Biological and Biomedical Journal

Journal homepage: https://bbj.journals.ekb.eg

Effect of *Moringa oleifera* leaves extract on biochemical, hematological and histopathological parameters in *Trypanosoma brucei* infected rats

Abdulganiyu M. Galadima[1], Grace O. Ezimaduakolam[1], Ibrahim Abubakar[2], Said S. Said[3], Sanusi U. Farouq[3]

[1]Department of Biochemistry, School of Biological Sciences, Federal University of Technology Owerri, Imo State, Nigeria.

[2]Department of Biology-Chemistry, Idris Koko Technical College, Farfaru, Sokoto, Nigeria.

[3]Department of Biochemistry and Molecular Biology, Faculty of Life Science, Federal University, Dutsin-Ma, Katsina, Nigeria.

ARTICLE INFO

Received: 18/1/2025

Revised: 30/7/2025

Accepted: 21/8/2025

Corresponding author:

Abdulganiyu M. Galadima, Ph.D

E-mail: amgaladima001@gmail.com

Mobile: (+2)348062922688

P-ISSN: 2974-4334

E-ISSN: 2974-4324

DOI: 10.21608/BBJ.2025.350281.1072

ABSTRACT

Trypanosomiasis remains an endemic disease in several societies affecting human and animals, causing a high mortality rate. *Moringa oleifera* has been used traditionally in the treatment of many diseases, including infections, cardiovascular diseases, hypertension, and cancers. This study aims to evaluate the effect of *M. oleifera* leaves extract (MOLE) on biochemical, hematological, and histopathological parameters in *Trypanosoma brucei* infected rats. The trypanosoma infection was induced in rats using the rapid matching method. Hematological parameters were analyzed using a standard hematology analyzer. Biochemical parameters were analyzed by ELISA and spectrophotometric methods. MOLE with concentrations of 400 and 800 mg/kg significantly ($p< 0.05$) reduced parasitemia level in the infected rats. The RBC, PVC, Hb, WBC, PLT, MCV, MCH, and MCHC contents were significantly ($p< 0.05$) increased in the infected treated rats. Treatment of the infected rats significantly ($p< 0.05$) decreased the level of ALT, AST, ALP, total bilirubin, urea, creatinine, total cholesterol, TAG, LDL-C, and VLDL-C. However, a significant ($p< 0.05$) increase in total protein, albumin, globulin, HDL-C, sodium, potassium, and chloride levels was observed in the infected treated rats. The adverse changes in the liver and kidney tissues of the infected rats were significantly ($p< 0.05$) reversed to normal hepatocytes and normal components of the portal triads in the treated rats. MOLE demonstrated anti-trypanosomal activity and diminished the adverse alterations in the biochemical, hematological and histopathological parameters induced by *T. brucei* in the rats.

Keywords: Anti-trypanosomal, Infection, *Moringa oleifera*, Parasite, *Trypanosoma brucei*

1. Introduction

Trypanosomiasis remains a major health problem for human and animals associated with high mortality and morbidity rates. Trypanosomosis is an endemic disease, especially in African countries, associated with many complications. Trypanosomiasis is mainly caused by *Trypanosoma brucei, T. congolense*, and *T. vivax* and transmitted via the activity of the vector, tse tse fly (Odeniran and Ademola, 2018). Trypanosomal infection is characterized by several symptoms, including severe anemia, loss of weight, infertility, and miscarriage (Adeyemi et al., 2012). Reports showed that about 70 million people have been affected by trypanosomiasis and its complications worldwide (Holanda-Freitas et al., 2020; WHO, 2021). However, it has been reported that about 3 million animals are die each year due to trypanosomal infection (Ogbadoyi et al., 2007). African countries recorded the highest

prevalence rate of trypanosomiasis due to the abundant availability of tsetse flies in the African region. In Nigeria, human and animals have been threatened by the trypanosomal infection and its complications, particularly in local communities. The spread of the disease to many local populous continues increasing in the country, causing high mortality rate (CFSPH, 2009).

Conventional treatments of trypanosomosis are associated with several challenges, including side effects of the drugs, toxicity, parasite resistance, and the lack of inadequate effective vaccine (WHO, 2021). Medicinal plants have highly effective, safe, and affordable treatments for trypanosomiasis and its complications. Many years ago, plants and herbs were used in the treatment of various diseases. Medicinal plants have significant importance in research and industry and serve as sources of bioactive compounds that are important in drug synthesis and development (Abubakar et al., 2021). Plants and herbs have been used in the treatment of many parasite infections and their complications. Medicinal plants are available in the local communities, produce few side effects, and cost less to purchase than conventional treatments. Medicinal plants demonstrated antioxidant properties (Ibrahim et al., 2024) and several pharmacological activities, including anti-ulcer (Abubakar et al., 2020a, b; Abubakar et al., 2021), anti-microbial (Falowo et al., 2018), and analgesic activity (Abubakar et al., 2024).

Moringa oleifera Lam is a monogeneric plant that belongs to the family Moringaceae and is widely available in the world (Abd et al., 2022). *M. oleifera* originates from the Northwest region of India and is widely available in the world, especially in African countries (Reham and Noorah, 2024). *M. oleifera* is commonly called "miracle tree" because of its local medicinal uses (Rode et al., 2022; Ali et al., 2021). *M. oleifera* has been used for the management of various diseases such as infections, cardiovascular complications, hypertension, cancers, bladder problems, and sores (Ishola et al., 2018). *M. oleifera* has nutritional value, medicinal properties, and industrial uses. Also, it has antioxidant properties and pharmacological activities due to its various phytoconstituents (Abd et al., 2020; Lin et al., 2021; Younis et al., 2022; Khalid et al., 2023). The roots, leaves, fruits, seeds, and flowers have been used for the treatment of cancers, paralysis, helminthic bladder problems, prostate problems, sores, and skin infections (Ishola et al., 2018). The leaves of the plant demonstrated antioxidant properties and cytoprotective effect against cellular peroxidation and apoptosis (Khalid et al., 2023; Lin et al., 2021).

