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Full Length Research Paper

Effect of *Faidherbia albida* on some biochemical parameters of rats infected with *Trypanosoma brucei brucei*

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Current drugs used in the management of African Trypanosomiasis are toxic and can encounter parasite resistance, hence the need for urgent, less toxic and readily available alternative source of trypanocide. The effect of hydro-alcoholic extract of the stem bark of *Faidherbia albida* in albino rats experimentally infected with *Trypanosoma brucei brucei* strain Lafia/CT/66/NITR was evaluated. The oral acute toxicity of the extract was determined in rats. Parasitaemia was determined and estimated daily while the packed cell volume (PCV), Total protein, albumin, globulin and free fatty acid were determined at days 5 and 10 post treatment. The results showed that the oral median lethal dose (LD_{50}) was greater than 5000 mg extract/ Kg body weight. There was prolongation of life in the treated groups when compared to the negative control. The administration of the extract led to a highly significant ($P<0.01$) increase in Total protein, albumin, and packed cell volume levels while the globulin and free fatty acid levels decreased significantly ($P<0.01$). It is suggested that the hydro-alcoholic extract of *F. albida* is trypanostatic and effective in the management of anaemia induced by *Trypanosoma brucei brucei* in rats

Key words: *Trypanosome brucei brucei, Faidherbia albida,* PCV, Free fatty acid.

INTRODUCTION

African Trypanosomosis is a disease caused by *Trypanosoma brucei, Trypanosoma gambiense, Trypanosoma vivax* and other species of the *Trypanosoma* genus and transmitted by tsetse fly (*Glossina morsitans sp)* (Maudlin, 2006). Symptoms become manifest 1- 3 weeks after a bite of the insect and these include fever, rash, swelling of the face and hands, headaches, fatigue, aching muscles and joints, itching skin, and swollen lymph nodes. Weight loss occurs as the illness progresses. Progressive confusion, personality changes, daytime sleepiness with night time sleep disturbances, and other neurologic problems occur after the infection has invaded the central nervous system. These symptoms become worse as the illness progresses. If left untreated, death will eventually occur after several years of infection (CDC, 2008). About 60 million people are exposed to human African Trypanosomosis (Lisa-Sanderson et al., 2007). Approximately 12,000 new cases of West African Trypanosomosis are reported to the World Health Organization each year (CDC, 2008). In 1998 alone, there were almost 40,000 reported cases, with a further 300,000 to 500,000 estimated to have remained undiagnosed (WHO, 2006). Although in the 1960's there seemed to be success in the eradication of this disease in Africa at large, lack of attention to this disease and its progression has resulted in its prevalence being about 50% in countries like the Democratic Republic of Congo, higher in incidence than the human immunodeficiency

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virus (WHO, 2006) . The disease kills some three million animals a year, at an annual loss of US$ 600 million to US$ 1.2 billion, according to FAO estimates (WHO, 2001). A further consequence of the underinvestment in the control of African Human Trypanosomosis is the limited number of drugs currently available for treatment, without which the disease is fatal (Bouteille et al., 2003). Current drugs used in the management of African sleeping sickness include suramin, eflornithine, melarsoprol, and nifurtimox (Peters, 2004). These drugs are toxic and can encounter parasite resistance (Bouteille et al, 2003). Despite the understanding of this disease, there is little or no interest in developing new drugs for its treatment due to lack of financial reward. It therefore seems rational to look at alternative source of remedy for this disease. Herbal remedies are known to have been used for the management of this disease. One of such is *Faidherbia albida* otherwise known as *Acacia albida* (Del) is of the family Mimosoideae. It is native to Southwest Africa, through West, North Africa to Egypt and East Africa. Common names include winter thorn and apple-ring acacia. The Hausa people of northern Nigeria call it 'Gawo' while in Fulfuldes it is called 'Chayski'. Phytochemical studies reveal that plants in this family contain tannins (Barry and McNabbs, 1999), which account for their use in making of dyes. In addition to this, Tijani et al (2008) reported the presence of alkaloids and saponins in the stem bark extract of *F. albida.*

