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Anti-trypanosomal activities and biochemical changes in *T. brucei* infected wistar rats following oral administration of aqueous stem bark extract of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides*

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Abstract

The toxicological and anti-trypanosomal potentials of aqueous stem bark extracts of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* in *Trypanosoma brucei* infected wistar rats were determined. The albino rats (30) were randomly assigned into six (A-F) groups, each of which contains five rats. Groups A (Control) and B (Negative control) received 1 ml of distilled water, groups C and D received 1ml each of the extracts, which is equivalent to 200mg/kg rat body weight of aqueous stem bark extract of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* respectively while groups E and F received 1ml each of Inositol Hexaphosphate (IP6) and Deferoxamine respectively as standard drugs. Parasite counts were made and recorded daily, the rats were later

sacrificed under slight anesthesia, 24 hours after the last treatment (day 12). The blood samples and vital organs were collected and used for the determination of biochemical assays of interest. The level of parasitemia was reduced in the *Z. zanthoxyloides* treated rats as at day 8 and 9 while there was no decrease in the level of parasitemia in the *P. erinaceus* group when compared with the control group. In the negative control rats, a progressive increment in the parasitemia level was recorded from the 4th day till the 8th day of infection before their death on the 9th day. Also, there were progressive increment in the parasitemia levels *of P. erinaceous* and *Z. zanthoxyloides* treated rats respectively before their death on the 8th day of infection, which is also the 4th day of treatment. A gradual reduction in parasitaemia in rats treated with *Z. zanthoxyloides* was however initiated before their eventual death on day 10. There was a significant difference in the average body weight, aspartate transaminase (AST), protein and albumin of *T. brucei* infected rats treated with aqueous stem bark extract of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* when compared with the controls. The aqueous extract of *P. erinaceus* stem bark causes significant increase in the weight of liver while no significant difference in the weight of kidney of *T. brucei* infected rats was recorded with respect to the control. Similarly, there was a significant increase in the liver and heart body weight ratios while there was no significant increase in the kidney body weight ratio as compared with that of the control. Also, the activity of alanine transaminase (ALT) in the serum of infected rats administered extracts of the two plants was not altered while it was significantly altered in heart. **T**hese results indicate that the two extracts caused mild alterations in the biochemical parameters studied at the studied concentrations (200mg/kg rat body weight). Of the two natural plants used, *Z. zanthoxyloides* is less toxic and produced the best pharmacological effect as such; it can be used in the management of trypanosomiasis more than *P. erinaceus*.

Keywords: *Pterocarpus erinaceus, Zanthoxyllum zanthoxyloides*, anti-trypanosomal, parasitemia, biochemical assay

Introduction

African trypanosomes cause trypanosomiasis, also known as sleeping sickness for which about 300,000 new cases are reported annually in some 36 developing African countries south of the Sahara where about 60

million people in some 200 locations are exposed to the risk of infection (Chretien and Smoak, 2005). This scourge remains a pressing challenge especially to African medical scientists for possible action plan that would include both preventive measures and treatment modalities (Okochi *et al*., 2003).

Available drugs are toxic, regimented and costly, generally non affordable by the poor (Kioy and Mattock, 2005; Moore, 2005), which make more evident the fact that researchers must continue in seeking inexpensive, readily available and less cumbersome approaches to the management and treatment of the disease (Jannin and Cattand, 2004; Moore, 2005). Hence, present trend is diverting towards the use of medicinal plants in the treatment of diseases and a number of such natural plant products have been credited with a lot of anti-trypanosomal potentials.

Pterocarpus erinaceus Poir (Fabaceae) is a medicinal plant used to treat several diseases. The whole plant, including stem bark, leaves; roots and resin have medicinal properties. The stem bark has been used in traditional medicine to treat inflammatory pathologies; such as rheumatism, gastric ulcer, ulcer, dermatitis (Nacoulma, 1996), it has also been known for its antimalarial property (Karou *et al*., 2003), antimicrobial activity (Nuhu *et al.*, 2000) and the mechanism underlying anti-gonadotrophic effects of the plant in pituitary cell culture (Benie and Thieulant, 2004) has been elucidated.

Zanthoxylum zanthoxyloides (Rutaceae) commonly called ‘toothache bark’ or ‘candle wood’ (English), Orin ata (Yoruba) is widespread in the West Tropical Africa occurring in Savanna and dry forest areas. The plant is also found in the Coastal areas (Iwu, 1999*). Z. zanthoxyloides* contains various secondary metabolites, which are known for their diverse biological properties (Adegbolagun and Olukemi, 2010). The plant is known for its antioxidative, anti-inflammatory, antisickling, antibacterial, antiviral, antihepatotoxicity, antiallegic, antitumoral and antihypertensive properties (Sofowora, *et al.*, 1975; Andersson *et al.*, 1996; Adesina, 2005).

