RESEARCH ARTICLE

Alkaloidal fraction of *Diospyros mespiliformis* protect against *Trypanosoma evansi*-mediated haematological and hepatic impairment in infected rats

Chizoba P. Agbadoronye*[1], Simon O. Abolarinwa[2], Bashir Lawal[3,4], Omonike S. Odeyemi[1], Adonis E. Irhue[5], Musa S. Achagwa[1], Muhammed Z. Ubabu[1], Victor E. Oigbochie[6], Sumayin A. Ngamdu[1],

[1]Nigerian Institute for Trypanosomiasis Research, Suleja Outstation, Niger State, Nigeria.
[2]Department of Animal Biology, Federal University of Technology, P.M.B 65, Minna, Nigeria.
[3]Department of Biochemistry, Federal University of Technology, P.M.B 65, Minna, Nigeria.
[4]Graduate Institute for Cancer Biology & Drug Discovery, Taipei Medical University, R.O.C
[5]Nigerian Institute for Trypanosomiasis Research, South South Zonal Office, Asaba, Nigeria.
[6]Farm Project Faculty of Agriculture, University of Benin, Benin City, Nigeria

ABSTRACT

The present study investigated the antitrypanosomal activities of crude and an alkaloidal fraction of *Diospyros mespiliformis* in *Trypanosoma evansi* - infected rats. A total of twenty-one (21) rats were divided into seven (7) groups of three (3) rats each. Groups 1-6 were infected with the T. evansi parasite and were treated with 100 and 200 mg/kg BW crude extract, 200 and 400 mg/kg BW of the alkaloid fraction of *D. mespiliformis,* 3.5mg/kg of berenil (standard control) and 0.2 mL/kg BW of normal saline (negative control) respectively. Group 7 serves as the normal control (non-infected and non-treated) rats. Results revealed that the crude extract at 400 mg/kg BW and alkaloid fraction at all doses tested (100 and 200 mg/kg BW) significantly ($P<0.05$) increased the RBC, PCV, MCH, MCHC, WBC, and reduced the elevated bilirubin when compared with the untreated control. The extract also significantly increased the reduced total proteins. In conclusion, an alkaloid from *Diospyros mespiliformis* ameliorative effect on *T. evansi*-induced biochemical and hematological alterations in rats, thus could be considered a novel agent for the development of a new drug against trypanosomiasis.

Keywords: *Diospyros mespiliformis, Trypanosoma evansi*, Biochemical parameters; Hematological parameters

Received: 28 May 29 2021, Revised: 10 June, 2021, Published: 15 June 2021

CONTACT Agbadoronye P.C: Nigerian Institute for Trypanosomiasis Research, Suleja Outstation, Niger State, Nigeria

Citation: Agbadoronye P.C, Abolarinwa S.O, Lawal B, Odeyemi, S.O, Irhue, A.E, Achagwa, S.M, Ubabu, Z.M, Oigbochie, V.E, Ngamdu, S.A. (2021). Alkaloidal fraction of Diospyros mespiliformis protect against Trypanosoma evansi-mediated haematological and heapatic impairment in infected rats. BIOMED Natural and Applied Science. 1(1);66-78

1.0 INTRODUCTION

Trypanosomosis is a group of diseases caused by flagellated protozoan parasites of the genus *Trypanosoma*, family Trypanosomatidae. They are widely distributed in Africa, South America, Asia, and Middle East [1]. African animal trypanosomiasis (AAT) is transmitted cyclically by tsetse flies (Glossina species) and mechanically by biting flies. Of the tsetse transmitted trypanosomes, three species namely *T. congolense, T.vivax,* and *T. brucei* comprise the major disease agents that affect livestock [1,2]. T. vivax and *T. evansi* can also be transmitted non-cyclically by biting flies and hence their distribution is much wider (extending to Asia and Latin America) than for the cyclically transmitted trypanosomes [3].

