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ANTITRYPANOSOMAL ACTIVITY OF *ANOGEISSUS LEIOCARPA* IN *RATTUS NORVEGICUS* (WISTER RATS) EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

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ABSTRACT
Trypanosomiasis is an important disease affecting livestock where infertility, abortion and loss in productivity leading to deaths are imminent in the infected herds. Therapeutic and prophylactic measures using trypanocidal drugs are hampered by drug resistance, toxicity, prolonged treatment regimens and antigenic variations. Hence the need to explore more alternate strategies such as the use of plants with the potential in the treatment of trypanosomiasis is indispensable. In vivo anti-trypanosomal activity of *Anogeissus leiocarpa* (African birch) was tested on *Rattus norvegicus* (wistar rats) infected with *Trypanosoma brucei brucei.* The experimental animals were grouped into seven groups of five rats per group. Doses of 200 mg/kg and 150 mg/kg of methanol crude extract of leaf and stem bark were administered orally to four groups while three served as control groups (ie. infected untreated, uninfected untreated and infected treated with standard drug/Diminazine aceturate). The level of parasitaemia, packed cell volume (PCV), total leukocyte counts (TLC) and differential leukocyte counts (DLC) were evaluated. Results of the phytochemical screening of the extracts revealed the presence of saponins, tannins, steroids, glycosides, alkaloids, flavonoids and anthraquinones. The mean parasitaemia of the treated groups was significantly lower ($P<0.05$) than the mean parasitaemia of the untreated groups. The groups treated with higher concentrations resist sudden drop in PCV and reduction in the level of parasitaemia. From the findings of this research, it could be concluded that treatment of T. brucei brucei infected rats with *A. leiocarpa* extract resulted in the improvement in PCV, TLC, neutrophils and lymphocytes, where group B (Stem at 200 mg/kg) prolonged the lives of the animals beyond the negative control group.

Keywords: *Anogeissus leiocarpa*, Antitrypanosomal activity, Phytochemistry

INTRODUCTION
Trypanosomiasis is an important disease affecting livestock. The disease usually results in loss in productivity of livestock, infertility, abortion and eventually deaths when left untreated (Maigari and Dabo, 2018). For decades, control and eradication programs against trypanosomiasis rely on therapeutic and prophylactic measures using trypanocidal drugs (Sani *et al*., 2020; Richards *et al*., 2021). Trypanocides often used in the treatment of Trypanosomiasis in ruminants include diminazene aceturate, isometamidium chloride, Ethidium bromide/chloride (*Homidium* and *Novidium* salts) as well as combination therapy. Regrettably, these drugs suffer resistance by trypanosomes (Richards *et al*., 2021).

On the other hand, previous attempts aimed at developing vaccines against African trypanosomiasis have also highlighted the difficulties in overcoming the immune evasion strategies that have been deployed by trypanosomes to enable them survive in host blood, including antigenic variation through serial expression of an abundant allelically excluded variable surface glycoprotein (VSG) or the rapid removal of surface-bound antibodies by hydrodynamic sorting (Richards *et al*., 2021).

Recent advances have indicated progress in the development of novel trypanocides (Richards *et al*., 2021). A good example of a promising trypanocide with a spectacular mode of action was benzoxaborole (Begolo *et al*., 2018) which has been shown to inhibit the trypanosome, cleavage and polyadenylation specific factor 3 (CPSF3) gene involved in mRNA processing (Wall *et al*., 2018), that diminishes the risks of cross-resistance. Benzoxaboroles can accumulate at a very high concentration in trypanosomes because the parent compound is a prodrug that is activated by enzymatic cleavage (Giordani *et al*., 2020). Disappointingly, recent reports have shown that *Trypanosoma brucei brucei* and *T. congolense* can render this drug ineffective by losing the genes that encode prodrug-activating enzymes (Richards *et al*., 2021).

Livestock farmers have also attributed treatment failure to poor quality of trypanocides. For instance quality analysis of diminazene aceturate and isometamidium chloride in West Africa, Togo, and Ethiopia, have shown that 51.9 %, 40 %, and 28 % of drugs, respectively, were not containing the correct dose of the active ingredients. Hence, effective treatment of African Trypanosomiasis may be inundated with the problem of noncompliance.

These worries coerce researchers to explore more alternate methods of developing a novel drug for African Trypanosomiasis. It is an established fact that, plants have provided the basis for traditional treatment of different types of ailments where they offer enormous potential sources of new chemotherapeutic agents. Plants have been more amenable to man's exploitation (Sani *et al*., 2020), in which safety, lack of adverse reactions and minimal side effects may be some important attributes to the potential use of phyto-medicine (Renckens and Dorlo, 2013).

