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Effects of Aqueous Leaf Extracts of *Loranthus micranthus* Linn. on Biochemical Profile of Wistar Rats Infected with *Trypanosoma brucei brucei*

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ABSTRACT

The incidence of trypanosomiasis remains a source of concern in the tropics and other parts of the world. Trypanosomiasis is a lethal disease which affects both man and animals. It is caused by a parasitic protozoon of the Genus Trypanosoma. Effects of aqueous crude leave extracts of *Loranthus micranthus* on the biochemical parameters of wistar rats infected with *Trypanosoma brucei brucei* were examined. Seventy-two adult-male rats were grouped into six (A-F) of 12 rats per group, comprising 3 replicate of 4 rats each. Blood samples were collected weekly for various biochemical parameters assay using standard methods. Liver markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the infected and treated rats and those of the infected and untreated control group showed marked significant increases ($p<0.05$) when compared with the standard drug and normal control groups. Similarly, data obtained showed that serum bilirubin, urea, creatinine and cholesterol levels were significantly high ($p<0.05$) in those animals treated with *L. micranthus* and in the negative control group. Serum albumin was significantly lower ($p> 0.05$) in the treatment groups with minimal increases in the group administered 800 mg/kg of the aqueous leaf extract. The results indicated that all the animals infected and treated with *L. micranthus* and the negative control group died from overwhelming parasitaemia unlike the case of those administered the standard drug. In conclusion, the aqueous leaf extract of *L. micranthus* may not be used as an anti-trypanosomal agent as well as hepato and nephro-protective agent.

***Keywords*:** Biochemical effects, Wistar rats, aqueous leaf extracts, *Loranthus micranthus*, *Trypanosoma brucei brucei*

Introduction

For many decades, trypanosomiasis has affected immensely the social and economic well-being of Africans in sub-Saharan regions and its incidence remains a source of worry in the tropics and the rest of the world.[1,2] The development of livestock industry in Sub-Sahara Africa has been greatly limited by the menace of African trypanosomiasis.[3] Due to their presence in the blood, these parasites produce several changes in the blood constituents.[4]*T. brucei brucei* is a single cell parasite that is transmitted by the tsetse fly which is the causative agent of sleeping sickness in both man and animals.[5,6]*T. brucei brucei* is the most widely distributed of the pathogenic animal trypanosomes and is the most important single cause of economic losses in camel rearing causing morbidity of up to 30 % in camel rearing areas.[7] The studies have shown that the parasite can infect all species of domestic livestock.

However, some drugs such as Suramin, melasoprol, eflonithine and pentamidine are examples of drugs used in the treatment of trypanosomiasis.[8] These drugs are expensive and not well distributed in the rural areas and have also been found to have adverse effects.[9]

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Thus, the need for the development of affordable, safe and effective drugs for the treatment of trypanosomiasis has resulted in the study of medicinal plants that have an anti-trypanosomal effect. Several herbal medicines have been discovered as good trypanosides.[10,11] But, this use of *L. micranthus* is yet to be authenticated.

African mistletoe (*L. micranthus*) is an obligate semi-parasite that depends on its host to obtain water and minerals but can carry out photosynthesis.[12] Trees such as *Kola acuminata, Kola nitida, magnifera indica, Azadirachta indica, Jatropha curcas* and *persia* sp are the host on which *L. micranthus* could grow.[13] It is commonly seen in the tropical rainforest region of Africa, Europe and America.[14] African mistletoes constitute several species with the *Loranthus* being the most common in Nigeria. This specie has been used traditionally by the people in Nigeria as an anti-cancer, anti-hypertensive, anti-diabetic and indeed as an all-purpose herb.[15] The herb has been analyzed and observed to contain many anti-nutrients.[16] It is used as alternative medicine in the treatment of epilepsy, headache, infertility, menopausal syndrome and rheumatism.[17] It is equally used in many parts of West Africa as an anti-microbial and antiplasmodial agent.[18]

