T. b. brucei* and administered with graded doses of *I. kudingcha* leaf methanolic extract.

Lesion/organ	Infected but untreated	Uninfected and untreated (normal rats)	Methanolic extract (infected and treated)		Diminazene (infected and treated)
			200 mg/kg	400 mg/kg	3.5 mg/kg
LIVER					
Periportal infiltration of inflammatory leucocytes	++	−	−	−	−
Multifocal aggregates of inflammatory leucocytes	−	−	−	−	+
KIDNEY					
Multifocal accumulation of inflammatory leucocytes	−	−	−	−	−
Tubular eosinophilic casts	−	−	−	−	−
BRAIN					
Mononuclear leucocytes	+++	−	−	−	−
Inflammation of brain	+++	−	−	−	−
HEART	−	−	−	−	−

not present (−), very mild (+), mild (++), moderate (+++), and severe (++++).

Table 8: Histopathological observations in organs of animals infected with *T. b. brucei* and administered with graded doses of *I. kudingcha* leaf chloroform extract.

Lesion/Organ	Infected but untreated	Uninfected and untreated (normal rats)	Chloroform extract (infected and treated)		Diminazene (infected and treated)
			200 mg/kg	400 mg/kg	3.5 mg/kg
LIVER					
Periportal infiltration of inflammatory leucocytes	++	−	+++	+	−
Multifocal aggregates of inflammatory leucocytes	−	−	+	−	+
KIDNEY					
Multifocal accumulation of inflammatory leucocytes	−	−	++	+	−
Tubular eosinophilic casts	−	−	++	+	−
BRAIN					
Mononuclear leucocytes	+++	−	−	−	−
Inflammation of brain	+++	−	−	−	−
HEART	−	−	−	−	−

not present (−), very mild (+), mild (++), moderate (+++), and severe (++++).

Table 9: Histopathological observations in organs of animals infected with *T. b. brucei* and administered with graded doses of combined leaf extracts of *I. kudingcha* (chloroform extract and methanolic extract).

Lesion/Organ	Infected but untreated	Uninfected and untreated (normal rats)	Combined extract (infected and treated)		Diminazene (infected and treated)
			200 mg/kg	400 mg/kg	3.5 mg/kg
LIVER					
Periportal infiltration of inflammatory leucocytes	++	−	−	−	−
Multifocal aggregates of inflammatory leucocytes	−	−	−	−	+
KIDNEY					
Multifocal accumulation of inflammatory leucocytes	−	−	−	−	−
Tubular eosinophilic casts	−	−	−	−	−
BRAIN					
Mononuclear leucocytes	+++	−	−	−	−
Inflammation of brain	+++	−	−	−	−
HEART	−	−	−	−	−

not present (−), very mild (+), mild (++), moderate (+++), and severe (++++).

The histopathological section of liver in the positive control administered with the standard drug (diminazene aceturate) showed multifocal aggregates of inflammatory leucocytes (Tables 7-9). Figures 5e and 6d show the photomicrograph of sections from the liver and brain.

4. DISCUSSION

Based on the results of acute toxicity studies, *I. kudingcha* extracts have shown no acute toxicity because no death was recorded at LD50 ≤ 5,000 mg/kg body weight. This observation corroborates a previous study on aqueous extract of *I. latifolia* where the maximum tolerable dose in rats was 168 g/kg [15]. However, this plant should be used with caution especially at high doses for a long period of time because the severities of histopathological changes observed in the liver and the kidney are suggestive of hepatotoxicity and nephrotoxicity. However, the extracts did not show any deleterious effect on the heart and the brain.

***Figure 5a*: A photomicrograph of the liver from animals administered 7% methanolic Tween 80 (normal control) showing normal hepatocytes arranged in chords around the central vein (V) radiating toward the portal triads (P). H&EX100.**
***Figure 5b*: A photomicrograph of the liver from *T. brucei* infected animals but untreated rat (negative control). Sections of the liver from the animals in this group showed a mild to moderate periportal infiltration of inflammatory leucocytes (arrow). Portal area (P), Central vein (V). H&EX100.**
***Figure 5c*: A photomicrograph of the liver from *T. brucei* infected animals administered methanolic extract of *I. kudingcha* (400 mg/kg body weight) showing normal hepatic lobules. The hepatic lobules showed normal hepatocytes (arrow) arranged in interconnecting chords around a central vein, radiating toward the portal areas. Central vein (V). H&EX400.**
***Figure 5d*: A photomicrograph of the liver from *T. brucei* infected animals administered chloroform extract of *I. kudingcha* (400 mg/kg body weight). Sections of the liver from animals in this group showed a mild periportal infiltration of inflammatory leucocytes (arrow). Hepatic artery (A); Hepatic vein (V); Bile duct (B). The above-mentioned structures are the components of the portal area/portal triad. H&EX400.**
***Figure 5e*: A photomicrograph of the liver from *T. brucei* infected animals administered 3.5 mg/kg b. w of diminazen aceturate. Sections of the liver from this group showed a few multifocal aggregates of inflammatory leucocytes (arrow). Portal area (P), Central vein (V). H&EX100.**

