Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

The effect of *Albizia gummifera* leaf ethanolic extract on *Trypanosoma brucei brucei* infected albino rats

Oloruntola, D.A.[1@]; Dada, E.O.[2] and Oladunmoye, M.K[2]

[1]Department of Medical Laboratory Science, University of Medical Sciences, Ondo City, Nigeria.
[2]Department of Microbiology, The Federal University of Technology, Akure, Nigeria.

KEYWORDS

Albizia gummifera.
Phytochemicals.
Public health.
Trypanosomiasis.
Zoonosis.

SUMMARY

Anti-trypanosomal activities of ethanolic extract of Albizia gummifera leaf (EAL) on Trypanosoma brucei brucei infected albino rats were evaluated. Thirty-six Albino rats were distributed into six groups: Group 1 (control); Group 2: (infected and not treated); Group 3: (infected and treated with 10mg/kg body weight diminazene aceturate); Group 4: (infected and treated with 500mg/kg body weight EAL); Group 5: infected and treated with 1000mg/kg body weight EAL; Group 6: infected and treated with 1500mg/kg body weight EAL. The parasitaemia level in the groups 3 and 6 was similar (P>0.05) to group 1, but lower (P<0.05) than the rest groups. The highest (P<0.05) chemo-suppression was being recorded in group 3, compared to the rest groups. The daily parasitemia levels increased in all groups, except for the control group, from day three post-infection until day ten post-infection, when it declined at varying rates in groups 1, 4, 5 and 6). The packed cell volume of the rats in group 2 group 4 were significantly (P<0.05) lower, compared to the control group on days 10 and 15 post-infection. On day five post-infection, the red blood cell counts were lower (P<0.05) in groups 2 and 4, compared to the control groups and other groups. The haemoglobin concentration of the rats in groups 2 and 4 were lower (P<0.05), compared to those in the control group. The aspartate transaminase level in groups 2 and 4 were significantly (P<0.05) higher, compared to the rest groups.

Efecto del extracto etanólico de hoja de Albizia gummifera en ratas albinas infectadas con Trypanosoma brucei brucei

ADITIONAL KEYWORDS

Albizia gummifera.
Fitoquímicos.
Salud pública.
Tripanosomiasis.
Zoonosis.

RESUMEN

Se evaluaron las actividades anti-tripanosomales del extracto etanólico de hoja de Albizia gummifera (EAL) en ratas albinas infectadas con Trypanosoma brucei brucei. Treinta y seis ratas albinas se distribuyeron en seis grupos: Grupo 1 (control); Grupo 2: (infectado y no tratado); Grupo 3: (infectado y tratado con 10 mg / kg de aceturato de diminazeno de peso corporal); Grupo 4: (infectado y tratado con 500 mg / kg de peso corporal EAL); Grupo 5: infectado y tratado con 1000 mg / kg de peso corporal EAL; Grupo 6: infectado y tratado con 1500 mg / kg de peso corporal EAL. El nivel de parasitemia en los grupos 3 y 6 fue similar (P> 0.05) al grupo 1, pero más bajo (P <0.05) que los demás grupos. La quimio-supresión más alta (P <0,05) se registró en el grupo 3, en comparación con los grupos de descanso. Los niveles diarios de parasitemia aumentaron en todos los grupos, excepto en el grupo de control, desde el día tres después de la infección hasta el día diez después de la infección, cuando disminuyeron a tasas variables en los grupos 1, 4, 5 y 6). El volumen de células empaquetadas de las ratas en el grupo 2 grupo 4 fue significativamente (P <0.05) más bajo, en comparación con el grupo de control en los días 10 y 15 después de la infección. El día cinco después de la infección, los recuentos de glóbulos rojos fueron más bajos (P <0.05) en los grupos 2 y 4, en comparación con los grupos de control y otros grupos. La concentración de hemoglobina de las ratas en los grupos 2 y 4 fue menor (P <0.05), en comparación con las del grupo de control. El nivel de aspartato transaminasa en los grupos 2 y 4 fue significativamente (P <0,05) más alto, en comparación con los demás grupos.

INFORMATION

Cronología del artículo.
Recibido/Received: 07.02.2021
Aceptado/Accepted: 10.10.2022
On-line: 15.10.2022
Correspondencia a los autores/Contact e-mail:
oloruntoladeborah@gmail.com

INTRODUCTION

Trypanosomiasis is a disease that affects both man and animals. Human African Trypanosomiasis, a nearly solely disease of the tropical region, is transmitted by the tsetse fly and caused by infection with *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* (Sutherland *et al*., 2015). According to WHO (2013), trypanosome parasite in the brain could cause a neurological breakdown, leading to a coma or death when adequate therapeutic measures are not given. Besides, the animal trypanosomiasis is also caused by pathogenic trypanosomes such as *Trypanosoma brucei*

spp, T. vivax, and *T. congolense* (Giordani *et al*., 2016). Valuable domestic animals such as ovines, bovines, caprines, suids, camelids, and equids can come down with the acute or chronic wasting disease, high morbidity, infertility and death, when infected with this Trypanosoma species and are not promptly treated (Giordani *et al*., 2016).

The use of drugs of choice (e.g. eflornithine, pentamidine, melarsoprol, arsobal, sumarin among others) for trypanosomiasis is costly, and these drugs are gradually losing their potency because of development of parasite resistance (Nwodo *et al*., 2015a). Subsequently, the search for an alternative to synthetic drugs; particularly phytochemical or plant parts derived drugs, for treating diseases such as trypanosomiasis is increasing (Mashi *et al*., 2019). Some plants have been proven to effectively treat various diseases (Mashi *et al*., 2019; Dada and Oloruntola, 2016). Some plants' trypanocidal activities have been reported (Toya, 2010; Nwodo *et al*., 2015b), but data and reports on trypanocidal effects *Albizia gummifera* is rear.