In recent times, herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nations. The practices of herbal medicines have produced results of proven efficacies compared to orthodox medicine.[19] However, the quest for better, safe and affordable drugs has triggered more research into herbal drugs, which led to the introduction of herbal preparations for therapeutic uses. In recent times, herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nations. Furthermore, the gains from herbal medicine, especially in developing countries, were inadequate due to lack of scientific basis, warranting that the quality and consistency of the alternative medicines be ascertained and maintained for their maximal

use and efficacy.[20] There is a paucity of information on the use of *L. micranthus* in the treatment of trypanosomiasis and that prompted this present research to study the biochemical effects of aqueous leaf extracts of *L. micranthus* linn. (*loranthaceae*) in rats infected with *T. brucei brucei*.

Materials and Methods

Collection and Preparation of L. micranthus extract

Fresh leaves of the mistletoe plant were procured from the forests around Obukpa, a community in Nsukka from the host tree *Kola accuminata* in July, 2019. The plant was identified by Mr. Alfred O. Ozioko of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, where the voucher specimen (LM-KA, 2019-1) was deposited. The leaves were collected and shade dried for two (2) weeks. The dried leaves were reweighed using an analytical balance and were reduced to fine powder using a milling machine (Honda: model 622, China).

The extraction was done using a previously described method.[21] About 500 g of the plant fine powder macerated in 1L of distilled water and allowed to stand for 24 h at 25 ^{0}C, with periodic shaking to increase the extraction capacity. The mixture was filtered using a 60 mm muslin cloth, and then concentrated to dryness in an oven at 45 ^{0}C. About 500 g of the extract was re-dissolved in normal saline and used to prepare the stock solution for oral administration to the animals according to their body weights.

Experimental animals

Seventy-two adult male rats weighing between 150- 250 g were obtained from the animal breeding facility of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were kept in stainless wire cages equipped with drinkers and fecal collecting trays, in a clean experimental animal house. The rats were fed commercial growers chick mash (18 % crude protein) and clean water, and allowed to acclimatize for 14 days before the study. The animals were allowed access to food and water *ad libitum* while, the droppings in the tray were removed daily.

Experimental Design

The rats were assigned into six groups (A, B, C, D, E and F) of 12 animals per group with each group containing 3 replicates of 4 rats each. Groups A, B, and C constitute the treatment groups while groups D, E and F were the test groups (negative, positive and normal control) respectively. Group A, B and C were administered 400, 800 and 1200 mg/kg respectively of the leaf extract of *L. micranthus*. The negative control (group D) also called the infected and untreated group and normal control groups (Group F) were administered 1 ml/kg of normal saline. The positive control (Group F) was infected and treated with a standard drug known as berenil at 1.5 mg/kg body weight. All the doses were administered orally once daily for 28 days using gastric gavage.

Collection of blood sample

Blood samples were collected before the start of the experiment (day 0) and at weekly intervals during treatment for various biochemical parameters. The blood samples were collected according to a previously described procedure[23] using a non-heparinized specimen bottle and then centrifuged at 4000 rpm for 10 minutes. Thereafter, the serum was carefully removed and used for various biochemical parameters.

Determination of Biochemical Parameters

The biochemical markers were analyzed using commercial enzyme kits (Randox Laboratories). The AST and ALT were determined using previously described method[24], while, ALP, total albumin, total serum cholesterol, serum urea and serum creatinine were determined using the respective methods [25-29]

Ethical considerations

The experimental animals were handled in accordance with the international guidelines for Handling of Laboratory Animals.[22]Ethical approval was sought from University of Nigeria Nsukka Institutional Research and Ethics Committee (IREC UNN) and an ethical approval number IREC215UNN was given.