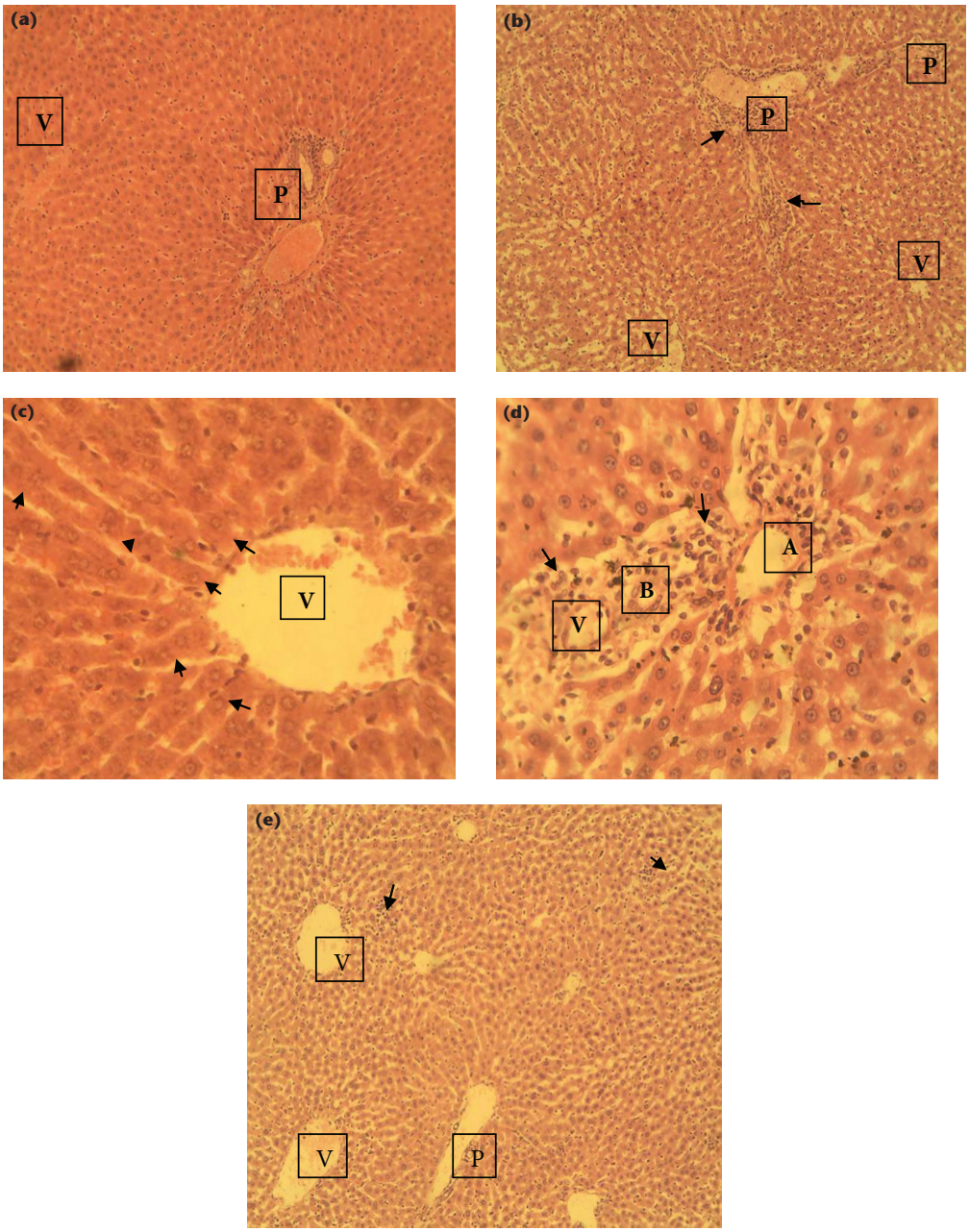
