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Original article

Effects of crude ethanolic extract of *Terminalia catappa* on the haematology parameters of red Sokoto goat sex perimentally infected with *Trypanosoma brucei brucei*

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ABSTRACT

The in-vivo activity of crude ethanolic extract of *Terminalia catappa* stem bark administered orally and intraperitoneally on red Sokoto goats experimentally infected with *Trypanosoma brucei brucei* was investigated. 15 male goats weighing between 8kg to 10kg were randomly grouped into 5 groups with three goats per group. Goats in all the 5 groups were infected intravenously with *T. b. brucei* and after demonstration of parasitaemia Groups 1 to 4 were administered crude ethanolic extract of *T. catappa* as follows: 50mg/kg orally to group 1, 100mg/kg orally to group 2, 50mg/kg intraperitoneally to Group3and 100mg/kg intraperitoneally to group4) while group5 served as untreated control. All treatments were given for 7 days. Result of phytochemical analysis shows alkaloids, saponins, terpenoids and steroid, increase in weight, Packed Cell Volume (PCV) and Red Blood Cells (RBC) indices after treatment in treated groups were also observed. It was concluded that *T. catappa* ethanolic extract given at a dose of 50 to 100mg/kg is effective and safe for the treatment of *T.b. brucei* infection in goats.

1. Introduction

Trypanosomes are protozoan parasites infecting a variety of domestic and wild animals as well as humans causing a debilitating disease. *Trypanosoma brucei gambiense* and *Trypanosoma rhodesiense* cause Human African Trypanosomosis (HAT), commonly called sleeping sickness, while *Trypanosoma brucei brucei,* is responsible for Animal Africa Trypanosomiasis (AAT), or Nagana in animals. In Africa, trypanosomosis is transmitted by tsetse fly of the Genus *Glossina* which is found in 37 countries of Africa (Denbarga et al., 2012). In these countries the disease poses a significant health challenge to humans and livestock (World Health Organisation, 2012). The wide spread occurrence of Trypanosomosis largely account for Africa's low livestock productivity and it is responsible for reducing the source of animal protein as well as contributing to food insecurity in the region (Malafaia and Talvani, 2011). Trypanosomiasis is transmitted biologically through the bite of a tsetse fly (*Glossina spp*.) but may also be transmitted through fomites and mechanical vectors including surgical instruments, needles, syringe and various biting flies (*Tabanids)*. Some species of trypanosomes are reported to be transferred between mammals through exchange of body fluids, such as in blood transfusion or sexual contact (Rocha et al., 2004).

Infection in humans is characterized within a few weeks by swollen lymph glands, aching muscles and joints, headaches and irritability. The second phase of the disease is characterized by involvement of the central nervous system with extensive neurological effects which if not treated can cause changes in personality, alteration of the biological clock (the cardiac rhythm), confusion, slurred speech, seizures and difficulty in walking and talking and finally, terminating in death (Mare, 2008). However, Nagana which results due to infection with *Trypanosomabruceibrucei* becomes apparent in infected animals in about 7 to 10 days. In such animals, the body temperature rises, heart and respiratory rates increase. The disease is also characterized by intermittent fever, odema, variable appetites, diarrhoea, body weakness, swelling of the eyelid, staggering, dull coats, lachrymation and emaciation (Anosa, 1988). In addition, an infected animal becomes anaemic and death may result.

2. Materials and methods

2.1. Sample plant

Terminalia catappa is a large plant belonging to the family *Combretaceae.* It is also known as "INDIAN ALMOND" or "TROPICAL ALMOND" while its common name is "fruit". In Nigeria, the Northerners call it "BAUSHE"; the Westerners call it "AYIN" or **"**GBE-FON" while the Easterners call it "EDO".

2.2. Sample collection

Fresh stem barks of *Terminalia catappa* were collected in polythene bags from Mando, Kaduna, Kaduna State between the months of June and July, 2014. During sample collection, relevant plant parts that could be useful in identification were also collected and taken to the habarium unit of the Department of Biological Science Laboratory, Ahmadu Bello University, Zaria. The plant was identified by a Taxonomist using appropriate keys and assigned a voucher number (1556). Plant stem barks collected were rinsed in distilled water to remove adhering dirt and later air dried for four weeks in the laboratory until it attained a constant weight. The sample of dried stem bark was grounded to fine powder using porcelain mortar and pestle. The resultant powder was stored in brown air-tight bottles at room temperature until required (Sofowora, 1993).