Statistical analysis

Data was analyzed using the GENSTAT (VSN International, Hemel Hempstead, Herts, UK). A One-way ANOVA was used to test the effect of treatment, while a Two-way ANOVA was used to determine the interactive effects of treatment and duration. DUNCAN was used to separate the means of different treatment groups. All results were presented as Mean ± SEM, while significant values were set at $p< 0.05$.

Results and Discussion

The weekly effects of aqueous extracts of *L. micranthus* on the serum AST, ALT, ALP, ALB and BIL levels of rats are presented in Table 1. Overall, there was a time dependent increase in the mean values of AST and ALT in all the treatment groups from week 1 to 3, Whereas group E recorded a significant increase and decrease ($p<0.05$) in week 1 and 2 in both parameters respectively. Similarly, there was a significant decrease and increase ($p<0.05$) in AST and ALT respectively on weeks 3 and 4 when compared with the baseline (week 0). However, there was an overall dose independent and significant increase ($p< 0.05$) in AST and ALT values of group A to D rats in weeks 1 to 3 when compared to the normal control group. A significant increase ($p<0.05$) in groups A-D when compared with the baseline (week 0) except in group E which increased significantly ($p<0.05$) at weeks 2 and 3 was observed in the mean values of ALP levels. There was an overall dose independent and significant increase in groups A-D at weeks 1, 2, and 3 when compared with the standard group and the normal control, while a significant increase ($p<0.05$) in week 2 was recorded in group E when compared with the normal control.

The results (Table 1) showed that at basal level, there were no significant differences among the mean values of the parameters investigated. Liver enzymes such as ALT, AST and ALP are usually released into the blood whenever the liver cells experience damage which frequently results in increased enzymatic activity.[30] The significant ($p<0.05$) increases observed in the activity of the liver enzymes such as ALT, AST and ALP when compared with the control group is an indication of liver damage. The observations made in this work suggests that parasitic protozoa and viral infections may be among the most common causes of liver injury and this results in AST and ALT being elevated to a greater degree than ALP. These elevated levels of liver enzymes ALT, AST and ALP were constant in the infected animals throughout the study. However, the group that was treated with the standard drug, diminazene aceturate (berenil), showed elevated levels of these enzymes but later stabilized to almost normal. This is an indication that the *L. micranthus* extract may have no hepato-protective properties in rats infected with *T. brucei brucei.* This is in line with a previous report, which reported increased ALT and AST levels in rats infected with *T. brucei brucei* and treated with ethanolic extracts of *Gardenia sokotensis*.[31] Also, this is at variance with a report [32], which ascertained significant decreases in the AST and ALT of normal male rats administered aqueous leaf extracts of *L. micranthus*, though, they reported elevated levels of serum ALP which also is in line with this present work.

Also, there was no dose-dependent and significant difference ($p> 0.05$) in the mean values of albumin in groups A to E except in group C which showed a significant increase at week 1 when compared with the normal control (Table 1). Although, significant increases ($p<0.05$) were observed in groups A, B and C at week 1, there was no level of significance ($p>0.05$) from weeks 2 to 4 when compared with week 0. However, significant increases ($p<0.05$) were recorded in group E at weeks 1, 3 and 4 when compared with week 0 whereas D did not show any significant difference ($p>0.05$) from weeks 1 to 4. Albumin is produced wholly in the liver and it plays an important role in regulating the flow of water between the plasma and tissue fluids through its colloidal osmotic pressure effects.[31] Consequently, any hepatic pathology could result in impairment of its production. A decrease in serum albumin level could also follow accelerated protein loss through the kidney or the gut.[33] Albumin has a normal half-life of 21 days on average and therefore, a reduction in serum albumin is usually not

evident early in the cause of liver damage.[34] In this present work, there was no significant difference in the total serum albumin levels when compared with the control. This work is at variances with the report of [31] which reported increases in the albumin levels of the infected animal though, not time dependent. This present investigation is in line with the work of [35] who observed significant increases in the serum albumin levels of rats infected with *T. brucei brucei* and treated with honey, which increase was attributed to the parasite infection.