The human infective parasites *T. brucei gambiense* and *T. brucei* rhodesiense are the causative agents of human African trypanosomiasis (HAT), commonly known as sleeping sickness. They are transmitted by tsetse flies. *T. b. gambiense* is found in west and central Africa while T. b. rhodesiense is found in eastern and southern Africa [4]. The third group of

trypanosome, *T. cruzi*, which is found mainly in Latin America, is a zoonotic infection which spread by blood-feeding triatomins [5]

Chemotherapy, the main means of controlling the disease is under threat due to parasite resistance. The current chemotherapy of HAT relies only on six drugs (Suramin, Pentamidine, Melarsoprol, Eflornithine, Arsobal, and Mel B), five of which were developed more than 30 years ago [6]. Others such as homidium, isometamidium, and diminazene aceturate are used in animal infections [7]. Each of these drugs has one or more of these challenges: expensive, highly toxic and need parenteral administration [6]. The continued use of the same trypanocides for years has resulted in drug resistance that has been largely responsible for the current chemotherapeutic failures [6,8]. The poor prospect for a vaccine due to antigenic variation of the parasite is further compounded by the unwillingness of the pharmaceutical industry to develop new compounds because of uncertain and unprofitable market or perhaps the localized nature of the disease [8]. These strongly suggest the need for further research into new drugs from natural resources which are perceived to be safe and therapeutically effective [9].

Medicinal plants have been used traditionally for the treatment of various parasitic and infectious diseases, and have received some scientific validation of their effectiveness [10,11]. In our previous study, we demonstrated that crude and an alkaloidal fraction of Diospyros mespiliformis (African Ebony) demonstrated significant antitrypanosomal activities and prolonged the survival days of *Trypanosoma Evansi* Infected Rats. The present study, therefore, aims to evaluate the effect of the crude and alkaloidal fraction of this plant on hematological and biochemical parameters of *T. evansi* infected rats.

2.0 Materials and Methods

2.1 Collection and identification of plant material

The leaves of *D. mespiliformis* were collected from the Kacha Local Government area of Niger State, Nigeria. The plant was identified and authenticated at the Department of Plant Biology, Federal University of Technology, Minna.

2.2 Experimental animals and Parasite

A total of twenty-one (21) rats weighing 125.65 ± 3.89 g were obtained from Animal Holding Unit, Department of Biochemistry, Federal University of Technology Minna, Nigeria. They were housed in clean cages with wood shavings as beddings under standard environmental conditions of temperature and relative humidity, 12 hrs daylight/night cycle) with access to commercial feed pellets (growers) and water *ad libitum*. The cages were cleaned regularly throughout the experimental periods (their beddings were changed every two days). Animals were kept in compliance with internationally accepted principles for human handling and use of laboratory animals in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997). *Trypanosoma evansi* was obtained from Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Kaduna state, Nigeria. The parasite was maintained in the laboratory by serial passage in rats

2.3 Extraction of Crude and Alkaloidal fraction of *D. mespiliformis*

The crude extraction *D. mespiliformis* leaf was carried out as reported in our previous study. The alkaloidal extraction was carried out as described by previous studies [12,13]. Briefly, the leaves of *D. mespiliformis* (50g) powder were moistened with 200 mL of 95% ethanol, alkalified with 200 mL of ammonia solution, and macerated for 24 hrs followed by extraction with ethanol. The ethanol extract was filtered, concentrated, and treated with 1.0 N hydrochloric acid. The filtrate was further alkalified with ammonia solution and the alkaloid was obtained by fractionation in a separating funnel using chloroform.

2.4 Experimental Design

A total of twenty-one (21) rats were divided into seven (7) groups of three (3) rats each. Groups 1-6 were infected with the T. evansi parasite according to the method described in the previous study and were treated with 100 and 200 mg/kg BW crude extract, 200 and 400 mg/kg BW of the alkaloid fraction of *D. mespiliformis,* 3.5mg/kg of berenil (standard control) and 0.2 mL/kg BW of normal saline (negative control) respectively. Group 7 serves as the normal control (non-infected and non-treated) rats.

2.5 Collection of blood sample

The procedure described by Yusuf *et al*. [14] was adopted for the preparation of serum. Briefly, the animals were anesthetized with diethyl ether and the blood was collected through the cardiac puncture into sample bottles and left for fifteen minutes to clot, and then centrifuged at 3000 rpm for 15 minutes in order to get the serum. The sera were stored in the refrigerator at -20°C for subsequent analysis. The whole blood was also collected into EDTA bottles for hematological analysis.

2.6 Biochemical Analysis

All biochemical analyses were conducted using the Randox Diagnostic kit (Randox Laboratories Ltd, Crumlin, UK). Alanine transaminase (ALT) was analyzed on the principle of the catalytic action of ALT on alanine and α – oxoglutarate to form pyruvate and glutamate [15]. Aspartate transaminase (AST) was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazine [16]. Serum total protein concentration was estimated based on the principle that cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a colored complex which absorbed maximally at 546 nm [17]. The bilirubin assay was based on the reaction between bilirubin and diazotized sulphanilic acid in an alkaline medium to form a blue colored complex which absorbed at 578 nm [18]. Albumin concentration was assayed based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-cresol sulphonephthalein (bromocresol green, BCG) [19]. The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance is directly proportional to the concentration of albumin in the sample.