Albizia gummifera (Fabaceae) is a plant native to Africa's wet lowland or upland and riverine forest (Mahlangu *et al*., 2017). Extracts of various parts of *Albizia gummifera* is being used for the treatment of malaria and stomach infections; while these extracts were also reported to possess antiplasmodial (Rukunga *et al*., 2007), and anti-microbial activities (Mbosso *et al*., 2010). Since synthetic and herbal drugs may affect the haematological and biochemical indices (Mashi *et al*., 2019), it is indispensable to evaluate these parameters during treatment. Therefore, this study aimed at investigation of the anti-trypanosomal effects of ethanolic extract of *Albizia gummifera* leaf.

MATERIALS AND METHODS

Collection of albizia gummifera leaves and extraction

Leaves of *Albizia gummifera* were collected from Afolu Farmstead, Ise-Ekiti (7°27′36″N5°25′12″E), Ekiti State, Nigeria. The leaf specimen was identified and authenticated by a Plant Scientist of the Department of Crop Soil and Pest Management, The Federal University of Technology, Akure, Nigeria. The collected leaves were cleaned with water, drained and dried under shade at ambient temperature for four weeks until they become crispy. After that, the leaves were ground with mortar and pestle into powder and kept in a glass jar until used. Five hundred grams of the leaf powder was macerated in 4.5 litres of 75% ethanol for three days (72 hours), filtered using millipore (pore size 0.7 µm) filter paper. After that, the extract was concentrated under a vacuum using a rotary evaporator (SCILOGEX SCI100-S 5L Rotary Evaporator, Vertical Coiled Condenser Manual Lift) at 35-40°C. The dried ethanolic extract of *Albizia gummifera* leaf (EAL) was kept at -20°C until use.

Phytochemical screening of the extract

The phytochemical analysis of EAL was carried out to quantify the flavonoids (Chang *et al*., 2002), alkaloids, tannin (Ezeonu *et al*., 2016), and saponins (Obadoni and Ochuko, 2002).

Trypanosome stock

Trypanosoma brucei brucei was obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria and was after that maintained in the Department of Microbiology, School of Life Science, The Federal University of Technology, Akure (FUTA), Nigeria, by continuous passaging the infected blood into the albino rats.

Experimental animals

Thirty-six albino rats weighting 94.72±0.57 g were housed under a normal standard laboratory condition, fed with grower's diet and clean water *ad libitum*. After acclimatization for two weeks, the animals were randomly distributed into the six experimental groups (6 rats/experimental group). Group 1: control; Group 2: infected and not treated; Group 3: infected and treated with 10mg/kg body weight (standard drug) diminazene aceturate; Group 4: infected and treated with 500mg/kg body weight EAL; Group 5: infected and treated with 1000mg/kg body weight EAL; Group 6: infected and treated with 1500mg/kg body weight EAL.

Trypanosome infection and treatment

Blood from confirmed parasitized rat was obtained by caudal puncture and dispensed into ethylenediaminetetraacetic acid (EDTA) sample bottle, and after that diluted with normal saline to serve as inoculum. The rats in groups 2, 3, 4, and 5 were infected peritoneally with 0.1ml of the inoculum contacting approximately 10^3 trypanosomes per ml of inoculum. The rats were administered their respective treatments orally using gavage needle when parasitaemia was detected (i.e. day five post infestation) and lasted for ten days.

Collection of samples and analysis

Procedures described by Parasuraman *et al.* (2010) were adopted in blood collection. Blood samples were collected from the saphenous vein at the back of the rats' hind leg on a 5-day interval to determine the parasitaemia level and haemogram. At the terminal stage of the experiment, the cardiac puncture was used for blood collection required for the parasitaemia level, haemogram and serum analyses. The blood samples for parasitaemia level and haemogram were collected into EDTA-bottle; while the plain bottle was used to collect blood for serum biochemical analysis.

The parasitaemia level was determined using the Rapid Matching method of Herbert and Lumsden (1976), while the average percentage chemo-suppression (CS) was calculated using the following formula (Dada and Oloruntola, 2016):

Chemosuppression = [(PNC-PSG)/PNC]*100

PNC: Parasitaemia level in the negative control; PSG: Parasitaemia level on the study groups.

The packed cell volume (PCV), red blood cells (RBC), haemoglobin concentration (HbC) and white blood cells WBC) were determined within two hours

post-collection following the procedures earlier described by (Shastry, 1983). The blood collected in the plain bottle was centrifuged, and its serum was collected into another plain bottle and frozen at -20ºC until use. The serum enzymes and biochemical (alanine transaminase, aspirate transaminase, urea, creatinine, and cholesterol) were determined with a Reflotron ® Plus BC79 (Roche Diagnostic, GombH Mannheim, Germany).

STATISTICAL ANALYSIS

All data were put through one-way analysis of variance using Statistical Package for Social Sciences (SPSS) version 20 using the following model: $Y_{ij}=m+f_i+E_{ij}$, where $Y_{ij=}$ the dependent observed variable's value, m=population mean, f_i= effect of ith treatment and E_{ij}=random error. The means were separated with Duncan multiple range test of SPSS.

