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Full Length Research Paper

Acute and subacute toxicity study of ethanolic extract of the stem bark of *Faidherbia albida* (DEL) A. chev (Mimosoidae) in rats

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***Faidherbia albida* (Del.) A. Chev (mimosoidae) is widely used in African traditional medicine (ATM) for management of fever, diarrhoea and human trypanosomiasis. Acute and sub-acute toxicity profiles of ethanolic stem bark extract of *F. albida* were evaluated in wistar albino rats. The acute toxicity was studied using the method of Lorke (1983). In the sub-acute toxicity study, four groups of six rats per group were used. The control group (1) received 10 ml normal saline/kg body weight while groups 2, 3 and 4 received oral daily doses of 125, 250 and 500 mg extract/kg body weight respectively for 21 days. The effects of the extract on clinical signs, feed and water intake, body weight changes, haematology, plasma biochemical parameters, relative organ weight (ROW) were evaluated. The oral LD_{50} of the extract was estimated to be greater than 5000 mg/ kg body weight. The extract produced slight increase in body weight of rats given 125 mg extract/kg body weight. However, dose-dependent highly significant ($P < 0.01$) decrease in body weight was observed at 250 and 500 mg/ kg-treated rats in weeks 2 and 3 of the study. Feed and water intake was not affected by the treatment. ROW for all organs was not affected by the treatment except significant ($P < 0.05$) increase in the testes of rats treated with 250 and 500 mg extract/kg body weight. Although the treatment elicited highly significant ($P < 0.01$) changes in the levels of the hepatic and some of the haematological parameters, they were within the normal reference range for rats. This study revealed that while the stem bark of the plant may be considered relatively safe when used sub-acutely, further investigation is needed to ascertain its effect on the male reproductive system as well as its effect on chronic administration.**

Key words: *Faidherbia albida,* toxicity, haematology, liver function, testes.

INTRODUCTION

Faidherbia albida (Del.) A. Chev (mimosoidae) also known as A*cacia albida* Del is a leguminous woody species distributed throughout the arid and semi arid lands of Africa. It is found in western, eastern and southern Africa (Vandenbelt, 1991). It is also found in the Middle East, Arabia and in Palestine (Wickens, 1969). It has been introduced into India, Pakistan, Nepal, Peru, Cyprus, Cape Verde and the Ascension Islands. *F. albida* is a large thorny tree that may reach 31 m in height with wide and rounded crown. The trunk is thicker at the base. The bark is light grey and smooth when young, but becomes cracked with age. *F. albida* is characterised by a long, deep taproot that can reach 7 m long. It possesses pairs of spines up to 2 cm. Its leaves are compound, bipinnate, blue green with 3-12 pairs of pinnae carrying 6-23 pairs of leaflets up to 12 mm long and 5 mm wide and the flowers are fragrant in dense cream-yellow spikes about 10 cm in length (Mulofwa, 1994). *F. albida* is valued for its green manure and fodder. It sheds its

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leaves at the beginning of the rainy season providing nutrients for new crops thus reducing the shade of the canopy. It is a nitrogen-fixing tree and therefore can enrich the soil and improve crop yield.