2.7 Haematological Analysis

Automated Haematologic Analyzer (Sysmex Haematology Systems, Sysmex America Inc., model no. KX-21N, Kobe, Japan) was used to determine the levels of hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), neutrophils, lymphocytes and platelets as described by Dacie and Lewis [20].

2.8 Statistical analysis

Data collected were subjected to statistical analysis using the statistical package for social science version 21.0 and expressed as mean ± standard error of the mean (SEM). Statistical significance of the results between groups was determined using one-way analysis of variance (ANOVA) followed by Duncans multiple range tests (DMRT). Differences in mean were considered to be significant at $p<0.05$.

3.0 Results

3.1 Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum aspartate transaminase activities in *T. evansi* infected rats.

The serum aspartate transaminase activities were significantly higher in *T. evansi* infected rats when compared with the normal control. Treatment with the crude and alkaloidal fraction

of *Diospyros mespiliformis* significantly reduced ($p<0.05$) the AST activities when compared with the untreated control. The AST activities in rats treated with alkaloid fraction were comparable ($p>0.05$) with the normal control but lower ($p<0.05$) than those treated with crude extract (Figure 1)

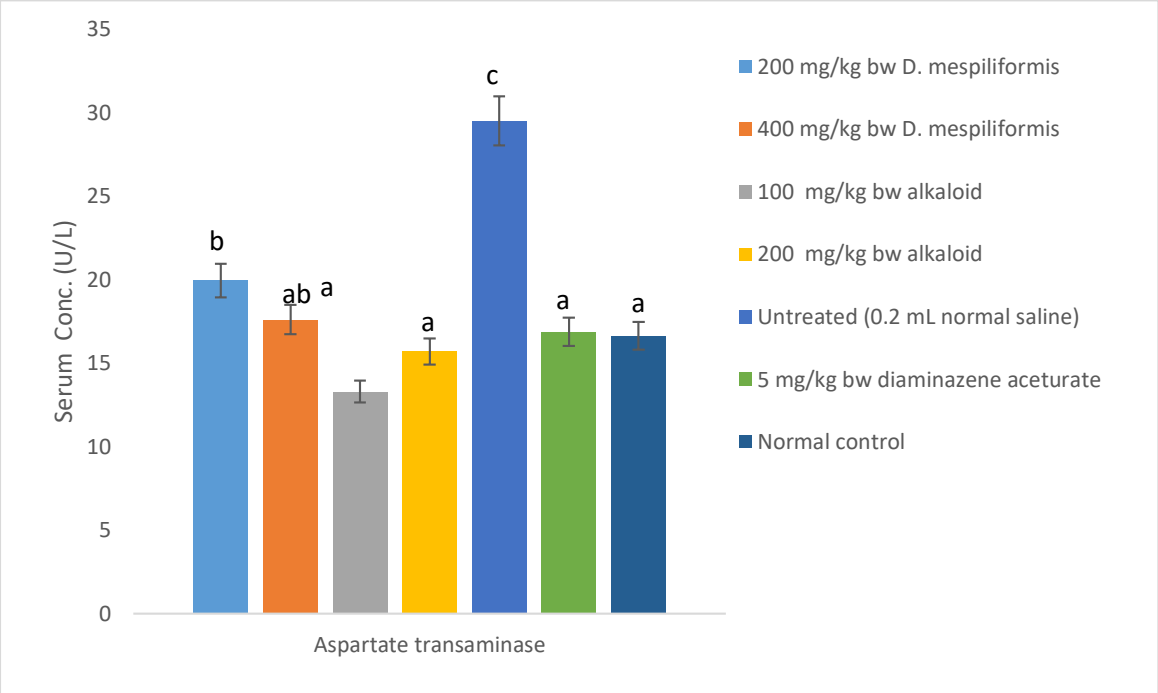

Figure 1: Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum activity of aspartate transaminase in *T. evansi* infected rats. Key: Each bar represents mean ± SEM of 3 determinations. Bars with different superscripts are significantly different ($p<0.05$).

3.2 Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum alkaline phosphatase activities in *T. evansi* infected rats.