RESULTS

EAL's phytochemical analysis divulged the existence of saponin, tannins, flavonoids, alkaloids, and cardiac glycosides, as shown in **Figure 1**. The effect of EAL treatment on the parasitemia level and percentage chemo-suppression in albino rats infected with *Trypanosoma brucei brucei* on day 15 post-infection is shown in **Table 1**. The parasitaemia level that was recorded in the groups treated with the standard drug and 1500mg/kg body weight EAL were statistically similar (P>0.05) to the control group, but lower (P<0.05) than the rest groups. The highest (P<0.05) chemo-suppression was being recorded in the group treated with standard drug, compared to the rest groups. The chemo-suppression percentage improves (P<0.05) significantly with an increase in the EAL dosage from 500mg/kg to 1000 mg/kg and 1,500 mg/kg.

Figure 2 shows *Albizia gummifera* ethanolic extract's effect on the parasitemia level per day (x10^{3}µ/ml) in albino rats infected with *Trypanosoma brucei brucei*. The daily parasitemia levels increased in the albino rats in all groups, except for the control group, from day three post-infection until day ten post-infection, when

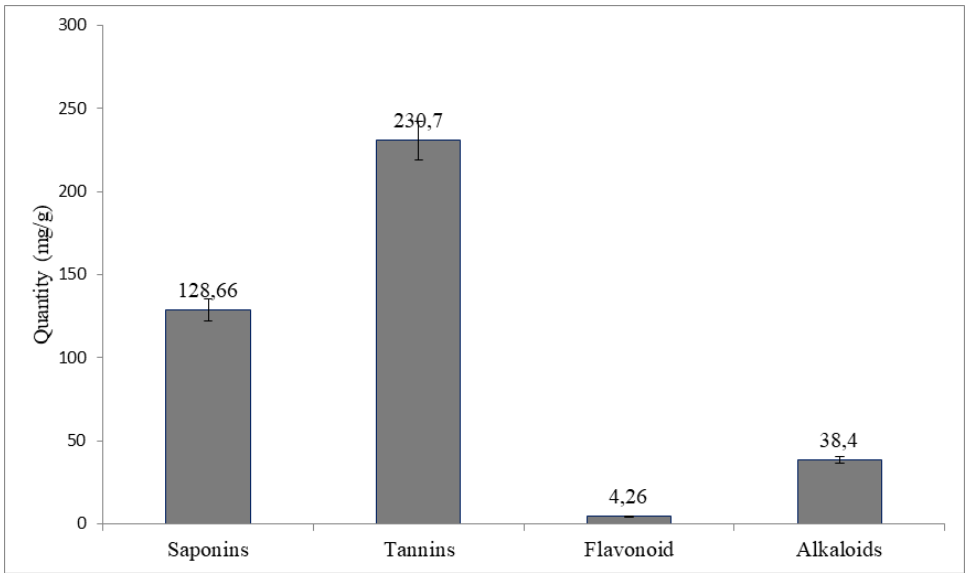

Figure 1. *Phytochemicals of ethanolic extract of Albizia gummifera leal (Fitoquímicos del extracto etanólico de Albizia gummifera leal).*

Table I. The effect of the ethanolic extract of *Albizia gummifera* leaf on the Parasitemia level and percentage chemo-suppression in albino rats infected with *Trypanosoma brucei brucei* days 15 post-infection (El efecto del extracto etanólico de hoja de Albizia gummifera sobre el nivel de parasitemia y el porcentaje de quimiosupresión en ratas albinas infectadas con Trypanosoma brucei brucei días 15 post-infección**).**

Group	Average parasitemia (x10^3/µml)	Chemo-suppression (%)
1	0.00±0.00^d	0.00±0.00^e
2	312.66±13.56^a	0.00±0.00^e
3	0.00±0.00^d	100.00±0.00^a
4	163.00±5.77^b	48.00±0.57^d
5	23.67±1.45^c	92.33±0.34^c
6	8.00±0.57^d	97.35±0.33^b
P value	0.00	0.00

Means in the same column with different letters are significantly different (P<0.05); Group 1: Normal control (not infected and not treated); Group 2: Negative control (infected and not treated); Group 3: Positive control (infected and treated with standard drug); Group 4: Infected and treated with 500mg/kg body weight EAL; Group 5: Infected and treated with 1000mg/kg body weight EAL; Group 6: Infected and treated with 1500mg/kg body weight EAL.