In northern Senegal, the leaves are boiled in water to make a cough mixture (Boury, 1962). The seeds can be boiled and eaten. The pods may be dried and ground into edible flour. They are said to have been used as fish poison and are worn as charm by African women and children to avert smallpox (Irvine, 1961). In Malawi, the root and bark are used as a poison to stupefy fish (Fanshawe, 1962). The stem bark exudes a gum which is sometimes collected in Nigeria and used for its emollient and emulsifying properties (Howes, 1949). The Pulaar people of Senegal use this gum as an aphrodisiac to treat 'impotence' (Kerharo and Adam, 1962 a). In Nigeria, an infusion of the bark is taken for fever, cough and to assist in child birth (Dalziel, 1937 and Singha, 1965). It is added to a potion to treat chest pain by the Fulanis of Nigeria (Jackson, 1973). A decoction of the bark is used in cleansing fresh wounds in a manner similar to that of potassium permanganate (Berhaut, 1975). Combined with other herbs, it is used to treat 'madness' (Kerharo and Adam, 1962 b, Berhaut, 1975). A decoction of the bark is also used as an emetic in fevers by the Masai people of East Africa, taken for diarrhoea in Tanganyika (Irvine, 1961) and for colds, haemorrhage, leprosy and ophthalmia in West Africa. A liniment made by steeping the bark is used for bathing and massage in pneumonia. The bark is employed in dental hygiene, strips used as dental floss in Namibia and its extract is employed in the treatment of toothache. In northern Nigeria, West Africa, the cattle-rearing nomads take a decoction of the stem bark orally for the management of the sleeping sickness (Trypanosomiasis).

Kubmarawa et al. (2007) reported its antimicrobial activity against *Pseudomonas aeruginosa, Bacillus subtilis* and *Salmonella typhii*. Ethanolic extract of the stem bark was shown by Tijani et al. (2008) to possess antipyretic, anti-inflammatory and anti-diarrhoeal properties in rats. Tijani et al. (2009) also reported the trypanostatic as well as anti-haemolytic effects of the extract in *Trypanosoma brucei brucei*-infected rats, thus explaining the basis for its use in folkloric medicine. The present studies were carried out to evaluate the sub-acute toxicity of *F. albida* in rats.

MATERIALS AND METHODS

Plant material

The leaves and stem bark of *F. albida* were collected from Gyamso ward in Toro Local Government Council of Bauchi State, Nigeria, in September, 2008. They were identified and authenticated by Mrs Jemilat Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine of National Institute for Pharmaceutical Research and Development (NIPRD). A voucher specimen (number NIPRD/H/6151) was deposited at NIPRD herbarium for future reference. The stem bark was cleaned, air-dried 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

200 g of the powdered stem bark were weighed and macerated in 2 l 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 dark brown extract. The percentage yield was calculated as:

Yield (%) = [wt of extract (g)/wt of plant material (g)] x 100

The crude extract was stored at -4°C until required for use. Aliquot portions of the crude extract residue were weighed and suspended with 2.5% tragacanth in distilled water for use on each day of the experiment.

Animals

Male wistar albino rats (200 - 250 g) obtained from Animal Facility Centre, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria were used in the study. The rats were fed standard laboratory diet, given water *ad libitum* and maintained under laboratory conditions of temperature 22 ± 1°C, relative humidity 14 ± 1% and 12 h light and 12 h dark cycle. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985) and NIPRD Standard Operating Procedure for Animal Care.

Acute toxicity study

Acute toxicity study was carried out using the method of Lorke (1983). In the first phase, nine rats randomly divided into three groups of three rats per group were given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. The rats were observed for signs of adverse effects and death for 24 h and then weighed daily for 14 days. Based on the results of the phase-one study, the procedure was repeated using another set of three rats randomly divided into three groups of one rat each, given 1600, 2900 and 5000 mg extract/kg body weight, respectively. For 14 days, the rats were observed for signs of toxicity which include but not limited to paw-licking, salivation, stretching, rubbing of nose on the floor and wall of cage, change in body weight and death. The surviving animals were sacrificed under chloroform anaesthesia, autopsied and examined macroscopically for any pathological changes. The number of deaths in each group within 24 h was recorded and the final LD_{50} values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

Sub-acute toxicity study

The sub-acute toxicity study was carried out using standard operating procedure on toxicity testing adopted by pharmacology and toxicology department, NIPRD. Twenty four rats were randomised into four groups of six rats each. Rats in control group were given normal saline (3 ml/kg), while those in the treatment groups received 125, 250 and 500 mg extract/ kg body weight via the oral route for 21 days. Water and feed intake were measured daily while body weights of the rats were determined twice weekly and at the end of the study (day 21). After 24 h (day 22), the rats were euthanized in an airtight glass chamber saturated with chloroform