2.3. Extract preparation

2.3.1. Preparation of crude ethanolic extract (CEE)

Plant extract was prepared using percolation method as described by (Garba and Okeniyi, 2012).). The procedure involves weighing 500g of powdered *Terminalia catappa* and percolating in 1500cm^3 of Ethanol for seven (7) days in a conical flask. The resulting extract was filtered and concentrated using a rotary evaporator at 40^oC to obtain crude ethanol extract (CEE).

2.3.2. Phytochemical screening of extracts

Phytochemical analysis of the extracts were conducted to determine the presence of secondary metabolites such as alkaloids, saponins, steroids, terpenoids and flavonoids using standard procedures as described by (Edeoga et al., 2005)

2.4. Experimental animals

15 male goats weighing between 8kg to 10kg were purchased from Afaka village market near Kaduna town in Kaduna State, Nigeria. The animals were transported immediately after purchase to the laboratory of Biological Science Department, Nigerian Defence Academy, Kaduna, where they were screened for ecto-parasites infestation, gastro-intestinal and haemo-parasitic infection by a Veterinarian. Animals found to be infected were treated. All the animals were kept for 2 months to acclimatize during which they were allowed access to water freely and access to feed composed of shaft of millet, sorghum grains and dried groundnut leaves.

2.5. Housing of animals

Animals were housed in cemented floor pens that were provided with a fly proof isolation unit with adequate ventilation throughout the experimental period. The pens were cleaned and disinfected with Izal daily.

2.6. Trypanosome stock

Blood sample was collected by cardiac puncture from a *T.b. brucei* infected mouse using EDTA coated syringe with needle in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University (ABU), Zaria, Nigeria. Blood collected was immediately diluted with physiological saline to obtain $1x10^3$ parasites (trypanosomes) per microscope field. 0.1ml of infected blood containing $1x10^3$ was inoculated intraperitoneally into four uninfected mice that were to serve as donor mice. Infection was monitored every morning by microscopic examination of blood samples taken from the tails of the four infected mice until parasitaemia was established.

2.7. Experimental infection of goats / grouping

Blood sample was collected from donor mice by cardiac puncture using an EDTA coated syringe and immediately diluted with physiological saline to serve as the innoculum. Each experimental goat was infected intravenously with 2ml of the inoculum containing at least 2 to 3 parasites per field. Goats in all the groups were monitored daily for onset of parasitemia through wet blood preparation that was examined microscopically. When parasitaemia was fully established in all groups, goats in groups 1 to 4 were treated for seven days by either oral or intraperitoneal administration of crude ethanolic extract stem bark of *T. catappa* at 50mg/kg and 100mg/kg doses respectively.

2.8. Experimental design/ grouping

Group I Crude ethanolic extract of *Terminalia catappa* administered intraperitoneally to goats (50mg/kg)
Group II Crude ethanolic extract of *Terminalia catappa* administered orally to goats (50mg/kg)
Group III Crude ethanolic extract of *Terminalia catappa* administered intraperitoneally to goats(100mg/kg)
Group IV Crude ethanolic extract of *Terminalia catappa* administered orally to goats (100mg/kg)
Group V Infected goats and untreated with crude ethanolic extract of *Terminalia catappa*

2.9. Statistical analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS) computer software, version 20. The Differences of mean analysis and standard deviation were carried out using Analysis of Variance (ANOVA) and Student T test where applicable. Probability of 0.05 ($P<0.05$) was considered significant.

3. Results and discussion

3.1. Phytochemical analysis of *T. catappa*

The result of phytochemical analysis of *T. catappa* crude ethanolic extract is presented in Table 1. Although the phytochemicals were not quantified, Alkaloids, saponins, terpenoids and steroids were detected in extract of *T. catappa* extracted by crude ethanolic extraction method. However, flavonoids were not detected in extracts of *T. catappa* processed by ethanolic extraction.

Table 1
Phytochemical constituent detected in *T. catappa* stem bark extracted using ethanolic extraction.

