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Original article

Effect of Extract of *Senna alata* (Candle bush Plant) on Some Haematological Indices of Wistar rats Infected with *Trypanosoma brucei brucei*

[1*]Kugama, M. A., [2]Tese, T., [2]Tamba, Z., [1]Omotainse, S.O., [3]Itodo, K. J., [2]Arumona, R., [2]Odebisi, K. O. and [4]Abutu, R. S.

[1]Human African Trypanosomiasis Research Department, Nigerian Institute for Trypanosomiasis Research, Kaduna.
[2]Vector and Parasitology Studies Research Department, Nigerian Institute for Trypanosomiasis Research, Kaduna.
[3]Department of Microbiology, Prince Abubakar Audu University, Anyigba, Kogi State
[4]Onchocerciasis Research Department, Nigerian Institute for Trypanosomiasis Research, Kaduna.

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ABSTRACT

This study was carried out to investigate the effect of *Senna alata* on some haematological indices of wistar rats infected with *Trypanosoma brucei brucei*. Sofowora methods were employed for phytochemical analysis to detect the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, phenols steroids/terpenes and glycosides. Results revealed the absence of steroids in all extracts, absence of saponins in petroleum ether and chloroform extracts, absence of flavonoids only in petroleum ether extracts and presence of free anthraquinones only in chloroform extract. Post infection treatment of animals stirred the emergence of parasitaemia by Day 3. Only wistar rats receiving 200mg/kgb.wt of chloroform extract survived by day 16. PCV and haemoglobin concentration (Hb) decreased significantly ($P<0.05$) for the infected not treated group. Also, Infected-treated groups recorded the lowest PCV (30.4%) and Hb (10.1g/dl) in wistar rats receiving aqueous extract on day 12. Highest change in WBC (from 6.2X 10^3 /µl to 1.32X 10^4 /µl) was recorded in group receiving 200mg/kgb.wt of chloroform extract.. These results thereby demonstrate ameliorative potentials of the extracts of *Senna alata* extract in some haematological indices of wistar rats infected with *Trypanosoma brucei brucei*. **Keywords:** Trypanosomiasis, Phytochemicals, Haematology, *Senna alata*

Corresponding author's email: meshackkugama@gmail.com, +2347038599888,

INTRODUCTION

African trypanosomiasis is a neglected tropical disease that affects humans and animals in rural areas of sub-Saharan Africa causing huge devastation in human development and livestock productivity. It is caused by a protozoan parasite and transmitted through the bite of an infected tsetse fly [1]. The flies are found in vegetation along water courses and lakes, forest edges and gallery forests, extending to vast areas of guinea savannah. The parasite lives extracellularly in the blood and tissue fluids in the mammalian host. The most important species responsible for the disease complex known as animal African trypanosomiasis include *Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax*. The human African trypanosomiasis is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* [2].

In spite of many decades of treatment and tsetse control efforts, three (3) million cattle reportedly die annually from AAT which prompted many researchers to work hard towards elimination of the disease [3,4]. African trypanosomiasis associated with *Trypanosoma brucei* species is one of the major factors responsible for rural underdevelopment in many sub-Saharan African countries. The disease has been linked to socio-economic burden of the affected countries where it is estimated to result in an annual economic loss of about $5 billion [5]. In both animals and humans, African trypanosomiasis is characterised with fever, anaemia, headache, generalized weakness, severe pruritus with scratching, skin lesions, mobile or rubbery lymphadenopathies, tenderness, oedema, malaise, weight loss, musculoskeletal pains and invasion of organs such as the brain, liver, adipose tissue, the skin and organs of the nervous system [6].

Plants are known to be rich source of raw materials in African traditional medicine and other parts of the developing world. There are more than 35, 000 plant species being used in various human cultures around the world for medicinal purposes [7,8]. Medicinal plants have long drawn history of use by human beings for cure of various ailments. More than 70% of the third world population now depend on traditional medicinal system, otherwise known as complementary or alternative system of medicine [9]. Also, studies have shown that some medicinal plants have anti-trypanosomal activity [10,11]. Natural products (plants and animals) are important sources of new drugs because their derivatives are extremely useful as lead structures for synthetic modification and optimization of bioactivity. Thus, traditional medicine remains popular for both historical and cultural reasons [12].