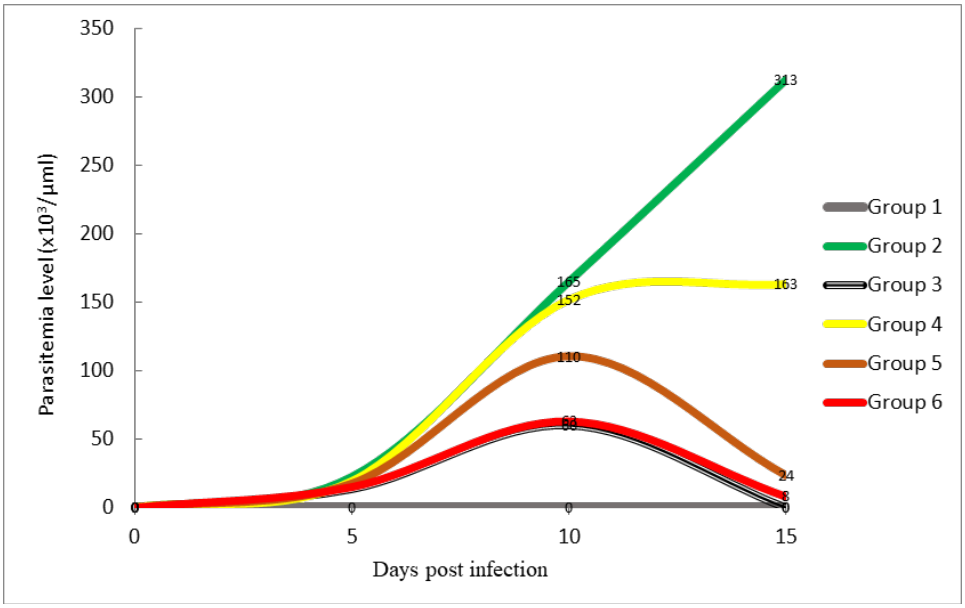

Figure 2. The effect of ethanolic extract of Albizia gummifera ethanolic leaf on the Parasitemia level/day (x103µ/ml) of albino rats infected with Trypanosoma brucei brucei. EAL: Ethanolic extract of Albizia gummifera leaf; Group 1: Normal control (not infected and not treated); Group 2: Negative control (infected and not treated); Group 3: Positive control (infected and treated with standard drug); Group 4: Infected and treated with 500mg/kg body weight EAL; Group 5: Infected and treated with 1000mg/kg body weight EAL; Group 6: Infected and treated with 1500mg/kg body weight EAL (El efecto del extracto etanólico de hoja etanólica de Albizia gummifera sobre el nivel de parasitemia / día (x103µ / ml) de ratas albinas infectadas con Trypanosoma brucei brucei. EAL: Extracto etanólico de hoja de Albizia gummifera; Grupo 1: Control normal (no infectado y no tratado); Grupo 2: Control negativo (infectado y no tratado); Grupo 3: Control positivo (infectado y tratado con fármaco estándar); Grupo 4: Infectados y tratados con 500mg/kg de peso corporal EAL; Grupo 5: Infectados y tratados con 1000mg/kg de peso corporal EAL; Grupo 6: Infectados y tratados con 1500mg/kg de peso corporal EAL).

it declined at varying rates in groups treated with the standard drug (group 1) and EAL (groups 4, 5 and 6).

Table 2 shows the effects of *Albizia gummifera* ethanolic extract treatment on the erythrogram of albino rats infected with *Trypanosoma brucei brucei*. The PCV of the rats infected and not treated (group 2) and infected and treated with 500mg/kg EAL (group 4) were significantly (P<0.05) lower, compared to the control group on days 10 and 15 post-infection; while the PCV of the rats treated with the standard drug (group 3), 1000mg/kg EAL (group 5), and 1500mg/kg EAL (group 6) were similar (P>0.05) to those in the control group. On day five post-infection, the RBC counts were lower (P<0.05) in groups 2 and 4, compared to the control groups and other treatment groups. However, on days 10 and 15 post-infection, the RBC counts of all the infected groups (groups 2 to 6) were significantly (P<0.05) lower, compared to the control group. However, the level of anaemia resulting from reduced RBC is more severe in groups 2 and 4. The HbC of the rats in groups 2 and 4 were significantly (P<0.05) lower, compared to those in the control group; while the HbC of the rats in groups 3, 5 and 6 were similar (P>0.05) to the control.

Figure 3 depicts the effects of *Albizia gummifera* ethanolic extract treatments on the WBC count of albino rats infected with *Trypanosoma brucei brucei*. On day 15 post-infection, the WBC counts of rats in groups 2, and 4 were lower (P<0.05) than groups 1, 3 and 6. The alanine transaminase, urea, creatinine and cholesterol levels were not significantly (P>0.05) different across the various groups; while the aspartate transaminase level in groups 2 and 4 were significantly (P<0.05) higher, compared to the rest groups (**Table 3**).

DISCUSSION

Myriad of plants have bioactive compounds that express medicinal properties (Oloruntola *et al*., 2018). The existence of phytochemicals such as saponins, tannins, flavonoids, alkaloids and cardiac glycosides in EAL suggests that the extract could produce therapeutic effects. For instance, the anti-trypanocidal activities of saponins (Ibrahin *et al*., 2013), flavonoids (Tasdemir *et al*., 2006), and alkaloids (Krstin *et al*., 2015) was reported.

Sundry reviews on plants and phytogens with trypanocidal properties have been published (Mbaya and Ibrahim, 2011; Ameenah and Mohamad, 2013). Precursory studies attributed the trypanocidal activity of phytogens to flavonoids and highly aromatic alkaloids (Nok, 2002; Omale and Joseph, 2011). It was relayed that phytogens has structure liable for creating radicals that may bring about the peroxidative destruction to trypanothione reductase that is very easily affected by alterations in redox balance (Umar *et al*., 2010). Therefore, phytogens exhibits antitrypanocidal activity by interfering with the redox balance of the parasite acting on the cellular defences against oxidative stress (Umar *et al*., 2010), interference of the phytogenic bioactive compounds with mitochondrial electron transport systems of the trypanosomes (Nok, 2002), and increasing the methylation of hydroxyl groups which results in the increased lipophilicity and consequent increased permeability of molecule across the parasites' membrane (Nwodo *et al*., 2015) are among suggested mechanism of action of phytogens bioactive compounds against trypanosomes. The reduction of

Table II. The effect of ethanolic extract of *Albizia gummifera* leaf treatment on the erythrogram of albino rats infected with *Trypanosoma brucei brucei* (El efecto del extracto etanólico del tratamiento de la hoja de *Albizia gummifera* en el eritrograma de ratas albinas infectadas con *Trypanosoma brucei brucei*).