	Phytochemicals				
Extraction method	Alkaloids	Flavonoids	Saponins	Terpenoids	Steroids
Crude ethanolic	+	-	+	+	+

+ present, - absent

3.2. Physical parameters of experimental goats

3.2.1. Clinical presentation

All the animals in all the groups (1 to 5) exhibited clinical signs of trypanosomes after 12 days of inoculation with the onset of parasitaemia. Before the commencement of *T. catappa* crude ethanolic extract administration, infected animals in all the groups showed sign of fever, dullness, weight loss and reduction in food intake. Deaths of two animals in group 5 (infected, but not treated) were recorded.

3.2.2. Goats rectal temperature

Although the rectal temperature of goats in all the 5 treatment groups were found to fluctuate slightly during treatment (Poi) when compared with pretreatment (Pi) rectal temperature, the difference was not statistically significant ($P<0.05$). in the treatment groups 1 to 4. Mean temperature fluctuation was between -0.03 and + 0.44 degrees Celsius (^{o} C). However, relatively higher mean rectal temperature variation (+1.5^{o}C) was recorded in the control (untreated) group when pre-infection rectal temperature was compared with post infection rectal temperature of goat.

3.2.3. Mean live weights of experimental goats

The mean weight (kg) of goats before infection (Pi) and after infection (Poi), and also during the experimental period is presented in Table 2. Animals in groups 1 to 4 did not show any significant change in weight when the Pi and Poi mean weights were compared ($P>0.05$). However, significantly higher mean weight change (4.6kg) was observed in the control group that were infected, but not treated ($P<0.05$).

Table 2
Mean physical condition of infected goats treated with crude ethanolic extract of *Terminalia catappa* and control group.

Index	Period	1	2	3	4	5
Mean temp.	Pi	39.13 (0.146)	39.96 (0.289)	39.27 (0.379)	38.93 (0.493)	38.47 (0.115)
(^{o}c) (±S.D)	Poi	39.30 (0.529)	38.93 (0.252)	39.30 (0.781)	38.90 (0.625)	39.97 (0.153)
Mean weight	Pi	8.8 (1.21)	7.8 (1.29)	10.0 (1.04)	8.4 (1.74)	8.4 (0.40)
(kg) (±S.D)	Poi	8.8 (1.27)	7.7 (1.39)	9.9 (0.91)	8.2 (1.59)	3.8 (0.21)

Pi = Pre infection, Poi= Post infection.

3.2.4. Parasitaemia profile

The effect of different concentration of crude ethanolic extract on the parasitaemia of *T.b. brucei* in infected goats is shown in Table 3. Parasitaemia was not detected in goats of groups 2 and 3 treated intraperitoneally with crude ethanolic extract at 50mgkg^{-1} and 100mgkg^{-1} respectively on day 4 of treatment. However, all the remaining groups given different concentrations of either crude ethanolic or ethyl acetate extracts orally or intraperitoneally showed significant reduction in parasitaemia by the 4th day of treatment.

3.2.5. Mean haematological index of goats

The result of the haematological study showed that there was a significant decrease in values of PCV, Hb and RBC after infection with *T.b. brucei* in all groups. The WBC values of all groups showed increased values after infection except in group 2 and 3. Although plasma protein values were not regular in all groups, they were sometimes higher and sometimes lower.

Table 3
Parasite clearance in goats treated with crude ethanolic extract of *T. catappa.*

Route of administration	Parasitaemia		Day		
	Extract	Dosage ($mgkg^{-1}$)	1	4	7
Oral	CE	50	++	+	-
	CE	100	++	+	-
Intraperitoneal	CE	50	++	-	-
	CE	100	++	-	-

CE= Crude ethanolic extract, += few trypanosomes per field, ++=several trypanosomes per field, -= absence of trypanosome in a field.

Table 4
Mean haematological indices of goats infected with *T.b. brucei* and treated with crude ethanolic extract of *T. catappa* and control.