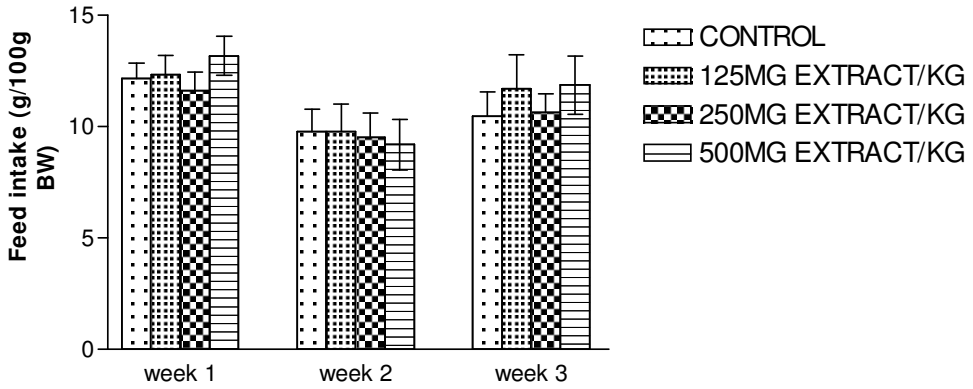


Figure 1. Effect of ethanolic extract of *F. albda* on weekly feed intake in rat (n = 6).

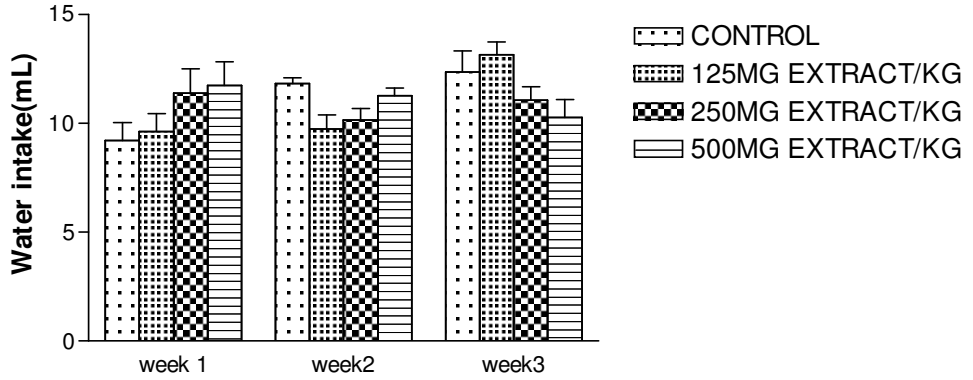


Figure 2. Effect of ethanolic extract of *F. albda* on water intake in rat (n = 6).

and after opening up the rats surgically, blood samples were collected by cardiac puncture into ethylenediaminetetraacetic acid (EDTA) bottles for the analysis of haematological parameters [white blood cell(WBC), red blood cells (RBC) haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LMP), monocytes (MXD), neutrophils (NEUT) and eosinophils (EOS)] using Sysmex KX-21N automated hematology analyzer (Sysmex America Inc, USA). Another portion of blood was collected into lithium heparin sample bottles, centrifuged to obtain the plasma and analysed for liver enzymes [alaninine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)], bilirubin, liver proteins, creatinine and urea levels using Hitachi 902 analyser (Roche Diagnostic, GmbH, Germany). The plasma was also analysed for electrolytes (sodium, potassium, chloride and bicarbonate ions) levels using Ilyte machine auto analyser. Different organs mainly the brain, heart, lungs, kidneys, liver, intestine, stomach, spleen and testes were removed and weighed. The relative organ-body weight ratio (ROW) of each rat was then calculated as:

ROW = wt of organ (g)/body wt of animal (g)

Statistical analysis

Results were expressed as the mean ± standard error of mean (SEM). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) followed by student's t-test. Differences in mean were considered to be significant when $P < 0.05$.

