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Toxicity and Anti-Trypanosomal Studies of Aqueous and Methanol Leaf Extracts of *Acacia Nilotica* on Trypanosoma Brucei-Induced Infection in Mice

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ABSRACT: African Animal Trypanosomiasis constitutes one of the greatest threats to the health of animals and socioeconomic status of people, particularly in developing countries. Chemotherapy, the main means of controlling the disease is limited due to parasite resistance and toxicity of the current anti-trypanosomal drugs. The development of a vaccine has been thwarted by antigenic variation of the parasite. Thus, plant extracts are one of the strategies being explored to address some of the problems encountered. The main objective of the current study was to investigate the toxicity and anti-trypanosomal activity of methanol and aqueous extracts of Acacia nilotica through in vivo assays against Trypanosoma brucei brucei. The chosen plants' healthy, fresh, matured leaves were air dried under shade, pulverized with mortar and pestle into powder, and passed through a 0.5mm mesh to standardize their particles. The plant samples were extracted using aqueous, methanol solvents. Qualitative and quantitative phytochemical analysis of the active chemical constituents of the extracts was conducted to determine saponins, flavonoids, tannins, terpenoids, steroids, and cardiac glycosides according to standard procedure. It was established that aqueous and methanol leaf extracts of the selected plants were safe in rats at dose levels of 1000, 3000, and 5000 mg/kg body weight for 72 hours. However, Sedation and abnormal movement were observed for both A. nilotica and Z. mucronata as manifestations of clinical toxicity at 5000mg/kg body weight. The fatal dose of all extracts exceeds the maximum dose of 5000mg/kg. Prolonged oral administration of the extracts for 21 days with A. nilotica extracts did not reveal major changes in body and organs weights, liver and kidney functions, the biochemical analysis showed a slight, non-significant increase in Alanine transaminase, Aspatate transaminase, and Alkaline phosphatase at (4000mg/kg) in rats, the average creatinine and urea levels were within the normal range, the relative organ weight and isolated organ are within the normal reference value. The photomicrographs of the liver and kidney sections showed mild histological changes. The in vivo assay showed the aqueous and methanol extracts from A. nilotica at 200mg/kg, 100mg/kg, and positive control (diminazine aceturate 3.5mg) reduced parasitaemia ($p < 0.05$), improved anaemia ($p < 0.05$), prevented body weight loss ($p < 0.05$) compared to the negative control. This study showed that the leaf of A. nilotica is safe and possess antitrypanosomal properties, suggesting that they may be a source of novel drugs for treatment of tropical diseases caused by trypanosomes.

KEYWORDS: Acacia nilotica, Trypanosomiasis, Toxicity, Histopathology, Trypanasoma brucie brucei.

INTRODUCTION

A protozoan parasite belonging to the genus Trypanosoma causes trypanosomiasis, a neglected tropical infectious disease with medical and veterinary significance in sub-Saharan Africa. Human African trypanosomiasis (HAT), often known as sleeping sickness, is caused by the species T. brucei brucei, whereas African Animal Trypanosomiasis (AAT), also known as Nagana in West Africa, is caused by T. b. rhodesiense and T. b. gambiense. [1, 2]. It is a parasitic infection that only manifests through the tsetse fly's geographic range [3]. Chemoprophylactic or chemotherapeutic drugs are primarily used to control trypanosomiasis [4]. Chemical relationships underlie the majority of these medications used to manage both human and animals [5]. In sub-Saharan Africa alone, trypanocides are reportedly administered up to 35 million times a year [6]. This amounts to only around one-third of the animals that are at risk for treatment. The shortage of medications, drug resistance, high cost, toxicity, and adverse reactions restrict the use of chemotherapy for African trypanosomiasis [8, 9]. For this reason, it's critical to look for less toxic, more affordable, readily available, and more effective chemotherapeutic drugs to treat the condition. Nigerians, like most Africans, rely on medicinal plants to heal a variety of ailments; while most of these plants are successful, there is a dearth of scientific research to support their claims. A great deal of work has gone into finding novel anti-trypanosomal medications, usually derived from plants, based on the knowledge of their traditional medicinal applications [10]. Nonetheless, little is known about the toxicity of herbal medicines and how they may affect the integrity of internal organs like the kidney and liver. Natural plant products have been marketed as safe; however, this can only be confirmed if toxicity tests employing cutting-edge scientific techniques have been completed.

Several studies demonstrated the ability of botanicals to treat trypanosomiasis [11]. For instance, after 60 minutes of incubation, a 50 mg/kg aqueous extract of *Peristrophe bicalyculata* immobilized 90% of *T. brucei brucei* in vitro [12]. In addition to *Saba florida* [13], other plants with antitrypanocidal activity against *T. brucei brucei* have also been discovered, including *Anchomanes difformis* [14], *Carissa spinarum* [15], *Lawsonia inermis* [16], *Anogeissus leiocarpa*, *Khaya senegalensis* and potash [17], *Garcinia kola* Nuts [18], and *Ranunculus Multifidus* [19]. *Acacia nilotica* is a plant with a vast geographic range that is usually found in western Africa, particularly Nigeria. It is both a notable decorative and medicinal species. Its use has been linked to a variety of biological activities, including those that are anti-diarrheal, anti-tuberculosis, and anti-leprosy. It has also been found to be a source of numerous active naturally occurring substances that might be used to create new drug targets [20]. This study evaluated the anti-trypanosomal activities and toxicological profile of Acacia nolitica leaf extracts in aqueous and methanol on trypanosoma bruicei-induced infection in mice.

EXPERIMENTAL WORK

Collection and Identification of Samples

Healthy, fresh, matured leaves of *A. nilotica* were collected from Gusau in Zamfara State, where the plants grow abundantly. The plant samples were placed in clean, sterile polythene bags and transported to the herbarium section of the Department of Biological Science, Nigerian Defense Academy Kaduna for identification and authentication. A. *nilotica* was identified with number NDA/BIOH/2024/01

Plant Sample processing and preparations

The leaves are washed and air-dried at room temperature for one week. The dried samples are processed through a grinder to create a fine powder and kept in air-tight containers at room temperature [21].

Extraction of plant materials

Distilled water and methanol weighing 1000 ml each was used to dissolve 100g powder of *A. nilotica.* The mixtures were kept at room temperature for 24 hours and 48 hours for distilled water before filtering using

Whatman filter paper No 1. Each filtrate was concentrated using a rotary evaporator at 40ºC. The filtrates were subjected to drying on a water bath at 25ºC to remove most of the solvents from the extracts [21].

Experimental animals and doses of extracts

Organization for Economic Co-operation and Development (OECD) guidelines 423 and 407 for toxicity assessment were employed for the Animal model [22]. Experimentations were carried out as previously observed by other studies with few adjustments [23]. A total of one hundred and five (105) Wister rats aged between 4-5 weeks old were purchased from the faculty of veterinary sciences, Ahmadu Bello University, Zaria. Male and female rats were kept apart to avoid any potential copulation. They were kept in cages made for laboratory rats, the animals were fed three times daily with growers' mash made from essential feed to make sure they did suffer starvation and they were given access to unlimited amounts of water. Animals were randomly selected and grouped into four groups (n=5) kept in their cages for seven (7) days before dosing to allow acclimatization to the laboratory conditions.

Acute toxicity assay

Twenty (20) rats were grouped into four groups including the control, each group consisted of five rats. This was achieved as already observed by other previous studies with few adjustments [23]. Animal groups were housed in separate plastic cages for ease observation and treatments. Both aqueous and methanolic leaf extracts of *Acacia nilotica* of doses 1000, 3000, and 5000 mg/kg were administered to rats orally to determine the LD_{50} of the extracts within 72 hours. Except for the control group which was not administered with extracts. After the administration, the animals were kept under close observation continuously for 1 hour for indications of acute toxicity, including the hypotensive response (dyspnea and lethargy) that the test extracts may cause at various doses intermittently for 4 hours and once every 24 hours for the next three (3) days. During this study period, daily clinical observations and records were made for food intake, water intake, respiration, eye color, movement, convulsion, aggressiveness, sedation, mortality, survival, and other abnormal habits.

The LD_{50} were calculated using the formula: $LD_{50} = \sqrt{(D\,o \times D\,100)}$

Where: D0 = Highest dose that gave no mortality

D100 = Lowest dose that produced mortality [24].

The lethality was followed by sub-acute toxicity assay.