The two plants have also been used locally across Africa to manage trypanosomiasis. Accordingly, in this study, we aimed to investigate the toxicity and anti-trypanosomal activity of aqueous stem bark extracts *of Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* in *T. brucei* infected wistar rats.

Material and methods

Chemicals and reagents

The assay kits for urea, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were products of RANDOX Laboratory Limited, Cumlin, UK. All other reagents used were of analytical grade and supplied by Sigma-Aldrich Inc., St. Louis, USA.

Sample collection and extraction

Fresh stem bark samples of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* were obtained from Ilorin, Kwara State, Nigeria. The plants were botanically authenticated by Mr. Bolu of the Herbarium unit of the Department of Plant Biology, University of Ilorin-Kwara State, Nigeria where voucher specimens were deposited. Their voucher numbers are UILH/002/599 and UILH/001/110 respectively.

The Fresh stem bark samples of *Z. zantholloides* and *P. erinaceous* were rinsed, dried at room temperature (25°C) and milled to powdery forms separately. 500g of each powdery plant materials were weighed and added separately to 5litres of distilled water each and left for 48 hours, while the mixtures was stirred at regular 8 hours interval. The mixtures were eventually filtered, first with a clean white handkerchief (cotton cloth) and then with a Whatman No. 1 filter papers to allow complete separation of residues from the filtrates. The filtrates were then concentrated in water bath at 40℃ to yield the needed final extracts.

Experimental Animals

Thirty (30) healthy adult male albino rats 140$\pm$10g were obtained from the Animal House of the Biochemistry Department of the University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in well ventilated plastic cages with saw dust as beddings and were fed on standard rat feed and allowed access to water *ad libitum*. The animals were acclimatized for a period of one week before being the commencement of the experiment. The animals were cautiously handled in accordance with the regulations set by the University of Ilorin Research Ethical Committee.

Animal Groupings

The experimental rats (*Rattus norvergicus*) were separated into six groups (A-F) of five male rats as shown below:

Group A: Positive control (Not infected, not treated) administered with 1 ml distilled water/day

Group B: Negative control (Infected, not treated) administered with 1 ml distilled water/day

Group C: infected and treated with 1ml of 200mg/kg body weight *P. erinaceous* stem bark extract/day

Group D: infected and treated with 1ml of 200mg/kg body weight *Z. zantholoides* stem bark extract/day

Group E: infected and treated with 1ml (5mg/kg body weight) of Inositol Hexaphosphate (IP6)/day (natural source)

Group F: infected and treated with 1ml (5mg/kg body weight) of Deferoxamine/day (synthetic source)

The administrations were carried out orally using cannula till the last day of the experiment, as dictated by the eventual death of the last surviving animal in the test groups.

Trypanosome stock

A stabilate of pleomorphic *trypanosome brucei* strain (*Trypanosoma brucei brucei*) was obtained from Nigeria Institute for *Trypanosomiasis and Onchocerciasis* Research (NITR), Kaduna and maintained in the Laboratory of Department of Biochemistry by serial passage into rats.

Parasite Inoculation into Donor Rats

Blood was collected by jugular puncture from a heavily infected rat and immediately diluted with phosphate buffer solution (PBS). The diluted infected blood contains about 2-5 parasites per field (microscopic field). 1ml of this blood was injected into two clean healthy rats intraperitoneally to serve as the donor. Parasitaemia was monitored every morning by

microscopic examination of the blood samples taken from the tail of the infected rats.

Infection of Experimental Animals

Blood was collected by jugular puncture with a syringe from a heavily infected rat and immediately diluted with phosphate buffer solution (PBS) to serve as inoculums. Healthy rats in each test group other than the control group were each infected intraperitoneally with 1ml of the inoculums containing about 100 trypanosome cells. The experimental animals were checked for infection from the third day post infection by checking their parasitaemia level. The animals were confirmed infected on the fourth day by the presence of about 3-5 parasites per field (microscopic field). The extracts were thereafter administered every morning and parasiteamia was monitored and recorded every three days by microscope examination till the end of the experiment (11th day).