RESULTS

The yield of the extract was 8.0% (w/w). In the acute toxicity study, the behavioural signs of toxicity observed in the rats at 2900 and 5000 mg extract/kg body weight were salivation, rubbing of nose and mouth on the floor of the cage and restlessness. Gross pathological study showed no abnormality in all the organs examined. Absence of death at all doses up to 5000 mg extract/kg showed that the LD_{50} of the ethanolic stem bark of *F. albida* is greater than 5000 mg extract/ kg body weight.

Effect of the extract on food and water intake

In the sub-acute toxicity study, there was no significant difference in the water and food intake in all the treatment groups when compared to the control (Figures 1 and 2).

Effect of the extract on body weight

The extract at 250 mg/kg body weight elicited a highly significant ($P < 0.01$) reduction in body weight in weeks 2 and 3. Significant ($p < 0.05$) reduction was observed in week 1 in the 250 mg extract/kg group and at 500 mg

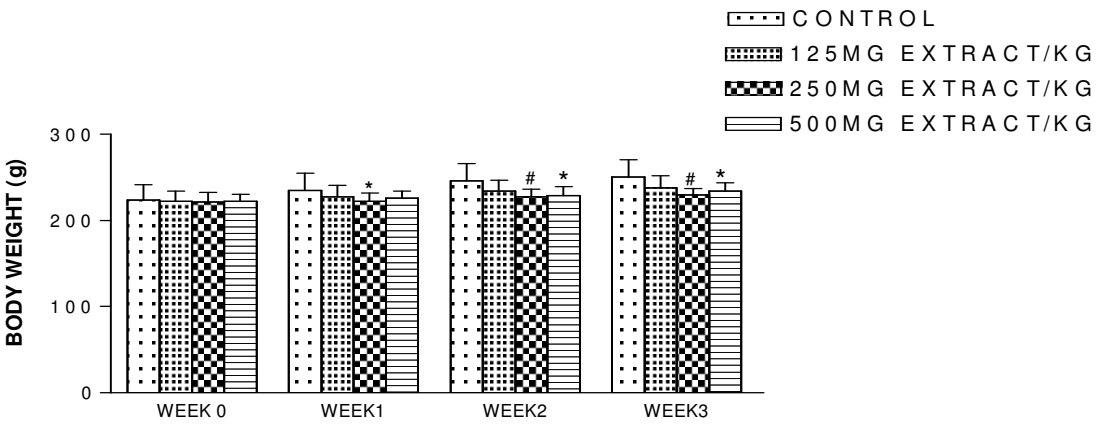


Figure 3. Effect of ethanolic extract of *F. albda* on weekly body weight of rat. n = 6; *significantly different from control at P < 0.05; #significantly different from control at P < 0.01.

Table 1. Effect of ethanolic extract of *F. albida* on haematological parameters in rats.

Parameter	Control	125 mg/kg	250 mg/kg	500 mg/kg
WBC	4.73 ± 1.18	7.42 ± 0.69	9.53 ± 1.03**	6.22 ± 0.99
RBC	7.12 ± 0.31	7.74 ±0.19	7.71 ± 0.15	8.02 ± 0.10
HGB	12.77± 0.59	13.65 ± 0.19	13.67 ±0.27	13.83 ± 0.18
HCT	45.67 ± 2.56	46.50 ±0.81	49.17 ± 0.93	47.67 ± 0.69
MCV	63.70 ±1.71	60.15 ± 2.42*	63.87 ± 1.24	59.53 ± 0.39*
MCH	17.93 ± 0.20	17.70 ± 0.20	17.73 ± 0.23	17.25 ± 0.18
MCHC	28.23 ± 0.61	29.43 ± 0.28	27.80 ± 0.36	29.33 ± 0.18
PLT	708 ± 23.79	675.17 ± 59.05**	737.33 ± 43.03**	860.83 ± 19.63**
LMP (%)	76.17 ± 2.92	66.33 ± 2.77**	69.50 ± 1.61**	75.00 ± 2.45
MXD (%)	1.67 ± 0.38	1.67 ± 0.45	1.67 ± 0.38	1.00 ± 0.24
NEUT (%)	20.50 ± 2.71	29.67 ± 2.31**	26.67 ± 1.47*	22.17 ± 2.52
EOS (%)	1.83 ± 0.44	2.17 ± 0.55	2.17 ± 0.28	1.83 ± 0.55

