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Toxicity Studies of the Crude Aqueous Root Extract of *Albizzia chevalieri* Harms in Albino Rats

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ABSTRACT: Aqueous root extract of *Albizzia chevalieri* has been reported to possess hypoglycaemic and hypolipidaemic effects. The current work investigated the LD_{50} of the crude aqueous root extract of *Albizzia chevalieri* and the effect of sub-chronic doses of the extract on liver and kidney function parameters of albino rats. The results indicated LD_{50} greater than 3000mg/kg body weight in albino rats observed for 72 hours. The result of the sub-chronic toxicity on liver function parameters showed significant ($P<0.05$) increase in serum total protein and globulin at doses greater than 2000mg//kg, which was reflected on the A:G ratio. Transaminases and alkaline phosphatase activities were also significantly ($P<0.05$) affected at doses greater than 2000mg/kg. Serum levels of urea were also increased significantly ($P<0.05$) but creatinine and uric acids were decreased significantly ($P<0.05$) at doses greater than 2000 mg/kg., Serum electrolytes were however not affected. The extract should therefore be use with care especially at doses greater than 2000 mg/kg.
Key words: *Albizzia chevalieri* root, LD_{50}, sub-chronic toxicity, albino rat.

INTRODUCTION

Herbal plants produce and contain a variety of chemical substances with varied physiological effects. They are huge reservoir of various chemical substances with potential therapeutic properties (Lewis and Elvin-Lewis, 1995). Herbal plants are being increasingly utilized to treat a wide variety of clinical diseases (Gupta *et al.,* 2004).

Herbs have been used by all cultures throughout history and thus, herbal medicine is the oldest form of health care known to mankind. It was an integral part of the development of modern civilization. Many drugs commonly used today are of herbal origin. Higher plants as source of medicinal compound continue to play a dominant role in maintenance of human health since antiquities (Suffness and Dowos 1982). The primary benefit of using plant derived-medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment (Iwu, 1994). However, it must be noted that not all medicinal plants are safe for consumption in the crude form. Some level of toxicity may arise as a result of potential toxic compounds they contain and pesticide application during cultivation (Amdur *et al.,* 1991 and Evans 1999). The therapeutic properties of medicinal plants used by traditional medical practitioners may be due to one or more of the many compounds of the plant material. These phytochemicals include complex carbohydrates, alkaloids, glycopeptides, terpenoids, tannins, cyanogens, peptides and amines, steroids, flavonoids, lipids, coumarins, sulphur compounds and inorganic ions among numerous others. Some of these compounds may be toxic, and thus the plants containing them, when consumed could confer varied levels of toxicity to the individual (Humphrey and McKenna, 1997). The growing interest in herbal medicine therefore demands toxicity risk assessment of the various indigenous preparations used in the treatment of diseases (Yakubu *et al.,* 2005).

The African continent is one of the continents endowed with the richest biodiversity in the world with food plants used as herbs, food and for therapeutic purposes (Iwu, 1994). One of such plants is *Albizzia chevalieri* (family Fabaceae). It is a large genus of tree, native to warm regions of the old world. It is usually

small trees or shrubs that grow up to 6m, occasionally 12m, with an open and rounded or umbrella shaped canopy, bark pale-grayish, corky and deeply creviced, dark brown slash. Twigs pubescent with 20-40 pairs leaflets each (Houerou, 2006). The plant is found in Southern Sahel, Northern Sudan, Savannas of Senegal to Chad and in parts of Northern Nigeria.

A. chevalieri leaves is widely used for the management of diabetes mellitus by traditional medicinal practitioners in some part of Nigeria and Niger Republic. Hypoglycaemic effects of the leaves (Saidu *et al.,* 2007a) and root (Saidu *et al.,* 2010) have been reported.

The present study investigated the acute and sub-chronic toxicity effects of the crude aqueous root extract of *A. chevalieri* in rats.

MATERIALS AND METHODS

Chemical and Reagent: All the chemicals and reagents used for thes study were of analytical grade. Assay kits were purchased from Randox Laboratories Ltd, Antrim, United Kingdom.

Plant Material: Fresh *A. chevalieri* root were obtain from Sanyinna, about 50km South of Sokoto, Nigeria. The plant material was identified by a Taxanomist in the Botany unit of the Department of the Biological Sciences, Usmanu Danfodiyo University Sokoto (UDUS) and voucher specimen were prepared and deposited in the Herbarium of the same Department. The root was dried in a shade, pulverized into a moderately coarse powder, using laboratory pestle and mortar and sieved with a $1mm^2$ sieve. The root powder was kept in a desiccator until required (Onoruvwe and Olorunfemi, 1998).

