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IN-VIVO ANTHELMINTIC AND TOXICITY STUDY OF CRUDE EXTRACTS OF *PROSOPIS AFRICANA* AGAINST *HAEMONCHUS CONTORTUS* IN GOATS

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ABSTRACT

In-vivo anthelmintic and toxicity study of *Prosopis africana* against *Haemonchuscontortus*in goats were evaluated for maximum tolerated dose (MTD) and therapeutic dose (TD) in this experiments. Two extracts, aqueous extract of stem bark (AESB) and aqueous extract of leaves (AELF) were obtained. Phytochemical constituents identified in the extracts include Tannins, Flavonoids, Alkaloids, Steroids, Cardiac glycosides and Saponins. In maximum tolerated dose and acute toxicity study, eighteen West African Dwarf goats (aged 5-6 months old) were used. Three dose regimes of 1000 $mgkg^{-1}$, 2000 $mgkg^{-1}$and 3000 $mgkg^{-1}$body weight for each type of extract was orally administered to three goats per dose with each dose being replicated thrice. Animals were observed for 48 hours for apparent signs of toxicity. Four weeks later they were re-examined for signs of delayed cytotoxic effects. At the same time, the goats were evaluated for egg per gram reduction. The results shows at the dose of 1000 $mgkg^{-1}$, there was no mortality and no evidence of toxicity in both gross and histopathological lesions. At the dose of 2000 $mgkg^{-1}$to 3000 $mgkg^{-1}$, various histopathological changes were observed, and at 3000 $mgkg^{-1}$ stem bark, showed highest reduction (94.2 %) in egg per gram. In this study, 1000 $mgkg^{-1}$ and 3000 $mgkg^{-1}$are considered as MTD and TD respectively.

Keywords: Anthelmintic, *Prosopis africana*, Goat, *Haemonchus*, Phytochemicals

INTRODUCTION

Parasitic helminths cause diseases that are of chronic and debilitating nature. They cause severe and have great economic consequences in farm animals than any single group of parasites; Among the helminths that affect livestock, *Haemonchuscontortus* is of major economic importance because they cause both clinical and sub clinical infections as well as enormous financial loses (up to $144 million annually) due to high mortality, poor reproductive performance, reduced growth and milk production, as well as lower wool production (Elelu*et al.*, 2016). The prevalence of helminth diseases especially *H.contortus*in Northern Nigeria is very high and the major control strategy adopted against it in Nigeria is the use of anthelminthics (Suleiman *et al.*,2014). However, the high cost of the anthelmintic has limited the effective control of the parasites (Maria leniraLeite-Browning, 2017). Control programs based mainly on the use of synthetic anthelmintics are no longer considered sustainable because of an increased prevalence of gastrointestinal nematode resistance, high cost to poor-farmers, and concerns regarding residue in food animals and possible environmental pollution. Alternative anthelmintics are thus required that are cheaper and probably safer for introduction into the farm production system. Traditional health practitioners in some parts of the world usually employ the extracts from the leaf, fruits, bark and roots of some medicinal plants as remedies for variety of human and animal ailments including helminthiasis. *Prosopis africana*(also called Iron wood) is plant known for its domestic and pharmacological uses (Dalziel, 1955). Almost all parts of the tree are used as medicine to mankind. The leaves and stem-bark are used as vermifuge for roundworms (Agbeja*et al.*, 2016).

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Teaching and Research Farm of the Department of Animal Science, College of Agriculture, Lafia. Lafia is located in the Southern Guinea Savannah Zone of Nigeria on Latitude $8^{0}35^{'}$N and longitude $8^{0}33^{'}$E. The average minimum temperature is 23^{0}C and maximum temperature is 36.9^{0}C, mean monthly relative humidity is 74%. The mean annual rainfall is 823mm, the mean monthly temperature is 35.06^{0}C (NIMET, 2019).

Collection, processing and extraction of plant materials

The *P.africana*leaves and stem-barkwere collected, washed in tap water and later with distilled water, air dried under shade and pulverized in a wooden mortar and pestle to obtain the powder. 200g of the powdered leaves and stem-barkwere soaked in 1000ml of distilled water for 24 hours at room temperature. The suspension was filtered and the filtrate evaporated to dryness using rotary evaporator and stored for use.