n = 6; *significantly different from the control at p<0.05; **significantly different from the control at P < 0.01.

extract/kg in the 2nd and 3rd weeks of the study (Figure 3).

Effect of ethanolic extract of *F. albida* on haematological parameters in rats

There was highly significant (p < 0.01) increase in the white blood cell count in the groups treated 250 mg extract/kg and a highly significant (p<0.01) reduction in the lymphocyte levels in the 125 and 250 mg extract/ kg groups. There was a highly significant (p<0.01) increase in platelet count in all the treated groups when compared with the control. There was a highly significant (p < 0.01) increase in the neutrophil count in the 125 mg group and a significant (p < 0.05) increase at 250 mg extract/kg. There were no significant changes for all the treated groups in red blood cells, packed cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, monocytes and eosinophils (Table 1).

Effects of ethanolic stem bark extract of *F. albida* on liver enzyme levels in rats

The extract produced a highly significant (p < 0.01) increase in the levels of aspartate and alanine transaminases at all dose levels and in the level of alkaline phosphatase at 125 and 500 mg extract/kg but there was no significant change in the levels of the total and direct bilirubin in all the treated groups (Table 2).

Effects of ethanolic stem bark extract of *F. albida* on liver proteins in rats

Significant (p < 0.05) increase in protein concentration

Table 2. Effects of ethanolic stem bark extract of *F. albida* on liver enzyme levels in rats.

Parameter	Control	125 mg Extract/kg BW	250 mg Extract/kg BW	500 mg Extract/kg BW
Aspartate transaminase (u/l)	273.17 ± 50.04	446.50 ± 118.41**	451.67 ± 119.19**	318.17 ± 62.72**
Alanine transaminase(u/l)	60.17 ± 5.45	90.50 ± 18.02**	132.17 ± 47.39**	79.67 ± 9.46**
Alkaline phosphatase (u/l)	109.17 ± 11.70	122.33 ± 5.36**	118.67 ± 9.53	128.17 ±9.07**
Total bilirubin(u/l)	3.12 ± 0.38	3.70 ± 0.24	3.88 ± 0.31	3.55 ± 0.18
Direct bilirubin(u/l)	1.52 ± 0.11	1.88 ± 0.08	1.92 ± 0.16	1.67 ± 0.13

n = 6; *significantly different from the control at p<0.05; **significantly different from the control at P < 0.01.

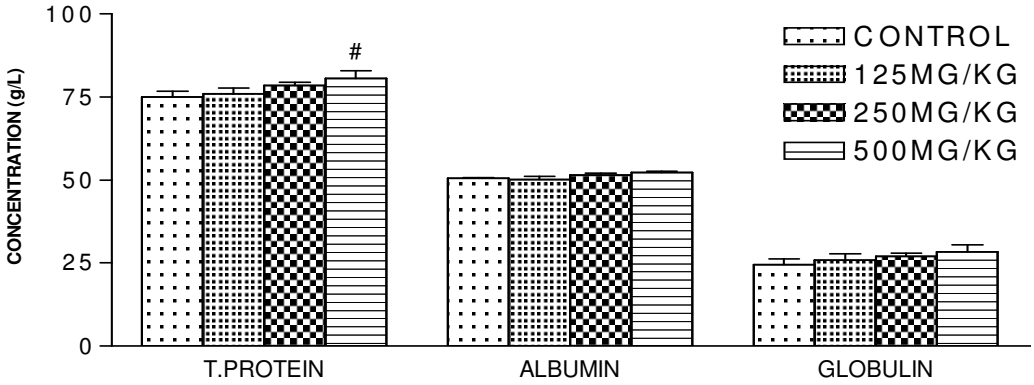


Figure 4. Effect of ethanolic extract of *F. albida* on liver protein in rat, n = 6; #significantly different from control at P < 0.01.

was produced in rats given 500 mg extract/kg while there was no significant difference in albumin and globulin in all the treated groups (Figure 4).