In Nigeria, *M.oleifera* is abundantly found in many local communities and is locally known as Zogale (Hausa), Odudu oyibo (Igbo), and Aweigbale (Yoruba). Herbalists in different local communities in Nigeria have been claiming the use of different parts of *M. oleifera* for the treatment of parasitic diseases. Studies should be conducted to scientifically validate the claims of local herbalists on the use of *M. oleifera* for the management of parasitic diseases. This study aims to evaluate the effect of *M. oleifera* leaves extract (MOLE) on biochemical, haematogical, and histopathological changes in *Trypanosoma brucei*-infected rats.

2. Materials and Methods

Collection and authentication of the plant sample

With the help of a local herbalist, *M. oleifera* L. leaves were collected from the Umudike forest located in Umudike community, Ikwuano Local Government Area, Abia state, Nigeria. The sample was authenticated at the Herbarium Unit, Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia, Nigeria. The voucher number (MOUAU/CVM/VPP/HERB/16/009) was deposited in the Herbarium Unit of the university.

Preparation of the plant extract

Preparation of the *M. oleifera* leaves extract (MOLE) was carried out according to the method described by Abubakar *et al.* (2022) with certain modifications. The fresh leaves were washed thoroughly with distilled water and then dried in a clean, ventilated laboratory for two weeks. The dried leaves were pulverized into fine powder using a pestle and mortar. Five hundred grams of the leaves powder were soaked in 1.5 liters of ethanol for two days with constant stirring at intervals of two hours. The extract was filtered

through Whatman filter paper, and the filtrate was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40 ^{0}C for three hours. The weight and percentage yield of the extract were 110.94 g and 22.19 %, respectively. The MOLE was stored at 4 ^{o}C until further analysis.

Experimental animals

A total of 25 male and female Wistar rats (age 8-10 weeks) weighing between 180- 200 g were used in this study. The animals were purchased from the Animals Laboratory Unit, Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, Abia, Nigeria. The rats were distributed into five aluminum cages, five rats in each, at a temperature of 22 ± 3 ^{o}C, relative humidity of 30-70%, and a 12/12 hours light-dark cycle. The rats were fed with standard grower pellets and had access to water *ad libitum*. Before the experiment, the rats were acclimatized for 7 days. The experiments were conducted based on the guidelines of the Organization for Economic Cooperation and Development (OECD) 423 for the care and use of laboratory animals (OECD: 2001/423/12-14).

Induction of *Trypanosoma brucei* infection

T. brucei was obtained from the Vom Veterinary Research Institute, Jos, Plateau State, Nigeria. The trypanosoma infection was induced in the rats using the standard rapid matching method as described by Herbert and Lumsden (1976) with some modifications. *T. brucei* was inoculated in the animals via intraperitoneal injection of 0.3 mL of normal saline containing 1 mL of the infected blood (3 x 10^6 per mL of blood). The rats were allowed for one week to get fully infected, after which the infection was confirmed. The level of parasitemia in the infected rats was monitored by microscopic examination of the rats' tail blood at 40x magnification using the Rapid Matching method. Monitoring of parasitemia was performed every 4 days for 12 days to reduce stress on the rats.

Experimental design

The male and female rats (n = 25) were distributed into five groups of five rats each. Group 1(Gp1) (normal control); the rats were not infected with *T. brucei*, and no extract administration. Gp2 (disease control); The rats were infected with *T. brucei* but no treatment was given. Group 3: The animals were infected with *T. brucei* and then treated with the standard drug, diminazene aceturate (20 mg/kg b.wt/day). Gp 4 and 5; The rats were infected with the parasite and then treated with 400 and 800 mg/kg b.wt, respectively. The rats were treated every day for 12 days. At the end of the treatment period, the rats were sacrificed, and blood samples were collected into EDTA and plain bottles for haematological and biochemical analyses, respectively. Liver and kidney tissues were collected in 10% formaldehyde solution for histopathological examination.

Hematological analysis

Determination of the hematological parameters; red blood cells (RBC), haemoglobin (Hb), white blood cells (WBC), platelet count (PLT), packed cellular volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) was performed according to the standard method of Woo (1970) using hematology analyzer (BC-2300 model, Mindray Medical Co., China) according to instructions of the manufacturer.

Biochemical analysis

The biochemical parameters; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (IFCC, 1980), total protein (Tietz, 1995), albumin (Bartholomew and Delany, 1964), total bilirubin and globulin (Tietz, 1995), urea (Ji and Bachmanov (2007), creatinine (Jaffe, 1986), sodium and potassium (AOAC, 1990), chlorides and bicarbonate (APHA, 2005), total cholesterol (TC) (Allain et al., 1974), triglycerides (Mcgowan et al., 1983), and high density lipoprotein-cholesterol (HDL-C) (NCPEP, 2001) were analyzed by ELISA, spectrophotometric, or flame colometric method using standard diagnostic kits (Randox Laboratories Limited, UK, Sigma-Aldrich, USA) according to the manufacturer's instructions.

Histopathological analysis

Liver and kidney tissues of the rats were examined for the histopathological changes using the standard method of Bing and Edward (2015). The tissue slices were fixed in 10% formaldehyde for two days. The specimens were dehydrated by placing them in a mixture of

ethanol and water (70–100%). The dehydrated tissues were then transferred to a mixture of equal volumes of alcohol and xylene for 24 hours and then cleared with two changes of xylene for 60 minutes. They were then infiltrated twice for 60 minutes each with molten paraffin wax in an oven at 60 ^{o}C. The solvent was evaporated, and the spaces within the tissues were filled with paraffin wax. The tissues were embedded in paraffin wax, trimmed and mounted on a wooden chuck, and then sectioned at 5µm thickness in the microtome. The sections (5 µm) were then floated on water, taken to a glass slide, and stained with haematoxylin and eosin stains. The slides were viewed under a light microscope at 40x magnification.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (Version 22, IBM Corp., Armonk, NY, USA). The level of significance between the groups was obtained by One-Way Analysis of Variance (ANOVA) using Dunnett's test and Student's t-test for post-hoc test for the means comparisons. The *p*-value less than 0.05 were considered statistically significant.