More so, there were time independent and significant increases ($p<0.05$) in bilirubin levels of animals in groups A and E at weeks 1, C and D at weeks 1 and 2 when compared with week 0 (baseline) (Table 1). Also, dose independent and significant increase ($p<0.05$) in the treatment groups A–E at week 1 were observed, whereas, significant increases ($p<0.05$) were observed in groups B at week 3, C at weeks 2 and 3, and D at week 2 when compared with normal control. Bilirubin being an output of metabolism begins to build up when the liver is not functioning properly or is out rightly damaged. Hepatitis, cancer of the pancreas and allergic reaction to a blood transfusion and obstructed bile ducts are other causes of increased bilirubin in the body.[36] High bilirubin levels means that the bile in the body is not being cleared from the body, it therefore clusters or and reflects first through the eyes which turns pale yellow before manifesting through the skin as jaundice. [3] The elevated level of bilirubin in the infected animals when compared with the control in this research could be a sign that there was liver malfunctioning and impaired hepatic excretory function in the infected animals when compared with the control groups. However, a drop observed in the bilirubin levels at week 2, and later rise at week 3 in those animals treated with *L. micranthus* could be an indication that the aqueous extract could not ameliorate the effects of trypanosomiasis.

Table 1: Effects of aqueous leaf extract of *L. micranthus* on AST, ALT, ALP, albumin and bilirubin of Rats on Weekly Basis