	Mean haematological indices (± S.D)									
	PCV (%)		Hb (dl)		RBC		WBC		Plasma protein	
Group	Pi	Poi	Pi	Poi	Pi	Poi	Pi	Poi	Pi	Poi
1	27.33 (3.055)	25.00 (3.000)	9.10 (1.015)	8.30 (1.000)	13.33 (2.367)	11.50 (1.808)	10.17 (0.723)	11.03 (1.332)	6.47 (0.036)	6.40 (0.100)
2	32.00 (6.557)	29.33 (5.859)	10.60 (2.265)	10.00 (2.364)	12.47 (0.874)	12.00 (0.623)	11.23 (3.099)	10.37 (1.011)	6.13 (0.808)	5.87 (0.503)
3	26.33 (3.215)	25.00 (6.245)	9.67 (1.739)	8.37 (2.028)	14.07 (1.007)	13.57 (1.787)	16.87 (2.686)	13.90 (3.151)	5.93 (0.945)	6.23 (1.137)
4	27.67 (4.726)	27.33 (6.351)	9.23 (1.570)	8.20 (1.587)	14.60 (0.794)	13.30 (1.015)	14.60 (2.816)	15.83 (1.258)	6.33 (0.945)	6.40 (0.889)
5 (control)	28.33 (4.041)	16.33 (1.528)	9.23 (1.365)	5.43 (0.513)	11.43 (2.255)	8.33 (1.929)	14.73 (3.564)	10.10 (2.498)	6.33 (0.945)	6.00 (0.720)

Pi = Pre infection, Poi = Post infection.

3.2.6. Leucocyte counts in goats infected with *T.b. brucei* and treated with different concentrations of *T. catappa* crude extract

Table 5 shows the mean leucocyte count of goats experimentally infected with *T. b. brucei* before and after treatment with extracts *of T. catappa*. The basophil counts were decreased in all groups after infection except in group 5 (control) which was the same both before and after infection. The eosinophil counts were higher in all groups before infection than after infection except in group 5 which was higher after infection. The monocytes count in all groups was lower after infection than before infection except in group 1 and 4 which were the same both before and after infection. The values of neutrophils were higher in all groups including the control after infection than before infection. Finally, the lymphocyte count was the same in group 3 before and after infection and was lower in all other groups including the control after infection compared to before infection.

Table 5
Mean leukocytes count of goats infected with *T.b. brucei* and treated with crude ethanolic extract of *T. catappa* and control.

	Mean differential count									
	Basophyl		Eosinophyl		Monocytes		Nucleocytes		Lymphocytes	
Group	Pi	Poi	Pi	Poi	Pi	Poi	Pi	Poi	Pi	Poi
1	02	01	03	01	02	01	30	43	63	54
2	02	01	03	03	02	02	30	40	63	54
3	02	01	04	02	03	01	30	35	61	61
4	02	01	04	03	02	02	31	38	61	56
5	02	02	03	04	03	02	30	34	62	59

Pi = Pre infection, Poi = Post infection.

The importance of ruminants in the economy cannot be overemphasized. They provide up to 30% of the meat supply in Nigeria (Omotainse et al., 2000). However, diseases have been reported to be a major constrain to ruminant prpofuction in Africa and Nigeria in particular. In this study, all goats with T*ypanosomabruceibrucei*, developed parasitaemia by the 12th day after inoculation. The developed clinical manifestation in infected goats in all the groups was similar to clinical manifestations of trypanosome infected animals. (Allam et al., 2011; Maina et al., 2013; Pathak, 2009; Adamu, 2009) have all listed characteristic manifestations of trypanosomiasis in animals to include fever, rise in temperature, weakness, emaciation and dullness of the coat. These manifestations were observed slightly in goats in group 1 to 4 before treatment with *T. catappa* extracts but were observed more in the control group which was not treated. The trypanocidal activity of *T. catappa* extracts recorded in the present study may be due to the action of one or more constituents present in the plant. Several works have either identified or isolated tannins or phenolic compounds (Shuaibu et al., 2008) flavonoids and Alkanoids (Umar et al., 2014) in plant that showed trypanocidal activities.