3. Results

Effect of MOLE on parasitemia level in *T. brucei*-infected rats

The effect of MOLE on parasitemia level in *T. brucei* infected rats is shown in Table (1). Prior to the treatment, the infected rats showed a highly significant ($p< 0.05$) level of parasitemia compared to the normal control. At days 8 and 12, treatment of the infected rats with MOLE (400 mg/kg, 800 mg/kg) and diminazene (20 mg/kg) significantly ($p< 0.05$) reduced the level of parasitemia in the rats compared with the disease control. However, at day 12 MOLE with a concentration of 800 mg/kg, significantly ($p < 0.05$) lowered the level of parasitemia in the rats, comparable to the standard drug diminazene (20 mg/kg) (Table 1).

Table 1. Effect of MOLE on parasitemia level in *T. brucei* infected rats

Treatment Group	Post-infection x 10^5	Day 4 x 10^5	Day 8 x 10^5	Day 12 x 10^5
Normal control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Disease control	5.53 ± 0.16$^{\#}$	5.63 ± 0.18$^{\#}$	5.78 ± 0.30$^{\#}$	5.82 ± 0.22$^{\#}$
Diminazene (20 mg/kg)	5.63 ± 0.17$^{\#}$	4.33 ± 0.15*	2.33 ± 0.12$^{*\#}$	1.00 ± 0.04$^{*\#}$
MOLE (400 mg/kg)	5.50 ± 0.15$^{\#}$	5.35 ± 0.15$^{\#}$	4.38 ± 0.14$^{*\#}$	2.23 ± 0.11$^{*\#}$
MOLE (800 mg/kg)	5.61 ± 0.23$^{\#}$	5.31 ± 0.14$^{\#}$	3.19 ± 0.13$^{*\#}$	1.16 ± 0.05$^{*\#}$

Values are expressed as mean ± standard deviation (n = 5). $^{\#}p< 0.05$ statistically significant ($p < 0.05$) compared with normal control, $^{*}p < 0.05$ statistically significant ($p < 0.05$) compared with disease control (One-way ANOVA) followed by Dunnett's multiple comparison test. MOLE; *M. olifera* leaves extract.

Table 2. Effect of MOLE on body weight of *T. brucei* infected rats

Treatment Group	Post-infection x 10^5	Day 4 x 10^5	Day 8 x 10^5	Day 12 x 10^5
Normal control	189.60 ± 1.72	190.80 ± 1.53	192.20 ± 1.68	192.80 ± 1.65
Disease control	163.80 ± 1.39$^{\#}$	162.80 ± 1.39	163.20 ± 1.46$^{\#}$	157.60 ± 0.81$^{\#}$
Diminazene (20 mg/kg)	162.20 ± 1.06$^{\#}$	167.00 ± 0.70$^{*\#}$	171.40 ± 1.03$^{*\#}$	191.00 ± 1.44*
MOLE (400 mg/kg)	160.00 ± 0.70$^{\#}$	164.20 ± 0.58$^{\#}$	167.20 ± 0.66$^{*\#}$	181.60 ± 1.83$^{*\#}$
MOLE (800 mg/kg)	160.40 ± 0.50$^{\#}$	167.40 ± 0.40$^{*\#}$	170.00 ± 0.70$^{*\#}$	190.00 ± 0.70*

Values are expressed as mean ± standard deviation (n = 5). $^{\#}p< 0.05$ statistically significant ($p < 0.05$) compared with normal control, $^{*}p< 0.05$ statistically significant ($p< 0.05$) compared with disease control (One-way ANOVA) followed by Dunnett's multiple comparison test. MOLE; *M. olifera* leaves extract.

Effect of MOLE on body weight of *T. brucei* infected rats

Table (2) shows the effect of the ethanolic leaves extract of *M. oleifera* on the body weight of *T. brucei* infected rats. Post *T. brucei* infection, the body weight of all the infected rats was significantly ($p< 0.05$) decreased compared with the normal control. Treatment of the rats with MOLE (400 mg/kg, 800 mg/kg) and diminazene (20 mg/kg) significantly ($p < 0.05$) increased the body weight of the rats. At day 12, the extract (800 mg/kg) demonstrated a significant ($p < 0.05$) increase in body weight of the rats comparable to the standard drug diminazene (20 mg/kg) (Table 2).

Effect of MOLE on renal function parameters in *Trypanosoma brucei-infected* rats

Table 5) indicates the effect of ethanol leaves extract of *Moringa oleifera* on liver function parameters in *Trypanosoma brucei* infected rats. In comparison with the disease control, a significant ($p < 0.05$) decrease in the level of urea and creatinine was observed in the extract (400 mg/kg, 800 mg/kg) treated rats. The concentration of sodium, potassium, and chloride in the extract (400 mg/kg, 800 mg/kg) treated rats was significantly ($p < 0.05$) increased compared with the disease control. However, no significance ($p > 0.05$) change was observed in the level of bicarbonate of the rats treated with the extract (400 mg/kg, 800 mg/kg) and the standard drug diminazene (20 mg/kg) (Table 3).

Effect of MOLE on lipid profile parameters in *Trypanosoma brucei* - infected rats

The effect of ethanol leaves extract of *Moringa oleifera* on lipid profile parameters in *Trypanosoma brucei* infected rats is shown in Table (4). The finding indicated that administration of the extract (400 mg/kg, 800 mg/kg) in the rats significantly ($p < 0.05$) decreased the level of total cholesterol, TAG, LDL-C, and VLDL-C while increasing the HDL-C level compared with the disease control. However, the rats treated with 800 mg/kg of the extract showed a significant ($p < 0.05$) decrease in total cholesterol, TAG, LDL-C, and VLDL-C levels and an increase in HDL-C level comparable to the standard drug Diminazene (20 mg/kg) (Table 6).