Effect of ethanolic extract of *F. albida* on renal indices of rats

No significant change was observed in urea, potassium, bicarbonate, sodium and chloride ions for all the treated groups (Table 3). However, there was a highly significant (P < 0.01) reduction in the concentration of creatinine in all the extract-treated groups (Table 3).

Effects of ethanolic stem bark extract of *F. albida* on relative organ weight (Row) of rats

There was a significant (P < 0.05) reduction in the row in the testes at 250 and 500 mg extract/kg body weight when compared with the control (Table 4).

DISCUSSION

The lack of death at oral treatment of over 5000 mg extract/kg body weight obtained suggests that the ethanolic stem bark extract of *F. albida* is practically non-toxic acutely (Cobett et al., 1984). It is therefore safe acutely for oral use in the ethno-therapeutic management of trypanosomasis, fever and diarrhoea. The high safety profile obtained may have been responsible for its wide spread use in different ethno-therapeutic interventions. The reduction in the body weight of the treated rats especially in weeks 2 and 3 at 250 and 500 mg/kg may not be due to the food and water intake as there was no significant decrease in these parameters among the treated groups. The decrease may have been due to direct systemic toxic effect of the extract resulting in muscle wastage of the rats. Lymphocytes are mediators of the specific immune response against pathogens, while neutrophils are responsible for phagocytosis (Sacher and McPherson, 1991). An increase in neutrophil count is associated with acute insult to the body whether in the form of infection or not. Use of drugs such as corticosteroids, histamine and epinephrine are known to cause an increase in neutrophils count (Sacher and McPherson, 1991). It is likely that the extract produced an effect similar to any of the above drugs. Platelets are responsible for haemostasis —a process aimed at reducing blood loss and repairing vascular injury (Dahlback, 2007). An increase in platelet count - thrombocytosis is usually a result of a reactive process or a myelo-proliferative disorder (Miller, 1996). The increase observed in the platelet count may indicate that the plant extract stimu-

Table 3. Effect of ethanolic extract of *F. albida* on renal indices of rats.

	Control	125 mg Extract/kg BW	250 mg Extract/kg BW	500 mg Extract/kg BW
CREATININE (µmol/L)	60.83 ±8.83	45.33 ±1.31**	47 ±2.54**	41 ±0.47**
UREA(mmol/L)	8.88 ±0.35	9.63 ±0.38	8.3 ±0.52	8.98 ±0.39
Na^+(mmol/L)	141.83 ±0.96	140.83 ±0.37	141.83 ±0.83	143.5 ±0.69
K^+(mmol/L)	5.31 ±0.52	6.5 ±0.39	6.05 ±0.82	6.43 ±0.83
HCO^-_3(mmol/L)	24.5 ±0.46	25.33 ±0.81	24.33 ±0.45	24.67 ±0.45
Cl^-(mmol/L)	102.17 ±0.93	102 ±0.47	102.33 ±1.04	103.5 ±1.07

Table 4. Effect of ethanolic stem bark extract of *F. albida* on Relative Organ Weight (ROW) in rats.