Sub-acute toxicity assay

The sub-acute toxicity assay was carried out with an animal model using rats. Twenty (20) rats were grouped into four (4) experimental units each had five rats placed in separate plastic cages for ease observation and treatments. Administered doses were adjusted from research previously conducted [23]. The doses of aqueous and methanolic leaf extracts of *Acacia nilotica* of 1000, 3000 and 4000 mg/kg were used to treat groups 1, 2, and 3, respectively, while Group 4 was not administered with extracts as it served as a control group. The dosage routine of the animals with extracts was repeatedly conducted once every day for 21 days. The dose administrations were done at twelve midday and made through orally, followed by clinical signs observations. Toxicity signs were recorded at the interval of 10 mins, 0.5 hr, 1 hr, 4 hrs, 8 hrs and 24 hrs immediately after dose administration. During this study period, daily clinical observations and records was made for food intake, water intake, respiration, eye color, movement, convulsion, aggressiveness, sedation, mortality, survival and any other anomalous were made. Animals were anaesthetized by ketamine 20:1 mg/kg intraperitoneal (20:1 mg/kg IP) injection to avoid pain during dissection. After anaesthesia reached depth, the animals were sacrificed. Kidney, liver, and spleen were removed for histopathological examination. These vital organs were preferred because they are the target organs involved in the detoxification and excretion of

the ingested harmful substances, which are more likely to manifest negative effects in the cells of the organs. The effects of extracts were studied and collected as histopathological data.

Serum collection and biochemical analysis

Blood samples were collected from the rats into non-heparinized tubes and then centrifuged at 3000 rpm for 10 min. The serum was carefully removed, put into sample bottles, and stored frozen for further biochemical analysis. The serum separated was analyzed to evaluate the hepatic enzymes; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) as described by [25, 26]. Likewise, the renal function tests such as serum urea; uric acid; creatinine; bilirubin; protein, and electrolytes (sodium, potassium, and chloride ions) were evaluated using the ready-to-use Kits from Sigma Aldrich based on the method described by [27].

Animal Weight Gained and Relative Organ Weight

The weight of each rat was taken as described by [25]. Each animal's liver, kidney, and spleen were carefully examined for gross pathological changes, and weighed. Relative organ weight (ROW) was calculated using the formula below and the organs were further taken for histopathological examination.

Relative Organ Weight = Absolute organ weight (g) X 100 Body weight of rat on sacrifice day (g) as described by [22].

Histopathological examination

Histopathological examination of the liver and kidney was initiated with the removal of organs from the body and stabilized using a fixative to prevent organ decay. It was dehydrated, cleaned, impregnated with wax, and embedded into paraffin wax for block formation. The block was then trimmed and sectioned using a rotary microtome, fixed onto the glass slide, and de-waxed by placing the slides on a water bath at 40ºC as described by [28, 29]. The slides were stained with iron hematoxylin and eosin, mounted on Canada balsam, and covered with a coverslip for viewing under the X10 and X40 objectives of the microscope.

Experimental design, trypanosome inoculation, and treatment

For each experiment, adult rats of both sexes weighing 150 -200g were grouped into six and each group had five rats. Rats from groups 1, 2, 3, 4, 5and 6 were infected intraperitoneally with 0.1ml of the inoculum containing about 1×10^6 trypanosomes/ml.

Group 1: Negative control; infected and untreated

Group 2: Positive control; infected and treated with Diminazene aceturate 3.5 mg/kg.

Group 3: Infected treated with extract (25 mg/kg).

Group 4: Infected treated with extract (50 mg/kg/).

Group 5: Infected treated with extract (100 mg/kg).

Group 6: Infected treated with extract (200 mg/kg).

Determination of Parasitaemia in experimental rats

Parasitaemia level was monitored at three-day intervals in blood obtained from the tail of infected rats. The number of parasites per ml of blood was determined microscopically at × 400 magnification using the “rapid matching” method by [30], and the number of trypanosomes per field was converted to antilog to provide the absolute number of trypanosomes per ml of blood [31]. To assess the anti-trypanosomal effect of the extracts, the level of parasitaemia (expressed as a log of an absolute number of parasites per millimeter of blood) in the animal was compared to that of the control animals. Animals that survived to the end of the experiment, with no parasite in their blood sample, were considered cured.

Determination of packed cell volume

Blood was obtained at five-day intervals by bleeding the tail vein of rats and filling three-quarters full heparinized capillary tubes with 50-60 of the blood. The filled tube was placed in the micro haematocrit centrifuge and spun at 12000 g for 5 min. The spinner tube was then placed into a specially designed scale, read, and expressed as a percentage [32].

Determination of Daily Weight Changes

The weights of the rats were monitored daily using an automated electronic scale. To weigh a rat, a round plastic container was placed on the scale and adjusted to zero followings which the animal was dropped inside the container and subsequently weighed [33, 34].

RESULT

Phytochemical compositions of aqueous and methanol leaf extracts of the selected plants

The results of the qualitative and quantitative preliminary phytochemical screening of aqueous and methanolic leaf extracts of *Acacia nilotica* were presented in table 1. The qualitative analysis indicates the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, cardiac glycosides, steroids, balsams and terpenoids glycosides in both aqueous and methanolic extracts. The quantitative analysis shows flavonoids with the highest concentration of 86.47±0.5 in aqueous extract and 67.05±0.2 in methanol extract, followed by cardiac glycosides with 28.20±0.2 in aqueous extract and 13.04±0.2 in methanol extracts, then carbohydrates, and tannins. Balsams have the lowest concentration of 06.12±0.3 in aqueous extract and 04.03±0.2 in methanol extract respectively.

Acute toxicity of Aqueous and Methanol Leaf Extracts of the Selected Plants

Acute toxicity of the aqueous and methanolic leaf extracts of *A. nilotica* was determined using the LD_{50} assessment. The administered doses of 1000, 3000, and 5000mg/kg of both aqueous and methanolic extracts did not reveal any mortality in the Wister rats within 72hours. However, sedation was noticed as a form of clinical toxicity manifestation for the rats administered with both aqueous and methanolic leaf extracts at 5000 mg/kg body weight (Table 10). Both extracts at 5000 mg/kg bring about a sedative sign to three out of five rats treated with aqueous extract and four out of five rats treated with methanol extract. observational signs witnessed an hour after administration. The clinical sign ceases to be visible within 2 hours.

Sub-acute toxicity of the Aqueous and Methanol Leaf Extracts of *A. nilotica*

The result of sub-acute toxicity assessment of both aqueous and methanolic leaf extracts of *A. nilotica* at the doses of 1000, 3000, and 4000 mg/kg from 1 to 21days were presented in table 13. The result revealed both extracts did not produce any mortality in the treated animals. However, neither the aqueous nor methanolic extracts showed any physical abnormality among the set clinical signs

Effects of Aqueous and Methanol Extracts of *A. nilotica* on Serum Biochemical Parameters for Hepatic Function of Rats

Figure 1 Present the results of aqueous and methanol leaf extracts of *Acacia. nilotica* on serum biochemical parameters for the hepatic function of rats. The result reveals the mean values of control groups (total protein 73.3±0.2; Albumin 35.2±0.3; Bilirubin 10.3±0.2) are lower than the mean value of total protein AqE 4000mg (80.4±0.3); MeE 4000mg (79.6±0.6), albumin AqE 4000mg (41.0±0.4); MeE 4000mg (40.2±0.2) and total bilirubin AqE 4000mg (11.2±0.3); MeE 4000mg (11.1±0.2) with no significant difference ($P<0.05$). Moreover, the mean values of control groups of ALT (26.2±0.3), AST (168.2±0.2) and ALP (94.2±0.2) are lower than the mean value of groups administered with the highest doses: AqE 4000mg (ALT 30.3±0.2; AST 173.0±0.3; ALP 96.6±0.1) and MeE 4000mg (ALT 31.0±0.3; AST 173.1±0.6; ALP 97.5±0.2). Although the

mean of the treated groups is high, it Shows no significant variations (P<0.05) and values are within the normal reference range.

Effect of Aqueous and Methanol Extracts of *A. nilotica* on Serum Biochemical Parameters for Renal of Rats

Figure 2 presents the results of aqueous and methanol leaf extracts of *A. nilotica* of some serum biochemical parameters for renal function test on rats. It shows the mean values of control groups; sodium (136±0.6), potassium (4.8±0.7), chloride (95.3±0.5), urea (3.6±0.3), Creatinine (90.3±0.1) and bicarbonate (25.3±0.2) is lower than those administered with the highest concentration of both AqE 4000 and MeE 4000mg/kg for Sodium; AqE 4000 (140.5±0.5); MeE 4000 (138.1±0.3), Potassium: AqE 4000 (4.9±0.3); MeE 4000 (5.0±0.3), Chloride: AqE 4000 (97.1±0.3); MeE 4000 (98.6±0.4), Urea AqE 4000 (7.5±0.4); MeE 4000 (7.3±0.4), creatinine AqE 4000 (99.2±0.5); MeE 4000 (98.5±0.6) and bicarbonate AqE 4000 (27.9±0.3); MeE 4000 (28.5±0.5). There was no difference and the values are within the normal range.