Preparation of Crude Extract: The powdered plant material was soaked in cold distilled water for 24 hours, after which it was filtered using a piece of clean, sterile, white Muslin cloth to remove debris and then through Whatman No. 1 filter paper. The percentage recovery was determined and the extract labeled crude aqueous extract and stored in small, capped plastic container at $+4^oC$ until required.

Experimental Animals: Fifty five albino rats, weighing 100-130g age 10-12 weeks were purchased from National Veterinary Research Institute Vom Jos, Nigeria and used for the studies. The rats were housed in metal cages in the Research Laboratory of Biochemistry Department, UDUS and allowed to acclimatize for three weeks. The rats were maintained on standard rats chows (Neimeth Livestock feed, Ikeja, Nigeria) *ad libitum* and allowed free access to drinking water.

Experimental procedure involving animals and their care were employed in conformity with guidelines for care and use of laboratory animals and the procedure approved by the Ethical Committee of UDUS, Nigeria.

Acute Toxicity Studies: The limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD) (2001), was employed. Aqueous extract of the plant (3000mg/kg body weight) were administered to 5 rats (one after the other) in a single oral dose and observed for 72 hours. Another group of five rats (control) received distilled water. The rats were observed for toxic symptoms such as weakness, loss of appetite, difficulty in movement, reaction to noise, and mortality.

Sub acute Toxicity Studies: A total of 25 albino rats were randomly divided into 5 groups of 5 rats each. Animals in groups 2, 3, 4, and 5 were orally administered 750, 1500, 2250 and 3000mg/kg body weight respectively, once daily for 28 days. Animals in Group 1 served as control group and received distilled water through the same route. The body weights of all the rats were recorded weekly throughout the experimental period. After 28 days of treatment the rats were fasted for 8 hours and anaesthetized in chloroform vapour and blood sample collected by cardiac puncture for biochemical analyses.

Determination of Biochemical Parameters: Serum total protein was determined by Biuret method as reported by Peter Jr. *et al*., (1982). Albumin was determined according to method of Doumas, *et al*., (1971) while globulin and A:G ratio were calculated. Aspartate Aminotranferase (AST) and Alanine Aminotransferase were estimated according to the method of Reitman and Frankel, (1957). Alkaline phosphatase was determined by Randox (Colorimetric) method of Rec (1972).

Bilirubin (Colorimetric method) was carried out according to method of Jendrasick and Grof, (1938) and Sherlock (1951). Urea was determined by the diacetylmonoxime method (Evans, 1968). Serum creatinine was estimated according to Jaffe's Reaction (Butler, 1975). Uric Acid was estimated according to the method of Collin and Diehl (1959) and Morin and Proxy (1973). Electrolyte (Na^+, K^+), were determined using flame photometry method as described by the Jenway manual/data book and Uriyo and Singh (1974). Serum bicarbonate level was estimated by back titrimetric method of Vogel (1964).

Statistical Analysis: Data were expressed as mean ± standard deviation (n=5). The results were analyzed using one way ANOVA. Post hoc was also conducted to determine the level of significant difference between each treatment and the control group using Tukey-Kramer Multiple Comparisons Test. Test significant was considered at $P < 0.05$.

RESULTS

The experimental rats treated with acute dose of 3000 mg/kg body weight of the root extract of *A. chevalieri* displayed no appreciable change in physical activity and displayed no apparent toxicity symptoms or mortality up to 72 hours post treatment, indicating that the LD_{50} of the crude extract in rats is greater than 3000 mg/kg.

Weekly weight changes of rats treated with sub-chronic doses showed that the extract did not affect growth of the rats significantly ($P>0.05$) (Table 1).

Liver Function: The result of serum total protein showed that, the extract caused gradual increase as the dosage increases from 750mg/kg to 3000mg/kg. Serum globulin also increased significantly ($P<0.05$) at high doses while the A:G ratio decreased significantly ($P<0.05$) as the doses increase (Table 2).

Activities of serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were affected at high doses (Table 3). The root extract significantly ($P<0.05$) increased the serum levels of bilirubin at high doses. The increase is more prominent in the unconjugated bilirubin (Table 4).

Kidney Function: Serum urea level of the rats treated with aqueous root extract of *A. chevalieri* were significantly ($P<0.05$) increased while serum creatinine and uric acid levels were decreased at high doses (Table 5). The electrolytes were however not affected by the extract (Table 6).

Table 1: Weekly weight changes (g) of rats administered different sub-chronic doses of aqueous root extract of *A. chevalieri*.

		Weeks			
Group	Initial weight	1	2	3	4
Control	182.10±22.03	183.00±8.98	182.75±20.89	186.25±0.96	195.00±5.72
750mg/kg	180.00±19.03	188.75±29.58	193.00±14.79	195.00±24.35	196.25±25.46
1500mg/kg	181.70±40.30	185.00±31.32	185.75±13.45	189.25±11.93	196.25±4.03
2250mg/kg	182.60±6.50	183.50±11.73	185.50±15.59	186.25±11.62	196.50±20.89
3000mg/kg	168.00±29.80	171.25±26.13	175.00±24.35	176.00±24.17	188.75±27.65

Values are expressed as mean ± Standard deviation of 5 rats treated for 28 days.