Phytochemical screening of the plant extracts

The aqueous extracts leaves and stem-bark of *P. africana* were subjected to phytochemical tests according to the method of Williams (2009).

Determination of maximum tolerated dose and therapeutic dose

In order to have a baseline dosage of the extracts, a maximum tolerated dose (MTD) and therapeutic dose (TD) of the extracts were determined according to the method of Dogar*et al.* (2012).

Experimental animals

Eighteen (18) West African Dwarf goats, averaging 5-6 months old were purchased and screened for nematode eggs. They were quarantined for one week and fed *ad-libitum* . Three dose regimes of 1000 $mgkg^{-1}$, 2000 $mgkg^{-1}$ and 3000 $mgkg^{-1}$body weight for each extract (leaves and stem-bark) were administered to three (3) goats per dose with each dose replicated thrice, they were observed for48 hours for apparent signs of toxicity. Four weeks later they were re-examined for signs of delayed cytotoxic effects. The dose that did not produce any sign of toxicity was considered as the maximum tolerated dose.

Gross and microscopic pathology (necropsy and tissue biopsy)

One (1) goat was salvaged from each MTD dosage and the visceral organs (liver, kidney, spleen and heart) were examined grossly and microscopically according to the method of (Choji*et al.*, 2015).

Experimental parasites *(Haemonchuscontortus)*

Adult *Haemonchuscontortus*were obtained from the abomasum of goats slaughtered in the abattoir. Female *Haemonchuscontortus* were identified and separated. The parasites were crushed together with vermiculite in a mortar, using pestle to obtain the eggs. The eggs together with the vermiculite were incubated at 27^{o}C in an incubator for 10 days after which the infective larvae (L_3) were harvested using Baerman's apparatus.

Experimental infection of goats with *Haemonchuscontortus*

Eighteen (18) goats were infected orally with 3000 $L_3$of*H.contortus*according to the techniques and recommendation of the World Association for the Advancement of Veterinary Parasitology (Coles, 1992). Thereafter the goats were evaluated for their worm burden expressed as egg per gram of faecal matter (epg). The dose that produced the highest reduction in epg four weeks after administration of the MTD range was considered to be the therapeutic dose for the extracts.

Statistical analysis

Data obtained from the faecal egg count reduction were analysed using the standard ANOVA procedures for a Completely Randomised Design using the General Linear Model of SAS (SAS, 2002). Treatment means were separated using Duncan's Multiple Range Test (Duncan 1955). A statistical probability of $P<0.05$ was the criterion for significance.

RESULTS AND DISCUSSION

Phytochemical screening and isolation of bioactive constituents

The comparative phytochemical constituents of *P.africana* aqueous extracts are presented in Table 1. The outcome of the phytochemical screening revealed that both the crude aqueous extracts of the leaves and stem-bark of the plant contain bioactive constituents (secondary metabolites) included Tannins, Saponins, Flavonoids, Steroids and Triterpenes, Cardiac glycosides and Alkaloids. These constituents were also isolated from stem bark and root of *Prosopis africana* by Kolapo *et al.* (2009). Similar phytochemical compounds were also isolated and reported by Burkill (1985) and Ezeke*et al.* (2010). Many Tannins and Alkaloids have been isolated from the leaves and stem-bark of *Prosopis* species (Burkill, 1985).

These active principles (secondary metabolites) have been reported to have anthelmintic and antimicrobial activities.

Table 1: Comparison of phytochemical active principles in both the aqueous crude stem bark extract and aqueous crude leaves extract.

Compound	AESB	AELF
Carbohydrates	+	+
Glycosides	+	-
Cardiac glycosides	+	+
Saponins	+	+
Steroids and tritepenes	+	+
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	-

Key = (+) = present, (-) = absent, AESB = Aqueous extract stem bark, AELF = Aqueous extract, leaves.

Determination of maximum tolerated dose (MTD) of the crude plant extracts

The outcome of the toxicity study and determination of maximum tolerated dose of the crude plant extracts is presented in Table 2. At a dose range of 1000$mgkg^{-1}$ to 3000 $mgkg^{-1}$, the aqueous crude extract of leaves and that of aqueous extract of stem-barkneither caused mortality, nor any visible behavioural or physical changes such as staggering,

salivation, diarrhoea, vomiting, restlessness and anorexia in the affected goats as the goats were stable before, before, during and after the oral administration of the extract. The goats remained active four weeks after administration thereby corroborating the findings AlShaibani*et al*. (2009).