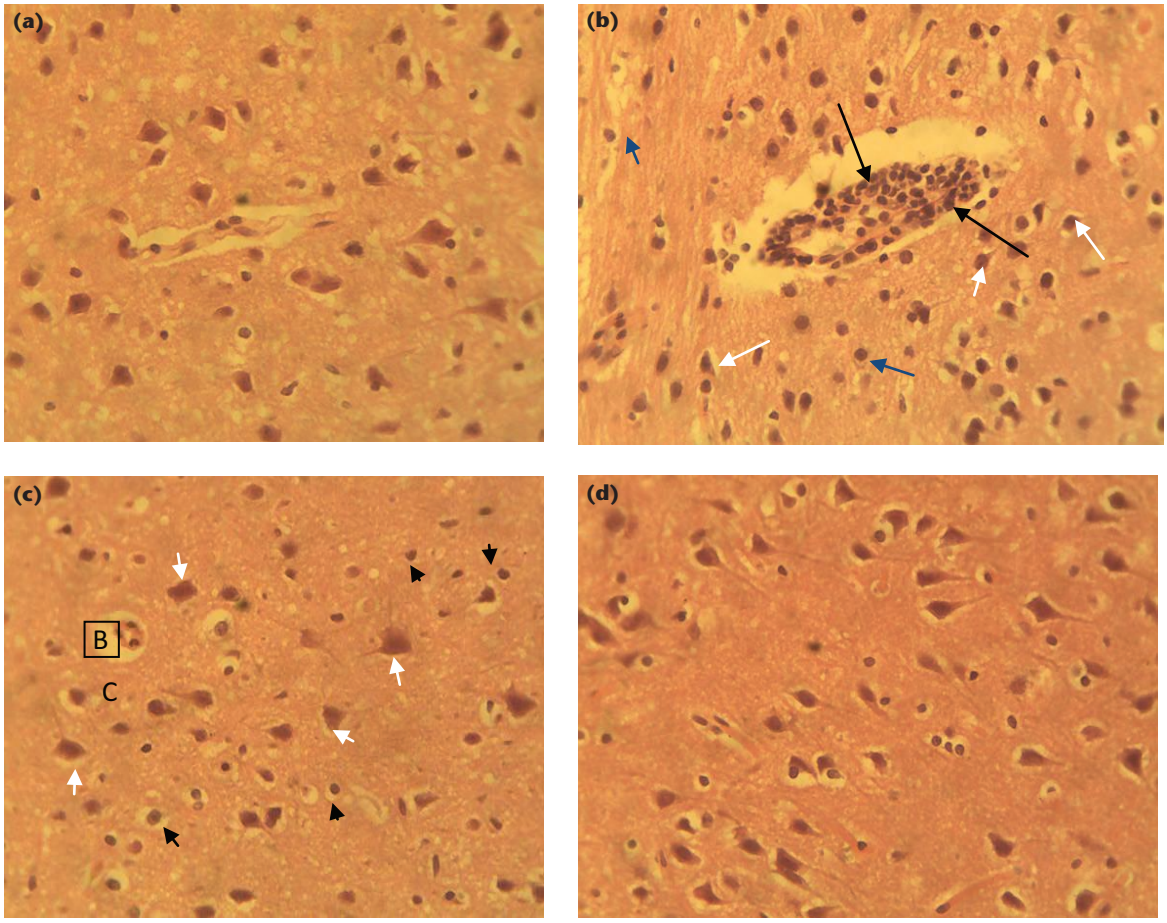