Body weight gain and relative organ weight of the rats administered with Aqueous and Methanol leaf Extracts of *A. nilotica*

Figure 3 presents the results of body weight gain and the relative weight of the isolated organs of rats administered with aqueous and methanol leaf extracts of *A. nilotica*. The highest body weight gain was recorded on group of rats administered with AqE 4000mg (14.04±1.06) and MeE 4000mg (15.02±1.09) and the least body weight gain was recorded among group of rats administered with AqE 1000mg (10.05±1.07) and MeE 1000mg (10.08±1.05) compared to control group with (10.02±1.05). Moreover, in the relative weight of the isolated organ, the highest weight was recorded among groups administered with AqE 4000mg with mean value: Spleen (0.34±0.13); Kidney (0.69±0.82); Liver (3.28±0.44) and MeE 4000mg with mean value: spleen (0.34±0.32); Kidney (0.69 9±0.62); Liver (3.28±0.36) compared to controls: spleen (0.32±0.20); kidney (0.67±0.01); liver (3.26±0.11). However, there is no variation on the mean values of both aqueous and methanol administered groups and the mean values of the control group.

Histopathology of liver and kidney of Rats treated with Extracts of A. nilotica

The result of histology of liver and kidney treated with Aqueous and Methanol Extracts of *Acacia nilotica* showed normal hepatocellular architecture with well-preserved hepatic cells, visible central veins, and no abnormalities for the control group and group treated with 1000mg/kg body weight (Plate 1 and 2). Rats treated with higher concentrations of 3000mg/kg and 4000mg/kg of the extract showed some histological changes such as mild dilation of sinusoids, and mild disorganization of hepatic cords (Plate 3 and 4). There was normal architecture of glomeruli and tubules of the kidney from the control group and the group treated with 1000mg/kg (Plate 5 and 6.). However, histopathological changes such as reduction of glomerulus cells were noted in the histoarchitecture of the kidneys of rats treated with 3000 and 4000mg/kg (Plate 7 and 8).

For methanol extracts, the result showed normal features of hepatocytes for the group treated with 1000mg/kg body weight of the extract (Plate 9). While the group treated with higher concentrations of 3000mg/kg and 4000mg/kg showed mild steatosis (Figures Plate 10 and 11). The group treated with 1000mg/kg showed slight mesenchymal proliferation (Plate 12) and the kidney of rats treated with 3000 and 4000mg/kg of the extract on the other hand showed mild tubules Proliferation (Plate 13 and 14).

Effects of Methanol and Aqueous extracts of *A. nilotica* on mean parasitemia (10^6) of rats infected with *Trypanosoma b. brucei*

The results of the mean group parasitaemia treated with various doses of Methanolic leave extracts of *A. nolitica* are presented in figure 4. Parasitaemia was established in the infected untreated group in 6 days post-infection and all other infected groups by 9 days. However, there was total clearance of parasites in the group

treated with the recommended dose of 3.5mg of diminazene aceturate by day 15 post-infection up to the end of the experimentation. Aparasitemia in the group treated 200mg by day 18 post-infection. However, a relapse parasitaemia was observed but with statistically significant reduction on 21 days $P< 0.05$ compared with groups treated 25mg, 50mg, 100mg and infected uncontrolled group. There was a significant increase in parasitemia in group treated with 50mg, 100mg. infected untreated group.

The results of the mean group parasitaemia treated with various doses of Aqueous leave extracts of *A. nolitica* are shown in figure 5. Parasitaemia was established in all the infected groups by 9 days Post infection except with infected untreated group in 6 days Post infection. There was total clearance of parasites in the group treated with recommended dose 3.5mg of diminazene diaceturate by day 9 post infection and group treated with 200mg by day 15 post infection up to the end of the experimentation. There was also aparasitaemia in the group treated 100mg by day 18 post infection. However, a relapse parasitaemia was observed on day 21 but with statistically significant reduction $P< 0.05$ compared with groups treated 25mg, 50mg, and infected uncontrolled group. There was significant increase in parasitaemia in groups treated with 25mg, 50mg, and infected untreated group.

Mean group packed cell volume (%) of *Trypanosoma b. brucei* infected rats treated with Methanol and Aqueous extract of *A. nilotica*

The results of the mean group PCV of *Trypanosoma b. brucei* infected Wister rats treated with methanol leaf extract of *A. nilotica* are shown in figure 6. By day 5 of Post infection, there were significant ($p < 0.05$) reductions in PCV of all the infected groups (25mg, 50mg, 100mg, 200mg DA 3.5mg and IU) when compared with Uninfected group. This reduction was later reversed in group treated with diminazene aceturate and 200mg but continued to decreased in all the other treated groups till the end of the experiment. Between days 10 and 20 of post infection, the mean PCV was significantly higher in all the extract treated group compared with infected untreated group.

However, the results of the mean group PCV of Trypanosoma b. brucei infected Wister rats treated with aqueous leaf extract of *A. nilotica* are presented in figure 7. By day 10 of Post infection, there w significant ($p < 0.05$) reductions in PCV of all the infected groups (25mg, 50mg, 100mg, 200mg DA 3.5mg and IU) when compared with Uninfected group. This reduction was later reversed in group treated with diminazene aceturate but continued to decreased in all the other treated groups till the end of the experiment. There was significant decrease of mean PCV of infected untreated group. Between days 10 to 20 of post infection compared to group treated with 200mg, 100mg and 50mg of the extract.

Mean group Body weight change (g) of *Trypanosoma b. brucei* -infected rats treated with Methanol and Aqueous leave extract of *A. nilotica*

The results of the mean group body weight changes of *Trypanosoma brucei brucei* infected rats treated with methanol leaves extracts of *A. nilotica* are presented in figure 8. The present finding showed that the extract improved the body weight of rat. From the first 15 days of the experiment the group treated with 200mg dose of the extracts and D.A 3.5mg significantly increase body weight. By day 20 of post infection, groups of rats treated with 50mg, 100mg, 200mg had significantly ($p < 0.05$) higher body weight than those in untreated group. There was no significant difference between group treated with high dose of 200mg of the extracts and the standard drug (DA 3.5mg) by day 20 post infection. The mean group body weight changes of *Trypanosoma brucei brucei* infected rats treated with aqueous leaves extract of *A. nilotica* are presented in figure 9. The present finding showed that the extract improved the body weight of rat except group treated 25mg and IU group. From the first 15 days of the experiment the group of rats treated with 50mg, 100mg, 200mg doses of the extracts and DA3.5mg significantly increase body weight. By day 20 of post infection, groups of rats treated with 50mg, 100mg, 200mg had significantly ($p < 0.05$) higher body weight than those in infected

untreated group. There was no significant difference between group treated with high dose of 200mg of the extracts and the recommended standard drug (DA3.5mg) by day 20 post infection.

Table 1: Phytochemical Contents of Aqueous and Methanol Extract of *Acacia nilotica*

Chemical Compounds	Aqueous Extract	Quantity	Methanol Extract	Quantity
Alkaloids	++	12.30±0.2	+	09.6±0.1
Flavonoids	+++	86.47±0.5	+++	67.5±0.2
Saponins	++	12.14±0.2	+	08.7±0.1
Tannins	++	19.02±0.3	+	12.3±0.4
Carbohydrates	++	19.07±0.4	+	16.4±0.5
Cardiac glycosides	+ +	28.20±0.2	+	13.4±0.2
Steroids	++	12.10±0.2	+	05.8±0.4
Balsams	+	06.12±0.3	+	04.3±0.2
Terpenoids	++	10.25±0.3	+	08.9±0.6

Table 2: Observations for Acute Toxicity of Aqueous and Methanol Extracts of *Acacia nilotica* on rats for 72 hours

Parameters for Assessment	Extracts (Mg/Kg)						
	Control	AqE 1000	AqE 3000	AqE 5000	MeE 1000	MeE 3000	MeE 5000
Food intake	N	N	N	N	N	N	N
Water intake	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N
Eye Color	N	N	N	N	N	N	N
Movement	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N
Aggressiveness	N	N	N	N	N	N	N
Sedation	N	N	N	Ab	N	N	Ab
Death/Survive	S	S	S	S	S	S	S

Key: AqE =Aqueous Extract; MeE=Methanol Extract; N =Normal; Ab =Abnormal; S=Survive; D =Death

Table 3: Assessments of Sub-acute toxicity of Aqueous and Methanol Extracts of *A. nilotica* on rats for 21 days

Parameters for Assessment	Extracts (Mg/Kg)						
	Control	AqE 1000	AqE 3000	AqE 4000	MeE 1000	MeE 3000	MeE 4000
Food intake	N	N	N	N	N	N	N
Water intake	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N
Eye Color	N	N	N	N	N	N	N
Movement	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N

Aggressiveness	N	N	N	N	N	N	N
Sedation	N	N	N	N	N	N	N
Death/Survive	S	S	S	S	S	S	S

AqE =Aqueous Extract; MeE=Methanolic Extract; N =Normal; Ab =Abnormal; S=Survive and D =Death