Table 3. Effect of MOLE on renal function parameters in *T. brucei* infected rats

Parameter	Normal control	Infected	Diminazene (20 mg/kg)	MOLE (400 mg/kg)	MOLE (800 mg/kg)
Urea (mg/dL)	20.38 ± 0.71	48.35 ± 2.32$^{\#}$	25.12 ± 0.91$^{*\#}$	31.02 ± 1.77$^{*\#}$	26.59 ± 0.92$^{*\#}$
Creatinine (mg/dL)	0.68 ± 0.07	1.31 ± 0.10$^{\#}$	0.72 ± 0.08*	0.95 ± 0.09$^{*\#}$	0.71 ± 0.08*
Sodium (mEq/L)	132.7 ± 0.68	122.07 ± 0.38$^{\#}$	130.78 ± 0.43*	126.40 ± 0.40$^{*\#}$	129.55 ± 0.41*
Potassium (mEq/L)	4.39 ± 0.18	3.02 ± 0.13$^{\#}$	4.29 ± 0.17*	3.97 ± 0.16$^{*\#}$	4.25 ± 0.16*
Chloride (mEq/L)	91.07 ± 1.56	81.35 ± 0.62$^{\#}$	89.88 ± 1.38*	87.72 ± 0.98$^{*\#}$	89.94 ± 1.40*
Bicarbonate (mmol/L)	19.80 ± 0.17	20.81 ± 0.20	20.13 ± 0.19	20.23 ± 0.19	19.96 ± 0.18

Values are presented as mean ± standard deviation (n = 5).$^{\#}p < 0.05$ statistically significant ($p < 0.05$) compared with normal control, $^{*}p < 0.05$ statistically significant ($p < 0.05$) compared with disease control (One-way ANOVA) followed by Dunnett's multiple comparison test. MOLE; *M. olifera* leaves extract.

Table 4. Effect of ethanol leaves extract of *Moringa oleifera* on lipid profile parameters in *Trypanosoma brucei* infected rats

Parameter	Normal control	Infected	Diminazene (20 mg/kg)	MOLE (400 mg/kg)	MOLE (800 mg/kg)
TC (mg/dL)	84.77 ± 0.63	103.27 ± 1.25$^{\#}$	86.23 ± 0.76*	91.20 ± 0.91$^{*\#}$	87.07 ± 0.80*
TAG (mg/dL)	80.23 ± 0.61	91.37 ± 1.03$^{\#}$	80.67 ± 0.68*	85.30 ± 0.64$^{*\#}$	81.47 ± 0.79*
HDL-C (mg/dL)	69.60 ± 2.63	52.03 ± 0.66$^{\#}$	67.33 ± 1.12*	60.83 ± 0.85$^{*\#}$	67.83 ± 1.94*
LDL-C (mg/dL)	20.12 ± 0.79	32.96 ± 1.74$^{\#}$	8.57 ± 0.22$^{*\#}$	14.11 ± 0.56$^{*\#}$	8.94 ± 0.24$^{*\#}$
VLDL-C (mg/dL)	16.05 ± 0.42	18.27 ± 0.52$^{\#}$	12.33 ± 0.31$^{*\#}$	16.26 ± 0.43*	13.29 ± 0.35$^{*\#}$

Values are presented as mean ± standard deviation (n = 5) $^{\#}p < 0.05$ statistically significant ($p < 0.05$) compared with normal control, $^{*}p < 0.05$ statistically significant ($p < 0.05$) compared with disease control (One-way ANOVA) followed by Dunnett's multiple comparison test. MOLE; *M. olifera* leaves extract, total cholesterol (TC), triglycerides (TAG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C)

Effect of MOLE on histopathological indices of the liver of the infected rats

Fig. 1 shows the effect of MOLE on histopathological indices of liver tissue of the infected rats. Normal hepatocytes, arranged in interconnecting cords around the central veins, and normal components of the portal triads were observed in the liver tissue of the normal control rats. Severe widespread piecemeal necrosis and marked infiltration of inflammatory leukocytes in the periportal areas of the hepatic lobules were observed in the liver tissue of the infected rats. The liver tissue of the rats treated with Diminazene (20 mg/kg), MOLE 400, and 800 mg/kg demonstrated normal hepatocytes arranged in interconnecting cords around the central veins and normal components of the portal triads (Fig. 1).

Effect of MOLE on histopathological indices of the kidney of the infected rats

The effect of MOLE on histopathological indices of kidney tissue of the infected rats is shown Fig. (2). Normal glomeruli in the Bowman's capsules surrounded by a sea of normal renal tubules were observed in the kidney tissue of the normal control rats. Multifocal, widespread glomerular sclerosis was observed in the infected rats. The kidney tissue of the rats treated with Diminazene (20 mg/kg), MOLE 400, and 800 mg/kg, exhibited normal glomeruli in the Bowman's capsules surrounded by normal renal tubules.