Erythrogram	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	P value
PCV (%)							
Day 0	51.44±1.02	51.17±0.94	51.26±1.11	50.72±0.98	50.53±0.95	51.19±1.05	0.98
Day 5	49.25±0.49	45.56±1.60	51.42±1.76	45.73±1.67	50.83±1.72	50.69±1.92	0.75
Day 10	50.48±0.93^{a}	44.65±1.93^{b}	51.44±1.23^{a}	45.43±2.07^{bc}	49.54±0.87^{ab}	50.57±0.94^{a}	0.02
Day 15	51.43±1.28^{a}	41.47±1.3^{c}	51.56±1.77^{a}	42.53±0.77^{b}	49.75±0.75^{a}	50.95±1.22^{a}	0.01
RBC (x10^{6}/mm^{3})							
Day 0	8.32±0.36	6.95±0.86	8.40±0.51	6.55±1.35	6.90±0.11	7.50±0.05	0.23
Day 5	8.28±0.26^{a}	6.70±0.05^{b}	7.90±0.23^{a}	6.81±0.00^{b}	7.60±0.40^{a}	7.78±0.04^{a}	0.01
Day 5	8.28±0.26^{a}	6.70±0.05^{b}	7.90±0.23^{a}	6.81±0.00^{b}	7.60±0.40^{a}	7.78±0.04^{a}	0.01
Day 10	8.32±0.21^{a}	6.59±0.17^{c}	7.50±0.23^{b}	6.72±0.06^{c}	7.15±0.08^{bc}	7.45±0.37^{b}	0.01
Day 15	8.45±0.26^{a}	6.01±0.05^{d}	7.77±0.02^{b}	5.95±0.08^{d}	7.25±0.08^{c}	7.64±0.15^{bc}	0.00
HbC (g/l)							
Day 0	17.15±0.34	17.06±0.31	17.08±0.37	16.91±0.31	16.84±0.32	17.06±0.34	0.98
Day 5	16.41±0.16	15.18±0.53	17.14±0.58	15.24±0.55	16.94±0.57	16.89±0.64	0.07
Day 10	16.83±0.31	14.88±0.64	17.14±0.41	15.14±0.68^{bc}	16.51±0.29^{ab}	16.85±0.31^{a}	0.02
Day 15	17.14±0.4	13.82±0.4	17.18±0.5	14.18±0.25^{b}	16.58±0.25^{a}	16.58±0.49^{a}	0.01

Means with different superscripts along the rows are significant (P<0.05); PCV: Packed cell volume; RBC: Red blood cell, HbC: Haemoglobin concentration; EAL: Ethanolic extract of *Albiziagummifera* leaf; Group 1: Normal control (not infected and not treated); Group 2: Negative control (infected and not treated); Group 3: Positive control (infected and treated with standard drug); Group 4: Infected and treated with 500mg/kg body weight EAL; Group 5: Infected and treated with 1000mg/kg body weight EAL; Group 6:Infected and treated with 1500mg/kg body weight EAL.

the parasitemia level and improved chemo-suppression percentage with increased EAL dosage from 500 mg/kg body weight - 1,500 mg/kg body weight suggests the bioactive components of EAL possess trypanocidal properties. The anti-trypanosomal activity of *Albizia species* has been reported earlier (Al-Musayeib *et al*., 2012). For instance, flavonoids, one of the bioactive compounds was earlier reported to cause in hibition of the energetic metabolism, cell proliferation, cytoadherence, proteion kinases and modulation of the

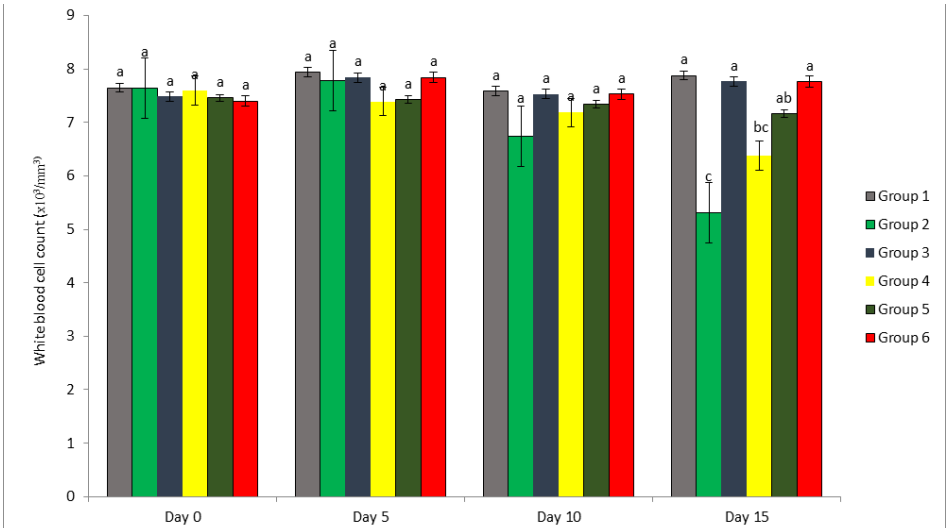

Figure 3. The effect of ethanolic extract of Albizia gummifera leaf on the white blood cell count of albino rats infected with Trypanosoma brucei brucei. EAL: Ethanolic extract of Albizia gummifera leaf; Group 1: Normal control (not infected and not treated); Group 2: Negative control (infected and not treated); Group 3: Positive control (infected and treated with standard drug); Group 4: Infected and treated with 500mg/kg body weight EAL; Group 5: Infected and treated with 1000mg/kg body weight EAL; Group 6: Infected and treated with 1500mg/kg body weight EA (El efecto del extracto etanólico de la hoja de Albizia gummifera en el recuento de glóbulos blancos de ratas albinas infectadas con Trypanosoma brucei brucei. EAL: Extracto etanólico de hoja de Albizia gummifera; Grupo 1: Control normal (no infectado y no tratado); Grupo 2: Control negativo (infectado y no tratado); Grupo 3: Control positivo (infectado y tratado con fármaco estándar); Grupo 4: Infectados y tratados con 500mg/kg de peso corporal EAL; Grupo 5: Infectados y tratados con 1000mg/kg de peso corporal EAL; Grupo 6: Infectado y tratado con 1500mg/kg de peso corporal EAL).

inflammatory response in pathogenic protozoa such as *Trypanosoma species* (Martinez-Castillo *et al*., 2018). Besides, variation in the parasitemia level curve/pattern in the various groups and in particular the declined parasitemia level in control and the groups treated with the varying dosages EAL in day ten post-infection shows that the dosage and chemical composition of any chosen drugs must be put into serious consideration when managing trypanosome infestation.