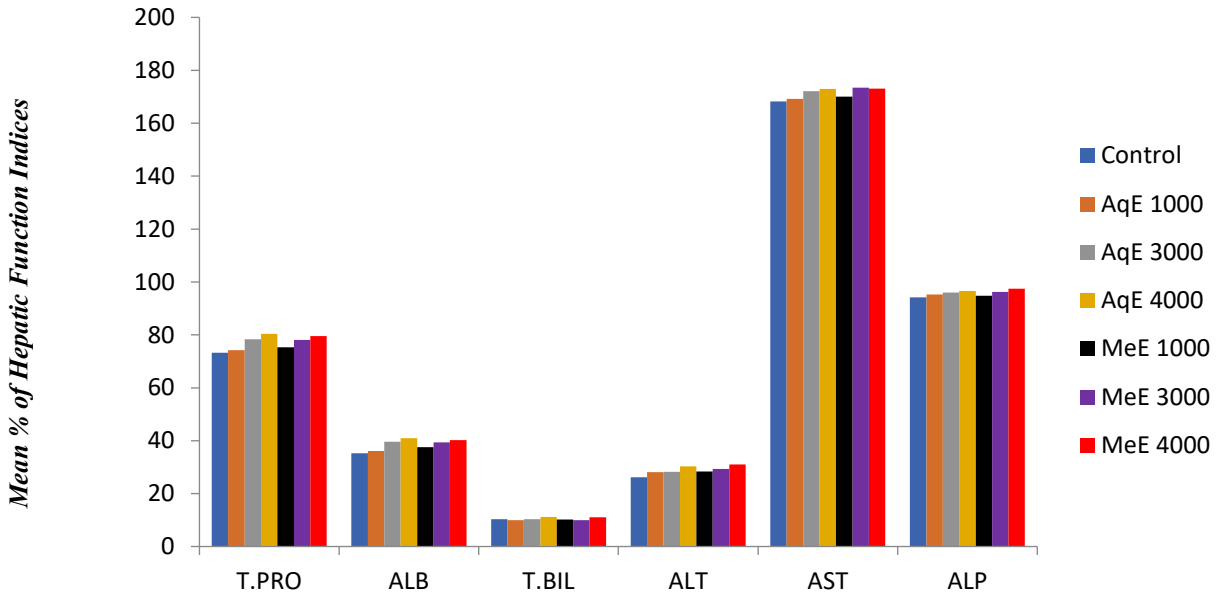

Figure 1: Effects of the Extracts of *A. nilotica* on Serum Biochemical Parameters for Hepatic Function Indices in Rats

Key: AqE (Aqueous Extract); MeE (Methanol Extract); T.Pro (Total protein); ABL (Albumin); T.Bil (Total bilirubin); ALT (Alanine transaminase); AST (Aspatate transaminase); ALP (Alkaline phosphatase).

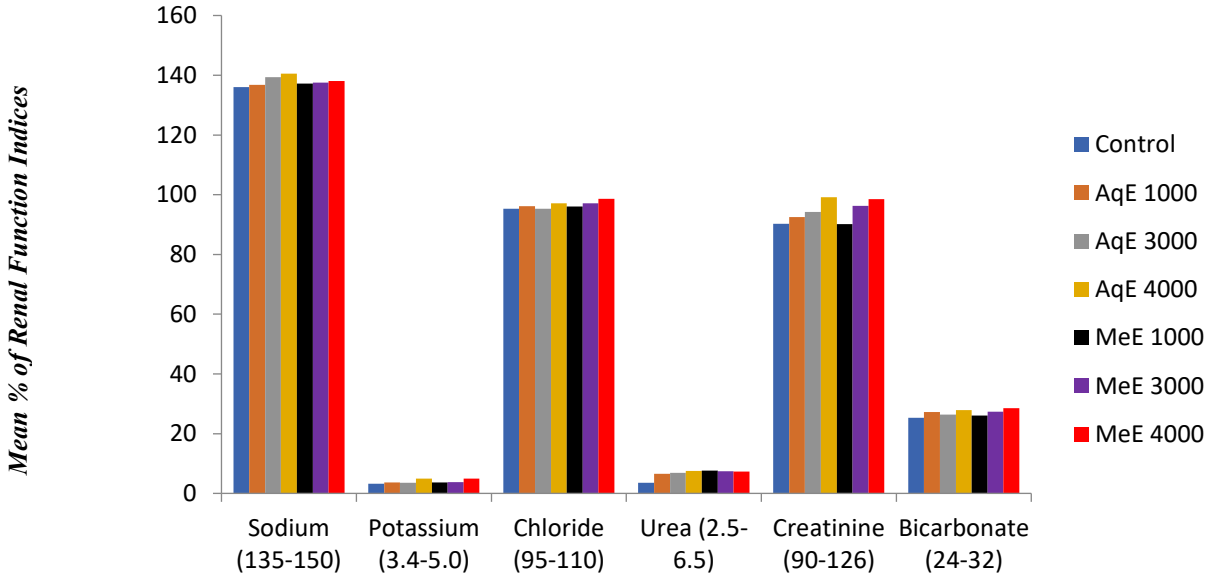

Figure 2: Effects of the Extracts of *A. nilotica* on Serum Biochemical Parameters for Renal indices in Rats

Key: AqE = Aqueous Extract; MeE= Methanol Extract.

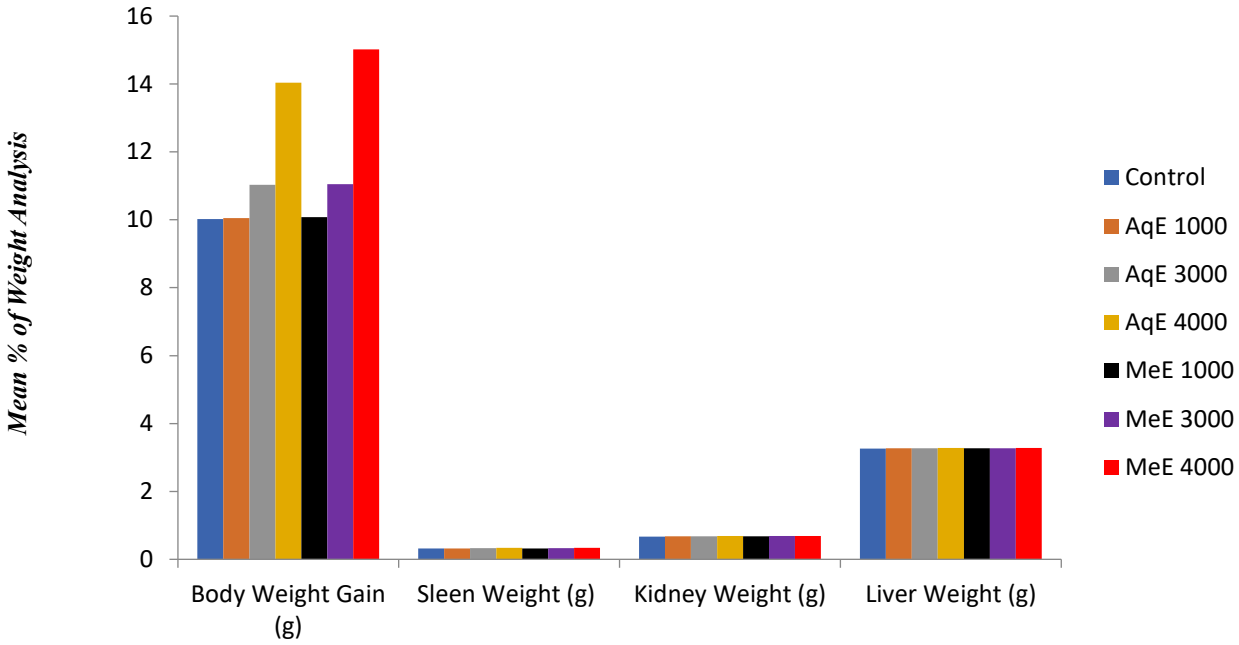

Figure 3: Effects of the extracts of *A. nilotica* on weight analysis in Rats
Key: AqE = Aqueous Extract; MeE= Methanol Extract.

Micrograph of liver and kidney of Rats treated with Aqueous Leave Extract of *A. nilotica*

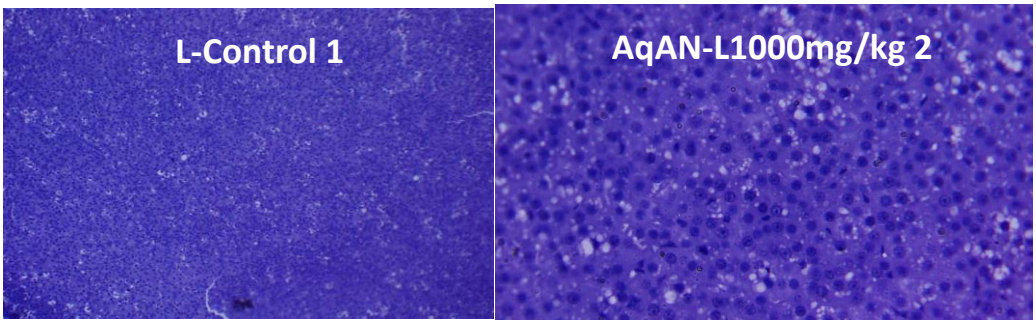

Plate 1: Micrograph of the liver from the control rat showing normal hepatocytes
Plate 2: Micrograph of the liver from rats treated with 1000mg/kg showing a preserved hepatic architecture, and many pyknotic nuclei.