Parameters	Groups	Duration (weeks)				
		0	1	2	3	4
AST (U/L)	A	110.00 ± 2.96^{2b}	171.33 ± 15.08^{1ab}	187.50 ± 3.17^{1b}	0.00 ± 0.00^{d3}	-
	B	117.67 ± 3.06^{3ab}	168.67 ± 19.04^{2ab}	224.50 ± 3.17^{1a}	151.50 ± 0.50^{2b}	-
	C	121.33 ± 3.09^{3a}	162.33 ± 8.03^{2ab}	215.00 ± 8.66^{1a}	162.00 ± 4.00^{2a}	-
	D	113.75 ± 2.15^{4ab}	180.00 ± 12.08^{2a}	215.00 ± 3.46^{1a}	155.50 ± 0.50^{3ab}	-
	E	115.58 ± 2.81^{3ab}	133.07 ± 8.18^{2b}	154.33 ± 1.85^{1c}	90.00 ± 2.082^{4c}	97.33 ± 8.41^{4b}
	F	116.42 ± 2.43^{1ab}	86.72 ± 1.36^{2c}	87.191 ± 3.26^{2d}	81.12 ± 2.81^{2c}	80.42 ± 3.72^{2a}
ALT (U/L)	A	38.50 ± 1.18^{2a}	93.33 ± 1.76^{1a}	95.50 ± 0.28^{2a}	0.00 ± 0.00^{3d}	-
	B	39.25 ± 1.54^{3a}	93.00 ± 1.73^{2a}	98.50 ± 0.28^{2a}	129.50 ± 0.50^{1a}	-
	C	39.67 ± 1.59^{3a}	97.67 ± 5.50^{2a}	98.00 ± 0.00^{2a}	125.00 ± 0.00^{1b}	-
	D	38.75 ± 0.78^{3a}	96.67 ± 0.88^{2a}	98.00 ± 0.00^{2a}	131.00 ± 1.00^{1a}	-
	E	38.83 ± 0.76^{2a}	25.00 ± 1.52^{3b}	30.00 ± 4.04^{3b}	56.00 ± 1.00^{1c}	59.33 ± 3.84^{1a}
	F	38.63 ± 0.75^{1a}	33.42 ± 1.88^{1b}	32.63 ± 2.13^{1b}	36.30 ± 2.68^{1c}	35.12 ± 2.87^{1b}
ALP (U/L)	A	66.50 ± 2.67^{3ab}	152.67 ± 5.04^{2a}	185.50 ± 6.63^{1a}	0.00 ± 0.00^{4e}	-
	B	63.08 ± 2.04^{4ab}	156.67 ± 4.05^{3a}	195.50 ± 3.17^{1a}	169.00 ± 1.00^{2a}	-
	C	52.17 ± 3.59^{3b}	158.00 ± 7.21^{2a}	191.50 ± 3.17^{1a}	150.00 ± 2.00^{2b}	-
	D	67.00 ± 2.24^{4a}	154.00 ± 6.24^{2a}	195.00 ± 2.88^{1a}	108.50 ± 0.50^{3c}	-
	E	65.00 ± 3.10^{3ab}	72.33 ± 10.69^{3b}	152.33 ± 10.65^{1b}	90.33 ± 4.91^{2d}	63.00 ± 4.04^{3a}
	F	62.03 ± 3.59^{1ab}	66.830 ± 3.74^{1b}	63.02 ± 6.43^{1c}	63.21 ± 1.75^{1d}	65.21 ± 3.04^{1a}
ALBUMIN (g/dl)	A	37.67 ± 1.00^{2a}	43.67 ± 3.38^{1bc}	31.50 ± 3.75^{2a}	0.00 ± 0.00^{3c}	-
	B	37.08 ± 0.82^{2a}	43.67 ± 1.76^{1bc}	38.50 ± 1.44^{2a}	43.50 ± 0.50^{2a}	-
	C	37.00 ± 0.69^{2a}	47.33 ± 1.76^{1ab}	36.00 ± 0.57^{2a}	40.50 ± 4.50^{2ab}	-
	D	37.17 ± 0.83^{12a}	40.33 ± 1.33^{1c}	33.00 ± 1.73^{2a}	$35.50 \pm 0 .50^{2b}$	-
	E	36.58 ± 0.80^{3a}	51.33 ± 0.33^{1a}	35.00 ± 1.73^{4a}	39.67 ± 0.33^{2ab}	41.33 ± 2.02^{2a}
	F	37.19 ± 0.82^{1a}	38.53 ± 0.33^{1c}	37.28 ± 1.36^{1a}	37.21 ± 0.24^{2ab}	65.21 ± 3.04^{1a}
BILIRUBIN (mg/dl)	A	5.20 ± 0.31^{2ab}	17.03 ± 4.43^{1a}	5.35 ± 0.83^{2cd}	0.00 ± 0.00^{d3}	-
	B	7.42 ± 94^{12a}	13.10 ± 3.67^{1a}	4.25 ± 1.06^{2d}	13.50 ± 0.10^{1a}	-
	C	5.07 ± 0.32^{3ab}	19.57 ± 5.33^{1a}	10.90 ± 1.61^{21b}	11.80 ± 0.80^{2ab}	-
	D	6.49 ± 0.85^{2ab}	17.17 ± 7.04^{1a}	16.00 ± 0.00^{1a}	9.60 ± 0.40^{12bc}	-
	E	5.53 ± 0.58^{2ab}	17.70 ± 0.49^{1a}	9.13 ± 2.30^{2bc}	8.33 ± 1.04^{2c}	5.57 ± 0.49^{2a}
	F	5.50 ± 0.31^{1ab}	5.11 ± 0.22^{1b}	6.01 ± 0.00^{1cd}	5.88 ± 1.10^{1c}	5.72 ± 4.12^{1a}

*Values with different alphabetic (lower case) superscripts differ significantly ($p<0.05$) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly ($p<0.05$) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM

However, the present study is not in line with the findings of [35] who observed that there were no significant changes in serum bilirubin of rats infected with *T. brucei brucei* and treated with honey.