The severity of trypanosome infection is connected to the degree of parasitemia and consequently, anaemia (Umar *et al*., 2000). Reports showed that packed cell volume could be used as an indicator of trypanosomal infection (Marcotty *et al*., 2008). Therefore, the relatively low PCV percentage recorded in the infected but not treated rats (group 2) and infected and treated with 500 mg/kg EAL (group 4) compared to the control and other groups in this study is indicative of the high level of severity of the trypanosomal infection in the rats. This result also divulged EAL's efficacy at 1,000-1,500 mg/kg body weight dosage in ameliorating or eliminating the adverse effects of trypanosomal infection in infected rats. This is supported by the reduced parasitemia level and improved chemo-suppression earlier recorded in the rats treated with the 1,000 and 1,500 mg/kg EAL in this study.

The reduced red blood cell counts recorded in all trypanosome infected rats (groups 2 to 6) on day 10 and 15 post-infection in this study compared to the control group may also be linked to the parasite activities in increasing the erythrophagocytosis. Anaemia also occurs in trypanosomiasis cases due to RBC – Trypanosoma adhesion (Boada-Sucre *et al.,* 2016). Free flagellums of the trypanosome glueing to RBC are supported by the emission of filopodia and filamentous material of parasitic origin (Boada-Sucre *et al.,* 2016). Therefore, the close contiguity between trypanosomes and RBC via sialic acid receptors bring about trauma to erythrocyte membranes at the point of contact (Mbaya *et al*., 2012). Besides, there occur changes in oligosaccharide make-up of the RBC surface, obvious membrane fusion, and mechanical injury caused by the motility of the trypanosomes in the bloodstream being caused by the biochemical, induced ultrastructural, and antigenic alteration of the RBC, which increases the erythrophagocytosis (Huson *et al*., 2009, Boada-Sucre *et al*., 2016). It was being observed that the level of anaemia (as a result of reduced RBC count) is more severe in groups 2 and 4, compared to the control and other groups in this study. This is supported by the haemoglobin concentration examination, which also revealed decreased haemoglobin concentration in the same group of rats (groups 2 and 4). Declined haemoglobin concentration is another feature associated with trypanosomal infection (Kagira *et al*., 2006).

Leukocytopenia in Trypanosome infection was reported in previous studies (Ndung'u *et al*., 2020; Kagira *et al*., 2006). In the same vein, the reduction of the white blood cells count, monocytes count, and granulocytes count on day 15 post-infection in groups 2, and 4 further indicates the severity of the parasitic infection in the aforementioned groups, compared to other groups and the possible active roles of these cells in the immunopathogenesis of trypanosomiasis. The observed leukocytosis post trypanosome infection could be attributed to leucophagocytosis due to leucocytes' antigen coating and declined white blood cell production (Kagira *et al*., 2006).

Serum biochemistry gives details about the physiological state of organs of organisms and, enzymes are very useful in detecting hepatic, muscular, pancreatic, skeletal and skeletal disorders (Oloruntola *et al*., 2018). For instance, the aspartate transaminase serum concentration is useful in diagnosing liver and biliary diseases and skeletal muscle diseases (Oloruntola *et al*., 2016). The elevated aspartate transaminase serum concentration reported in the groups of rats infected with *Trypanosoma brucei brucei* (group 2) and those infected with *Trypanosoma brucei brucei* infected and subsequently treated with 500 mg/kg body weight EAL (group 4) suggests possible liver damage and myocardial infarction, as a consequence of the *T. brucei brucei* infection (Akinseye *et al*., 2020). Bosschaerts *et al*. (2009) reported that uninhibited inflammation linked with the persistence of myeloid cells/macrophages (M1) cells is also a prime cause of liver lesion observed in trypanosusceptible animals. The elevated aspartate transaminase serum concentration recorded in the *T. brucei brucei* infected rats and treated with 500 mg/kg body weight EAL suggests that the 500 mg/kg body weight EAL therapy was not efficiently preventing this earlier mentioned pathogenic feature.

Table III. The effect of ethanolic extract of *Albizia gummifera* leaf on the serum biochemical and enzymes activities of albino rats infected with *Trypanosoma brucei brucei* (El efecto del extracto etanólico de la hoja de *Albizia gummifera* en las actividades bioquímicas y enzimáticas séricas de ratas albinas infectadas con *Trypanosoma brucei brucei*).

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	P value
Alanine transaminase (I.U/l)	20.06	24.36	20.17	24.08	20.06	20.51	0.21
Aspertate transaminase (I.U/l)	42.29^{b}	54.61^{a}	44.05^{b}	53.52^{a}	42.49^{b}	42.63^{b}	0.01
Urea (mg/dl)	14.26	16.21	14.36	16.79	14.58	14.29	0.98
Creatinine (mg/dl)	0.36	0.53	0.42	0.45	0.42	0.40	0.26
Cholesterol (mg/dl)	18.15	19.59	19.06	18.67	19.98	18.26	0.98

Means with different superscripts along the rows are significant (P<0.05); EAL: Ethanolic extract of *Albizia gummifera* leaf; Group 1: Normal control (not infected and not treated); Group 2: Negative control (infected and not treated); Group 3: Positive control (infected and treated with standard drug); Group 4: Infected and treated with 500mg/kg body weight EAL; Group 5: Infected and treated with 1000mg/kg body weight EAL; Group 6: Infected and treated with 1500mg/kg body weight EAL.