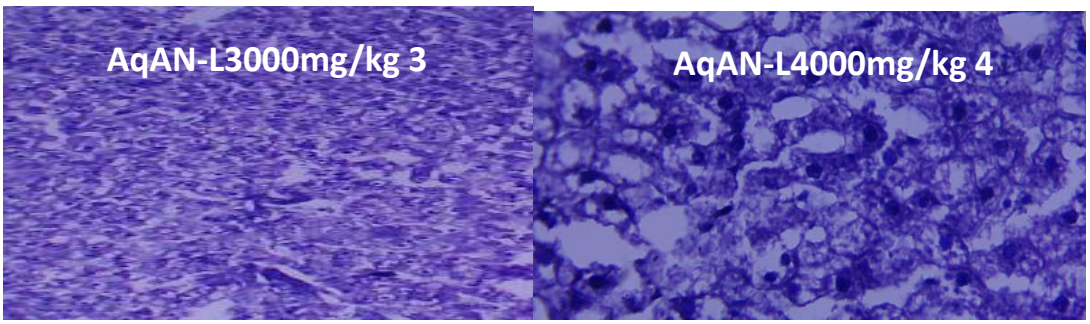

Plate 3 and 4 Micrograph of the liver from rats treated with 3000mg/kg and 4000mg/kg showing mild dilation of sinusoids, and mild disorganization of hepatic cords.

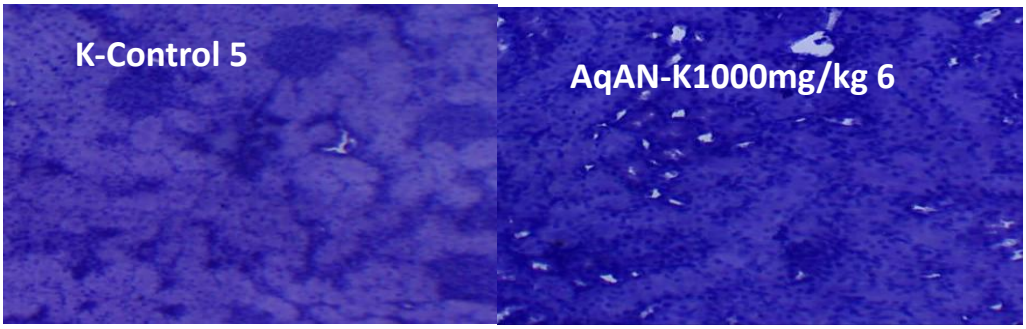

Plate 5 and 6: Micrograph of the kidney from the control rat showing the normal architecture of glomeruli and tubules

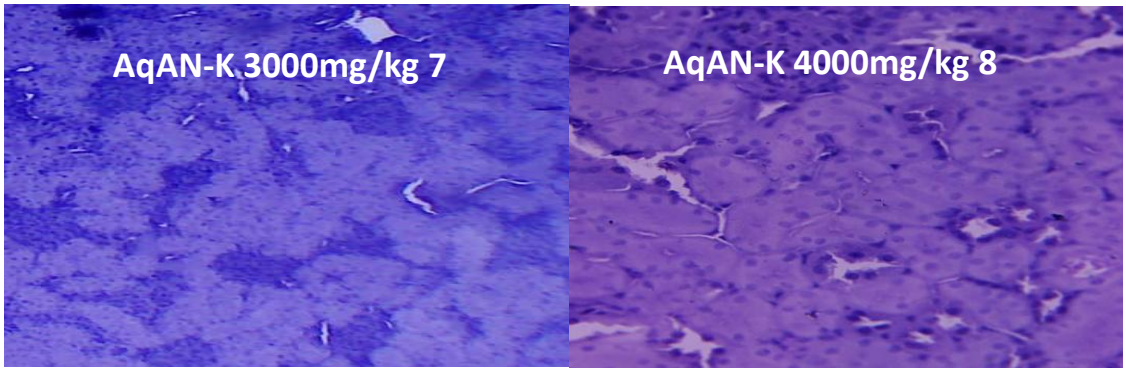

Plate 7 and 8: Micrograph of the kidney from rats treated with 3000mg/kg and 4000mg/kg showing mild reduction of glomerulus cells

Micrograph of liver and kidney of Rats treated with Methanol Leave Extract of *A. nilotica*

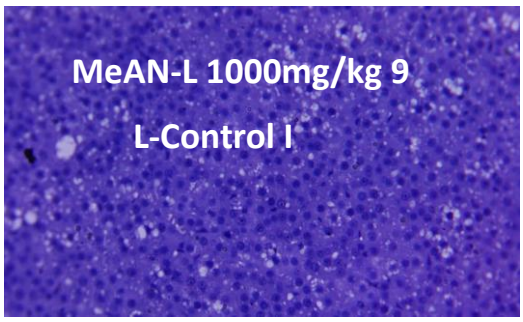

Plate 9: Micrograph of the liver from rats treated with 1000mg/kg showing normal features of hepatocytes

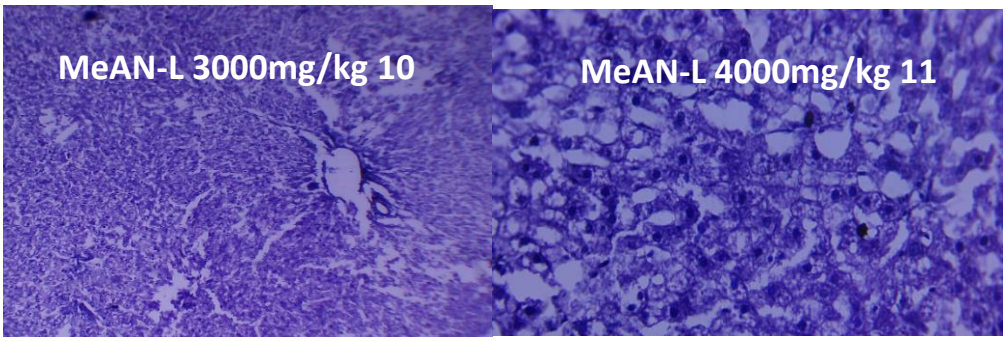

Plate 10 and 11: Micrograph of the liver from rats treated with 3000mg/kg and 4000mg/kg showing hepatocytes with mild steatosis

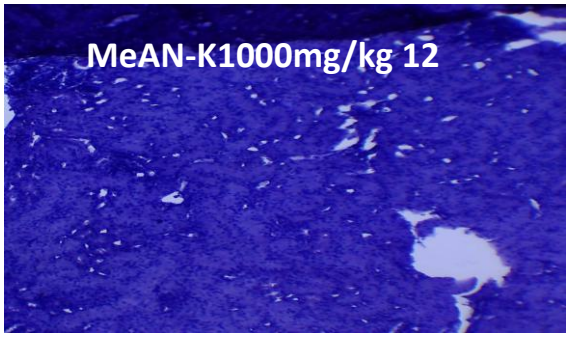

Plate 12: Micrograph of the kidney from rats treated with 1000mg/kg showing mild mesenchymal proliferation

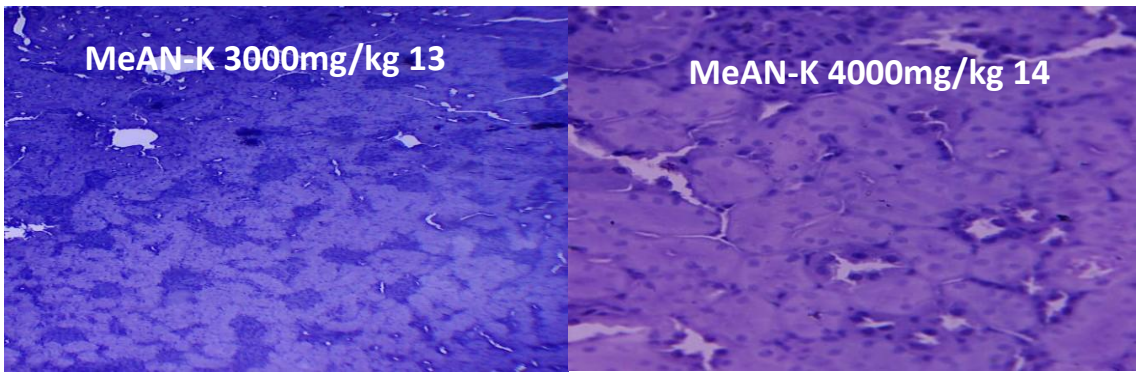

Plate 13: Micrograph of the kidney from rats treated with 3000mg/kg showing mild mesenchymal proliferation

Plate 14: Micrograph of the kidney from rats treated with 4000mg/kg showing mild tubules and moderate mesenchymal proliferation.