In folkloric medicine, it is used in fevers by the Masai people of Kenya as well as for diarrhoea in Tanganyika **(**Irvine, 1961**)**. These activities have been reported by Tijani et al. (2008). It is also used for colds and hemorrhage. A liniment, made by steeping the bark, is used for bathing and massage in pneumonia. The bark infusion is used for difficult delivery, and is used as a febrifuge for cough (Irvine, 1961). Kubmarawa et al. (2007) reported the antimicrobial activity of *F. albida* against *Pseudomonas aeruginosa, Bacillus subtilis and Salmonella typhii*. In northern Nigeria, especially among the cattle-rearing nomads, a decoction of the stem bark is taken orally for the management of the sleeping sickness. This study was conducted to investigate the claim on the use of *Faidherbia albida* stem bark in the management of Trypanosomiasis infection.

MATERIALS AND METHODS

Plant material

The leaves and stem bark of *F. albida* were collected in the month of January from Gyamso Ward in Toro Local Government Council of Bauchi State, Nigeria. They were identified and authenticated by Mrs. Jemilat Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine, NIPRD. A voucher specimen (number NIPRD/H/6151) was deposited at NIPRD herbarium for future reference. The stem bark was cleaned, air-dried at room temperature for 7 days and pounded into fine powder using mortar and pestle. The powder was stored in an airtight container and kept in a cool, dry place.

Extract preparation

Two hundred grams of the powdered stem bark was weighed and soaked in 2000 ml of water and ethanol in ratio 1:1 for 48 h. The mixture was filtered using muslin cloth followed by Whatman filter Paper (No. 1) . The resultant filtrate was evaporated to dryness on steam bath to give a yield of 8.0% of the extract. The crude extract was stored at -4^{o}c until required for use.

Animals

Male Wistar rats (150 – 200 g) obtained from Experimental Animal Department, University of Jos were used in the study. The rats were fed with grower's mash, water *ad libitum* and maintained under laboratory condition of temperature 22 ± 1^{o}C, relative humidity 14 ± 1 and 12 h light: 12 h dark cycle. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985).

Acute toxicity (LD_{50}) study

Acute toxicity study was carried out using the method of Lorke (1983) . Briefly this study was carried out in 2 stages. In the first stage, nine rats randomly divided into three groups of three animals each were administer with the extract at 10, 100 and 1000 mg /kg body weight respectively. In the second stage three rats were each, administered with the extract at 1600, 2900 and 5000 mg /kg body weight, respectively. At each stage, the administration was by oral route and rats were observed for signs of adverse effects which include but not limited to salivation, rubbing of nose and mouth on the floor of the cage and restlessness and mortality for 24 h and subsequently for 14 days. The surviving animals were sacrificed, autopsied and examined for any gross pathological changes.

Trypanosome inoculation

Trypanosoma brucei brucei strain Lafia/CT/66/NITR obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Nigeria was used for the study. The organism maintained by serial passage in rats was inoculated into the donor rats. A heavily infected blood sample from donor rats was diluted with physiological saline to give 1 x 10^{7} parasites per ml to obtain inocula. Healthy rats (I - V) of 6 animals each were then inoculated with 0.1 ml of the diluted blood sample.

Administration of the extract

Group I rats were given 1000 mg extract/kg body weight 24 h post parasite inoculation**,** group II rats were given1000 mg extract/kg body weight 48 h post parasite inoculation. Group III rats received 1000 mg extract/kg body weight at establishment of infection while groups IV and V rats served as the negative and positive control respectively and were not treated with the extract. Treatment was daily for 5 consecutive days.

Therapeutic monitoring of extract

Development of Parasitaemia in these rats was checked daily by wet blood film prepared from tail blood at x40 magnification. The number of parasite seen per field under the microscope was counted as described by Herbert and Lumsden (1976). Total plasma Proteins and albumin concentrations were measured biuret and bromocresol green methods (Tietz, 1976), respectively on days

Table 1. Effect of *Faidherbia albida on parasite count in rats infected with Trypanosoma brucei brucei*.