CONCLUSIONS

In this study, it was demonstrated that 1500mg/kg EAL body weight reduced the parasitemia level and that the increase in the dosage of the EAL from 500mg/kg EAL to 1000 mg/kg EAL and 1,500mg/kg EAL improves the chemo-suppression percentage in the experimental rats. The results revealed that anaemia, leukocytopenia, liver and myocardial infarction are possible post-Trypanosoma parasite infection effects or signs in the experimental rats. These aforementioned pathological effects could be prevented or ameliorated by 1000 mg/kg EAL and 1,500mg/kg EAL therapy.

BIBLIOGRAPHY

Akinseye, OR, Mustapha, A, & Angela, AN 2020, Biochemical indicators in trypanosomiasis infections. *Journal of Analytical and Pharmaceutical Research*, vol.9, no. 1, pp. 11-14.

Al-Musayeib, NM., Mothana, RA., Al-Massarani, S, Matheeussen, A, Cos, P, & Maes, L 2012, Study of the *in vitro* Antiplasmodial, antileishmanial, and anti-trypanosomal activities of medicinal plants from Saudi Arabia. *Molecules*, vol. 17, no.10, pp. 11379-11390.

Ameenah, G, & Mohamad, FM 2013, African flora as potential sources of medicinal plants: Towards the chemotherapy of major parasitic and other infectious diseases—A review. *Jordan Journal of Biological Science*, vol. 6, pp.77–84.

Boada-Sucre, AA, Spadafor, MSR, Tavares-Marques, LM, Finol, [HJ,] & Reyna-Bello, A, 2016, *Trypanosoma vivax* adhesion to red blood cells in experimentally infected Sheep. *Pathology Research International*, vol. 2016, pp.4503214.

Bosschaerts, T, Guilliams, M, Stijlemans, B, De Baetselier, P, & Beschin, A, 2009, Understanding the role of monocytic cells in liver inflammation using parasite infection as a model. *Immunobiology*, vol. 214, no. 9-10, pp. 737-747.

Chang, CC, Yang, MH., Wen, HM, & Chern, JC, 2002, Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, vol. 10, no.3, pp. 178-182.

Dada, EO, & Oloruntola, DA 2016, *In vivo* Antiplasmodial activity of ethanolic leaf extract of *Tithonia diversifolia* (Hemsl.) A.Gray against *Plasmodium berghei* Nk65 in infected Swiss Albino mice. *Journal Applied Life Science International*, vol. 8, no. 3, pp. 1-8.

Ezeonu, CS, & Ejikeme, CM 2016, Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New Journal of Science,* vol. 2016,pp. 1-9. https://doi.org/10.1155/2016/5601327

Giordani, F, Morrison, LJ, Rowan, TG, De Koning, HP, & Barrett, MP 2016, The animal trypanosomiases and their chemotherapy: a review. *Parasitology*, vol. 143, no. 14, pp. 1862-1889.

Herbert, WJ., & Lumsden, WHR 1976, Trypanosoma brucei: A rapid "matching' method for estimating the host's parasitemia. Experimental Parasitology, vol. 40, no. 3, pp. 427-431.

Huson, LE, Authié, E, Boulangé, AF, Goldring, JP, & Coetzer, TH, 2009, Modulation of the immunogenicity of the *Trypanosoma congolense* cysteine protease, congopain, through complexation with alpha (2)-macroglobulin. *Veterinary Research,* vol. 40, no.6, pp.52.

Ibrahim, MA, Aliyu, AB, Meduteni, K, & Yunusa, I 2013, Saponins-rich fraction of Calotropis procera leaves elicit no anti-trypanosomal activity in a rat model. *Asian Pacific Journal of Tropical Biomedicine*, vol. 7, no. 7, pp. 569-572.

Kagira, JM, Thuita, JK, Ngotho, M, Mdachi, R, Mwangangi, DM, & Ndung'u, JM 2006, Haemagology of experimental Trypanosoma brucei rhodesiense infection in vervet monkey. *African Journal of Health Science*, vol.13, pp.59-65.

Krstin, S, Peixoto, HS, & Wink, M 2015, Combination of alkaloids affecting different molecular targest with the Saponin digitonin can synergistically enhance trypanocidal activity against *Trypanosoma brucei brucei*. *Anitimcribial Agents and Chemotheraphy*, vol. 59, no. 11, pp. 7011-7017.

Mahlangu, ZP, Botha, FS, Madoroba, E, Chokoe, K, & Elgorashi, EE 2017, Anti-microbial activity of *Albizia gummifera* (J.F. Gmel.) C.A.Sm leaf extracts against four Salmonella serovars. *South African Journal of Botany*, vol. 108, pp.132-136.

Marcotty, T, Simukokoo, H, Berkvens, D, Vercruysse, J, Praet, N, & Van de Bossche, P 2008, Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia. *Preventive Veterinary Medicine*, vol. 87, no. 3-4, pp. 288-300.

Martinez-Castillo, M, Pacheco-Yepez, J, Flores-Huerta, N, Guzman-Tellez, P, Jarillo-Luna, RA, Cardenas-Jaramillo, LM, Campos-Rodriguez, R, & Shibayama, M 2018, Flavonoids as a natural treatment against *Entamoeba histolytica. Frontiers in Cellular and Infection Microbiology,* vol. 8, pp. 209. doi: 10.3389/fcimb.2018.00209. www.frontiersin.org.