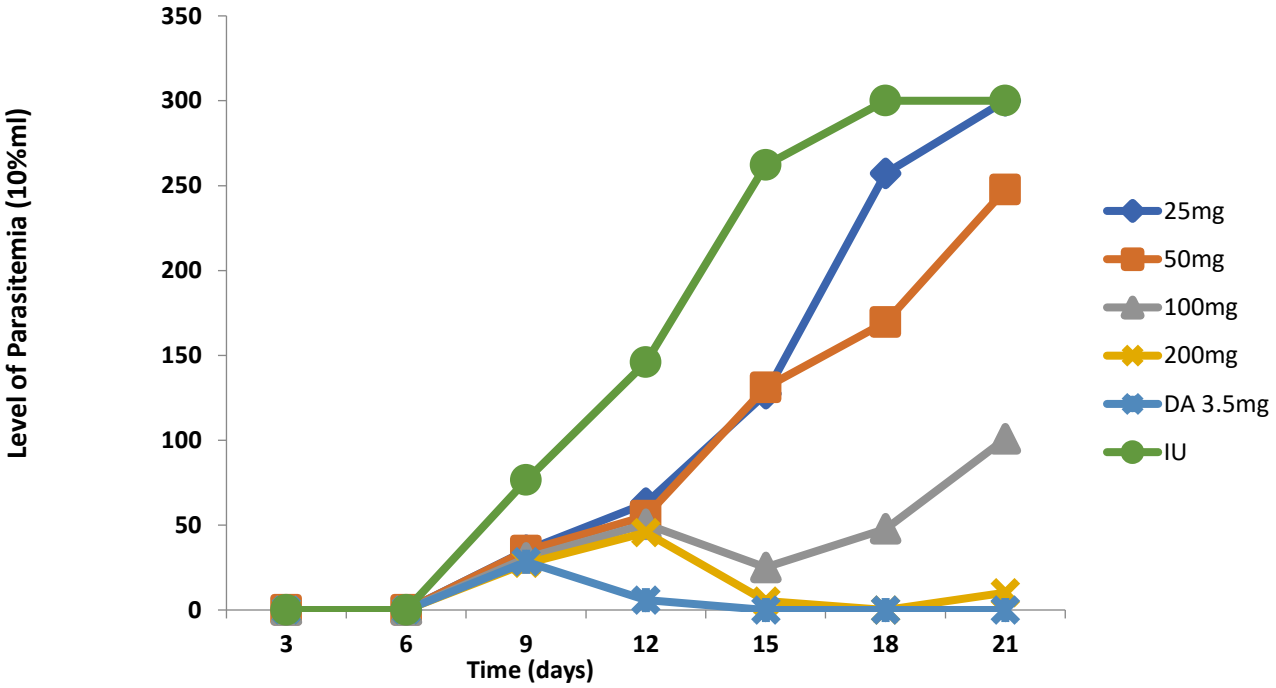

Figure 4. Effects of Methanolic leaves extracts of *A. nilotica* on mean parasitemia (10^6) of Rats infected with *Trypanosoma b. brucei*

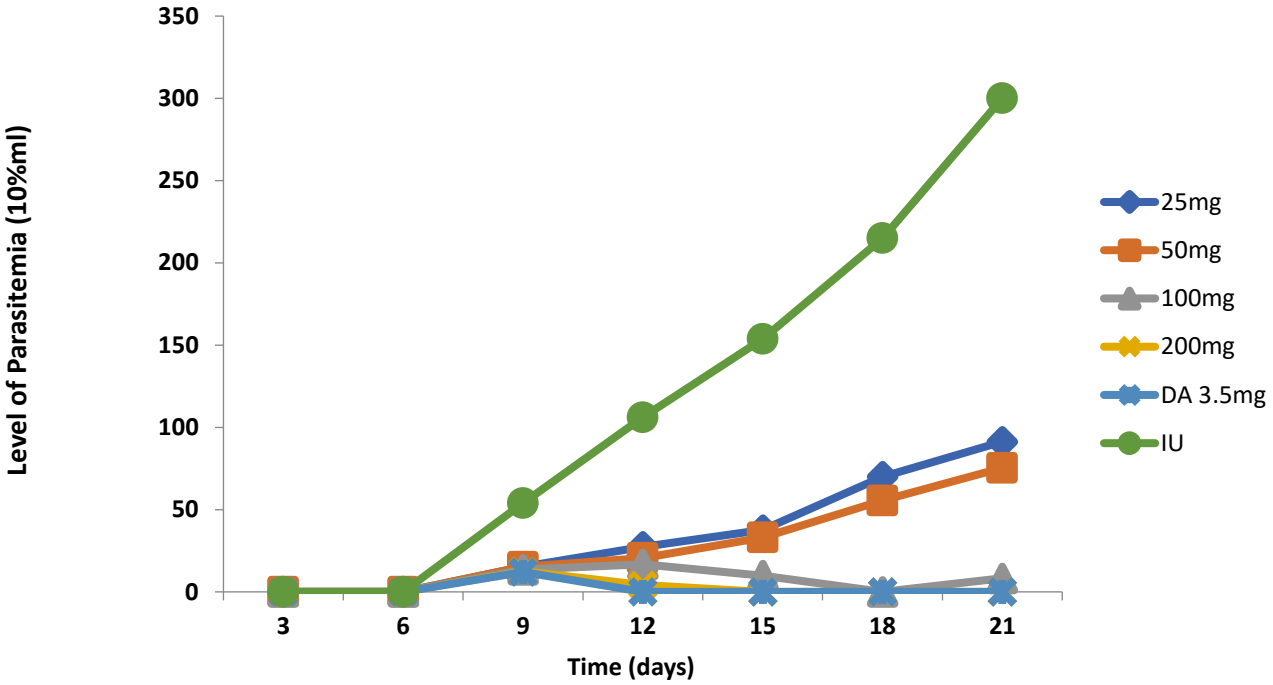

Figure 5. Effects of Aqueous leaves extract of *A. nilotica* on mean parasitemia (10^6) of Rats infected with *Trypanosoma b. brucei*

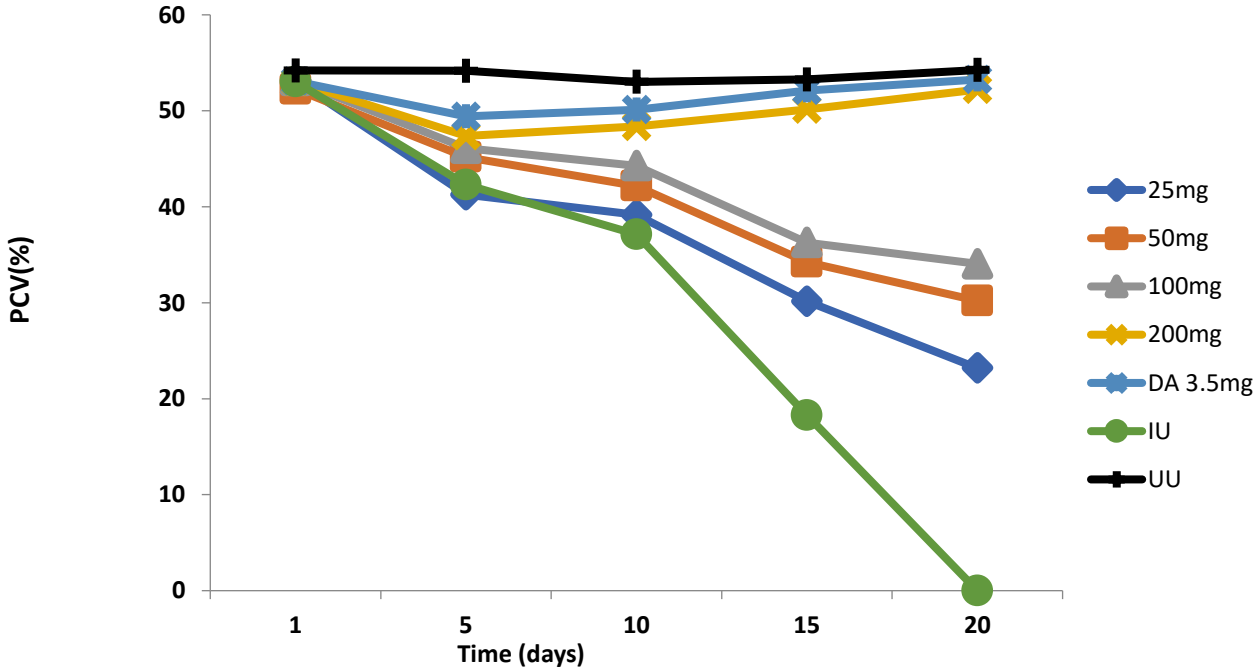

Figure 6. Effects of Methanolic leave extracts of *A. nilotica* on mean packed cell volume (%) of Rats infected with *Trypanosoma b. brucei*