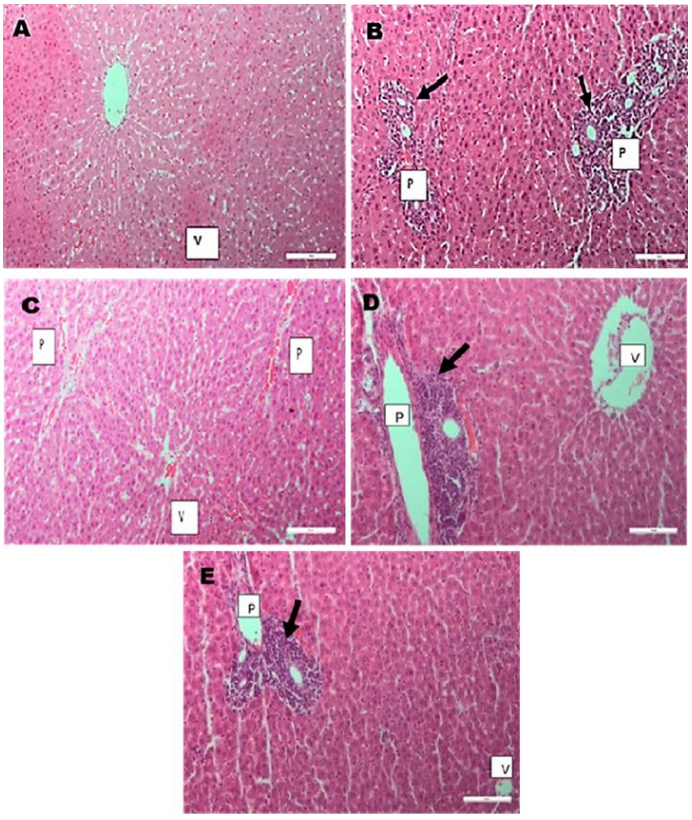

Fig. 1. Effect of ethanol leaves extract of *Moringa oleifera* on histopathological indices of the liver of the infected rats. Normal control (A) showed hepatocytes surrounded central vein (V), disease control (B) showed marked infiltration of inflammatory leuckocytes (arrows) in the peripheral areas (P) of hepatic lobules. Infected group treated with Diminazene aceturate (C), MOLE 400mg/kg (D), and MOLE 800mg/kg (E) showed normal hepatocytes arranged in interconnecting cords around the central veins (V) and normal components of the portal triads (P).

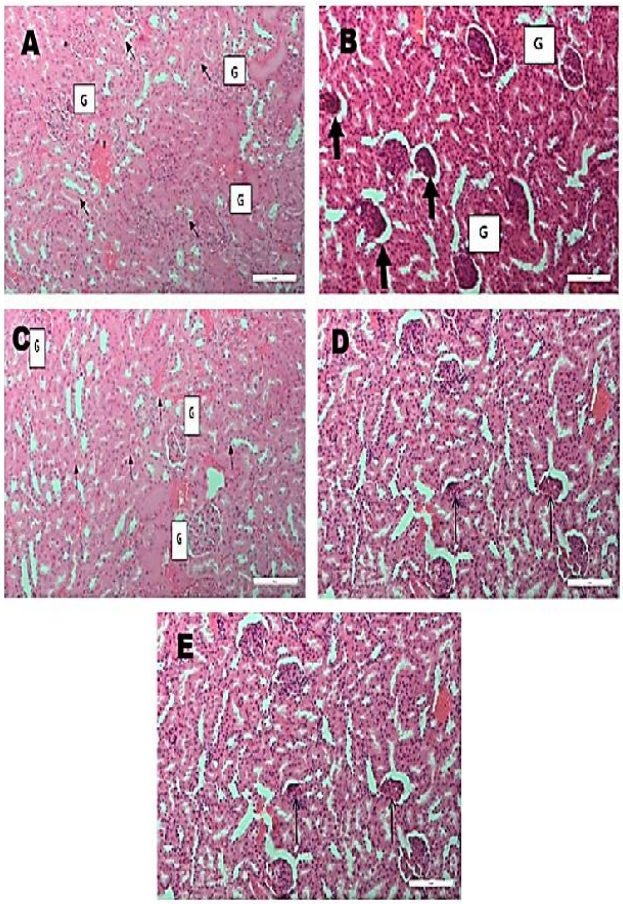

Fig. 2. Effect of MOLE on histopathological indices of the kidney of the infected rats**.** Normal control (A) shows Normal glomeruli (G) in the Bowman's capsules surrounded by a sea of normal renal tubules (arrow). Infected group (B) shows multifocal, widespread glomerular sclerosis (arrow). Infected rats treated with diminazine, MOLE 400 and 800 mg/kg (C, D,E, respectively) show normal glomeruli in the Bowman's capsules surrounded by normal renal tubules (arrow).

4. Discussion

In this study an elevated levels of parasitemia were observed in all the infected rats after the parasite inoculation before the treatments. Similar studies reported high levels of parasitemia in Trypanosoma sp. infected rats (Ukachi et al., 2015; Sunday and Hassana 2010; Lawal et al., 2007). Fluctuations in the levels of parasitemia in Trypanosoma sp. infected animals could be attributed to a continuous cycle of trypanosome replication and destruction (Barry and McCulloch, 2001). Administration of the *Moringa oleifera* leaves extract and Diminazene significantly reduced the parasitemia levels in the treated rats. Similar results have been reported in the relevant studies following administration of other plants' extracts in the Trypanosoma sp. infected rats (Ibrahim et al., 2012; Abu and Uchendu, 2011; Shuaibu et al., 2008). Results of the present study showed significant decrease in body weight of the *T. brucei* infected untreated (disease control) rats. The decrease of the body weight of the infected rats could be due to the progression of the infection. Treatment of the rats with *Moringa oleifera* leaves extract and diminazene significantly increases the body weight of the rats. This finding is in agreement with the study by Fajinmi et al. (2021), who reported a decrease in the body weight of *T. congolense-infected* rats.

In this study, high levels of RBC, PVC, Hb, WBC, PLT, MCV, MCH, and MCHC were observed in the infected rats treated with *Moringa oleifera* leaves extract and diminazene**.** The result of this study is in agreement with the similar study by Nwoha and Omamegbe (2015), who reported a highly significant level of haemoglobin, PCV, and RBC in *T. brucei*-infected rats treated with trypanocidal drug. Also, a relevant research by Morrison et al. (2010) showed an elevated level of hemoglobin and PCV in *T. brucei-infected* treated donkeys. A significantly high level of MCH and MCHC was reported in infected donkeys treated with Novidium (Barry and McCulloch, 2001). However, similar to this finding, low levels of hemoglobin and PCV were observed in *T. congolense*-infected untreated rats (Fajinmi et al., 2021; Saleh et al., 2009). Levels of certain hematological parameters, such as hemoglobin, red blood cells, and packed cell volume, determine the degree of anemia in the biological system. Anemia is characterized by a low level of hemoglobin and packed cell volume (Fajinmi et al., 2021; Deschamps et al., 2016). Low levels of haemoglobin and PCV in parasite-infected rats could be attributed to hemolysis due to high parasitemia levels, destruction of red blood cells and proliferation of the parasite infection (Fajinmi et al., 2021).