The serum alkaline phosphatase activities were significantly higher in *T. evansi* infected rats when compared with the normal control. Treatment with the crude and alkaloidal fraction of *Diospyros mespiliformis* significantly reduced ($p<0.05$) the ALP activities when compared with the untreated control. The ALP activities in rats treated with alkaloid fraction at 100 and 200 mg/kg bw were comparable ($p>0.05$) with the normal control but lower ($p<0.05$) than those treated with crude extract (Figure 2).

3.3 Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum alkaline phosphatase activities in *T. evansi* infected rats.

The serum concentrations of total proteins were significantly lower in *T. evansi* infected rats when compared with the normal control. Treatment with the crude and alkaloidal fraction of *Diospyros mespiliformis* significantly increased the ($p<0.05$) serum concentrations of total proteins when compared with the untreated control (Figure 3).

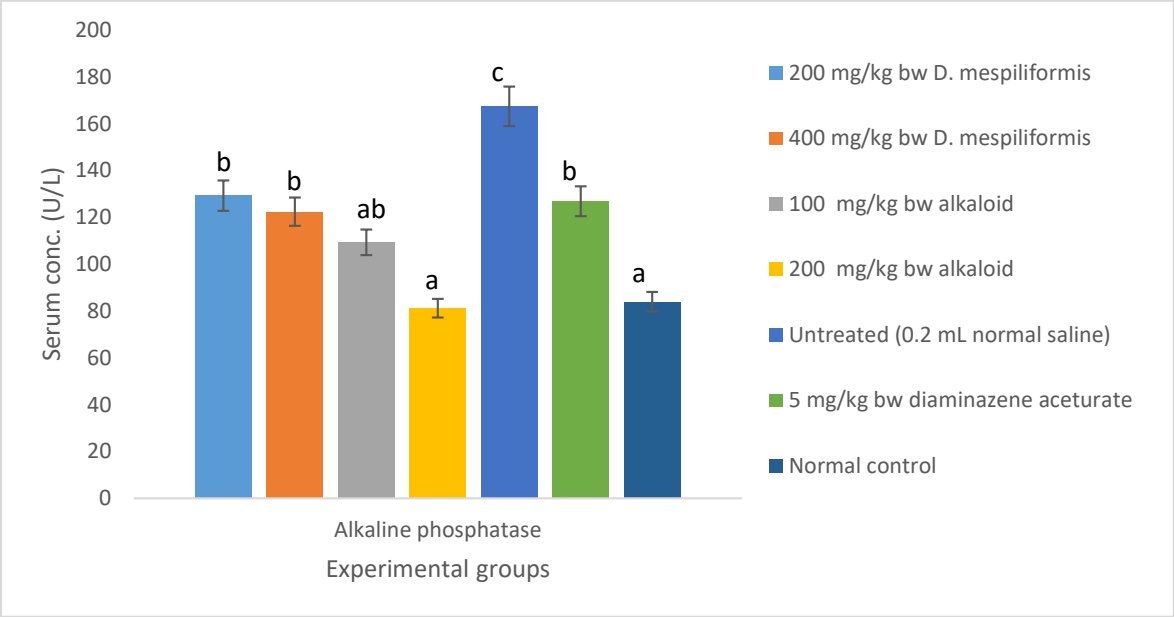

Figure 2: Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum activity of alkaline phosphatase in *T. evansi* infected rats. Key: Each bar represents mean ± SEM of 3 determinations. Bars with different superscripts are significantly different ($p<0.05$).