Organs	Control	125 mg Extract/kg BW	250 mg Extract/kg BW	500 mg Extract/kg BW
HEART ($X10^{-3}$)	3.32± 0.008	3.32± 0.12	3.59± 0.11	3.32± 0.088
LUNG ($X10^{-3}$)	6.67± 0.13	7.42± 0.28	8.26± 0.45	7.68± 0.699
KIDNEY ($X10^{-3}$)	7.23± 0.21	5.8± 0.81	7.05± 0.13	6.89± 0.56
INTESTINE ($X10^{-3}$)	6.08± 0.74	5.55± 0.34	5.29± 0.56	5.03± 0.58
LIVER ($X10^{-3}$)	30.54± 0.94	29.27± 0.89	32.59± 1.02	30.53± 1.63
TESTES ($X10^{-3}$)	16.99± 2.4	13.82± 1.70	12.47± 0.47*	11.62± 0.56*
STOMACH ($X10^{-3}$)	9.52± 0.73	12.46± 1.14	10.81± 1.06	12.68± 1.60
BRAIN ($X10^{-3}$)	5.7± 0.51	5.69± 0.32	6.38± 0.27	6.67± 0.40
SPLEEN ($X10^{-3}$)	3.14± 0.6	2.07± 0.20	3.57± 0.50	1.86± 0.18

n=6; *- significantly different from control group at $p<0.05$.

lates the biosynthesis of clotting factors by the liver and may therefore be useful in the treatment of haemorrhage. These observations may however not result in any adverse effect since the platelet count is still within the normal range for rats (Mitruka and Rawnsley, 1993). The mean corpuscular volume (MCV) is an erythrocyte index that measures the average volume of erythrocytes (Morris and Davey, 1996). It is decreased in microcytic anaemia and increased in macrocytic anaemia (Herfindal and Gourley, 2000). The observed decrease in MCV values may also not indicate that the extract elicits haemolytic anaemia since the values obtained in this experiment falls within the reference range for MCV in laboratory. That this is not indicative of anaemia is shown in the increases observed in the red cell count and the packed cell volume. Tijani et al. (2009) reported that this plant stimulates erythropoiesis in cases of anaemia. Alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are markers of liver function. These enzymes are only released in significant amounts into the bloodstream from the cytosol and subcellular organelles when hepatic injuries occur (Lu, 1996). ALT is more hepato-specific than AST because it is more sensitive to hepatic damage (Herfindal and Gourley, 2000) and there is a good correlation between its level and severity of hepatic necrosis (Lu, 1996). Increase in ALP level is also observed in bone disorders involving osteoblastic activity. However, an elevation in ALP activity between 2 and 8 fold the normal value usually is the first clue to intrahepatic and extra hepatic cholestasis (biliary obstruction) (Moss, 1987, Young and Holland, 1995). The observed increases in the values of ALT and AST in this study fall within the normal range, while AST was slightly higher than the upper limit of the experimental animal values (Picus et al., 1996). The significant increase in the total plasma protein concentration observed at the highest dose used for the study may indicate that the liver function was not adversely affected by the extract. This observation is further confirmed by the report of an earlier study by Tijani et al. (2009) which showed that the ethanolic stem bark extract of *F.albida* significantly increased the serum protein level in rats infected with *T. brucei brucei*. It may therefore be suggested that the extract increases the protein synthesis function of the liver in cases of injury resulting in enhanced haemostasis, prevention of ascites and deposition of fats in the liver. Reduction in creatinine level is observed in cases of muscle wasting as seen in malnutrition (Pincus, 1996). The highly significant ($P < 0.01$) reduction in creatinine concentration in all the extract-treated groups indicates that the extract does not exert deleterious effect on the renal function and that the decrease in body weight of rats at 250 and 500 mg extract/kg body weight might be

due to muscle wasting possibly due to toxic effect of the extract. Reduction in the relative organ weight for the testes may be due to deleterious effect of the extract with possible adverse effect on its physiological function of spermatogenesis.

Conclusion

These results suggest that ethanolic stem bark extract of *F. albida* is relatively safe when used sub-acutely in rats. In view of the adverse effect of the extract on the testes, it is recommended that a reproductive study be carried out on male rats. Clinical assays have to be done as to confirm the low toxicity of the extract in humans.

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