Traditional medicine has demonstrated potency of being the best choice to obtain a variety of drugs. This discovery necessitate that such plants should be investigated to better understand their properties, safety and efficiency [12]. The prospect of immunization against the disease appears difficult. The key to this paradox lies in the phenomenon of antigenic variation. The drugs used for treatment of human and animal trypanosomiasis are limited. Some of the current drugs in use are toxic and cumbersome in addition to being expensive [13]. *Senna alata* which is commonly called candle bush plant is an important medicinal plant which contains antimicrobial activity. The leaf extract has been reported to be useful in the treatment of heart failure, gonorrhea, abdominal pains, odema and malaria fever. Leaf extracts of *Senna alata*

contain glycosides, saponins, flavonoids, and tannins [14]. However, to the best of our knowledge, information on the effect of *Senna alata* on haematological indices of wistar rats infected with trypanospomes is not documented. Therefore, this study aimed at examining the effect of *Senna alata* on the haematological indices of wistar rats infected with *Trypanosoma brueci brucei.*

MATERIALS AND METHODS

Collection and authentication of plant materials

The plant *Senna atata* was harvested in Zaria, Kaduna state, Nigeria. It was authenticated by a botanist in the herbarium, Department of Biological sciences, Ahmadu Bello University, Zaria and given a voucher number 906. The leaves were dried at 28^0C (room temperature), pulverized in a mortar and sieved to obtain a fine powder and then stored in an airtight container with proper labeling.

Plant Extraction

One thousand grams (1000g of powdered) plant material was weighed and extraction was done in light petroleum ether (60 – 80^0C). The petroleum ether was concentrated invacuo to obtain a product referred to as petroleum ether extract (PEE). The marc (Petroleum ether defatted residue) was extracted with ninety-five percent (95%) methanol and the solvent recovered invacuo to obtain crude methanol extract (CME). One hundred gram (100g) of the CME was dissolved in water and partitioned four times with equal portion of chloroform in one thousand meals(1000ml) separatory funnel. The chloroform portion was pooled and concentrated invacuo to obtain a product referred to as the chloroform extract (CE). The remaining portion of the aqueous methanol fraction was again partitioned four times with equal portion of butanol. The butanol portion was pooled and concentrated invacuo to obtain what is referred to as the butanol extract (BE). The remaining portion of the reconstituted aqueous methanol portion was concentrated invacuo and subsequently referred to as aqueous residue (AR).

Experimental Animals

In this study, forty-eight wistar rats (*Rattus rattus novergicus*) were purchased from the animal house of Nigerian Institute for Trypanosomiasis Research, Kaduna. The animals were housed in standard rat cages, maintained on standard pellet diet from Vital Plc, Jos, Nigeria and water *ad libitum*.

Infection of Animals

A number of 10^4 parasites per meal were introduced intraperitoneal into rats in 0.1-0.2 ml blood/PBS solution. Number of parasite/ml was estimated using the method of Herbert and Lumsden [15].

Phytochemical Screening of crude extract of *Senna alata*

Method of Sofowora was used to detect the presence of anthraquinones, flavonoides, alkaloids, saponin, glycosides and tannins steroids and carbohydrates [16].

Determination of Packed cell volume

The PCV is the volume of red blood cells (RBCs) expressed as a fraction of the total volume of the blood. The microhaematocrit method was used [17].

Determination of Haemoglobin concentration

The level of Heamoglobin concentration was determined according to the method of Cheesbrough [17].

The concentration of hemoglobin was calculated according to the following formula:

$$\text{Hb concentration (g/dl)} = \frac{\text{absorbance of tested sam}}{\text{absorbance of standard}}$$

Determination of White Blood Cell Count

Improved Neubeur haemocytometer (counting chamber) was used to count white blood cells (WBCs) under the light microscope [18].

Calculation: Number of cells counted divided by volume of one square ($0.1mm^3$) multiplied by number of squares counted and multiply by dilution factor (20 for WBC count).

Animal grouping

Fort-eight albino rats grouped into eight groups of six rats each were used for the screening. The animals were inoculated with approximately 10^4 trypanosomes and infection was allowed to establish. Five groups served as extract test (petroleum ether extract, crude methanolic extract, chloroform fraction, butanol fraction and aqueous residue fraction) and sixth as positive control (i.e reference drug Diminazene aceturate). A seventh group served as negative control (untreated) and eight as uninfected and untreated. The extract was dissolved in Tween 20, then distilled water and administered orally. Treatment with extract and reference drug commenced on 3th day of post infection.

Ethical Clearance

Ethical clearance for the use of laboratory animals was obtained from Kaduna State Ministry of Agriculture and Forestry, Kaduna.

Data Analysis

Data analysis was performed using Statistical Package for Social Science (SPSS), version 20.0. To compare the results obtained from different groups, one-way ANOVA was employed.