Anogeissus leiocarpa, commonly called African birch, widely distributed in northern Nigeria with a wide range of values including medicinal properties, economic importance and traditional uses (Maigari *et al*., 2017). Previous works reported that *A. leiocarpa* possess enormous pharmacological activities including antibacterial (Aliyu and Sani, 2011), antidiabetic (Mann *et al*., 2008), antiplasmodial (Mann *et al.* 2010), antifungal (Maigari *et al*., 2017), leishmanicidal, antimalarial, anthelmintic and antioxidant (Ahmad, 2014) activities. For instance, the *in-vitro* antibacterial activity of *A. leiocarpa* extracts against *Escherichia coli* and *Staphylococcus aureus* has been reported (Aliyu and Sani, 2011). Potent antifungal activity against *Aspergillus nigar* and *Rhizoctonia* species by the crude extracts of *A. leiocarpa* has also been documented (Maigari *et al*., 2017). Similarly, several studies on the use *of A. leiocarpa* in the treatment of trypanosomiasis have been published (Victor, 2013; Ahmad, 2014; Mukhtar *et al*., 2017). The present study is aimed at assessing the *in vivo* antitrypanosomal activities of *A. leiocarpa* in Wistar rats infected with *T. brucei brucei.*

MATERIALS AND METHODS

Collection of Plant Material

Leaves and stem (bark) of *Anogeissus leiocarpa* were collected from Danmaliki town, Kumbotso Local Government Area (LGA) of Kano State. All the plant samples were obtained with the help of traditional healers. They were all authenticated at the Department of Plant Biology, Bayero University Kano, Nigeria. A voucher specimen was deposited in the herbarium with the herbarium accession number BUKHAN 0029.

Preparation of Plant Extracts

The *A. leiocarpa* leaves and bark were air dried at room temperature, separately reduced to coarse forms using mortar and pestle, and subsequently evaporated slowly to dryness on a hot water bath (100^{o}C), to give a concentration of 50mg/ml dry matter.

Determination of Phytochemical Constituents

The phytochemical analysis of the plant extracts was carried out as described by Aliyu and Sani (2011) to test for the presence of alkaloids, tannins, flavonoids, tarpenens, saponins, quinines, phenols and glycosides.

Purchase, Maintenance of Experimental Animals and Parasites Inoculation

Adult *Rattus norvegicus* (Wister rats) of mixed sexes, weighing between 120 – 150 g were purchased from the Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University, Kano. The experimental animals were housed and managed, following the Animals in Research: Reporting *in vivo* Experiment (ARRIVE) (Kilkenny *et al*., 2010), as well as Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) (Smith *et al*., 2015) guidelines for reporting animal research.

Trypanosoma brucei isolates was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), North Central Headquarter, Vom, Plateau State, Nigeria. The parasite was characterized using standard trypanosome detection methods according to the description of Dabo and Maigari (2017). The parasite was then passaged onto donor rats and maintained in the laboratory by the continuous passage in Wistar rats of mixed sexes (120–150g) until a parasitaemia of approximately 10^5 parasites/ ml was reached. The rats were infected with 0.01 ml of blood containing about $1x10^3$ trypanosomes. The trypanosome count was carried out using the "rapid matching" method as described by Herbert and Lumsdein (1976).

Analysis of Haematological Parameters

Blood samples were collected daily before and after treatment from the tip of the tail of each animal for the determination of packed cell volume (PCV) and total/differential leucocyte counts. The parasitaemia was also estimated daily by the rapid matching method of Herbert and Lumsdein (1976) in which the number of trypanosomes per microscopic field was compared with those of the standard logarithmic table.

Experimental Design

The rats were divided into 7 groups (A - G) of 5 animals each. Treatments A, B, C, D and E rats were each inoculated with 1×10^6 *T. brucei* in 0.2ml normal saline, while F were not infected. With the establishment of parasitaemia, animals in treatments A, B, C, D and G were respectively treated with the extract orally for 14 consecutive days at different dose levels per kg body weight as follows:

1. Treatment A: Leaf extract (150 mg/kg)
2. Treatment B: Leaf extract (200 mg/kg)
3. Treatment C: Stem extract (150 mg/kg)
4. Treatment D: Stem Extract (200 mg/kg)
5. Treatment E: Infected, untreated
6. Treatment F: Uninfected, untreated
7. Treatment G: Infected, treated with *Diminazine aceturate* (Suramin®)