Table 2 showed the weekly effects of aqueous extracts of *L micranthus* on the urea, creatinine and serum cholesterol levels of rats. There was no overall dose-dependent and significant difference ($p>0.05$) in serum urea levels in all the treatment groups at weeks 1 to 4, except at week 1 where all the groups showed significant increases ($p<0.05$) when compared with the normal control (Table 2). Similarly, groups A, B and D showed a significant increase ($p<0.05$) in the duration of treatment at week 1 while a significant increase ($p< 0.05$) was observed in group C at week 3 when compared with week 0. However, there was no significant difference ($p> 0.05$) in the duration of treatment in groups E when compared with week 0. High blood urea is usually associated with an excess breakdown of proteins in the blood, increased catabolism of tissue proteins and reduced urea excretion.[37] Urea is a biochemical marker used to ascertain kidney function. High blood urea is an indication of kidney dysfunction thereby making it almost unable to discharge waste timely.[38] Under such conditions, toxins produced by the patient's body will build up and therefore become a threat to other body organs. In kidney failure, blood urea levels accelerate rapidly when the glomerular filteration rate decline to 50 % of the normal range. Thus, elevated blood urea is an indication of serious illness and various symptoms such as fluid retention, fatigue/tiredness, reduced appetite, poor concentration, insomnia will be exhibited by the patient. However, the observed increases in weeks 1 and 3 of the treatment groups when compared with the control as shown in table 2, indicates that the extracts could not control the effects of the parasite which may have caused the increase in urea levels of the animals in these weeks when compared with the control and those treated with the standard drug. This work corroborates the work of [38]and [31] although, they worked on the plant *Gardenia sokotensis*. Also, it disagreed with [39] who observed a significant decrease in the serum urea level of rats fed with *Mucunapruriens*.

There was no significant difference ($p> 0.05$) recorded in mean values of creatinine levels in the treatment groups (A-C) from week 1 to 3 when compared with the normal control, whereas group D and E significantly increased and decreased ($p< 0.05$) respectively at week 1 as shown in table 2. In the duration of treatment, group A, B and D significantly increased ($p< 0.05$) at week 1 while group C showed significant increase ($p< 0.05$) at week 3 when compared with the baseline (week 0). However, there is an observed significant difference ($p< 0.05$) in group E at weeks 1 and 2 when compared with the baseline. Creatinine is a waste product of metabolism and is transported through the bloodstream to the kidneys. The kidney filters out most of the creatinine into urine the GF. Any impairment to the kidney will results in an increase in reatinine level in the blood due to its poor clearance by the kidney. However, abnormal high levels of creatinine could lead to kidneys failure.[40]

The findings of this present study is at variance with [41] whose work an *Mucuna* flagellipes found that the aqueous extracts did not affect the serum chemistry of albino rats.

Overall, there was a significant increase ($p> 0.05$) in the effect of treatment on cholesterol levels in all the groups at week 1 and C, D and E at week 2 when compared with the normal control (Table 2). Also, significant increase ($p> 0.05$) was observed in the duration of treatment in groups A ,B and C from weeks 1 to 3 while group D recorded a significant increase ($p> 0.05$) in week 2 and group E in weeks 1 and 2 when compared with the baseline (week 0). High cholesterol levels such as LDL have been implicated in cardiovascular diseases. Fatty acids carried by LDL become oxidized and may cause injury to blood vessels. Atherosclerosis sets in when white blood cell move into the lining and arterial walls. Gradually, they transform into foam cells which accumulates fats and cholesterol. Substances such as calcium collect at this same site, and slowly but surely atherosclerotic plaque forms. [42] From the results obtained, there were elevated levels of cholesterol in the treatment groups when compared with the control group.