RESULTS

Phytochemical Analysis of crude extract of *Senna alata* Leaves

The results for qualitative phytochemical constituents of extracts of *Senna alata leaves* are summarized on Table 1. The results indicate that flavonoids were absent only in petroleum ether extract while saponins were absent in both petroleum ether and chloroform extracts. Carbohydrates, cardiac glycosides, triterpenes and alkaloids were present in all the extracts, anthraquinones were present only in chloroform extract while steroids were absent in all the extracts.

Effect of Extracts of *Senna alata* leaves on Packed cell volume of Wistar rats infected with *Trypanosoma brucei brucei*

The effect of extracts of *Senna alata* leaves on packed cell volume (PCV) of wistar rats infected with *Trypanosoma brucei brucei* s is presented on Table 2. The results indicate a significant ($p < 0.05$) decrease in PCV on the 3rd day in groups challenged with parasites and treated with extracts with Butanol extract recording the highest fall in PCV. PCV decreased significantly ($p < 0.05$) from 43.8 to 38.1% for groups treated with 200mg/kgbwt and 400mg/kgbwt of

butanol extracts respectively. The decrease in PCV was significantly ($p < 0.05$) higher in groups that received 400mg/kg b.wgt except for chloroform extract where the difference was not statistically significant ($p > 0.05$). For the group treated with 200mg/kg b.wgt of methanol extract, PCV decreased significantly ($p < 0.05$) from 42.5% on day 0 to 33.6 % on day 9. Administration of chloroform extract caused a decrease in PCV from 44.1 and 41.5% to 30.7 and 31.9% on day 15 and day 12 for groups that received 200mg/kg b.wgt and 400mg/kg b.wgt respectively, the decrease in PCV was also statistically significant ($p > 0.05$). Administration of aqueous extract recorded a decrease in PCV from 39.4 and 40.5% in group that received 200mg/kg b.wgt and 400mg/kg b.wt respectively to 30.4% on day 12.

Effect of Extracts of *Senna alata* leaves on Hemoglobin concentration of Wistar rats infected with *Trypanosoma brucei brucei*

The effects of extracts of *Senna alata* leaves on haemoglobin concentration (Hb) of wistar rats infected with *Trypanosoma brucei brucei* is presented on Table 3. The results indicate a statistically significant ($p > 0.05$) decrease in Hb on the 3rd day of post infection for all infected rats with administration of extracts generating a statistically significant ($p < 0.05$) drop in Hb in groups that received 400mg/kg b.wgt than 200mg/kg b.wgt except in the group treated with chloroform extract. The highest decrease in Hb was recorded in group that received 200mg/kg b.wgt of butanol extract, Hb dropped from 14.5 to 12.7 g/dl while in group receiving 400mg/kg b.wt of butanol extract, it dropped from 14.3 to 11.8 g/dl; they recorded decrease in Hb concentrations in infected rats treated with the butanol extracts was statistically significant ($p < 0.05$). For the group treated with 200mg/kgb.wt of methanol extract, Hb dropped from 14.2 g/dl on day 0 to 11.2 g/dl on day 9. When chloroform extract was administered, Hb dropped from 14.7 and 13.8 g/dl to 10.3 and 10.6 g/dl on day 15 and day 12 for groups that received 200mg/kgbwt and 400mg/kgbwt respectively. Administration of aqueous extract recorded a significant ($p < 0.05$) decrease in Hb from 13.1 and 13.5 g/dl in groups that were treated with 200mg/kg b.wgt and 400mg/kg b.wgt respectively to 10.1 g/dl on day12.

Effect of Extracts of *Senna alata* leaves on White Blood Cell Count of Wistar rats infected with *Trypanosoma brucei brucei*

The effects of extracts *Senna alata* leaves on white blood cell count (WBC) on *Trypanosoma brucei brucei* infected albino rats is presented on Table 4. The results indicated that on day 3, there was a statistically significant ($p < 0.05$) increase white blood cell in all infected groups with groups that were treated with 200mg/kg b.wgt of extracts recording a significantly ($p < 0.05$) increase in white blood cell than groups that received 400mg/kg b.wgt of petroleum ether and methanol extracts while groups that received 400mg/kg b.wgt recorded a significantly ($p < 0.05$) increase in white blood cell counts compared to group that received 200mg/kg b.wgt of the chloroform, butanol and aqueous extracts. The highest increase in white blood cell was recorded in group that received 400mg/kg b.wgt of butanol extract where white blood cell count was raised from 6.5 X 10^3 /µl to 9.6 X 10^3 /µl. Administration of 200mg/kgbwt of methanol extract generated an increase in white blood cell from 6.7 X 10^3 /µl to 12.6 X 10^3 /µl on day 9; the increase was statistically significant ($p < 0.05$).