***Figure 6a*: A photomicrograph of the brain from animals administered 7% methanolic Tween 80 (normal control) showing normal neuronal and glial cells in the grey matter. H&EX400.**

***Figure 6b*: A photomicrograph of the brain from *T. brucei* infected animals but untreated rat (negative control). Sections of the brain from this group showed normal neurons and normal glial cells. However, aggregation of mononuclear leucocytes around some capillaries in grey matter (arrow) was observed. This is known as perivascular cuffing, and it is consistent with inflammation of the brain (encephalitis). Neuronal cell (white arrow), Astrocyte (blue arrow), Blood capillary (BC). H&EX400.**

***Figure 6c*: A photomicrograph of the brain from *T. brucei* infected animals administered extracts of *I. kudingcha* (400 mg/kg body weight). Sections of the brain from this subgroup did not show any change from the normal histo-architecture. It showed normal neuronal and glial cells in the grey matter. Neuronal cells (white arrow), Astrocytes (black arrow). Astrocytes are the most predominant of the glial cell population in the grey matter. Blood capillary (BC). H&EX400.**

***Figure 6d*: A photomicrograph of the brain from *T. brucei* infected animals administered 3.5 mg/kg b. w of diminazen aceturate. Sections of the brain showed normal neurons (arrow) and normal glial cells in the grey matter. H&EX400.**



The results of phytochemical screening of *I. kudingcha* revealed that the methanolic extract of the plant is a potential medicinal plant with appreciable high levels of phytochemicals. The tolerable level of the plant showed that it may contain very low level or no toxicants. This is evident from the acute toxicity carried out in this study. The results of this study compared favorably with others who showed that medicinal plants contain bioactive components and that they are safe for medical treatment [16-19].

Observations in the infected animals treated with the extracts showed that the effect of the methanolic plant extract is dose dependent and reduced parasitemia better than the chloroform plant extract. This had earlier been reported by [20]. Although the level of parasitemia reduced significantly four days post treatment in animals treated with the methanol extract, parasitemia increased significantly afterwards. The reason for the failure of the administered dose to clear the parasites in the blood could be that a high level of parasitemia was attained before commencement of treatment and also the possibility of the parasites to sequester or hide within the tissues to escape destruction by the *I. kudingcha* extract; similar result was reported by [21]. However, histological studies on the organs of infected and treated animals showed that the plant extracts possess both

curative and protective effect against the damages in the liver and kidney caused by trypanosomiasis in the hemolymphatic stage. The extracts were also able to protect the brain from accumulation of mononuclear leucocytes observed in the negative (infected not treated) control animals, which is often associated with encephalitis in trypanosomiasis infection. The observed accumulation of leucocytes in the brain may have been caused by the parasite infecting the brain that is characteristic of *T. b. rhodisiense* or *T. b. gambiense* infection in man. This observation is not impossible because experimental model of human African trypanosomiasis (HAT) in mice infected with *T. brucei brucei* had been reported [22]. The protective effect of the methanolic extract on the brain is validated by its effect on the blood stream parasite that can be regarded to be dose dependent. It is also interesting to know that toxicological studies revealed no pathological damages in the brain and heart of uninfected animals. With these protective properties, *I. kudingcha* plant may be a potential source of drug that could be used in the treatment of trypanosomiasis both at the hemolymphatic stage and meningo-encephalic stages of infection owing to the fact that most of the current drugs used in the treatment of trypanosomiasis have the set back of undesirable side effects or high toxicity [7].

The result of the phytochemical analysis of the plant reveals the presence of alkaloids and saponins in both extracts (methanol and chloroform). Tannins were observed to be present at lower concentration in the methanol extract but not detected in chloroform extract, while flavonoids were undetected. Alkaloids were reported by [23] to have anti-trypanosomal activity by potentially inhibiting its respiration. However, bioactive compounds exhibit interference with the redox balance of the parasites activity either on the respiratory chain or on the defenses against reactive oxygen species (oxidative stress). Some agents act by binding with the kinetoplast DNA of the parasite [24]. Micronized purified flavonoid fraction administered alone or with diminazene had also been reported by Kobo *et al*. [25] to improve the anemia caused by *T. b. brucei* infection in wistar rats. Hence, the anti-trypanosomal efficacy observed in this plant extract may be attributed to the active phytochemicals that might be acting singly or synergistically.

This study is the first to report on the effect of *I. kudingcha* on histopathological damages caused by the trypanosome parasite under *in vivo* studies. Part of the limitations of this study is the inability to determine what is responsible for the protective and curative properties of the plant. It is suggested that further research should be conducted to study the *in vitro* activities of the plant against Trypanosomes and identification of the phytochemical responsible for the therapeutic activity.

Author Contributions

Soniran OT designed the study, supervised the *in vivo* studies on parasitemia, and wrote the manuscript; Ngele KK contributed to the design of the study and proofread the manuscript; Alisa CO sourced for the plant from China and supervised the phytochemical screening of the plant and also proofread the manuscript; Omoboyowa DA supervised the histological studies and also proofread the manuscript; and Agu NH conducted the *in vivo* studies on parasitemia and histological studies.

Conflict of Interest

None.

Funding

None.

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