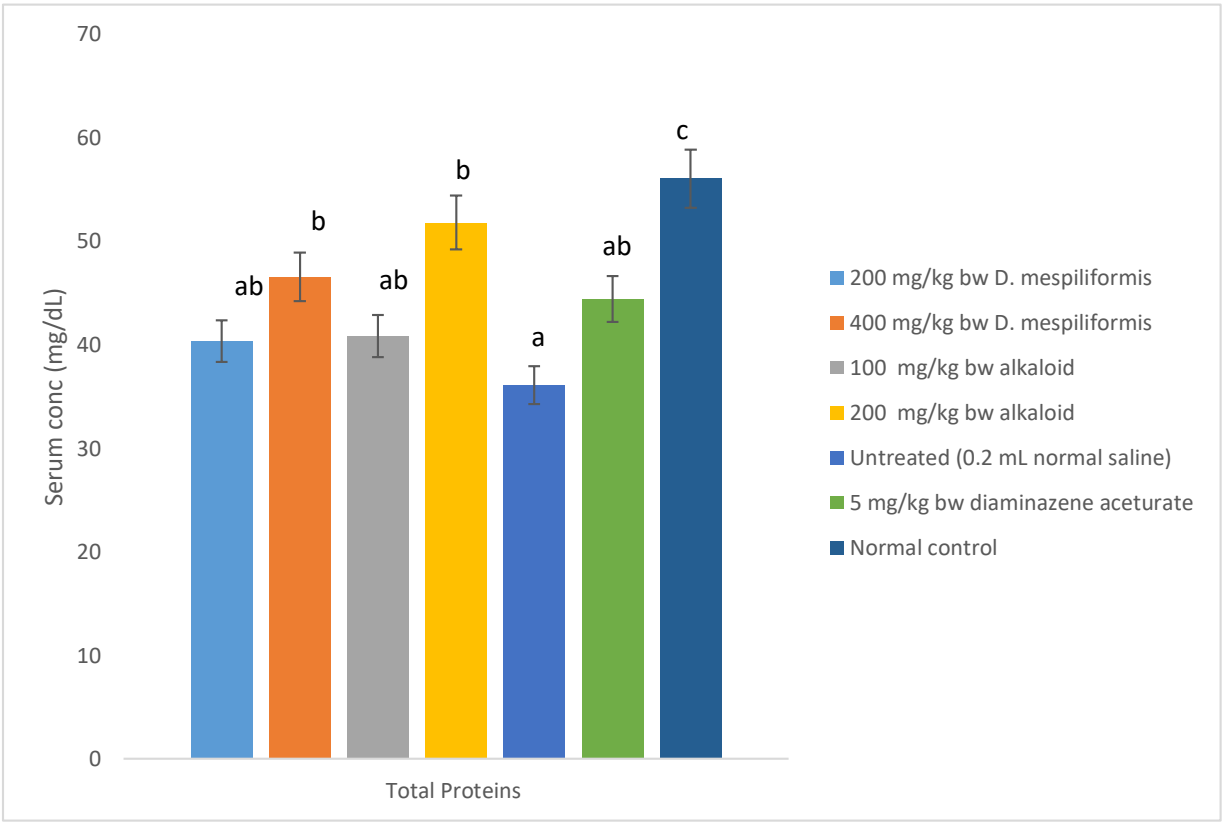

Figure 3: Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum concentration of total proteins in *T. evansi* infected rats Key: Each bar represents mean ± SEM of 3 determinations. Bars with different superscripts are significantly different ($p<0.05$).

3.4 Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum bilirubin concentration in *T. evansi* infected rats.

The serum concentrations of bilirubin were significantly higher in *T. evansi* infected rats when compared with the normal control. Treatment with the crude and alkaloidal fraction of *Diospyros mespiliformis* significantly decreased the ($p<0.05$) serum concentrations of bilirubin when compared with the untreated control. Rats treated with crude extract (200 and 400 mg/kg bw) and alkaloidal fraction (100 and 200 mg/kg b w) were not significantly different from each other (figurre 4)