Blood collection, isolation and homogenization of tissues of interest

Collection of sample for biochemical analyses was as described by (Sulaiman and Adeyemi, 2010). Briefly, the blood and tissues of interest were collected from each rat after every sacrifice. They were sacrificed by anaesthetizing in a jar containing cotton wool soaked with diethyl ether. When they became unconscious, blood samples were collected by jugular puncture into EDTA sample bottles. The blood samples were centrifuged at 1000rpm for 15mins and 1ml of the serum was collected in appropriately labelled sterile bottles, containing 4ml of distilled water. The rats were then dissected and organs of interest (kidneys, heart and liver) were collected, weighed and preserved separately in 5ml of 0.25M sucrose solution before their eventual homogenization (1:5 w/v) and centrifugation to aspirate the filtrates into sterile sample bottles till needed.

Statistical Analysis

Data were expressed as mean of five replicates ± standard error of mean (S.E.M). The data were subjected to analysis of variance and Turkey's multiple range tests using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $P<0.05$.

Results

Parasitemia

Table 1 shows the average daily parasitemia in *T. brucei* infected wistar rats. The control rats have no parasite count recorded in their blood. In the negative control rats, a progressive increment in the parasitemia level was observed from the 4th day till the 8th day of infection before their death on the 9th day. Also, there were progressive increment in the parasitemia levels *of P. erinaceous* and *Z. zanthoxyloides* treated rats respectively before their death on the 8th day of infection, which is also the 4th day of treatment. A gradual reduction in parasitaemia in rats treated with *Z. zanthoxyloides* was initiated before their death on day 10.

Average Body Weight

Table 2 shows the effects of *P. erinaceous* and *Z. zanthoxyloides* extracts on average weights of *T. brucei* infected rats over three weeks of the experiment. In the first week, the average weight of the negative control group (139.7) and that of other groups (*Z. zanthoxyloides*: 131.9; IP6: 132.6 and deferoxamine: 113.4) were significantly lower ($P<0.05$) in weight compared with the positive control group (151.8), which is not significantly different from *P. erinaceous*: 149.2.

In the second week, which was the infection and treatment week, there was no significant difference ($P>0.05$) between the control group (151.3) and the negative control group (150.3). However, the average weight of *P. erinaceous* group (162.4) was significantly increased and is higher ($P<0.05$) when compared with the average weight of both control groups. The increase in average weight recorded in the other three groups (*Z. zanthoxyloides*, 136.2, IP6, 139.3 and deferoxamine, 123.9) were significantly lower ($P<0.05$) compared with the control groups.

In the third week, the average weight of positive control group (150.5) remain significantly higher ($P<0.05$) compared with the negative control group (135.3) and other experimental groups (*P. erinaceous* 124.5, *Z. zanthoxyloides* 133.0, IP6 131.9 and 117.9), with deferoxamine (117.9) having the least average weight.

Organs' Weight

Table 3 shows the effects of *P. erinaceous* and *Z. zanthoxyloides* extracts on weight of organs. For the liver, there was no significant difference (P>0.05) in its weight in groups D (6.400) and E (6.420) when compared with that of the positive control (5.963). Liver weight in groups B (7.193), C (7.750) and F (7.213), which were not significantly (P>0.05) different from each other, were slightly higher than found in the positive control group.

For the kidney weight, there was no significant difference (P>0.05) between the control (1.273) and the other experimental groups (1.330, 1.307, 1.207, 1.117 and 1.267).

For the heart's weight, there was no significant difference (P>0.05) between the positive control group (0.560) and the IP6 treated group (0.577). However, there was significant increment (P<0.05) in the other experimental groups (0.860, 0.697, 0.683, 0.623) compared with the positive control. The highest heart weight recorded was in the negative control group (0.860).

Percentage Organ-body Weight Ratio

Table 4 shows the effects of *P. erinaceous* and *Z. zanthoxyloides* extracts on Organ Body-Weight ratios. For the liver, there was a significant increase (P<0.05) in all the groups compared with the control group. IP6 and Deferoxamine treated groups recorded the highest liver body-weight ratio.

For the kidney, there was no significant difference (P>0.05) between the control (0.813), the *P. erinaceous* (0.813) and *Z. zanthoxyloides* (0.880) groups. However, kidney body-weight ratio in other groups were significantly increased (P<0.05) compared with the control.

For the heart, there was also a significant decrease (P<0.05) in other experimental groups compared with the two control groups, with the highest value recorded in the heart of the negative control group.

Aspartate Transaminase (AST)

Table 5 shows the AST Activity in the serum and selected tissues of rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*. The AST activity was significantly higher (P<0.05) in the serum of negative control (245.77) rats when compared with the control (137.00). However, there was a significant reduction (P<0.05) in the AST activities in the serum of rats treated with the two extracts (51.3 and 33.3), with the least AST activity recorded in the serum of *Z. zanthoxyloides.* No significant difference in those treated with the two standard drugs (107.0 and 97.5) when compared with the control (137.0).