Table 2: Serum proteins (g/dl) and albumin:globulin (A:G) ratio of rats treated with crude extract of *A. chevalieri* root for 28 days.

Group	Protein*	Albumin	Globulin*	$A:G*$
Control	6.29±0.20	3.06±0.07	3.03±0.21	1.01±0.37
750mg/kg	6.55±0.27	3.38±0.03	3.16±0.25	1.08±0.08
1500mg/kg	6.78±0.22	3.60±0.08	3.19±0.28	1.14±0.12
2250mg/kg	7.86± 0.12[a]	3.56±0.01	3.70±0.12[a]	0.95±0.04
3000mg/kg	8.33±0.14[a]	3.34±0.02	4.01± 0.17[a]	0.86±0.08[a]

Values are expressed as mean ± Standard deviation of 5 rats.;* ANOVA indicated p<0.05; [a] values are significantly (P<0.05) different from the respective control

Table 3: Some serum enzymes activities (U/L) of rats treated with crude aqueous extract of *A. chevelieri* root for 28 days.

Group	Aspartate Aminotransferase*	Alkaline Phosphatase*	Alanine Aminotransferase*
0.00mg/kg	55.81±0.13	60.67±3.38	17.60 ± 0.44
750mg/kg	60.74 ± 0.24	85.31 ± 3.68[a]	20.88 ± 0.51
1500mg/kg	63.12 ± 0.70	102.12 ± 1.95 [a]	24.27 ± 0.55 [a]
2250mg/kg	64.49 ± 1.26	109.85 ± 2.31 [a]	27.40 ± 0.55 [a]
3000mg/kg	88.66 ± 0.29[a]	117.58 ± 3.15 [a]	39.00 ± 0.53 [a]

Values are expressed as mean ± Standard deviation of 5 rats.;* ANOVA indicated p<0.05; [a] values are significantly (P<0.05) different from the respective control

Table 4: Serum Bilirubin (µmol/l) levels of rats treated with crude aqueous extract of *A. chevalieri* root for 28 days.

Group	Total Bilirubin *	Direct Bilirubin	Unconjugated Bilirubin *
0.00mg/kg	17.71± 2.06	13.69 ±1.01	4.02±2.49
750mg/kg	16.73± 1.10	11.84± 1.00	4.89±1.15
1300mg/kg	15.14 ±1.18	10.73± 0.83	4.41±1.32
2250mg/kg	20.66 ±1.35[a]	10.17± 0.93	10.50±1.96 [a]
3000mg/kg	21.65 ±1.10 [a]	9.99 ±1.01	11.66±1.16 [a]

Values are expressed as mean ± Standard deviation of 5 rats.;* ANOVA indicated p<0.05; [a] values are significantly (P<0.05) different from the respective control

Table 5: Some Kidney function parameters of rats treated with crude aqueous root extract of *A. chevalieri* for 28 days.

Group	**Urea(µmol/l)***	**Creatinine(µg/dl)***	**Uric acid(mg/dl)***
Control	6.36 ±0.55	276.11± 4.22	3.30±0.05
750mg/kg	7.35±0.17[a]	244.44± 4.39 [a]	1.98±0.07 [a]
1500mg/kg	7.78± 0.22 [a]	215.47± 5.69 [a]	2.10±0.08 [a]
2250mg/kg	8.89± 0.13 [a]	214.38± 6.11 [a]	1.37±0.08 [a]
3000mg/kg	9.72± 0.23 [a]	163.89 ±4.39 [a]	1.09±0.03 [a]

Values are expressed as mean ± Standard deviation of 5 rats.;* ANOVA indicated p<0.05; [a] values are significantly (P<0.05) different from the respective control.

Table 6 Serum Electrolyte levels (mmol/l) of rats treated with crude aqueous extract of *A. chevalieri* root 28days.

Group	Sodium	Potassium	Bicarbonate*
Control	149.80± 0.84	6.12±0.58	27.40±0.55
750mg/kg	148.00± 1.00	5.90±1.00	26.00±0.71
1500mg/kg	147.20 ±1.10	5.68±0.08	23.40±0.55
2250mg/kg	146.60± 0.55	5.82±0.08	24.80±1.30
3000mg/kg	145.60± 0.54	5.64±0.11	22.40±1.14[a]

Values are expressed as mean ± Standard deviation of 5 rats.;* ANOVA indicated p<0.05; [a] values are significantly (P<0.05) different from the respective control

DISCUSSION

The root extract of *A. chevalieri* have been reported to possess significant hypoglycaemic and hypolipidaemic effects in alloxan induced diabetic rats (Saidu *et al.*, 2010). It is however an established fact that some plant extracts could be inherently dangerous, containing naturally occurring toxins which may be cytotoxic

(Humphrey and McKenna, 1997). Accordingly most of the herbal preparations do not have drug regulatory approval to demonstrate their safety and efficacy (Seth and Sharma, 2004). It is therefore pertinent to establish the safety of medicinal plant preparations through toxicological assessments. Liver, being the primary organ for the detoxification and distribution of drugs, and the kidney, the major excretory organ, could be assessed to establish the safety of a substance (Guptan *et. al,* 1994).