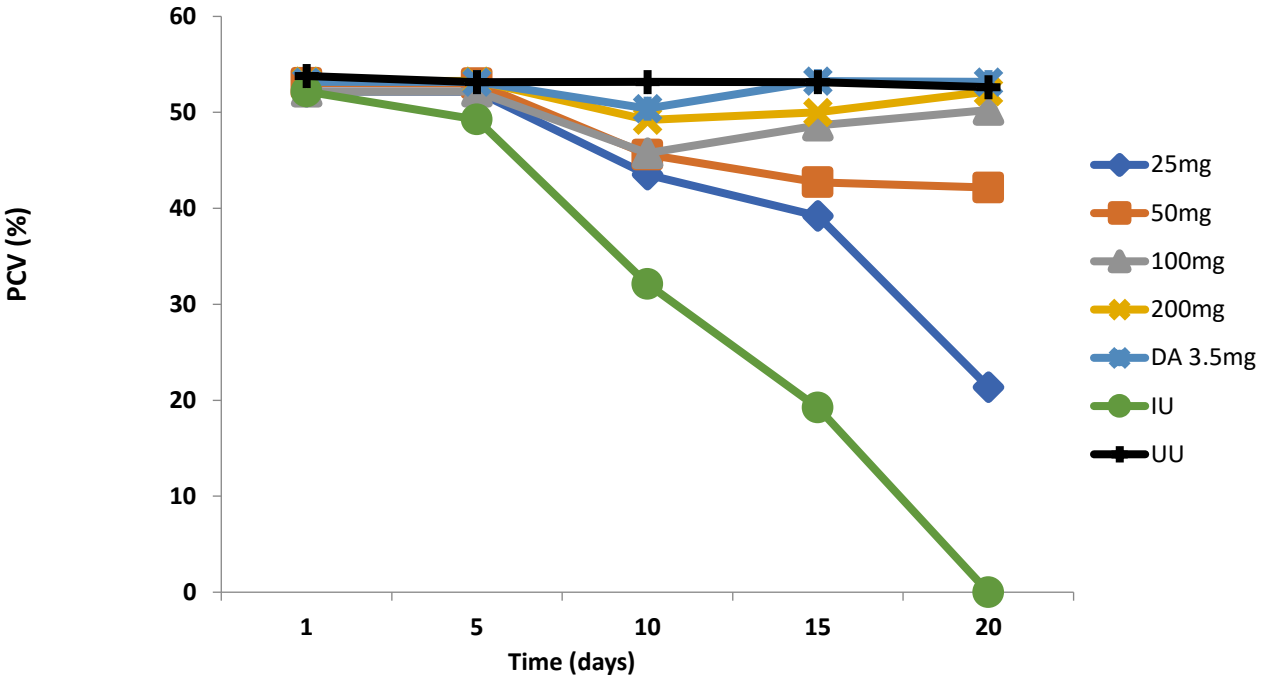

Figure 7. Effects of Aqueous leave extract of *A. nilotica* on mean packed cell volume (%) of Rats infected with *Trypanosoma b. brucei*

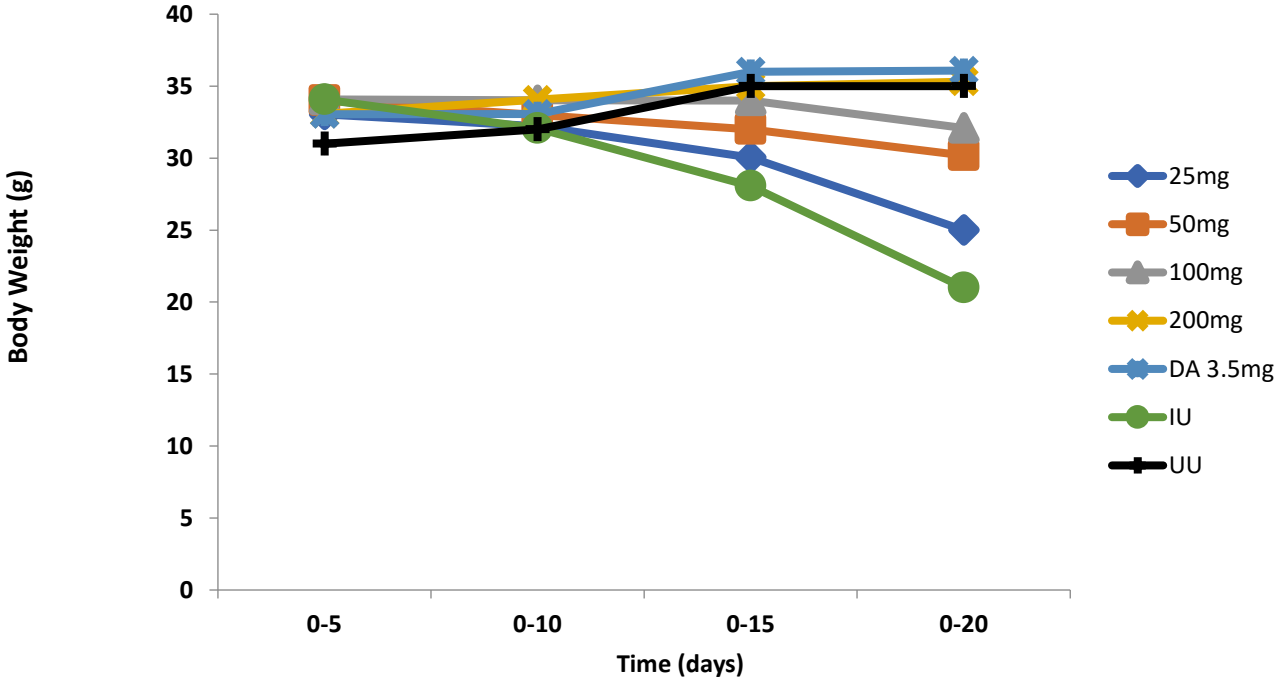

Figure 8: Effects of Methanol leave extracts of *A. nilotica* on Mean group Body weight change (g) of Rats infected with *Trypanosoma b. brucei*

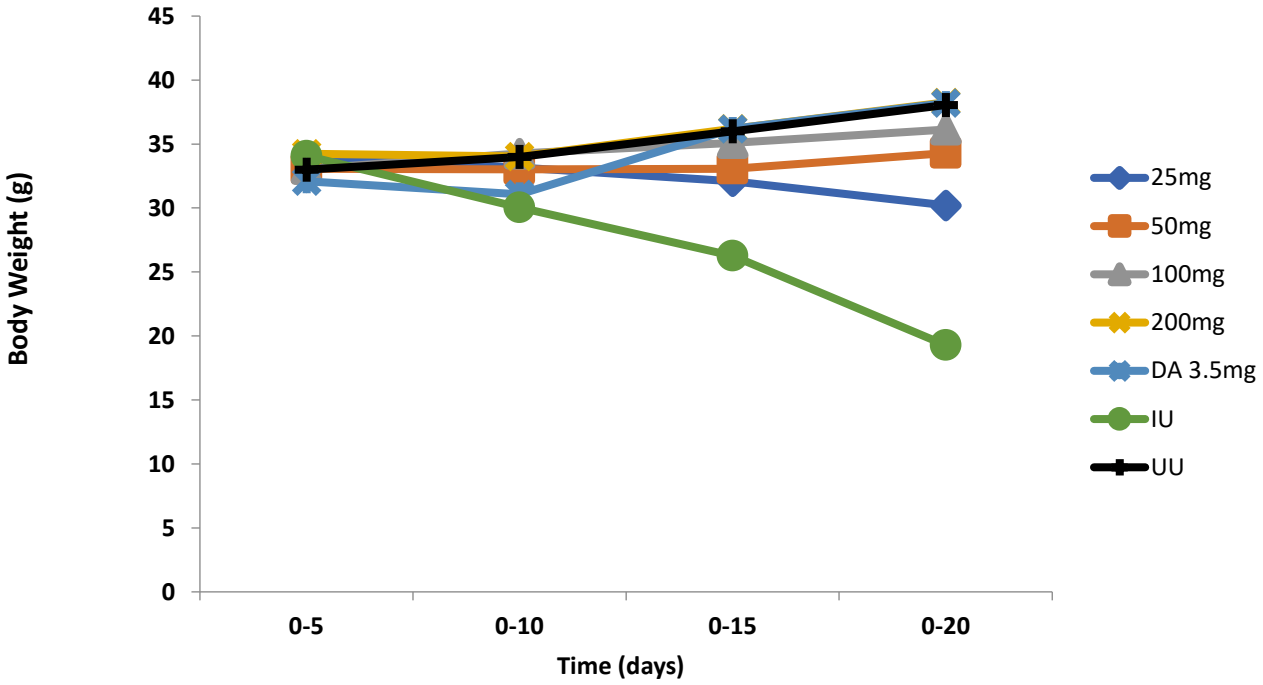

Figure 9: Effects of Aqueous leave extract of *A. nilotica* on Mean group body weight change (g) of Rats infected with *Trypanosoma b. brucei*

DISCUSSION

Medicinal plants are widely distributed within the plant kingdom [35]. The use of medicinal plants for their pharmacological properties is being reported in different countries. [35,36]. Plant-derived phytochemicals have been used as the foundation for treating a wide range of illnesses [37]. Accordingly, it is necessary to get scientific confirmation of the general toxicity of the plants and their compounds. Therefore, understanding their safety and any potential adverse effects is crucial to using them effectively in disease management [38, 39]. Table 1 displays the findings of the initial phytochemical screening, both qualitative and quantitative screening on *A. nilotica* extracts in methanol and aqueous forms. Previous studies indicate that the phytochemical metabolites that have been identified are linked to a range of biological activities, including anti-bacterial, anti-fungal, anti-viral, anti-helminthic, anti-allergens, anti-inflammatory, antidiarrheal, anti-septic, anti-fungal properties, anti-parasitic, anti-irritant, anti-oedema, and antitussive, and they also reduce bleeding, improve wound healing, alleviate epilepsy, and treat viral, cold, cough, and acute bronchial disease[40, 41, 42, 43]. Variations in the phytochemicals obtained could result from the extraction solvents involved.