The AST activity was significantly lower (P<0.05) in the liver of negative control rats (100.0) when compared with the control (153.5). Also, there were significant reductions in the AST activities of the liver of the experimental rats (107.8, 108.1, 98.3 and 103.5) compared with the control while no significant differences were recorded when compared with the negative control (100.0).

The AST activity was significantly lower (P<0.05) in the heart of negative control rats (229.0) when compared with the control (275.2). Also, there were significant reductions (P<0.05) in the AST activities in the hearts of other experimental groups (56.7, 28.7, 81.6 and 56.8) compared with the control (275.2). AST activities in the hearts of groups treated with both extracts are not significantly different from each other (72.32, 78.93) but are higher than recorded in groups administered the two standard drugs.

Alanine Transaminase (ALT)

Table 6 shows the ALT Activity (U/I) in serum, liver and heart of rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*. In the serum, there was a significant increase (P<0.05) in the ALT activity of the negative control rats (87.86) compared with the positive control (23.33) and other experimental groups. *P. erinaceous* (21.07), *Z. zanthoxyloides* (16.25) and IP6 (24.05) treated rats have no significant difference (P>0.05) in ALT activities when compared with the control. Similarly, a significant increase (P<0.05) in ALT activity was also recorded in the rats treated with deferoxamine (39.70), compared with the control.

In the liver, there was a significant reduction (P<0.05) in the ALT activities of four of the groups compared with the control (111.30). However, there was no significant difference (P>0.05) in ALT activities in the *P. erinaceous* (105.7) and *Z. zanthoxyloides* (96.01) treated rats as well as the negative control rats compared with the control group. The IP6 (86.96) and deferoxamine (82.09) treated rats also showed significant reduction (P<0.05) when compared with the control (111.30).

In the heart, there was no significant difference (P>0.05) in the ALT activity of the IP6 (49.88) compared with the control (57.44). However, all other treated groups (72, 78, 79) and the negative control group (98.99) showed significant increase (P<0.05) in ALT activity when compared with the control group (57.44).

Alkaline Phosphatase (ALP)

Table 7 shows the alkaline phosphatase activity in the serum and some tissues of rats administered aqueous extracts of *Z. zanthoxyloides* and *P. erinaceous*. In the serum, there was significant decrease (P<0.05) in ALP activity of the negative control (6.284) when compared with the control (8.468). However, the *P. erinaceous* (10.008) and deferoxamine (9263) treated rats exhibited the highest and the most significant increase (p<0.05) in their ALP activities respectively when compared with the control (8468). The IP6 treated rats (7.685) however showed no significant difference (P>0.05) with control (8.468), both of which are significantly higher than the *Z. zanthoxyloides* treated group (1.793).

In the liver, the most significant reduction (P<0.05) in the ALP activity was recorded in the liver of the negative control group (10.939) compared with the other groups. A significant increase (P<0.05) in the IP6 treated rats (19.312) compared with the positive control group (14.924). There was however no significant difference (P>0.05) between the other experimental groups and the positive control group.

In the kidney, there was no significant difference (P>0.05) in the ALP activities between the various groups and the control group (21.515).

Total Protein

Table 8 shows the protein concentration in the serum and liver of rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*. In

the serum, there was significant reduction (P<0.05) in the protein concentration of the negative control group (20.44) and other treated groups (16.90, 2.76, 3.85, 18.24) in comparison with the control group.

In the liver, there was significant reduction (P<0.05) in the protein concentration of the negative control group (23.12) in comparison with the positive control group (45.15), the significant reduction (P<0.05) was also recorded by the *P. erinaceous* (36.53) and *Z. zanthoxyloides* (24.89) treated rats. However, there was no significant difference (P>0.05) between the IP6 treated rats (42.22) and the control group (45.15), but there was a significant increase (P<0.05) in the deferoxamine treated rats (51.99) compared with the control group (45.15).

Albumin

Table 9 shows the Albumin Concentration in the serum and liver of rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*. In the serum, there was a significant increase (P<0.05) in the albumin concentration of the negative control rats (1.789) compared with the control (1.458). *P. erinaceous* (1.111) and *Z. zanthoxyloides* (0.962) treated rats showed a significant reduction (P<0.05) when compared with the control. The IP6 treated rats (1.496) had no significant difference (P>0.05) with the positive control (1.458) while no significant difference (P>0.05) between deferoxamine treated rats (1.799) and the negative control (1.789).

In the liver, there was a significant decrease (P<0.05) in the albumin concentration of the negative control rats (0.443) compared with the control (1.592). *P. erinaceous* (0.924) and *Z. zanthoxyloides* (0.808) treated rats showed a significant reduction (P<0.05) in the albumin concentration when compared with the control (1.592), whereas the IP6 treated rats had the highest liver albumin concentration compared with the control. There was also no significant difference between the deferoxamine treated rats (1.305) and the control (1.592).