The result of the current study showed that the LD_{50} of the crude aqueous extract of the plant was found to be greater than 3000mg/kg, which may be accepted as safe (OECD 2001). Other workers have reported different LD_{50} values for different plant extracts. The oral (rat) LD_{50} of ethanol extract of *Vitex leucoxylon* leaf (>3000mg/kg), cold water infusion extract of the same plant (1050mg/kg), ethanolic extracts of *Ailanthus excelsa* (1000mg/kg), *Toddalia asiatica* (350mg/kg) and *Araucaria bidwilli* (250 mg/kg) have been reported (Dahanukar *et. al*, 2000). The LD_{50} of *Boerhavia diffusa* has been reported to be >2000mg/kg body weight in both mice and rats (Orisakwe *et. al,* 2003). *A. chevalieri* leaf extract was also reported to be greater than 3000 mg/kg in rats (Saidu *et al.,* 2007b)

In the current work there was a progressive increase in the body weights of the rats treated with different subchronic doses of the root extract of *A. chevalieri.* This may be an indication that the drug does not affect the feed utilisation ratio of the animals. The body weights of animals treated with sub-chronic doses of aqueous extracts of *Boerhavia diffusa* (Orisakwe *et. al,* 2003) and *A. chevalieri* leaf (Saidu *et al*., 2007b) were also reported to increase progressively.

Increased plasma total protein concentration observed in the current work at high doses may be due to dehydration and/or increased plasma immunoglobulin concentration due to infection. Accordingly, serum globulin concentration and albumin:globulin ratio were significantly ($p<0.05$) affected. This may be an indication that higher doses of the extract might have predisposed the animals to infection resulting in increased serum globulin and a decreased A:G ratio.

The enzymes, Aspartate Aminotranferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) showed progressive increase in activities at all doses administered, with most significant effect at the 3000mg/kg. These findings imply that the extract, may at these doses, affect the liver. Serum ALT and AST are useful indices for identifying inflammation and necrosis of the liver (Tilkian *et. al,* 1979). ALT has its highest concentration in the liver with kidney and skeletal muscles having lesser activity of the enzyme. ALT measurements are however more liver specific than the AST and its activity is usually greater than AST activity at early or acute hepatocelluar disease (Whitby *et. al,* 1989). AST on the other hand tend to be released more than the ALT in chronic liver diseases such as cirrhosis (Whitby *et. al,* 1989).Liver, bone placenta and intestine are clinically important sources of the plasma activity of ALP. The activity of this enzyme is increased in many clinical states; the most important being bone and liver diseases. Accordingly, serum ALP is a useful diagnostic, screening and follow up tools of cholestatic hepatobiliary lesions (Wolf, 1978). Cholestasis is the main, if not the only liver disease responsible for increased plasma alkaline phosphatase activity. Thus, a normal alkaline phosphatase activity, in the presence of abnormal levels of other liver function parameters, may be suggestive of liver pathology other than obstruction (Tilkian *et. al,* 1979).

Elevated Bilirubin is an indication of liver cell impairment. The gradual increase in the serum levels of Unconjugated Bilirubin may be an indication that there the rats had liver function impairment, resulting in diminished ability of hepatocytes to conjugate bilirubin. Bilirubin is a useful index of the excretory function of the liver. In the liver, bilirubin is conjugated with glucoronic acid in a reaction catalysed by bilirubin –UDP-glucuronyltransferase which renders it soluble and subsequently excreted into the bile.

The extract of the root of *A. chevalieri* causes a significant increase in serum urea without adversely affecting the serum levels of uric acid, creatinine or the electrolytes. This may be indicative that the extract may have no any

adverse effect on the kidney. This assertion may be due to the fact that creatinine is usually a more accurate marker of kidney function than urea. Although elevated levels of creatinine, urea and uric acid are positive risk of renal impairment. A lower blood level of creatinine does not indicate impairment. The increased in serum urea levels recorded in the present study may be due to factors other than kidney problem (Baker *et al*., 2001).

Although the root extract have been reported to possess significant hypoglycaemic effect in alloxan induced rats (Saidu *et al.*, 2010), the results of the sub-chronic effect on the liver function parameters indicated that the root extract be use with caution especially at higher doses.

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