Evaluating the safety of plant extracts for human and animal use is a fundamental requirement to continue the development of new therapies within the framework of regulatory, preclinical, and clinical guidelines [44]. The acute toxicity of the aqueous and methanol extracts from *Acacia nilotica* was ascertained (Table 2). there was no mortality for groups that received extract doses of 1000, 3000, and 5000mg/kg for a 72-hour period. However, for the animals given both extracts, sedation was observed.

These findings suggest that the fatal doses for aqueous and methanolic leaf extracts for *A. nilotica* must exceed the maximum dose of 5000 mg/kg body weight. The fact that animals given such large dosages did not die or exhibit any severe toxic symptoms might be a key indicator of the plant extracts' relative safety. [45] Previously conducted studies that indicate substances having an LD50 between 50 and 500 mg/kg body weight are considered highly toxic, those having an LD50 between 500 and 1000 mg/kg body weight but not exceeding 1000 mg/kg are considered moderately toxic, and those having an LD50 exceeding 1000 mg/kg body weight

are considered relatively safe (no toxicity). The sedation signs and aberrant movements observed in rats treated with 5000mg/kg in this study were probably due to flavonoids present in the extracts. The aberrant movements were suggestive of neurological deficits. It is known that plants high in flavonoids and alkaloids produce neurological defects in animals [46]. Sedation ensures that toxicants like rotenoids (flavonoids), are frequently responsible for the bioactivities [47,48].

Histopathological analyses were also conducted to rule out any potential complications that might be occurring at the cellular level, even though the physical characteristics of the rats indicated none of the doses (1000, 3000, and 5000 mg/kg) of aqueous and methanolic leaf extracts were lethal. The sub-acute study showed that 1000, 3000 and 4000 mg/kg/day of the aqueous and methanolic extract in adult rats was well tolerated (Table 3). In this study, the biochemical analysis showed a slight, non-significant increase in Alanine transaminase, Aspatate transaminase, and Alkaline phosphatase at high doses (5000mg/kg) in rats treated with Aqueous and methanol extracts in *A. nilotica* compared to control. While rats treated with lower and medium doses were able to decrease the enzyme level and protein, bilirubin, and albumin levels of the treated rats, and the control are within the normal level. Therefore, the variations are not attributed to the toxic effects of the extracts. This result corroborated earlier research [49] who stated that the absence of rising alanine transaminase, aspartate transaminase, and alkaline phosphatase is indicative of normal liver function. The average creatinine and urea levels were within the normal range, although there was slight increase compared to the control in all the group rats treated with aqueous and methanolic extracts. These results suggest that *A. nilotica* has no disastrous effects but rather a protective effect on the kidney. The results of this study show a normal state of renal function, which is consistent with the other findings [50], who previously reported that measurements of urea, creatinine, and uric acid can be used simultaneously to assess kidney function; normal levels indicate a lower risk of problems with the renal system. The kidneys' integrity was maintained and their function was unaffected by extracts in either aqueous or methanolic form [51, 52, 53]. The differential dosage concentrations of *A. nilotica's* aqueous and methanolic leaf extracts appeared to impact body weight increase and detached organ weight of liver, kidney, and spleen. The more substantial body weight gain in the rats may be related to increased metabolic activity. This finding was supported by other researchers [54, 55]. The photomicrographs of sections of the liver, and kidney of rats treated orally with aqueous and methanolic leaf extracts of *A. nilotica* at doses of 1000, 3000, and 4000mg/kg for 21 days showed mild histological changes. The absence of major histological change is consistent with the biochemical parameters measured. This indicates that *A. nilotica* leave extracts at doses of 1000, 3000, and 4000mg/kg for a short duration might have no toxic effect. A similar result was also obtained in the toxicity studies of polyherbal formulations in rats where no significant toxic effects were observed on the physical signs and symptoms, weight gain, food intake, biochemical parameters, and histopathology of vital organs [56, 57]. It may be reasoned that *A. nilotica* extracts do not exhibit hepatotoxicity and nephrotoxicity sufficient to significantly impact the functioning of the liver and kidney in light of the disparity between the biochemical and histological results.

The prepatent time of *Trypanosoma b. brucei* in rats treated with aqueous and methanolic leaf extracts was 9 days for *A. nilotica.* In a comparable experimental study using *T. congolense*, another type of animal trypanosome, there was a record of 3 days prepatent time after the parasite had been introduced in the mice [58]. The length of the prepatent period is determined by the strain of the parasites and the host's immune status. The findings indicated that the aqueous and methanol leaf extracts from *A. nilotica* eliminated the parasites from the bloodstream of the infected rats with 200mg/kg. In comparison, lower doses (100mg/kg, 50mg/kg, and 25mg/kg) greatly reduced the parasite's load. Still, they did not eliminate parasites from the infected rats' bloodstreams, compared to the negative control. The reduction in parasitemia reported was dose-dependent. The presence of important phytochemical substances (flavonoids, alkaloids, saponins, tannins, steroids,

glycosides, and terpenoids) in the extracts provides a logical explanation for the antitrypanosomal effects demonstrated in this study. Similar research suggested that flavonoids potentiate trypanocidal activities [59]. Additionally, it has been demonstrated that flavonoids successfully lower animal mortality, histopathological damage, and parasite burdens [60, 61, 62, 63]. In addition to their immunomodulatory functions, flavonoids have been demonstrated to exhibit strong antiprotozoan efficacy against a wide range of pathogens, including Trypanosoma spp. These pathogens' production of several heat shock proteins is downregulated by flavonoids [64]. Alkaloids have been demonstrated to have anti-trypanosomal properties [65].

The mean group PCV of *Trypanosoma b. brucei* infected rats treated with methanol and aqueous leaf extract of *A. nilotica* revealed that by day 5 post-infection, there were reductions in PCV of all the infected groups. The reduction was later reversed in the group treated with the highest dose of extract (200mg) and the standard drug (diminazene aceturate) but continued to decrease in all the other treated groups till the end of the experiment. The improvement in PCV in the treatment group and prevention of subsequent decline reported with both extracts, particularly at the end of the experiment when parasitemia levels were very low, may indicate an antitrypanosomal action of the extracts. The increase in PCV might be related to a decrease in parasite proliferation, suppression of toxic compounds produced by trypanosomes, or scavenging of trypanosome-associated free radicals [66]. In this study, the experimental animals infected with *T. brucie brucie* manifested weight loss. However, treatment with the methanol and aqueous leaf extracts of *A. nilotica* and diminazene aceturate prevented the loss of body weight associated with parasitemia compared to negative controls. The reduction in parasitemia brought on by treatment may have contributed to the weight gain by lowering the parasite burden in the bloodstream which otherwise would lead to depressed appetite and food intake during fever peaks. This observation aligns with previous research [67, 68] suggesting a decrease in body weight in trypanosomiasis is linked to an increase in parasitemia, reduced food intake, disrupted metabolic function, and hypoglycemia. However, it differs from [69], who found that the standard dose of diminazene aceturate (3.5 mg/kg) could not prevent weight reduction, thus, it failed to eliminate parasitemia.

CONCLUSION

Toxicity studies revealed extracts of *A. nilotica* were not toxic at dose levels of 1000, 3000, and 5000mg/kg of body weight of rats. However, sedation and abnormal movements were demonstrated at 5000mg/kg of aqueous and methanol extracts of *A. nilotica* and *Z. mucronata*. The extracts do not exhibit hepatotoxicity and nephrotoxicity sufficient to significantly impact the functioning of the liver and kidney in light of the disparity between biochemical and histological results. Also, the body and associated organ weight are unaffected by different dose concentrations of the extract. *In vivo* assay showed extracts of *A. nilotica* have varying effects as in reduced parasitemia levels, prevented the decline in packed cell volume, and prevented body weight loss of rats infected with *T. b. brucie.* Aqueous extract had the highest efficacy compared to methanol extract. Accordingly, they have the potential to contribute in controlling Animal African Trypanosomiasis.

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