Urea

Table 10 shows urea concentration in the serum and kidney of rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*. In the serum, there was a significant increase (P<0.05) in the urea

concentration of negative control (8.411) and *P. erinaceous* (10.450) groups when compared with the control (6.885). However, there was no significant difference (P>0.05) between the IP6 (6.270) and deferoxamine (6.201) groups when compared with the control. The *Z. zanthoxyloides* (1.793) however showed a significant reduction (P<0.05) compared with all the groups.

In the kidney, there was a significant decrease (P<0.05) in the urea concentration of negative control (21.15), *P. erinaceous* (22.09), IP6 (13.48) and deferoxamine (14.58) groups when compared with the control (26.57). However, there was no significant difference (P>0.05) in the urea concentration of *Z. zanthoxyloides* treated group (24.16) and the control (26.57).

Table 1: Effect of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* on the parasitemia of *trypanosoma brucei* infected rats

	Days										
Groups	1	2	3	4	5	6	7	8	9	10	11
A (Control)	0	0	0	0	0	0	0	0	0	0	0
B (Negative control)	0	0	0	2.33	6.33	7.50	9.00	11.00	D	-	-
C (*P. erinaceus*)	0	0	0	5.33	8.67	14.00	16.00	D	-	-	-
D (*Z.zanthoxyloides*)	0	0	0	4.33	9.00	13.5	11.00	9.50	7.50	D	
E (Inositol hexaphosphate)	0	0	0	5.00	8.33	12.67	11.33	9.67	9.00	5.00	D
F (*Deferoxamine*)	0	0	0	4.67	9.67	14.00	14.00	10.00	12.00	10.00	D

Values are expressed as mean ± SEM of 5 replicates. D: death.

Table 2: Effect of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* on the average weight of *trypanosoma brucei* infected rats

	Average body weight (g)		
Groups	Week 1	Week 2	Week 3
A (Control)	151.8 ± 0.342a	151.3 ± 2.054a	150.5± 2.204a
B (Negative control)	139.7 ± 1.939b	150.3 ± 2.638a	135.3± 0.882b
C *(P. erinaceus)*	149.2 ± 2.888a	162.4 ± 1.481b	124.5± 2.500b
D (*Z.zanthoxyloides)*	131.9 ± 1.064b	136.2 ± 1.958c	133.0± 1.000b
E (Inositol hexaphosphate)	132.6 ± 3.733b	139.3 ± 2.934c	131.9± 2.899b
F (Deferoxamine)	113.4 ± 2.330c	123.9 ± 2.288d	117.9 ± 5.068c

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05)

Table 3: Effect of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* on some organs weight in *trypanosoma brucei* infected rats

	Organ weight (g)		
Groups	Liver	Heart	Kidney
A (Control)	5.96±0.83a	0.56±0.09a	1.27±0.15a
B (Negative control)	7.19±0.57b	0.86±0.03c	1.33±0.06a
C (*P. erinaceus)*	7.75±0.93b	0.70±0.01b	1.31±0.00a
D (*Z.zanthoxyloides)*	6.40±0.67a	0.68±0.06b	1.21±0.12a
E (Inositol hexaphosphate)	6.42±0.81a	0.58±0.15a	1.18±0.19a
F (Deferoxamine)	7.27±0.69b	0.62±0.11b	1.27±0.05a

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05)

Table 4: Effect of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* on some percentage organ-body weight ratio in *trypanosoma brucei* infected rats

	% organ-body weight ratio		
Groups	**Liver**	**Heart**	**Kidney**
A (Control)	3.77±0.07a	0.35±0.01a	0.81±0.02a
B (Negative control)	4.98±0.23b	0.58±0.07b	0.93±0.04b
C *(P. erinaceus)*	4.72±0.13b	0.44±0.05c	0.81±0.09a
D *(Z.zanthoxyloides)*	4.63±0.36b	0.49±0.03c	0.88±0.10a
E(Inositol hexaphosphate)	5.53±0.41c	0.48±0.10c	0.98±0.12b
F(Deferoxamine)	5.78±0.42c	0.50±0.09c	1.01±0.05b

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05)

Table 5: Effect of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* on the activity of aspartate transaminase (AST) in *trypanosoma brucei* infected rats