Chloroform extract administered at 200mg/kg b.wgt and 400 mg/kg b.wgt generated an increase in white blood cell from 6.2X 10^3 /µl and 6.7X 10^3 /µl to 12.2X 10^3/µl and 12.9X 10^3 /µl on day 12 and 15 respectively; the increase was statistically significant ($p < 0.05$). Aqueous extract at doses of 200mg/kg b.wt and 400mg/kgbwt recorded an increase in white blood cell from 6.4X 10^3 /µl and 6.8X10^3/µl to 13.2X10^3/µl and 12.6X10^3/µl respectively on day 12; the increase in white cell counts in infected rats treated with the aqueous extract was statisitically significant ($p < 0.05$).

Table 1: Phytochemical Constituents of Extracts of *Senna alata* Leaves

Phytochemical Constituent	Pet. Ether	Meth.	Chloro.	But.	Aq.
Carbohydrates	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Steroids	_	_	_	_	_
Triterpenes	+	+	+	+	+
Flavonoids	_	+	+	+	+
Saponins	_	+	_	+	+
Alkaloids	+	+	+	+	+
Anthraquinones	_	_	+	_	_

KEY: + = presence of component; - = absence of component

Table 2: Effect of Extracts of *Senna alata* Leaves on Packed Cell volume of *Trypanosoma brucei brucei* Infected Wistar Rats

Day	Packed Cell Volume (%)												
	NC	DC	SD	Petroleum Ether		Methanol		Chloroform		Butanol		Aqueous	
				200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg
0	43.2	39.6	41.4	42.3	46.1	42.5	40.7	44.1	41.5	43.4	42.8	39.4	40.5
3	42.7	39.8	40.1	40.5	42.27	40.1	37.0	42.5	41.2	38.1	35.4	37.8	38.3
6	43.3	D	39.7	D	D	38.2	D	40.4	39.7	D	D	37.2	36.1
9	42.9		40.3			33.6		36.3	39.7			36.6	33.2
12	41.4		38.8			D		30.9	31.9			30.4	30.4
15	41.8		42.2					30.7	D			D	D
18	42.9		41.3					D					
21	42.6		41.0										

NC: Normal control; DC: Disease control; SD: Standard Drug (3.5mg/kg of diaminazane aceturate); D: death of

Table 3: Effect of Extracts of *Senna alata* Leaves on Haemoglobin Concentration of *Trypanosoma brucei brucei* Infected Wistar Rats

Day	Haemoglobin concentration (g/dl)												
	NC		DC	SD		Pet. Eth		Met		Chloro.		But	Aq.	
				200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg
0	14.4	13.2	13.8	14.1	15.4	14.2	13.6	14.7	13.8	14.5	14.3	13.1	13.5
3	14.2	12.6	13.4	13.5	14.2	13.4	12.6	14.2	13.7	12.7	11.8	12.6	12.8
6	14.4	D	13.0	D	D	12.7	D	13.5	13.2	D	D	12.4	12.0
9	14.3		13.4			11.2		12.1	13.2			12.2	11.1
12	13.8		12.9			D		10.3	10.6			10.1	10.1
15	13.9		14.1					10.2	D			D	D
18	14.3		13.8					D					
21	14.2		13.7										

NC: Normal control; DC: Disease control; SD: Standard Drug (3.5mg/kg of diaminazane aceturate); D: death of rats

Table 4: Effect of Extracts of *Senna alata* Leaves on White Blood Cell Count of *Trypanosoma brucei brucei* Infected Wistar Rats

Day	White Blood Cell count (X 10^3 /µl)											
	NC	DC	SD	Pet. Eth		Met		Chloro.		But		Aq.	
				200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg
0	6.8	6.5	6.4	6.8	6.5	6.7	6.8	6.2	6.7	7.0	6.5	6.4	6.8
3	6.7	9.1	8.9	9.2	9.4	9.4	9.4	8.9	9.0	9.8	9.6	8.9	9.4
6	7.1	D	8.7	D	D	10.4	D	9.4	9.8	D	D	10.0	10.2
9	6.8		7.5			12.6		10.1	10.6			12.1	11.5
12	6.7		7.7			D		10.7	12.1			13.2	12.6
15	6.9		7.4					13.2	D			D	D
18	6.5		7.4					D					
21	6.8		7.1										

NC: Normal control; DC: Disease control; SD: Standard Drug (3.5mg/kg of diaminazane aceturate); D: death of rats

DISCUSSION

The study revealed different types of phytochemicals present in the petroleum ether, methanol, butanol, chloroform and aqueous extracts of *Senna alata* which should be responsible for its medicinal values. This is in line with the findings of Mordi who revealed that there are many different secondary metabolites such as saponins, alkaloids, flavonoids, anthraquinones, tannins, terpenes, steroids, carbohydrates found in different parts of *Senna alata* [19]. It was reported that different solvents have different solubility capacities for different phytoconstituents, hence the differences in the activities of the various extracts [20]. This could account for the reason why some phytochemicals are absence in some extracts and presence in others. The phytochemical screening revealed the presence of anthraquinones in chloroform extract only. Other factors such as solvent to plant material ratio, particle size of plant material, temperature, extraction method, seasons, geographical location and soil type also influence the phytochemical constituents of an extract [21].