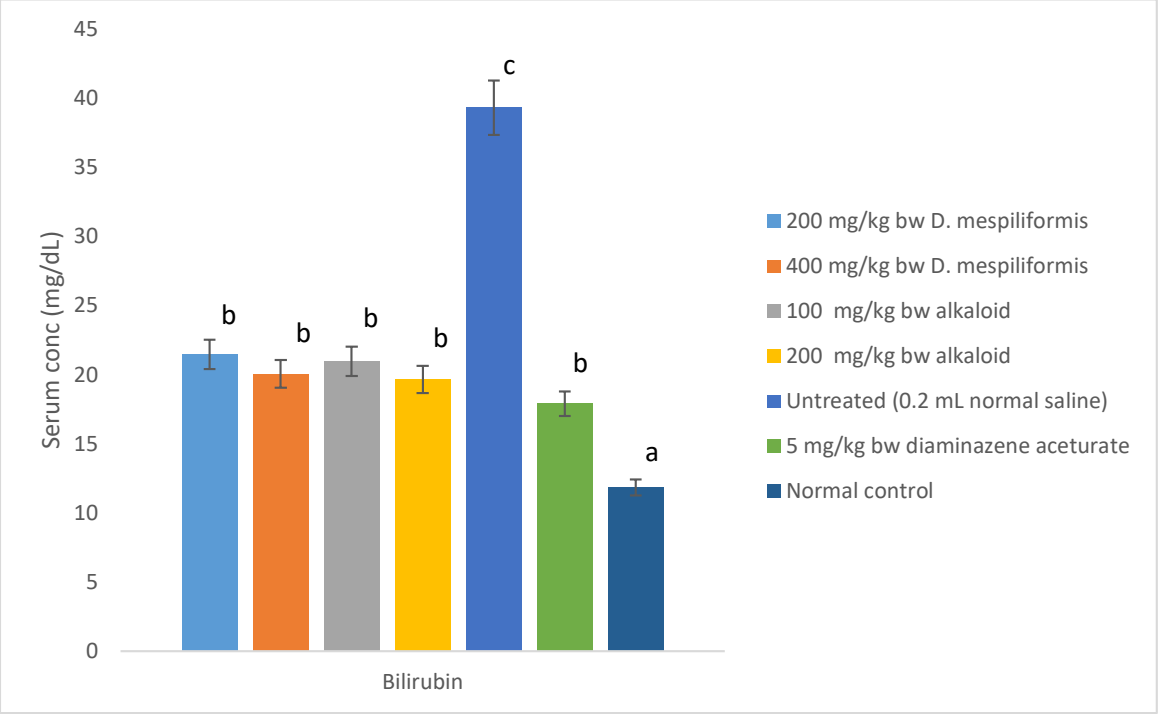

Figure 4: Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum concentration of bilirubin in *T. evansi* infected rats. Key: Each bar represents mean ± SEM of 3 determinations. Bars with different superscripts are significantly different ($p<0.05$).

3.5 Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum albumin concentrations in *T. evansi* infected rats.

The serum concentrations of albumin were significantly lower in *T. evansi* infected rats when compared with the normal control. Treatment with the crude and alkaloidal fraction of *Diospyros mespiliformis* significantly increased the ($p<0.05$) serum concentrations of albumin when compared with the untreated control. Rats treated with 400 mg/kg bw crude extract and 200 mg/kg bw alkaloid fraction had higher concentrations of albumin when compared with the lower doses (figure 5).

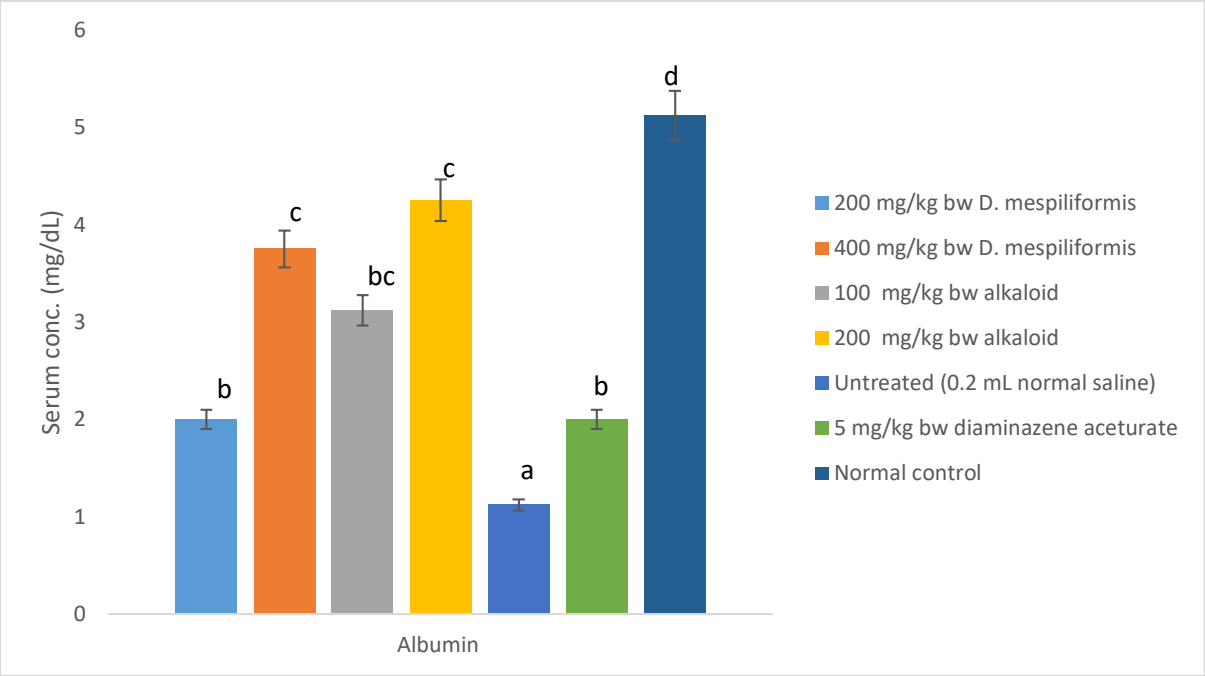

Figure 5: Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on serum concentration of albumin in *T. evansi* infected rats. Key: Each bar represents mean ± SEM of 3 determinations. Bars with different superscripts are significantly different ($p<0.05$).