	Aspartate transaminase activity (U/I)		
Groups	**Serum**	**Liver**	**Heart**
A (Control)	137.00±20.08a	153.50±9.54a	275.20±19.97a
B (Negative control)	245.70±0.67b	100.00±19.53b	229.00±2.01b
C (*P. erinaceus)*	51.30±20.08c	107.80±0.75c	56.66±13.19d
D (*Z.zanthoxyloides)*	33.34±3.14c	108.10±13.06c	28.69±17.69d
E (Inositol hexaphosphate)	107.00±10.79a	98.26±4.02b	81.59±10.67c
F (Deferoxamine)	97.53±54.45a	103.50±3.99b	56.81±25.73d

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05)

Table 6: Effect of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* on the activity of alanine transaminase (ALT) in *trypanosoma brucei* infected rats

	Alanine transaminase activity (U/I)		
Groups	Serum	Liver	Heart
A (Control)	23.33 ± 2.42a	111.30 ± 2.99a	57.44 ± 1.98a
B (Negative control)	87.86 ± 2.52b	97.80 ± 7.20b	98.99 ± 6.77b
C (*P. erinaceus)*	21.07 ± 6.19a	105.70 ± 1.02a	72.32 ± 5.88c
D (*Z.zanthoxyloides)*	16.25 ± 2.99a	96.01 ± 1.23b	78.93 ± 6.83c
E (Inositol hexaphosphate)	24.05 ± 0.77a	86.96 ± 0.10c	49.88 ± 19.28a
F (Deferoxamine)	39.70 ± 21.79c	82.09 ± 8.35c	79.64 ± 2.47c

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different ($P<0.05$)

Table 7: Effect of *Pterocarpus erinaceous* and *Zanthoxyllum zantholoides* on the activity of alkaline phosphatase (ALP) in the serum and some tissue *of trypanosoma brucei* infected rats

	Alkaline phosphatase (U/I X10^3)		
Groups	Serum	Liver	Kidney
A (Control)	8.468 ± 0.695a	14.924 ± 3.039a	21.515 ± 0.044a
B (Negative control)	6.284 ± 0.050b	10.939 ± 2.688b	21.389 ± 0.094a
C (*P. erinaceus)*	10.088 ± 5.635c	14.943 ± 2.205a	22.311 ± 0.342a
D (*Z.zanthoxyloides)*	1.793 ± 0.335d	15.156 ± 1.181a	21.730 ± 0.110a
E (Inositol hexaphosphate)	7.685 ± 3.024a	19.312 ± 0.704c	22.052 ± 0.298a
F (Deferoxamine)	9.263 ± 4.110c	15.265 ± 2.649a	22.155 ± 0.307a

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05).

Table 8: Effect of *Pterocarpus erinaceous* and *Zanthoxyllum zantholoides* on the concentration of protein in *of trypanosoma brucei* infected rats

	Protein concentration (mg/ml)	
Groups	Serum	Liver
A (Control)	31.78±0.00a	45.15±4.047a
B (Negative control)	20.44±0.014b	23.12±1.353b
C *(P. erinaceus)*	16.90±11.090c	36.53±2.138c
D (*Z.zanthoxyloides)*	2.76±0.813d	24.89±1.058b
E(Inositol hexaphosphate)	3.85±1.952d	42.22±2.289a
F(Deferoxamine)	18.24±4.782c	51.99±11.410d

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05).

Table 9: Effect of *Pterocarpus erinaceous* and *Zanthoxyllum zantholoides* on the concentration of Albumin (ALB) in serum and liver albumin in *trypanosoma brucei* infected rats

	Albumin (g/dl)	
Groups	**Serum**	**Liver**
A (Control)	1.458±0.441a	1.592±0.198a
B (Negative control)	1.789±0.032b	0.443±0.171b
C *(P. erinaceus)*	1.111±0.058c	0.924±0.225c
D (*Z.zanthoxyloides)*	0.962±0.062c	0.808±0.113c
E(Inositol hexaphosphate)	1.496±0.187a	2.209±0.683d
F(Deferoxamine)	1.799±0.458b	1.305±0.612a

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05).

Table 10: Effect of *Pterocarpus erinaceus* and *Zanthoxyllum zanthoxyloides* on the concentration of urea in *trypanosoma brucei* infected rats

	Urea (U/I)	
Groups	**Serum**	**Kidney**
A (Control)	6.885 ± 2.307a	26.570 ± 4.140a
B (Negative control)	8.411 ± 0.335b	21.150 ± 0.766b
C (*P. erinaceus*)	10.450 ± 0.444c	22.090 ± 0.399b
D (*Z.zanthoxyloides)*	1.793 ± 0.339d	24.160 ± 3.752a
E(Inositol hexaphosphate)	6.270 ± 2.330a	13.480 ± 0.654c
F(*Deferoxamine*)	6.201 ± 2.553a	14.580 ± 2.821c

Values are expressed as mean ± SEM of 5 replicates. Values in each column with different letters are significantly different (P<0.05).