In the current study, high levels of ALT, AST, ALP, and total bilirubin were found in the *T. brucei*- infected untreated rats. The finding of this study is in line with the similar study in

which elevated level of AST, ALT, and ALP in *T. brucei* -infected untreated rats was observed (Awekew et al., 2017). Yang et al. (2014) observed an elevated level of ALT in *T. brucei* -infected rams, which may be attributed to the liver destruction due to the parasite infection. A similar finding showed a high level of AST in *T. brucei*-infected gilts (Allam et al., 2011; Barry and McCulloch, 2001). In the present finding, the level of ALT, AST, ALP, and total bilirubin was significantly decreased in the infected treated rats. The result of this study is in agreement with the results of relevant studies, which showed a significant decrease in AST, ALT, ALP levels in the infected treated rats (York, 2017). The high levels of AST, ALT, and ALP in the infected rats could be due to liver damage due by the parasite infection. High levels of ALT are an indicator of muscle disorders, inflammatory problems, and hepatic disorders characterized by necrosis or changes in cell membrane permeability (Kaneko et al., 2008). This finding showed low levels of total protein, albumin, and globulin in the infected untreated rats. Administration of *Moringa oleifera* leaves extract and diminazene in the infected rats significantly increased the levels of total protein, albumin, and globulin in the rats. A significant decrease in albumin level was observed in animals infected with other trypanosoma species (Herrera et al., 2002).

In this study, inoculation of *T. brucei* in the rats increased the levels of urea and creatinine while decreasing the levels of sodium, potassium, and chloride in the infected rats. The high level of urea and creatinine was significantly decreased following the treatments of the rats. Elevated levels of urea and creatinine were reported in *T. congolense* infected untreated cattle (Anosa, 1988). The high significant level of creatinine and urea observed in this study indicated that *Trypanosoma brucei* infection causes adverse effects on the rats' kidney tissue and the normal renal function. However, administration of *Moringa oleifera* leaves extract and diminazene significantly increased the levels of sodium, potassium, and chloride in the rats. In the present study, elevated levels of total cholesterol, TAG, LDL-C, and VLDL-C, and low HDL-C levels were observed in the infected untreated rats. A significant decrease in the concentration of total cholesterol, TAG, LDL-C, and VLDL-C, coupled with a significant increase in HDL-C level, were observed in the rats treated. Biryomumaisho et al. (2003) reported a decrease in total cholesterol level in the infected goat breeds. In this study, it was observed that the liver and kidney tissues of the infected rats have been altered by the *Trypanosoma brucei* infection. Treatment of the rats with *Moringa oleifera* leaves extract and diminazene significantly diminished these adverse changes to normal hepatocytes and normal components of the portal triads.

5. Conclusion

The *Moringa oleifera* leaves extract exhibited anti-trypanosomal activity and diminished the adverse changes in biochemical, hematogical, and histopathological profile of the *Trypanosoma brucei* infected rats. The *Moringa oleifera* leaves extract has therapeutic properties against *Trypanosoma brucei* infection hence, hence validating the traditional use of the plant in the treatment of the parasite infection. Further study should be done to isolate and characterize the active compound(s) responsible for the anti-trypanosomal activity of the plant.

Conflict of Interest

The authors declared that there is no conflict of interest.

5. Reference

Abd EME, Alqhtani AH, Swelum AA, El-Saadony MT, Salem HM, Babalghith AO, Taha AE, Ahmed O, Abdo M, El-Tarabily KA. 2022. Pharmacological, nutritional and antimicrobial uses of *Moringa oleifera* Lam. leaves in poultry nutrition: An updated knowledge. Poult. Sci., 101(9): 102031.

Abd HH, Ahmed HA, Mutar TF. 2020. *Moringa oleifera* leaves extract modulates toxicity, sperms alterations, oxidative stress, and testicular damage induced by tramadol in male rats. Toxicol. Res. 9: 101–106.

Abu AH, Uchendu CN. 2011. In-Vivo Trypanocidal Activity of Hydroethanolic Extract of Hymenocardia acida Stem Bark in Rats. Vet World, 4: 113–116.

Abubakar I, Abubakar MG, Aliyu JD, Ibrahim S, Abdullahi Z, Zubairu A, Umar AS. 2024. Analgesic effect of ethylacetate fraction of

the methanol leaf extract of Hannoa klaineana in rats. J. BioSci. Biotechnol. 13(2): 155-161.

Abubakar I, Danyaya JA, Abdullahi Z, Zubairu A, Sahabi AU, Ahmad F. 2022. Phytochemical Screening, Nutritional and Anti-nutritional Composition of Aqueous Rhizome Extract of *Curcuma longa*. J Biotechn Biochem. 8(2):1–9.

Abubakar I, Muhammad HY, Shuaibu YB, Abubakar MG, Hassan SW. 2021. Anti-ulcerogenic Activity of the Fractions of Methanol Leaves Extract of *Hannoa klaineana* in Wistar Rats. International Journal of Pharma and Biosciences, 12(2): 27–40.

Abubakar I, Muhammad HY, Shuaibu YB, Abubakar MG. 2020a. Anti-Ulcer Activity of Methanol Extract of the Leaves of *Hannoa klaineana* in Rats. J Phytopharmacol. 9(4):258-264.