3.6 Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on hematological parameters in *T. evansi* infected rats.

T. evansi infected rats showed a significant decrease in Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC) when compared with normal control. Treatment of *T. evansi* infected rats with the crude extract at 400 mg/kg BW and alkaloid fraction at all doses tested (100 and 200 mg/kg BW) significantly ($P<0.05$) increased the RBC, HB, PCV, MCH, MCHC, RBC and WBC count when compared with untreated control. MCV, neutrophils and eosinophils in all treated groups were not significantly ($p>0.05$) different from the untreated and the control groups (Table 1)

Table 1: Effect of crude and alkaloidal fraction of *Diospyros mespiliformis* on hematological parameters in *T. evansi* infected rats

				Alkaloid fraction		Crude Extract	
	Positive	Negative	Normal	100 mg/kg bw	200 mg/kg bw	200 mg/kg bw	400 mg/kg bw
HB	10.83±0.23^{b}	7.61±0.66^{a}	10.90±0.63^{a}	11.80±0.67^{b}	10.65±0.85^{b}	9.01±0.89^{b}	11.50±0.53^{b}
PCV	33.90±0.21^{b}	21.89±2.83^{a}	31.06±1.43^{b}	35.87±3.45^{b}	32.98±3.09^{b}	22.50±1.73^{a}	38.78±2.34^{b}
MCV	45.98±1.23^{a}	46.98±3.40^{a}	41.09±3.90^{a}	47.20±2.45^{a}	48.09±4.33^{a}	44.50±3.92^{a}	47.06±5.93^{a}
MCH	22.89±0.34^{b}	13.82±1.82^{a}	26.09±2.03^{b}	20.00±2.34^{b}	24.50±3.94^{b}	26.50±2.09^{b}	22.50±0.89^{b}
MCHC	31.89±1.34^{b}	21.13±4.32^{a}	38.05±3.09^{b}	32.00±3.45^{b}	37.50±4.34^{b}	33.3±2.34^{b}	35.50±2.34^{b}
RBC	5.90±0.32^{b}	2.83±0.04^{a}	5.00±0.89^{b}	5.20±0.07^{b}	5.55±0.06^{b}	5.85±0.08^{b}	2.00±0.87^{a}
PLC	472.32±5.43^{b}	259.78±12.98^{a}	278.09±7.32^{a}	290.89±7.54^{a}	291.98±9.45^{a}	480.76±7.90^{b}	370.89±9.38^{a}
TWBC	5.24±0.21^{bc}	3.41±0.89^{a}	4.40±0.01	6.10±0.83^{c}	6.30±0.67^{b}	3.78±0.33^{b}	6.10±0.90
N	32.83±2.34^{a}	32.35±3.84^{a}	30.00±2.31^{a}	35.40±0.73^{a}	36.87±2.35^{a}	37.78±3.09^{a}	39.00±2.03^{a}
L	34.34±2.34^{a}	38.90±4.32^{a}	60.00±6.32^{c}	71.50±3.45^{d}	45.55±4.56^{b}	64.98±5.98^{c}	55.00±3.09^{bc}
E	22.34±2.34^{a}	21.34±2.83^{a}	25.00±0.98^{a}	23.00±0.93^{a}	29.98±1.34^{a}	21.87±0.78^{a}	26.00±1.03^{a}
RDW+	30.45±3.43^{a}	32.98±3.04^{a}	43.20±2.83^{b}	44.45±2.45^{b}	48.87±3.45^{b}	48.99±3.89^{b}	48.50±3.90^{b}

Values are mean ± SEM of 3 determinations. The values along the same row with different superscripts are significantly different ($p<0.05$). Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), platelet count (PLT), lymphocytes (L), eosinophils (E), and neutrophils(N).

4.0 DISCUSION

Assessment of hematological parameters can be used to determine the extent of infection and effects of medicinal plant extracts on the blood constituents of an animal [21,22]. Such analysis is relevant to risk evaluation because changes in the hematological system are highly predictive for human toxicity when data are translated from animal studies [23,24]. The significantly decreased levels of red blood cells (RBC), PCV, MCH, MCHC, and RBC of the infected untreated rats may probably be due to pathological effects of trypanosomosis that presents anemia as a major manifestation [25,26]. This is consistent with the findings of different authors that reported a decrease in hematological values in rats infected with trypanosome [27,28].