RESULTS AND DISCUSSION

Phytochemical Constituents of *Anogeissus leiocarpa*

The results of the screening of phytochemical constituents of the stem bark of *A. leiocarpa* revealed the presence of Saponins, Tannins, Steroids, Glycosides, Alkaloids, Flavonoids and Anthraquinones, while leaf extract contained all except Flavonoids and Anthraquinones (Table 1). The result obtained reaffirms the earlier claims by the previous studies that extracts of *A. leiocarpa* contains several metabolites. Previous studies revealed the presence of alkaloids, glycosides, phenols, steroids, tannins, anthraquinones, saponins and flavonoids (Kaboré *et al*., 2010; Mann *et al.* 2010; Aliyu and Sani, 2011; Abedo *et al*., 2013; Mukhtar *et al*., 2017). Abedo *et al.* (2013) noted that, the active compounds isolated from this plant have been shown to be mainly triterpenes and ellagic acid derivatives (flavonoids and phenolic compounds). This was more garishly established by Mukhtar *et al*. (2017), who maintains that, these phytochemicals have been proven to be the driving force behind the pharmacological activities of *A. leiocarpa.*

Table 1: Phytochemical Composition of *Anogeissus leiocarpa*

Phytochemical Constituents	Stem	Leaves
Saponin	+	+
Tannin	+	+
Steroids	+	+
Glycosides	+	+
Alkaloids	+	+
Flavonoids	+	_
Anthraquinones	+	_

Key: (+) present; (-) Absent

Activities of Crude Leaf and Stem Extracts of *Anogeissus leiocarpa* on Packed Cell Volume

The crude leaf and stem extracts of *A. leiocarpa* in relation to PCV of the rats infected with *T. b. brucei* revealed that, Treatment D (Stem at 200mg/kg) resists sudden drop in PCV when compared to the infected untreated group (Table 2). This shows that higher concentration has more effect on the PCV thus prolonging the lives of the experimental animals. Among the feature that caused severity of trypanosome infection include anaemia, which is linked to severity and level of parasitaemia (Umar *et al*., 2007). Anaemia in Trypanosomosis is haemolytic in that, the red blood cells are removed from circulation by expanding mononuclear phagocytic system (Maigari *et al*., 2018). Hence, the degree of anaemia, as estimated by measuring PCV, could be used as a useful indicator of animal Trypanosomosis (Maigari and Dabo 2018).

Activities of Crude Leaf and Stem Extracts of *Anogeissus leiocarpa* on Parasitaemia

The crude leaf and stem extracts of *A. leiocarpa* affects the parasitaemia level as presented in Table 3. The level of parasitaemia in Treatment D (stem at 200 mg/Kg) was lesser than that of infected untreated, infected treated with other concentrations as well as infected treated with standard drug. This also shows that higher concentration of the extract has more effect on the parasitaemia, thus, prolonging the lives of the experimental rats, although the activity varied among the different parts of the plant. Stem bark of the plant showed higher concentration of the active components. The active components in the stem bark of *A. leiocarpa* were hydrolysable tannins, and may be responsible for the high anti-trypanosomal activity.

Table 2: Activities of Crude Leaf and Stem Extracts of *Anogeissus leiocarpa* on Packed Cell Volume (PCV)

Group	PCV%		
	Pre-Treatment	Post Treatment	Survival days
A (Leaf at 150mg/kg)	42.5 ± 0.5	35.5^{b} ± 2.1	7 – 8
B (Leaf at 200mg/kg)	43.0 ± 4.4	37.0^{b}±0.5	7 – 9
C (Stem at 150mg/kg)	42.5 ± 0.5	35.5^{b}± 2.1	7 – 11
D (Stem at 200mg/kg)	42.5 ± 0.5	39.0^{a}± 1.0	9 – 12
E (Infected, untreated)	42.0 ± 1.0	29.5^{b} ± 0.5	5 – 6
F (Uninfected, untreated)	41.5 ± 0.5	39.5^{b} ± 0.5	> 14
G (10mg/kg Diminazine aceturate)	43.7 ± 4.5	37.5^{b} ± 1.5	7 – 14

Superscripts with different letters are indicating significant difference between the mean values (±standard deviation) along the rows (P=0.00484)

Table 3: Activities of Crude Leaf and Stem Bark Extracts of *Anogeissus leiocarpa* on Parasitaemia

	Parasitaemia		Survival days
Group	**Pre-treatment**	**Post treatment**	
A (Leaf at 150 mg/kg)	0.49 ± 0.08	324.65^{b}±73.5	7 – 8
B (Leaf at 200 mg/kg)	0.45 ± 0.05	228.02^{b}±23.2	7 – 9
C (Stem at 150 mg/kg)	0.38 ± 0.1	188.55^{b}±62.7	7 – 11
D (Stem at 200 mg/kg)	0.30 ±0.1	94.81^{a}±7.9	9 - 12
E (Infected, untreated)	0.57 ± 0.07	449.65^{b} ±51.6	5 – 6
F (10mg/kg Diminazine aceturate	0.30 ± 0.1	188.24^{b} ±30.7	7 – 11

Superscript with different letters are indicating significant difference between values along the rows (P=0.003916).