Abubakar I, Muhammad HY, Shuaibu YB, Abubakar MG. 2020b. Gastro-protective Effect of Ethylacetate Fraction of the Leaves of *Hannoa klaineana* on Aspirin and Histamine-Induced Gastric Ulcer in Rats. Int J Pharmacol Toxicol. 8(1): 70–77.

Adeyemi OS, Akanji MA,Ekanem JT. 2012. Ethanolic extract of Psidiumguajava influences protein and bilirubin level in *Trypanosoma brucei* infected rats. J Biol Sci., 12:111–6.

Ali RA, Perna S, Riva A, Petrangolini G, Peroni G, Nichetti M, Iannello G, Naso M, Faliva MA, Rondanelli M. 2021. Novel Insights on Anti-Obesity Potential of the Miracle Tree, *Moringa oleifera* : A Systematic Review. Journal of Functional Foods, 84: 104600.

Allain CC, Poon LS, Chan CSG. 1974. Enzymatic determination of total serum cholesterol. *Clinical chemistry*, 29: 537 – 541.

Allam L, Ogwu D, Agbede RI, Sackey AKB. 2011. Hematological and serum biochemical changes in gilts experimentally infected with *Trypanosoma brucei*. Vet Arhiv., 81(5): 597609.

American Public Health Association (APHA). 2005. Standard methods for examination of water and wastewater. 21st Edn., Washington, DC., ISBN: 0875530478, pp: 2-61.

Anosa VO. 1988. Haematological and biochemical changes in human and animal trypanosomiasis. Part II. Rev Elev Med Vet Pays Trop., 41:151-164

AOAC. 1990. Official Methods. Minerals, Official Methods of analysis, Washington, DC, USA.

Awekew A, Fetene B, Eshetu E. 2017. Review on serum biochemical changes in ruminants infected with major trypanosome species. Int J Adv Res Biol Sci., 4: 26 – 35.

Barry JD, McCulloch R. 2001. Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. Adv Para. 49: 1-70.

Bartholomew RJ, Delany AM 1964. Proceedings of Australian Association of Clinical Biochemists, 1: 64.

Bing QH, Edward CY. (2015). Chemical and Physical Fixation of Cells and Tissues: An Overview. In: Yeung ECT, et al. (eds.), Plant Microtechniques and Protocols, Springer International Publishing Switzerland.

Biryomumaisho S, Katunguka-Rwakishaya E, Rubaire-Akiiki CM. 2003. Serum biochemical changes in experimental *Trypanosoma congolense* and *Trypanosoma brucei* infections in East African sheep. Vet Arch., 3:167-180.

Burcin A, Sehkar O, Eda C, Gulsum E, Umar FM, Ozlem S, Refiye Y, Aysen Y. 2024. *Moringa oleifera* hydroalcoholic leaf extracts mitigate valproate-induced oxidative status in the extraorbital lacrimal gland in a rat model. Experimental Eye Research. 248: 110104.

Centers for Disease Control and Prevention, African Trypanosomiasis, Epidemiology and Risk Factor. 2022. https://www.cdc.gov/ parasites/slee pingsickness/epi.htm1#.

CFSPH. (2009). African Animal Trypanosomia-sis. The Center for Food Security and Public Health (CFSPH), Iowa State University. www.cfsph.iastate.edu.

Deschamps JY, Desquenes M, Darso L, Ravel S, Bossard G, Charbonneau M, Garaud A, Roux FA. 2016. Refactory hypoglaemia in a dog infected with *Trypanosoma congolense*. Parasite, 23: 1-7.

Fajinmi AO, Faleke OO, Magaji AA, Chafe UM, Kassim MA, Musa UB, Ormaga MT, Tese T, Hussaini M. 2021. Haematological changes in wistar rats experimentally infected with *Trypanosoma congolense* and *Trypanosoma brucei* brucei obtained from North-west Nigeria. Sokoto Journal of Veterinary Sciences, 19(2): 7380.

Falowo AB, Mukumbo FE, Idamokoro EM, Lorenzo JM, Afolayan AJ, Muchenjev V. 2018. Multifunctional application of *Moringa oleifera* Lam. in nutritionand animal food products: a review. Food research international, 106: 317-334.

Herbert WJ, Lumsden WHR. 1976. *Trypanosoma brucei*: A Rapid, "Matching" method for Estimating the host parasitemia. Exp Parasitol., 40:427–31.

Herrera HM, Alessi AC, Marques LC, 2002. Experimental Trypanosoma evansi infection in South American coati (Nasuanasua): haematological, biochemical and histopathological changes. Acta Trop., 81:203-210.

Holanda-Freitas IT, Cupertino MC, Cardoso dos Santos E, Oliveira L, Geller M, Siqueira-Batista R. 2020. Human African Trypanosomiasis: Current Standing and Challenges. J. Trop. Pathol., 49(3): 133 – 148.

Ibrahim H, Ogbadoyi E, Adamu K, Bello M, Yemisi I. 2012. Evaluation of Antitrypanosomal Activity of Ethyl Acetate Extract of Adansonia digitata Seed Extract in T. b. brucei Infected Albino Mice. Int J Drug Res Tech, 2: 454 – 460.

Ibrahim IB, Abubakar I, Ibrahim S, Adiya ZSG, Buhari HB, Shehu SR. 2024. Phytochemicals Screening, Proximate composition and Anti-oxidants analysis of Italian *Citrus paradisi* Fruits. J. Trop. Pharm. Chem. 8(1): 2087-7099.

International Federation of Clinical Chemistry (IFCC) 1980. Physicochemical quantities and units in clinical chemistry. Journal of Clinical Chemistry and Clinical Biochemistry, 18: 829 – 854.

Ishola IO, Yemitan KO, Afolayan OO, Anunobi CC, Durojaiye TE. 2018. Potential of *Moringa oleifera* in the treatment of benign prostate hyperplasia: Role of antioxidant defence systems. Med. Princ. Pract., 27: 15–22.