Discussion

Average daily parasitemia in *T. brucei* infected wistar rats administered with aqueous stem bark extract of *P. erinaceous* and *Z. zanthoxyloides*

Parasitaemia has been shown to correlate with severity of infection (Sulaiman *et al*., 2010, Sulaiman *et al*., 2012, Ekanem and Yusuf, 2008). Previous reports have shown that 50% parasitemia is an indication of significant extract's activity (Legros *et al*., 1999). Some researchers have shown that treatment failure is possible in cases of massive parasitemia at the time of the therapeutic intervention (Tijani *et al.,* 2009). The death of the rats treated with *P. erinaceous* on day 4 post-treatment, depicts that the stem bark extract of *P. erinaceous* had no trypanocidal effect, the rats in this group astonishingly died before the death of the negative control group, which suggests some toxic effects of the extracts on them. This result is in accordance with the one reported by Mann and Emmanuel (2012) that the crude extract of the stem bark of *P. erinaceous* had no trypanocidal effect. The failure of the crude extract of the stem bark of *P. erinaceous* to show any trypanocidal action depicts that the anti-trypanosomal activities are lacking in the stem bark as opposed to the claims by the traditional healers, which according to the existing literature

is used in Nupe ethnomedicine in the management of sleeping sickness in Nupeland (Mann *et al.,* 2009). Also, the *Z. zanthoxyloides* treated groups died a day before the two standard drugs depicting its close proximity to the effectiveness of the two standard drugs used. This result is also in support of work done by Mann and Emmanuel (2012), which earlier suggested that the stem bark extract of *P. erinaceous* exhibit mild anti-trypanosomal activities.

Effects of aqueous stem extract of *P. erinaceous* and *Z. zanthoxyloides* on weight of organs in *T. brucei* infected rats

Organ weight is a useful marker of cellular swelling, atrophy or hypertrophy (Amresh *et al*., 2008). The weight of the heart and liver of all experimental groups were significantly higher than recorded in the positive control group except for the liver of inositol hexaphosphate and *Z. zanthoxyloides* groups as well as in the heart of inositol heaxaphosphate.The weight of the heart of the negative control group is higher than recorded for other groups The absence of an effect or a major increase on the weight of isolated kidney suggests that the extracts and standard drugs used did not cause any form of swelling, atrophy nor hypertrophy on the kidneyas No significant increase or decrease was recorded for kidney of experimental animals.

Effects of aqueous stem extract *of P. erinaceous* and *Z. zanthoxyloides* on percentage organ-body weight ratio of *T. brucei* infected rats

Organ-body weight ratio is a useful marker of cellular swelling, atrophy or hypertrophy (Amresh *et al.,* 2008). Therefore, the absence of a significant effect on the computed organ-body weight ratios in some of the organs suggests that the extracts and the standard drugs used did not cause any major form of swelling, atrophy and hypertrophy on the organs, especially in the kidney. Higher percentage organ body ratio recorded in all the experimental groups than that of the positive control group as well as other changes in the levels of biochemical indices following the administration of chemical compounds, including plant extracts, could be an earlier event preceding gross morphological changes in the organ(s). This however is not always the case in some other instances (Aboyade *et al.,* 2009).

Aspartate transaminase (AST) activity in the serum and selected tissues of *T. brucei* infected rats administered aqueous extracts *of P. erinaceous and Z. zanthoxyloides*

The measurement of the activity of some enzymes could be used to evaluate for cellular toxicity arising from exposure to chemical compounds including drugs (Adeyemi and Akanji, 2011). The aminotransferases (ALT and AST) are 'markers' of liver damage and can thus be used to assess liver cytolysis (Pramyothin *et al.*, 2006). Although AST is commonly included clinically as part of a diagnostic liver function test, AST has a broader clinical utility since it may also be elevated in diseases affecting other organs, such as the heart or muscles in myocardial infarction, also in acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases and trauma (GenWay Biotech Inc). The decreased AST activities in the liver and heart of rats treated with *Z. zanthoxyloides* and *P. erinaceaous* extracts respectively depicts that the tissues have been compromised by the extracts and the AST might have leaked into the serum.

