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Acute and Sub-chronic Toxicity Studies of Crude Aqueous Extract of *Albizzia chevalieri* Harms (Leguminosae)

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Abstract: The current study reports the toxicological studies of the crude aqueous leaf extract using albino rat models. The *in vivo* effects of acute and sub-chronic doses of the extract on liver function and kidney function parameters were studied. The results indicated that the LD_{50} of the extract is > 3000 mg kg^{-1} body weight. There were no significant differences ($p>0.05$) in weight changes of the animals on different doses of the extract during both the acute and sub-chronic toxicity tests. The biochemical parameters of the animals on different doses of the extract were not significantly ($p>0.05$) different, except the ALT and AST that, in non-dose dependence, showed significant differences ($p<0.05$) in both test models. Serum globulin level of the animals on different doses during the sub-chronic test was also significantly different ($p<0.05$). These results indicated that the crude extract of *A. chevalieri* may be relatively safe for human consumption.

Key words: Albizzia chevalieri, crude aqueous extract, toxicity

INTRODUCTION

A number of plants with acclaimed anti-diabetic properties are being studied in different laboratories throughout the world, especially in developing countries. This became more apparent following WHO (1994) recommendations regarding the need to develop and evaluate better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta-cell destruction, promoting beta-cell regeneration or repair and interrupting pathways leading to the various complications of diabetes. These recommendations, the cost and side effects of most orthodox hypoglycaemic agents, stimulated an increase demand for natural products with anti-diabetic activity that have fewer side effects (Kameswara *et al*., 1999). The most promising of such products are of plant origin (WHO, 1994).

Indigenous remedies for the management of diabetes mellitus have been in use as far back as the 6th century (Kameswara *et al*., 1999). The NAPRALERT database, for example, listed over 1200 species of plants representing 725 genera in 183 families including marine algae and fungi with hypoglycaemic activity. Over half of these plants have been used ethnopharmacologically in traditional medicine as antidiabetics and about 50% have been studied experimentally (IBISmedical.com, 2000).

Many plant materials have been studied for antidiabetic properties. These include aqueous extract of the root of *Tinospora cordifolia,* which is used in India (Stanely *et al*., 2000), banana flowers (*Musa Sapientum*) also used in India (Pari and Maheswara, 1999), extract of *Picrorrhiza kurroa* used in Kashmir (Joy and Kuttan, 1999), *Momordica cymbalaria* fruits and *M. charantia* used in indo-Pakistani subcontinent (Akhtar *et al*., 1981; Kameswara *et al*., 1999; IBISmedical.com, 2000). Others are *Hygophyllum gaetulum* extract (Jaouhari *et al*., 2000), juniper berries (*Juniperus communis*)

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(Medina, 1994), water decoction of *Psacalium decompositum* (Alarcon-Aguilar *et al*., 2000), *Medicago sativa* used in South Africa (Gray and Flatt, 1997), *Punica granatum* flowers (Jafri *et al*., 2000), *Parmentiera edulis* fruit (Perez *et al*., 2000), *Citrullus colocynthis* fruit (Abdel-Hassan *et al*., 2000), *Azadirachta indica*, *Gymnema sylvestre*, *Catharatus roseus* and *Ocimum sanctum* (Chattopodhyay 1999), *Strophanthus hispidus*, *Tetracera alnifolia*, *Carica papaya*, *Harungana madagascariensis*, *Dialium guineense*, *Terminalia macroplera*, *Spondias mombin*, *Dioscorea cayenensis*, *Spigelia anthelmia*, *Triumfetta rhomboidea*, *Sphenostylis sterocarpa*, *Momordica charanatia*, *morinda lucida*, *Urena lobata*, *Triphochiton scleroxylon*, *Irvingia grandifolia* and *Lonchocarpus cyanescens.* (Onoagbe *et al*., 1999). *Albizzia chevalieri* has also been reported to have significant hypoglycaemic activity (Saidu *et al*., 2007). These activities were increased when the active agent was fractionated with column chromatography on silica gel (Saidu *et al*., 2007).

The hypoglycaemic properties of these plants and indeed the medicinal properties of plants used by traditional medical practitioners may be due to one or more of the many arrays of chemical constituents 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. Some plants are therefore inherently dangerous, containing naturally occurring toxins, often with cytotoxic, carcinogenic effects or some other toxic properties (Humphrey and McKenna, 1997). *Carica papaya*, which is a known hypoglycaemic plant (Onoagbe *et al*., 1999; Lohiya *et al*., 1999), for example, is reported to induce sterility in experimental animals (Lohiya *et al*., 1999; 2000). The histopathological changes observed in the organs of the rats treated with raw larva of *Cirina forda* (Westwood) also suggested that the plant is toxic (Akinnawo *et al*., 2005).