	Group I	Group II	Group III	Group IV	Group V
Days 0-4	0	0	0	0	0
Day 5	4.47±1.42**	4.47±1.42**	11.40±2.31**	23.77±6.56	0
Day 6	78.95±0.00**	128.15±20.09	162.23±26.78**	132.95±13.15	0
Day 7	90.8±4.85**	157.45±16.12**	206.29±13.16**	260.13±6.71	0
Day 8	137.74±8.08**	98.45±7.96**	183.19±6.67**	343.85±13.32	0
Day 9	167.44±4.05**	167.38±12.07**	211.65±11.75**	450.42±13.55	0
Day 10	196.94±0.00	137.74±8.08	167.05±20.80	0	0
Day 11	225.17±8.10	187.15±4.00	211.28±20.66	0	0
Day 12	294.65±8.02	235.35±0.00	240.62±11.78	0	0
Day 13	431.27±15.64	431.27±15.644	382.26±45.51	0	0
Day 14	0	469.58±0.00	408.74±37.85	0	0
Day 15	0	469.58±0.00	431.27±15.64	0	0

** Significantly different from the infected untreated control (group IV) at p<0.01

Table 2. Base line values of some biochemical parameters of rats infected with *Trypanosoma brucei brucei.*

Groups	Protein conc(mg/dL)	Albumin conc (mg/dL)	Globulin conc (mg/dl)	Free fatty acid conc	PCV values
I	6.95±0.06	4.80±0.08	2.15±0.02	0.61±0.00	41±0.01
II	6.9±0.04	4.80±0.08	2.10±0.04	0.61±0.01	40±0.12
III	6.85±0.02	4.85±0.20	2.0±0.04	0.63±0.02	41±0.04
IV	6.95±0.06	4.85±0.20	2.10±0.04	0.63±0.02	41±0.06
V	6.40±0.00	4.05±0.02	2.20±0.04	0.61±0.002	40±0.01

5 and 10 post treatment .The plasma globulin concentration was calculated as the difference between total protein and albumin concentrations. The determination of the Free Fatty Acids levels was carried out as described by Koichi (1977). Packed Cell Volume (Kelly, 1977),

Statistical analysis

Results were expressed as mean ± SEM. All parameters were analyzed statistically using Student t-test. P value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Acute toxicity

The behavioral signs of toxicity observed in the rats at 2900 and 5000 mg extract/kg body weight in the course of the study were salivation, rubbing of nose and mouth on the floor of the cage and restlessness. The animals gained weight throughout the study duration. No death was recorded in the rats at all the doses used. Gross pathological study showed no abnormality in all the organs examined. The oral LD_{50} was therefore greater than 5000 mg/kg body weight.

Anti-trypanosomiasis study

The extract significantly (P<0.01) reduce the Parasitaemia level time dependently in all the treated groups when compared with the negative control throughout the duration of observation (Table 1).

There was a highly significant (p<0.01) time dependent increase in total protein, albumin and globulin levels in all the treated groups on days 5 and 10 post treatment of the study when compared with the infected untreated control (Tables 2 - 4). The free fatty acid levels decreased significantly (p<0.01) in all the treated groups on days 5 and 10 post treatment of the study when compared with the infected untreated control as shown (Tables 2 - 4) . The PCV value increased significantly (p<0.01) in the treated groups and the positive control on days 5and 10 post treatment when compared to the negative control. The absence of death at dose level of 5000 mg extract/kg body weight shows that the extract of *F. albida* is practically non –toxic in rats (Lorkes, 1983; Matsura, 1975; Corbett et al., 1984) and is therefore safe for use. The results obtained from our study on the anti-trypanosomiasis effect of stem bark extract of *F. albida* showed that the extract possess trypanostatic effects, since it slowed down the rate of proliferation of the trypanosome parasite. This may be due to the ability of the extract to inhibit prostaglandin biosynthesis as has been reported by Tijani et al. (2008). Previous work by Allison (1978) has shown that inhibitors of prostaglandin synthesis reduces parasitaemia and shortens the time to

Table 3. Effect of Faidherbia albida on some biochemical parameters of rats infected with *Trypanosoma brucei brucei* on day 5 post treatment

Groups	Protein conc(mg/dL)	Albumin conc (mg/dL)	Globulin conc (mg/dl)	Free fatty acid conc	PCV values
I	7.15±0.02**	5.10±0.04**	2.05±0.06**	1.26±0.004**	39±0.03**
II	6.75±0.02**	3.90±0.04**	2.85±0.02**	1.57±0.0**	38±0.05**
III	6.05±0.02**	3.55±0.10**	2.50±0.12**	1.83±0.00**	38±0.08**
IV	5.8±0.00	0.15±0.02	5.65±0.02	6.2±0.00	25±0.02
V	6.4±0.08**	4.40±0.00**	2.20±0.00**	0.62±0.04**	40±0.03**

n=6
** Significantly different from the infected untreated control (group IV) at p<0.01.