Alanine Aminotransferase (ALT) and aspartate transaminase (AST) Activity in the serum and selected tissues of *T. brucei* infected wistar rats

The measurement of ALT levels in human serum has proved to be a valuable indicator of liver function in clinical settings (Huang *et al.*, 2006). The aminotransferases (ALT and AST) are 'markers' of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al.*, 2006). The reduction in the activity of the ALT from the liver of rats treated *with Z. zanthoxyloides* and *P. erinaceaous* respectively without the corresponding increase in the serum enzyme could be due to inhibition of the enzyme activity by components of the extract, as supported by Akanji *et al.* (1993), inactivation of the enzyme molecule *in situ* (Umezawa and Hooper, 1982), or depletion of important molecules required by the enzyme for maximum activity (Yakubu *et al.*, 2003). The loss in the activity of these enzymes in the liver will adversely affect carbohydrate and amino acid metabolism, thereby affecting energy production (Akanji *et al.*, 2013).

Alkaline phosphatase activities of selected tissues of *T. brucei* infected rats administered aqueous extract of *P. erinaceous* and *Z. zanthoxyloides*

In humans, ALP is present in all tissues throughout the body, but is particularly concentrated in liver, bile duct, kidney, bone, intestinal mucosa and the placenta (Tamas *et al.*, 2002). ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974) and is often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Akanji *et al*., 1993). The enzyme transfers ions and metabolites across the cell membrane. The maintenance of the activity (no significant difference) of ALP in the liver and kidney of *Z. zanthoxyloides* and *P. erinaceous* treated rats compared with the positive control could be a consequence of activation of the enzyme or an increase in the rate of the synthesis of the enzyme, induced by the components of the extracts. Furthermore, as supported by Mayne (2005), the increase in the ALP activity in the serum of the *P. erinaceous* treated rats could be a consequence of leakage of the enzyme from other tissues apart from liver and kidney or a reduced rate of clearance of the enzyme from the serum. This increase could indicate an enhanced functional activity of the organs (liver and kidney) (Brain and Kay, 1927), since there will be an increase in the rate of ion transport across the cell membranes in these tissues.

Protein Concentration in the serum and liver of *T. brucei* infected rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*

The concentration of total protein is a useful 'marker' of secretory, synthetic and excretory functioning of the liver (Yakubu and Musa, 2012). The increase in the total protein content suggests a compromise of the synthetic ability of the liver (Akanji *et al.*, 2013). The extracts might have increased the functional activity of the liver by interfering with the metabolism (anabolism) of total protein. However, the animals did not recover fully from the assault of the extracts, since the levels of the total protein were not restored back to the positive control value.

Albumin concentration in serum and liver of *T. brucei* infected rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*

Albumin is the major plasma protein. Molecular weight is approximately 66.3kDa. It is synthesized exclusively in the liver. A falling concentration in chronic liver disease suggests a clinically significant deterioration in liver function 'decompensation' (ACB, 2012). The concentration of albumin is also a useful 'marker' of secretory, synthetic and excretory functioning of the liver and kidney (Yakubu and Musa, 2012). Thus, the decreased albumin concentration in the liver of rats treated with *Z. zanthoxyloides* and *P. erinaceaous* extracts respectively depicts that the extracts have compromised the liver and some of the liver albumin might have leaked into the serum.

Urea Concentrations in the serum and kidney of *T. brucei* infected rats administered aqueous extracts of *P. erinaceous* and *Z. zanthoxyloides*

The significance of the enzyme urease includes: to serve as a virulence factor in human and animal infections of the urinary and gastrointestinal tracts, play role in recycling of nitrogenous wastes in the rumens of domestic livestock (Mobley and Hausinger, 1989; Banerjee and Aggarwal, 2013). The significant reduction ($P<0.05$) of urea observed in the kidney of *P. erinaceous* treated rats depicts the extract has compromised the kidney integrity and some of the urea have moved into the serum, which might be responsible for the increased urea concentration in the serum. The kidneys regulate the excretion of urea and reabsorption of electrolytes into the blood. Filtration occurs at the glomeruli while reabsorption takes place in the renal tubules (Mayne, 2005). When there is compromise of normal glomerular function, substances normally cleared by the kidneys such as urea accumulate in the biological fluid. The presence of an effect on the levels of serum urea of *P. erinaceous* treated rats suggests that the normal excretion of these biomolecule by the kidney was being adversely affected by the extracts.

Conclusion

The aqueous extract of *Z. zanthoxyloides* stem bark reduced the level of parasitemia while the aqueous stem bark extract of *P. erinaceous* did not, contrary to the belief of those using it locally to manage trypanosomiasis,

Z. zanthoxyloides aqueous stem bark extract has anti-trypanosomal activity. **T**hese results indicate that the two extracts caused mild alterations in the biochemical parameters monitored at the studied concentrations (200mg/kg rat body weight). Of the two natural plants used, *Z. zanthoxyloides* is less toxic and produced the best pharmacological effect as such; it can be used in the management of trypanosomiasis more than *P. erinaceus*.

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