Table 2: Determination of maximum tolerated dose of aqueous crude stem bark and leaves extract of *Prosopisafricana*

Dose ($mgkg^{-1}$)	No of goat	Mortality	Observation	Inference
Leaves				
1000	3	0	NAD	NAD
2000	3	0	NAD	NAD
3000	3	0	NAD	NAD
Stem-bark				
1000	3	0	NAD	NAD
2000	3	0	NAD	NAD
3000	3	0	NAD	NAD

Key: NA = normal activity, NAD = no abnormality detected.

The effect of MTD of the extracts on egg per gram reduction is presented on Table 3. It can be seen that at the MTD of 1000 $mgkg^{-1}$ 2000 $mgkg^{-1}$ 3000 $mgkg^{-1}$ stem bark caused -7.4 %, 64.2 %, 94.2 % reduction in epg while leaves produced -7.5 %, 69.9 % and 82.2 % at week four post treatment. Therefore 94.2% produced by stem bark conforms to the standard set by WAAVP of 90 % and above. Based on this and the physical response of the goats to the extract, 3000 $mgkg^{-1}$ was chosen as the experimental treatment dose as recommended by Lorke (1983).

Table 3: Effects of MTD of the plant extracts on experimental animals (Goat) during the study

		Stem bark			leaves		
MTD dosage (mg/kg)	Exposure time (wkg)	Total epg	Mean + SE	%reduction epg	Total epg	Mean + SE	%reduction epg
1000	0	5610	1870 ±69.12	0	4572	1524±342.03	0
	1	6380	2126.7±92.59	-13.7	5402	1800±338.87	-18.1
	2	5905	1968.3 ± 104.08	-5.3	4830	1610±143.74	-5.6
	3	4030	1343.3 ± 73.3	28.2	3180	1060±156.76	30.5
	4	6025	2008.3 ±89.01	-7.4	4910	1636±377.82	-7.5
2000	0	24120	8040 ±182.21	0	22325	7441.67±123.57	0
	1	11625	3875±313.22	51.8	95871	3190.33±206.31	57.1
	2	10200	3400 ± 880.81	57.7	8265	2755.00±460.3	63
	3	9205	3068.3 ± 145.81	61.8	7321	2440.3±99.48	67.2
	4	8646	2882 ±149.15	64.2	6704	2234.6±87.45	69.9
3000	0	40520	13506.67 ±481.76	0	30416	10138±232.8	0
	1	20346	6782±303.74	49.8	17250	5750±239.4	43.3
	2	8202	2734 ± 102.04	80	8930	287.6±112.7	70.6
	3	4170	4170 ± 543.08	89.7	7270	2423.4±99.3	76.1
	4	2361	787±277.14	94.2	5412	1804±70.0	82.2

Percentage egg per gram was calculated using the formula N-n/Nx100 where, N = mean weekly 0 epg before administration, n = mean epg post administration, D = number of goats used.

Effect of MTD on histopathological findings and acute toxicity study

The result of the histopathological investigations of the crude plant extracts on various organs and tissues in goats are presented in the Plates below:

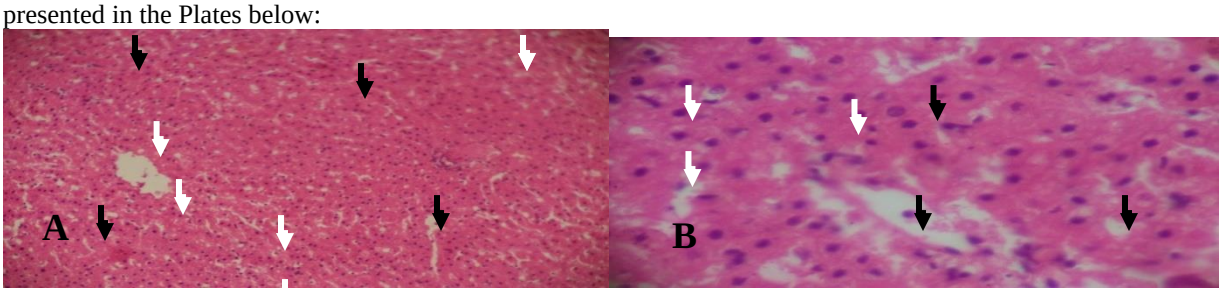

Plate 1A: *Kidney of a goat administered 2000 mg/kg body weight of stem-bark showing severe tissue degeneration (White arrows) and loss of both nuclear and cytoplasmic components (Black arrows). X100.* ***Plate I B:*** *Kidney of goat administered 3000 mg/kg body weight stem-bark showing compacted glomeruli (White arrows) with atrophied cellular demarcations (Black arrows). X400*

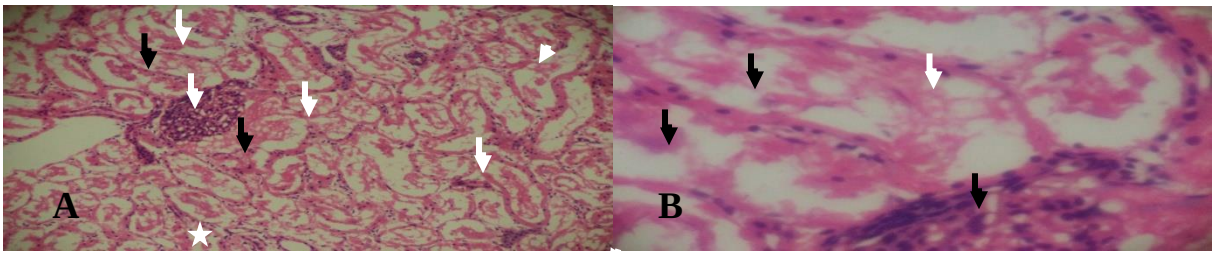

Plate II A: *Heart of a goat administered 3000 mg/kg stem-bark showingpyknosis (White arrows) and presence conc nsed nuclei (Black arrows). X100;* ***Plate II B****: Liver of goat administered 3000 mg/kg stem-bark showing hepatomegaly (White arrows) and massive reduction in sinusoidal space (Black arrows). X400*

The result of the finding has shown that at 1000 $mgkg^{-1}$stem-bark and leaves , there were no detectable histopathological changes in the kidneys, spleen, liver and heart. At the dose rate of 2000$mgkg^{-1}$stem-bark and leaves there were various histopathological changes ranging from severe atrophy, tissue degeneration and congestion. The histopathology findings on various sections revealed some evidences of toxicity of the plant extract especially at a much higher doses. The finding revealed toxic effect on the kidney at both 2000 $mgkg^{-1}$ and 3000 $mgkg^{-1}$ dose levels. However in the heart and liver, toxicity was only observed at the 3000 $mgkg^{-1}$ dose. The histopathology lesions seen in the kidney might suggest that this extract is majorly excreted via the kidney. Also the cause of this lesion may or may not be connected with the active principles responsible for the anthelmintic effect since this effect is only seen at the point of excretion of the extract. The toxicity appeared to be dose dependant, it may be inferable that 1000 $mgkg^{-1}$ should be the MTD. On the other hand, 3000 $mgkg^{-1}$ gave higher efficacy (94.2 %) reduction in feacal egg count. This suggests therefore that 3000 $mgkg^{-1}$ may be considered as the therapeutic dose. However, based on the outcome of the histopathology, caution must be taken not to give the 3000 $mgkg^{-1}$ as a single dose. It may be given in three divided doses within a short period of seven days.

Conclusion and recommendation

The current study has shown promising *in-vivo* Anthelmintic activity because the active principles (secondary metabolites) which have been reported to have Anthelmintic activities in medicinal plants have also been detected in this plant. In this study therefore, the Anthelmintic effects observed may be attributed to the presence of Tannins, Saponins, Cardiac glycosides, Steroids, Triterpenes and Flavonoids earlier identified through phytochemical screening. *P. africana*, although known for its domestic and pharmacological purposes. The plant has an Anthelmintic property with no evidence of toxicity if given at lower doses. At higher doses, its Anthelmintic efficacy can be compared with other synthetic drugs if given at repeated doses.

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