The Presence of alkaloids, saponins, terpenoids and steroids in *T. catappa* could therefore be responsible for the trypanocidal activity of the plant extract on *T.b. brucei.* According to (Sofowora, 1993), the presence of secondary metabolites in plants produces some biological activities in man and animal, and is responsible for its potential use as herbs and also as drugs (Mann et al., 2008). Rise in temperature due to fever and reduced weight, emaciation among other symptoms have been associated with trypanosomiasis. Baracos et al. (1987) speculated that rise in temperature observed in parasitic infection may be due to parasite load which results in inflammatory processes leading to the release of pyrogens. Although there was rise in temperature in treated groups, yet the temperature was within normal. The insignificant difference in temperature between Pre-infection (Pi) and Post infection (Poi) of goats in all groups treated with crude extracts of *T. catappa* either orally or intraperitoneally could be due to the trypanocidal effect of the extracts which probably hindered the proliferation of parasite to a level that lead to generation of pyrogens and subsequent rise in temperature as was not treated in goats in control group.

Weight loss usually observed in parasitic infection has been attributed to the depletion of food reserve in the host of parasite (Adamu et al., 2009). In addition, there could be reduced feed intake by parasitized host leading to weight loss. Maina et al. (2013) reported that *T.b. brucei* infection is accompanied by a decrease in body weight and an elevation of body temperature in experimental rats. Although goats in all the infected and treated groups had reduced weight when the Pi and Poi weights were compared, yet the difference was insignificant since most of the groups maintained a constant weight despite parasitaemia. This however was not the case with the control (infected untreated) group which showed steady weight loss as the days increased. For a drug to be able to produce its intended clinical effect, it must first be able to reach its site of action in the body at an effective concentration. Although there are wide varieties of routes of drug administration, certain special routes may provide better therapeutic outcomes in addition to physical and chemical properties of the drug (Sim, 2015).

Crude ethanolic extract of *T. catappa* at dose of 50mgkg^{-1} and 100mgkg^{-1} BW was found to clear *T.b. brucei* in treated goats by the 4th day of treatment using the intraperitoneal route. The efficiency of intraperitoneal route of drug administration over the oral route using the same dosage (50mgkg^{-1} and 100mgkg^{-1}) is to be expected since oral drug administration has several drawbacks which include variable absorption of drugs, poor bioavail ability due to first pass metabolism and variable drug concentration in the body which could lead to therapeutic failures.

The effectiveness of ethanolic extract could be associated with the solubility and availability of the phytochemical processes. There was a significant change in the haematological indices in the treated and untreated groups. The Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cell (RBC) of infected goats decreased after infection indicating anaemia (Jenkins et al., 1980; Abenga et al., 2002). The mean PCV value of the goats before infection compared with during and after treatment was increased indicating the effect of the plant extract on the PCV of the goats. This supports previous works of Alayande et al. (2011); Omotainse et al. (2000); Igweh and Onabanjo (1989) and Allam et al. (2011). Several theories have been put forward to explain this increase in PCV among which is the postulation that dead and dying trypanosomes release hemolytic factors into the animal's blood (Anosa and Kaneko, 1983; Emmanuel et al., 1999; Esievo, 1983; Mellors and Samad, 1989). As parasites divide, increasing blood parasite population, and consequently giving rise to more dead parasites, more of the hemolytic factors are produced leading to increased destruction of erythrocytes, and hence the reduction in PCV. The observed reduction in cell counts is consistent with the observed anaemia. The same trend was exhibited in the haemogloblin values with decrease in average haemoglobin values after infection.

The values of Red Blood Counts (RBC) counts of goats were recorded before and after infection since the RBC can be used as an index to know the severity of a disease Alayande et al. (2011). Thus, the values of RBC of all the groups were reduced after infection and this could be as a result of the ingestion of the blood by the parasites (Losos and Ikede, 1972) and this gradually increased after treatment with the plant extract at both concentrations. The treatment of goats in group 1 to 4 with the plant extract which started on the 12th day post inoculation were able to clear the parasites in the blood on the 4th day of treatment resulting to a complete reversal of the haematological indices. White Blood Cell (WBC) counts of the treated groups showed a slight rise in values after infection which could be an indication that the goats employed immunological response in order to fight the foreign bodies (the parasites) (Mc Corrie et al., 1980; Mare, 2000). This could be the reason why the infected and untreated goats despitetheir loss of appetite and weakness of the body, survived till the 19th day post inoculation when the first untreated goats died.

4. Conclusion

Administration of crude ethanolic extract of *Terminaliacatappa* to *T.b. brucei* infected goats increased haematological indices and also reduced the level of parasitaemia in infected goat which makes the plate a potential source of herbal remedy for the treatment of trypanososmiasis.

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