Table 2: Effects of Aqueous Leaf Extract of *L. micranthus* on urea, creatinine and cholesterol levels of rats

Parameters	Group	Duration (weeks)				
		0	1	2	3	4
	A	4.07 ± 0.27^{2a}	8.87 ± 0.61^{1a}	3.80 ± 0.05^{2a}	0.00 ± 0.00^{3b}	-
	B	4.57 ± 0.91^{2a}	8.13 ± 1.63^{1a}	5.45 ± 2.04^{12a}	3.85 ± 0.05^{2ab}	-
	C	4.53 ± 0.31^{2a}	7.10 ± 1.01^{12a}	4.80 ± 1.38^{2a}	10.25 ± 05.75^{1a}	-
UREA (mg/dl)	D	4.68 ± 0.40^{2a}	7.90 ± 1.41^{1a}	3.75 ± 0.43^{2a}	5.50 ± 1.50^{2ab}	-
	D	4.27 ± 0.21^{12a}	8.50 ± 4.65^{1a}	5.37 ± 0.47^{12a}	3.80 ± 0.15^{2ab}	3.73 ± 0.24^{2a}
	F	4.21 ± 0.43^{1a}	4.44 ± 0.21^{1b}	4.49 ± 0.20^{1a}	4.33 ± 0.13^{1ab}	4.31 ± 0.11^{1a}
	A	65.75 ± 4.49^{2a}	163.00 ± 9.01^{1ab}	67.00 ± 10.97^{2a}	0.00 ± 0.00	-
	B	73.25 ± 2.62^{2a}	150.33 ± 24.90^{1ab}	84.00 ± 37.52^{2a}	88.50 ± 0.50^{2ab}	-
	C	74.12 ± 6.99^{2a}	111.00 ± 14.17^{12ab}	99.00 ± 32.33^{2a}	172.00 ± 75.00^{1a}	-
CREATININE	D	77.58 ± 3.96^{23a}	165.00 ± 20.21^{3c}	65.00 ± 18.47^{3a}	103.50 ± 0.50^{ab}	-
(mg/dl)	E	76.17 ± 4.50^{2a}	43.30 ± 20.21^{3c}	126.00 ± 14.97^{1a}	67.00 ± 8.08^{2ab}	86.67 ± 8.96^{2a}
	F	73.50 ± 6.21^{1a}	74.23 ± 3.11^{1b}	76.22 ± 5.32^{1a}	74.10 ± 4.757^{1ab}	$75.31 \pm 3.72_1{}^{a}$
	A	0.44 ± 0.03^{2b}	1.57 ± 0.29^{1b}	1.80 ± 0.05^{1b}	0.00 ± 0.00^{b3}	-
	B	0.65 ± 0.06^{4a}	1.11 ± 0.20^{3ab}	2.15 ± 0.25^{1ab}	1.75 ± 0.50^{2a}	-
	C	0.63 ± 0.08^{3ab}	1.17 ± 0.12^{21ab}	2.70 ± 0.00^{1a}	1.75 ± 0.85^{2a}	-
CHOL(mmol/l)	D	0.75 ± 0.07^{2a}	1.07 ± 0.08^{2ab}	2.60 ± 0.14^{1a}	0.98 ± 0.03^{2ab}	-
	E	0.60 ± 0.05^{3ab}	1.87 ± 0.23^{2a}	2.80 ± 0.41^{1a}	0.80 ± 0.06^{3ab}	0.77 ± 0.14^{3a}
	F	0.64 ± 0.08^{1a}	0.74 ± 0.29^{1c}	1.30 ± 0.04^{1b}	0.80 ± 0.05^{1ab}	0.71 ± 0.50^{1a}

*Values with different alphabetic (lower case) superscripts differ significantly ($p<0.05$) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly ($p<0.05$) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM.

The elevated levels of cholesterol were highest at week 2 for all treatment groups with group E (standard drug group) having the highest level when compared with the control groups.

Conclusion

This study has vividly shown that the rats infected with *T. brucei brucei* had a severe and adverse effect on the biochemical indices. These findings indicated that aqueous extract of mistletoe *(L. micranthus)* do not possess anti-trypanosomal properties as well as hepato and nephro-protective activities. Thus, it has therefore shown that the aqueous leaf extract of *L. micranthus* may not be suitable for the management of trypanosomiasis as well as hepato and nephro-toxicity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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