The current study reports the *in vivo* acute and subchronic effects of the crude aqueous leaf extract of *A. chevalieri on* the serum biochemical indices of liver and kidney functions in albino rats.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents used for this study were of analytical grade. Assay kits were used for the assay of biochemical parameters and purchased from Randox Laboratories Ltd, Antrim, United Kingdom.

Plant Materials

A. chivalieri was obtained from a suburb, about 50 km south of Sokoto, Nigeria in February 2004 and identified by a Taxonomist from the Botany unit of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. Voucher specimen was prepared and deposited in the Herbarium of the same Department with voucher No: UDUS/VS/04/09. The leaves were sun dried, ground using laboratory pestle and mortar and sieved with a 1 mm 2 sieve. The powdered leaf was kept in plastic bags in a desiccator until required.

Preparation of Crude Extracts

The powdered plant material was soaked in cold distilled water at 20% (w/v) for 24 h The extract was filtered through several folded clean white muslin piece of cloth to remove debris. The filtrate was then filtered through a whatman No 1 filter paper. The final filtrate was evaporated in a drying cabinet set at 40°C (Harborne, 1973) and the amount of solute per gram dried powdered leaf calculated. The evaporated extract was then reconstituted in distilled water at 30% (w/v). The reconstituted extract, labelled crude aqueous extract was stored in small-capped plastic containers at +4°C until required. This was used for both the acute and sub-chronic toxicity tests.

Animals

Male Adult rats were used for this aspect of the research. Apparently healthy animals were purchased from National Institute for Trypanosomiasis Research (NITR) VOM, near Jos Plateau state Nigeria. The animals were allowed to acclimatise to the laboratory environment for a week during which they were allowed free access to clean water and food.

Acute Toxicity Study

The animals were grouped into six groups of five animals each and labelled A_C, B_C, C_C, D_C, E_C and F_C, respectively. The crude aqueous extract was administered in single oral doses of 500, 1000, 1500, 2200 and 3000 mg kg^{-1} body weights of the animals in groups B_C, C_C, D_C, E_C and F_C, respectively. Animals in group A_C were administered 0.5 mL of distilled water through the same route and served as control. The animals were weighed before and 72 h after the administration of the drug. The animals were observed for toxic symptoms such as weakness or aggressiveness, food refusal, loss of weight, diarrhoea, discharged from eyes and ears, noisy breathing and mortality for 72 h (Fielding and Metheron, 1991; Vijayalakshmi *et al*., 2000).

Sub-Chronic Toxicity Study

There were 6 groups of 5 animals each labelled S_C, T_C, V_C, X_C, Y_C and Z_C. The crude water extract was administered orally in 5 gradations of 150, 300, 500, 1000 and 1500 mg kg^{-1} body weight per day for 28 days to groups T_C, V_C, X_C, Y_C and Z_C animals, respectively. The control group (group S_C) received 0.5ml of distilled water, under similar experimental conditions and through the same route and for the same numbers of days. The body weight changes were monitored throughout the experimental period on weekly basis. Similarly, the animals were observed for manifestation of toxicity and mortality as in acute toxicity test.

At the end of the observation periods in both the sub-chronic and acute toxicity tests, the animals were anaesthesized by dropping each of the animals successively in a transparent plastic jar saturated with chloroform vapour. The animals were then removed from the jar and blood samples collected from the animals through cardiac puncture. The blood samples were allowed to clot, centrifuged and serum collected and used for biochemical analyses.

Liver Function Tests

The following tests were conducted to investigate derangements in the liver function of animals used for both acute and sub-chronic toxicity studies: Serum total bilirubin was estimated by a modification of Jendrassik and Grof method (AACC, 1982). Serum total protein and albumin were determined using Biuret method (Cheesbrough, 1991) and Bromocresol Green (BCG) binding method (Cheesbrough, 1991), respectively. Serum globulin level was calculated as the difference between serum total proteins and globulins. Albumin/Globulin (A/G) ratio was estimated from the values obtained for the albumin and globulin. Serum AST and ALT activities were determined using Reitman-Frankel method (Cheesbrough, 1991). Alkaline phosphatase activity was estimated in the serum by the nitrophenyl phosphate method of Bessey (Cheesbrough, 1991).