Mashi, JA, Sa'id, AM, Bello, F, Yakasai, HM, Bello, B, &Idris, RI (2019). Biochemical indices and haematological studies of Ethyl acetate extract of Persea Americana leaf in Albino rats. *Asian Journal of Research in Biochemistry*, vol. 4, no. 4, pp. 1-10.

Mbaya, A, Kumshe H, & Okwudiri-Nwosu, C 2012, The mechanism of anaemia in trypanosomosis: a review. In: Silverberg D., editor. *Anemia.* In Tech. Pp. 269–282.

Mbaya, AW, & Ibrahim, UI 2011, *In vivo* and *in vitro* activities of medicinal plants on haemic and humoral trypanosomes: A review. *International Journal of Pharmacology*, vol. 7,pp. 1–11.

Mbosso, EJT, Ngouela, S, Nguedia, JCA, Beng, VP, Rohmer, M, & Tsamo, E 2010, *In vitro anti-microbial activity of extracts and compounds of some selected medicinal plants from Cameroon. Journal of Ethnopharmacology*, vol. 128, no. 2010, pp. 476-481.

Ndung'u, K, Murlla, GA, Thuita, JK, Ngae GN, Auma, JE, Gitonga, PK, Thungu, DK, Chemuliti J & Mdachi, RE 2020, Defferential virulence of Trypanosoma brucei rhodesiense isolates does not influence the outcome of treatment with anti-trypanosomal drugs in the mouse model. *Plos One,* vol. 15, no. 11, pp. e0229060.

Nok, AJ 2001, Azaanthraquinone inhibits respiration and in vitro growth of long slender blood stream forms of T. congolense. *Cell Biochemistry and Function*, vol. 20, pp. 205-212.

Nwodo, NJ, Ibezim, A, Ntie-Kang, F, Adikwu, MU, & Mbah, CJ, 2015b, Anti-trypanosomal activity of Nigerian plants and their constituents. *Molecules*, vol. 20, pp.7750-7771.

Nwodo, N, Okoye, F, Lai, D, Debbab, A, Kaiser, M, Brun, R & Proksch, P 2015a, Evaluation of the *in vitro* trypanocidal activity of methylated flavonoid constituents of *Vitex simplicifolia* leaves. *BMC Complementary and Alternative Medicine*, vol. 15, pp. 82. doi: 10.1186/s12906-015-0562-2.

Obadoni, BO, & Ochuko, PO 2002, Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta states of Nigeria. *Global Journal of Pure and Applied Science*, vol. 8, no. 2, pp. 203-208.

Oloruntola, OD, Agbede, JO, Ayodele, SO, & Oloruntola, DA 2018, Neem, pawpaw, and bamboo leaf meal dietary supplementation in broiler chickens: Effect on performance and health status. *Journal of Food Biochemistry*. Vol. 42, no. 2, pp. e12723.

Oloruntola, OD, Ayodele, SO, Agbede, JO, & Oloruntola, DA 2016, Effect of feeding broiler chicken with diets containing *Alchornea cordifolia* leaf meal and enzyme supplementation. *Archivos de Zootecnia*, vol. 65, no. 252, pp. 489-498.

Omale, J, & Joseph, JE 2011, Comparative evaluation of trypanocidal activities of *Cissus multistriata* and *Saba florida* (benth) leaf extracts. *Journal of Bioscience and Technology*, vol. 2,pp. 197–204.

Parasuraman, S, Raveendran, R, & Kesavan, R 2010, Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutic*, vol. 1, no. 2, pp. 87–93.

Rukunga, GM, Muregi, FW, Tolo, FM, Omar, SA, Mwitari, P, Muthaura, CN, Omlin, F, Lwande, W, Hassanali, A, Githure, J, Iraqi, FW, Mungai, GM, Kraus, W, & Kofi-Tsekpo, WM, 2007, *The antiplasmodial activity*

of spermine alkaloids isolated from Albizia gummifera. *Fitoterapia*, vol. 78, no. 7-8, pp.455-459.

Shastry, GA, 1983, Veterinary clinical pathology (2nd ed.). New Delhi, India: CBS Publishers and Distributors.

Sutherland, CS, Yukich, J, Goeree, R, & Tediosi, F 2015, A literature review of economic evaluations for neglected tropical disease: Human African trypanosomiasis (Sleeping sickness). *Plos Neglected Tropical Disease*, vol. 5, no. 9(2), pp. e0003397. doi:10.1371/journal.pntd.0003397.

Tasdemir, D, Kaiser, M, Brun, R, Yardley, Schmidt, T.J., Tosun, F, & Ruedi, P, 2006, Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrobial Agents and Chemotheraphy*, vol. 50, no. 4, pp.1352-1364.

Toya, NB 2010, Immunobiology of African trypanosomes: Need of alternative intervention. *Journal of Biomedicine and Biotechnology*, vol. 2010, pp.389153.

Umar, IA, Ibrahin, MA, Fari, NA, Isah, S, & Balogun, DA 2010, In-vitro and –vivo anti-trypanosoma evansi activities of extracts from different parts of *Khaya senegalensis*. *Journal of Cell and Animal Biology*, vol. 4, no. 6, pp. 91-95

Umar, IA, Toh, ZA, Igbalajobi, FI, Igbokwe, IO, & Gidado, A 2000, The effect of orally administered vitamin C and E on severity of aneamia in *Trypanosoma brucei* – infected rats. *Tropical Veterinarian*, vol. 18, pp. 71-77.

WHO 2013, Control and surveillance of human African trypanosomiasis. World Heal Organ Tech Rep Ser: 1237. http://www.ncbi.nlm.nih.gov/pubmed/24552089.