The increase in the levels of these hematological components in rats treated with the crude and alkaloidal fraction of *Diospyros mespiliformis* is an indication that the extract prevented further parasite-induced damage to the RBC, it could also be an indication of hematopoietic properties of the plant extract [29]. In concordance with the present study, Luka *et al*., [30] reported that hematological parameters (packed cell volume (PCV), hemoglobin concentration (Hb), red blood cells (RBC), white blood cells (WBC), MCV, (MCHC and differential leucocyte counts (DLC) were significantly modulated ($p>0.05$) after administration of *Diospyros mespiliformis* to Wister rats [30]. It, therefore, becomes reasonable to argue that *Diospyros mespiliformis* have the potentials to inhibit the destruction of RBC and also enhance hematopoiesis trypanosome infection.

The biochemical indices monitored in the liver and serum are useful 'markers' for assessing tissue damage [31-33]. The liver which plays a vital role in the intermediary metabolism of biomolecules and drugs could also be affected by the toxic side effects of these drugs and diseases [13,34]. AST are markers of liver damage and can be used to assess liver cytolysis during parasitic infection [35]. The increase in AST activities in the infected untreated rats may be related to liver inflammation and is an indication of abnormal function of the liver. Alkaline phosphatases are often used to assess the integrity of the plasma membrane and endoplasmic reticulum. The alteration in serum ALP activities in *T. evansi* infected untreated rats suggested that the integrity and functionality of the endoplasmic reticulum and plasma membrane have been compromised by the trypanosome infection [36]

The elevation of these enzyme levels recorded here agrees with earlier reports from natural and experimental infected animals [35]. The results suggest probable infiltration of vital body organs and inflammation particularly of the liver, muscles, and kidneys by *T. evansi*. Elevated enzyme levels may also result from the effect of trypanosome lyses resulting from the host's defense mechanisms [37]. Rats treated with the alkaloid fraction drug shows satisfactory activities of biomarker enzyme which were an indication of preserved organ integrity.

The total proteins, albumin, and bilirubin play major roles in assessing the integrity of the kidney and liver [38]. *Trypanosoma parasite* has a varying effect on total protein as hypoproteinemia as well as hyperproteinemia [39]. The present study, however, showed significant hypoproteinemia in serum of *T. evansi* infected untreated rats. This significant decrease may be due to the mobilization of defensive enzymes (which are known proteins) to counter the effect of trypanosome-induced oxidative stress which was consequently ameliorated by the crude and alkaloidal fraction of *Diospyros mespiliformis.*

The decrease in albumins and total proteins reported in this study could lead to overhydration which is injurious to cellular homeostasis. This will harmfully compromise the normal metabolic activities of the liver and consequently the health of the animals [39,40]. Bilirubin is an endogenous anion product of hemoglobin degradation of the red blood cell. The high-level bilirubin in untreated rats is an indication of impaired liver function as reported by Kumar et al. [41]. The improvement in the concentrations of bilirubin, albumin, and total proteins in

rats treated with the crude and alkaloidal fraction of *Diospyros mespiliformis* is an indication of the reduced pathological effect of the parasite in the presence of the extract.

5.0 Conclusion

These results suggest that *T. evansi* infection-induced anemia and biochemical alterations in infected rats. However, treatment with a crude and alkaloidal fraction of *Diospyros mespiliformis* exhibited a hematopoietic effect and protective effect against biochemical alterations.

Consent for publication: Not applicable

Availability of data and material: All data are presented in the manuscript

Competing interests: The authors declared no conflict of interest exist

Funding: This study received no external funding

Authors' contributions: All authors participate in the execution of this project. All authors read and approved the final manuscript.

Acknowledgments: The authors would like to appreciate the technical staff of Biological Sciences and Biochemistry Laboratory Federal University of Technology Minna, for their kind assistance

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Citation: Agbadoronye P.C, Abolarinwa S.O, Lawal B, Odeyemi, S.O, Irhue, A.E, Achagwa, S.M, Ubabu, Z.M, Oigbochie, V.E, Ngamdu, S.A. (2021). Alkaloidal fraction of Diospyros mespiliformis protect against Trypanosoma evansi-mediated haematological and heapatic impairment in infected rats. BIOMED Natural and Applied Science. 1(1);66-78