Hematological parameters are useful indices that can be employed to assess the potentials of plant extracts in living systems. They can also be used to describe blood relating functions of chemical compound/plant extract. Such type of laboratory investigations has been reported to be highly sensitive, accurate, and reliable and it remains the bedrock of

ethical and rational research, disease diagnosis, prevention and treatment [21].

Anaemia is a constant feature of trypanosome infections and its severity has been linked to the level of parasitemia. It has been established that the measurement of anaemia gives a reliable indication of the disease status and productive performance of trypanosome infected animals. Reports of leucocytosis due to lymphocytosis are shown at the onset of trypanosomiasis while leucopaenia is always seen at terminal stage of the infection. These are usually due to wax and wear syndrome on the animal immune system caused by the ever-changing variable surface glycoprotein of the infecting trypanosomes [22]. However, the administration of *Momordica balsamina* pulp and *Securidaca longipenduculata* prevented leucocytosis due to lymphocytosis thus prolonging the survival of the wistar rat beyond the death of the infected, untreated control [23]. Similar result was seen in this study when using *Senna alata* due to the body employing its immune arsenals to fight the invading parasites in the process of immune response enhance the production of more white blood cells.

The low PCV observed in the infected group may be as a result of acute haemolysis due to growing infection. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione on the surface of the red blood cell. Severity of anaemia usually reflects the intensity and duration of parasitaemia [24].

Findings from this study indicated that in untreated rats infected with *T. brucei brucei,* there was significant decrease in packed cell volume. This was earlier reported by researchers that anaemia is a principal clinical symptom of trypanosome infection [22]. Packed cell volume has been variously utilized as an indirect measure of red blood cell count and, thus, as an index for the anemic status of an individual. Therefore, the decreased PCV values observed in the *T. brucei* infected-untreated control were indicative of progressive anemia. Trypanosomes are reported to metabolize the sialic acids that constitute the membrane of red cells resulting in their increased susceptibility to lyses, and the attendant anemia. Severe anemia impairs oxygen transport and cellular metabolism and could ultimately result in death [24]. The present result indicated that the petroleum ether extract of *Senna alata* did not prevent, ameliorate or reverse the trypanosome associated decline in packed cell volume of the infected rats. This decline in PCV values in *T. brucei* infected rats treated with petroleum ether extract of *Senna alata* may be connected with the level of saponin contents during the determination of phytoconstituents. Saponins have been reported to possess hemolytic properties [25].

It is also possible that this extract possesses some antioxidant activities that could scavenge *Trypanosoma brucei brucei* generated free radicals which are implicated in the development of anaemia and are capable of causing oxidative stress during trypanosomiasis infection. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by

binding with the kinetoplast DNA of the parasite [26].

Increase in white blood cells (WBC) was also observed in rats after infection and administration of *Senna alata*. Similar result was observed with mistletoe, although the increase was not statistically significant. The crucial role of WBC in defending the body against infection and tissue damage is well known. This supports previous reports that mistletoe and some commonly prescribed medicinal plants contains agents that stimulate the production of leucocytes. This suggests that the extract may have immune boosting effect on the animals. Such effects may also be due to increase in vascular permeability [27]. Immune boosters are usually recommended to strengthen and harmonize degenerative body systems and assist the immune system to fight invading agents such as bacteria, viruses and protozoans [28].

Authors Contribution

KMA and TT conceptualized the study. TT, TZ, OKO, OSO and AR designed the study. IKJ, TT, AR, ARS, KMA and TZ participated in Laboratory work and data collection.TT, KMA, OSO and IJK performed the data analysis; TZ, OKO, OOJ, IKJ and AR interpreted the data. TT, ARS and OKO prepared the first draft of the manuscript, reviewed by KMA, TZ, ARS and AR. All authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

Authors declared no conflict of interest.

Ethics Approval and Informed Consent

Ethical approval for the use of laboratory animals was obtained from the Kaduna state ministry of Agriculture and Forestry.

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