Activities of Extracts of *Anogeissus leiocarpa* on Total and Differential Leucocytes Counts

The activities of crude leaf and stem bark extracts of *A. leiocarpa* on Total and differential leucocytes as presented in Table 4 indicated that, the stem extract at higher concentration had more effect on the lymphocytes as there was no leucocytosis due to lymphocytosis (an increase in number of the white blood cells). Lymphocytosis can occur in the healing phase of infectious diseases, during chronic antigenic stimulation due to infectious agents, neoplasia, and hypoadrenocorticism (Maigari *et al.*, 2018). Some of the mechanisms underlying leukocytosis in animals include the increased release of WBC from the bone marrow, decreased emigration into the tissues, and a shift of cells from the marginal into the circulatory pool (Webb and Latimer, 2011; Maigari *et al.*, 2018). Reports of leukocytosis due to lymphocytosis are shown at the onset of trypanosomiasis while leucopenia is always seen at terminal stage of the infection (Maigari *et al.*, 2018). These are usually due to wax and wear syndrome on the animal immune system caused by the infecting trypanosome. The administration of the stem bark extracts of *A. leiocarpa* at 200 mg/kg prevented leukocytosis due to lymphocytosis, thus prolonging the survival of the rats beyond the death of infected, untreated control.

Neutropenia (reduction in neutrophils) was also not observed at 200 mg/kg of stem extract compared to infected untreated and other groups. Neutropenia occurs in the first days of severe, acute inflammation, including sepsis, mastitis, peritonitis, metritis, enteritis, and pneumonia (Maigari *et al.*, 2018); and can be caused by viral rickettsial, protozoal and fungal infections, bone marrow disease, toxins, neoplasia, or idiosyncratic drug reactions (Webb and Latimer, 2011; Roland *et al.*,2014; Maigari *et al.*, 2018). Effect of extract as seen in this research led to decrease in coating of the progenitor cells resulting to improvement in neutropenia when compared with the infected, untreated group. Furthermore, these observations may have led to the prolongation of lives by 4, 1, 3 and 3 days of the experimental groups, respectively.

Animals treated with 150 mg/kg/day died earlier than those treated with 200 mg/kg/day. This suggests that the antitrypanosomal effect of the extract might be dose dependent, as higher dosage may mean higher concentration of the active phytochemical components (Sani *et al.*, 2020), which could be responsible for the survival of the rats treated with 200 mg/kg/day for fourteen (14) days.

Table 4: Activities of Crude Leaf and Stem Bark Extracts of *Anogeissus leiocarpa* on Total and Differential Leucocyte Counts

Group	TLC($\times 10^3$)	Lymphocytes	Neutrophils	Survival days
A (Leaf at 150mg/kg)	*6.2 ±1.2 **11.4 ± 0.9	*62.0±3.0 **72.5±0.5	*33.5±3.5 **26.5 ± 2.1	7 – 8
B (Leaf at 200mg/kg)	*7.7 ±0.2 **9.1 ± 0.8	*67.3±3.8 **75.0 ±7.2	*31.7±4.1 **21.0 ±2.0	7 – 9
C (Stem at 150mg/kg)	*6.7±0.7 **11.0 ± 2.0	*66.5±2.1 **70.0 ± 2.8	*33.5±2.1 **29.0 ± 1.4	7 – 11
D (Stem at 200mg/kg)	*9.3 ± 0.4 **8.3 + 1.1	*74.0±5.3 **73.0 ± 6.2	*28.5±0.5 **30.0 ± 2.0	9 -12
E (Infected, untreated)	*6.2 ± 0.3 **10.2 ± 0.7	*60.7 ± 4.7 ** 71.7± 6.0	*39.0 ± 4.3 ** 25.5 ± 3.5	4 – 5
F(Uninfected/untreated)	*8.7 ± 0.5 **9.0 ± 1.5	*74.6 ± 0.9 ** 76.7 ± 1.5	*25.0 ± 1.2 **22.7 ± 0.6	> 14
G (10mg/kg Diminazine aceturate)	*6.7 ± 0.8 **7.0 ± 0.7	*66.0 ± 9.5 ** 67.3 ± 5.8	*28.5 ± 0.5 ** 35.0 ± 1.0	8 – 12

Keys: *pre treatment; **post treatment

CONCLUSION AND RECOMMENDATIONS

It could be concluded that treatment of *T. brucei brucei* infected rats with *A. leiocarpa* extract resulted to the improvement in PCV, TLC, Neutrophils and lymphocytes. Stem bark at 200mg/kg prolonged the lives of the animals beyond the untreated, consequently reaffirming the folkloric claims that *A. leiocarpa* extracts have ethnomedicinal properties, and thus could be use in the management of African animal Trypanosomiasis.

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