Table 4. Effect of *Faidherbia albida* on some biochemical parameters of rats infected with *Trypanosoma brucei brucei* on day 10 post treatment.

Groups	Protein conc (mg/dL)	Albumin conc. (mg/dL)	Globulin conc (mg/dl)	Free fatty acid conc. (mg/dl)	PCV values
I	6.90±0.04	4.10±0.04	2.80 ±0.45	0.96±0.004**	37±0.09
II	6.4±0.08	3.95±0.02	2.45±0.10	0.99±0.004**	36±0.13
III	5.8±0.00	3.10±0.04	2.70±0.04	1.01±0.08	34±0.07
IV	DEAD	DEAD	DEAD	DEAD	DEAD
V	6.4±0.04	4.2±0.08	2.20±0.04	0.63±0.004**	41±0.05

n=6
** Significantly different from the infected untreated control (group IV) at p<0.01

KEY:
I Rats in the group received 1000 mg extract/kg body weight 24 h post parasite inoculation II
Rats in the group received 1000 mg extract/kg body weight 48 h post parasite inoculation III Rats
in the group received 1000 mg extract/kg body weight at the establishment of infection.
IV Rats in the negative control were infected with the parasite but were not treated with the extract.
V Rats Positive controls were neither infected nor treated with the extract. Treatment was daily for 5 consecutive days.

to parasite wave remission. There have been reports of decreased plasma albumin concentrations in several trypanosome infections (Anosa, 1988) and this has been linked to either plasma expansion, proteinuria (Bruiju, 1987) or hepatocellular damage (Saror, 1980). The decrease in serum total protein could be attributed to a decrease in serum albumin probably from decreased hepatic biosynthesis. This may be explained by the fact that, according to Adah et al. (1993), *T. brucei* induced an elevation of serum transaminases. This may indicate that *T. brucei* infection causes reduction in liver function which includes protein synthesis. The extract elevated the albumin level in the treated groups which may suggest the ability of the extract to prevent hepatocellular damage caused by the trypanosome infection. The elevated albumin level may account for low level of free- fatty acid observed in all the treated groups because free fatty acid normally does not exist in free state in the blood but complexed to serum albumin (Catherine et al., 2006). It has been reported that the free fatty acid attains haemolytic level only when the serum albumin binding capacity is exceeded (Tizard et al., 1978).It has earlier been shown that severe haemolytic crisis that occurs in Trypanosomiasis is caused by surface active lipids which are cytolytic and free fatty acid has been grouped among such agents especially linoleic acid (Tizard et al., 1978).The significant increase in packed cell volume (PCV) values of all infected and treated rats irrespective of time of treatment may in part be due to the ability of the extract to reduce the level of circulating surface active agents such as the free fatty acids especially linoleic acid that has been implicated in lysis of red blood cells. Previous work by Bisalla et al. (2007) reported a PCV value of 23% in animals infected with Trypanosomiasis which is consistent with our results as the PCV values of the infected untreated rats reduced to 25%. However, the decrease in PCV values of the extract treated groups was insignificantly different from the positive control, it is therefore suggested that the extract prevented lysis of the red blood cell induced by circulating free fatty acids level. *F. albida* does possess trypanostatic effect as evident in the reduced rate of proliferation of parasitaemia in all the extract treated groups. In addition it prolonged the life of trypanosome-infected rats by reducing the accumulation

of free fatty acids in circulation implicated in lysis of red blood cell leading to anaemia a leading cause of eventual death from Trypanosomiasis. It is therefore effective in the management of Trypanosomiasis related anemia thus providing scientific evidence for its folkloric medicinal use in the management of Trypanosomiasis.

Conclusion

This study has demonstrated that Faidherbia albida possesses trypanostatic as well as anti-haemolytic effects in *Trypanosoma brucei brucei* infected rats.

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