Ishola IO, Yemitan KO, Afolayan OO, Anunobi CC, Durojaiye TE. 2018. Potential of Moringa oleifera in the treatment of benign prostate hyperplasia: Role of antioxidant defence systems. Med. Princ. Pract., 27: 15–22.

Jaffe MZ. 1986. Physiological chemistry, 10: 391.

Ji H, Bachmanov AA. 2007. Differences in postingestive metabolism of glutamate and glycine between C57BL/6ByJ and 129P3/J mice. Physiology and Genomics, 31(3): 475 – 482.

Kaneko JJ, Harvey JW, Bruss LM. (2008). Clinical Biochemistry of Domestic Animals (pp. 854-858). USA: Elsevier Inc. 6th Edition.

Khalid S, Arshad M, Mahmood S, Siddique F, Qazalbash UR, Ranjha M, Lorenzo JM. 2023. Extraction and quantification of Moringa oleifera leaf powder extracts by HPLC and FTIR. Food Anal. Methods, 16: 787–797.

Khalid, S. et al. (2023). Extraction and quantification of *Moringa oleifera* leaf powder extracts by HPLC and FTIR. Food Anal. Methods, 16: 787–797.

Lawal IA, Ameya F, Ajanusi OJ, Jatau ID, Musa B. (2007). Distribution and morphological forms of *Trypanosoma brucei* in tissues/organs of experimentally infected albino rats. Proceedings of 44th Nig. Vet. Med. Assoc. (NVMA) Ann. Cong. November, 15-18, (pp. 257-260), Delta State, Nigeria.

Lin X, Wu L, Wang X, Yao L, Wang L. 2021. Ultrasonic-assisted extraction for flavonoid compounds content and antioxidant activities of India Moringa oleifera L. leaves: Simultaneous optimization, HPLC

characterization and comparison with other methods. J. Appl. Res. Med. Aromat. Plants, 20: 100284.

Mcgowan MW, Artisis JD, Strindberg DR. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clinical chemistry, 29: 538 – 542.

Morrison LJ, McLellan S, Sweeney L, Chan CN, MacLeod A, Tait A, Turner CMR. 2010. Role for parasite genetic diversity in differential host responses to *Trypanosoma brucei* infection. Inf Imm. 78(3): 1096-1108.

National Cholesterol Education Program Expert Panal (NCEPP) 2001. Third report of the national cholesterol education program (NCEP). Expert on detection, evaluation and treatment of high blood cholesterol in adult. NIH publication Bethesda, national heart, Lung and blood institute, pp. 451 – 476.

Nwoha RIO, Omamegbe JO. 2015. Comparative haematological changes in rats experimentally infected with *Trypanosoma brucei* brucei and treated with imidocarb dipropionate and diminazene aceturate. Res J Vet Sci. 8: 36-41.

Odeniran PO, Ademola IO. 2018. A meta-analysis of the prevalence of African animal trypanosomiasis in Nigeria from 1960 to 2017. Parasit. Vector. 11:280.

Ogbadoyi EO, Abdulganiy AO, Adama TZ and Okogun JI. 2007. In Vivo Trypanocidal Activity of Annona senegalensis Pers. Leaf Extract Against *Trypanosoma brucei* brucei. Journal of Ethnopharmacology, 112: 85-89.

Reham MA, Noorah SA. 2024. The protective effect of Moringa oleifera leaves extract on paracetamol hepatotoxicity in male rats. Journal of Biomedical Science and Engineering, 17(3): 72 – 82.

Rode SB, Dadmal A, Salankar HV. 2022. Nature's gold (*Moringa oleifera*): Miracle properties. Cureus, 14(7): e26640.

Saleh MA, Bassam MA, Sanousi SA. 2009. Oxidative Stress in Blood of Camels (Camelus dromedaries) Naturally Infected with Trypanosoma evansi. Vet. Parasitol, 162: 192-199.

Shuaibu MN, Wuyep PT, Yanagi T, Hirayama K, Ichinose A. 2008. Trypanocidal Activity of Extracts and Compounds from the Stem Bark of *Anogeissus leiocarpus* and Terminalia avicennoides. Parasitol Res, 102: 697703.

Sunday EA, Hassana S. 2010. Antitrypanosomal Property of Some Extracts of Different Parts of *Moringa oleifera*, Lam. Electronic Journal of Biology, 6(1): 19-23.

Tietz NW. 1995. Clinical Guide to Laboratory Tests. Philadelphia, PA: WB Saunders. 3rd ed., pp. 624–625

Ukachi EI, John OI, Samuel OO, Agunna EE, Cynthia EO, Atu AA, Gloria NE, Alexander IG. 2015. Antitrypanosomal and Antioxidant Activities of *Moringa oleifera* Lam Leaf Extracts. Journal of Pharmaceutical, Chemical and Biological Sciences, 3(1): 17-23.

Woo PTK. 1970. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. Acta Tropical, 27: 384-386.

World Health Organisation, WHO. (2004). World Health Report 2004, Statistical annex.

World Health Organization, Trypanosomiasis, human African (sleeping sickness). Privacy Legal Notice 2021. https://www.who.int/newsroom/factsheets/detail/trypanosomiasis-humanafrican (sleeping sickness)

Yang X, Schnackenberg LK, Shi Q, Salminen WF. 2014. Hepatic toxicity biomarkers. In: Gupta RC, ed. Biomarkers in Toxicology. Waltham, MA: Academic Press; 2014:241-260.

York MJ. 2017. A Comprehensive Guide to Toxicology in Nonclinical Drug Development (ed. Faqi AS). 2nd ed. Amsterdam, The Netherlands: Elsevier; 2017: 325-374.

Younis N, Khan MI, Zahoor T. 2022. Phytochemical and antioxidant screening of Moringa oleifera for its utilization in the management of hepatic injury. Frontiers in Nutrition, 9: 1078896.