Renal Function Tests

Serum creatinine was measured by modified alkaline picrate method (Cheesbrough, 1991). Urease-Berthelot colorimetric method was used for the assay of serum urea (Cheesbrough, 1991). Serum uric acid (Jung and Parekh, 1979) was assayed by a method based on the oxidation of uric acid by Fe (III) in an acid medium.

Serum Electrolytes

Serum Na^+ and K^+ were measured using flame emission spectrophotometer (Cheesbrough, 1991). Bicarbonate ion was estimated using the titrimetric method in which a known amount of concentrated HCl was added to a fresh serum sample. Shaking the sample expels CO_2 liberated. H^+ remaining was back titrated with NaOH using phenol indicator (Cheesbrough, 1992).

Statistical Analysis

The results are presented as mean±standard error of the mean of five animals. The results were analysed using analysis of variance (ANOVA). Post Hoc Tests Multiple Comparisons using LSD was utilized to identify differences in means. SPSS windows version 10 was employed for the analysis.

RESULTS

The results of the LD_{50} calculation is presented in Table 1. The Arithmetic Method of Karbar (Aguiyi *et al*.,1996; Dede and Dogara, 2004) was used for the calculation. The results indicated that the crude aqueous extract of the leaves of *A. chevalieri* had LD_{50} of > 3000.00 mg kg^{-1} body weight.

The acute effects of the aqueous extract on the weight of the animals are presented in Fig. 1. The results indicated no significant difference between the animals treated with the different doses of the extracts.

Table 1: The LD_{50} calculated by the method of Karbar

Groups	Dose (mg kg^{-1})	Dose difference (Dd)	No. of animals	No. of dead	Death (%)	Mean (Md) dead	DD X Md
A_C	0	00	5	0	0	0	0
B_C	500	500	5	0	0	0	0
C_C	1000	500	5	0	0	0	0
D_C	1500	500	5	0	0	0	0
E_C	2200	700	5	0	0	0	0
F_C	3000	800	5	0	0	0	0
Total		3000					0

LD_{50} was calculated from the table above as

$$LD_{50} = LD_{100} - \frac{Dd \times Md}{N}$$

Where LD_{50} = Dose that caused 50% mortality
LD_{100} = Dose that caused 100% mortality (in this case >3000 mg kg^{-1})
N = No. of animals per group
Dd×Md = Dose difference multiply by the mean death

$$LD_{50} = (>3000) - \frac{300 \times 0}{5}$$

> 3000 – 0
> 3000 mg kg^{-1} body weight

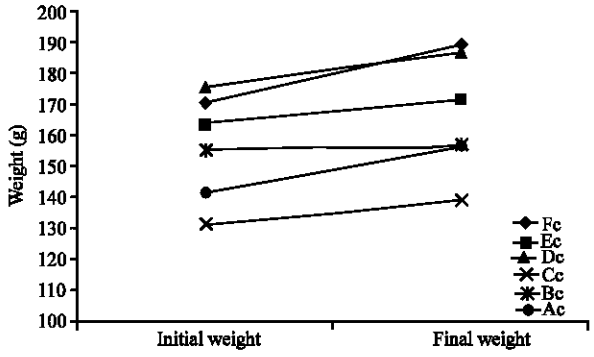

Fig. 1: Weight changes of rats treated with acute oral doses of aqueous extract of *A. chevalieri*

Table 2: Effect of acute toxicity of the crude aqueous extract of *A. chevalieri* on Serum proteins levels of rats treated with acute oral doses

		Parameters			
Groups	Dose (mg kg^{-1})	Total protein (g dL $^{-1}$)	Albumin (g dL^{-1})	Globulin (g dL^{-1})	A:G ratio
A_C	0	5.63±0.46	4.88±0.21	1.17±0.58	3.35±1.45
B_C	500	6.56±0.81	5.30±0.48	1.26±0.53	4.90±1.45
C_C	1000	6.24±0.39	4.50±0.31	1.74±0.41	3.58±1.23
D_C	1500	6.85±0.50	4.18±0.17	2.75±0.50*	1.68±0.29*
E_C	2200	6.74±0.29	4.12±0.19	2.62±0.15*	1.58±0.09*
F_C	3000	6.18±1.41	5.90±0.74	1.97±0.43	3.60±0.39

*Values bearing asterisks differ significantly ($p<0.05$) from the control (group A_C)

Table 3: Liver enzymes activities (U/l) of rats treated with acute oral doses of aqueous leaf extract of *A. chevalieri*

		Parameters		
Groups	Dose (mg kg^{-1})	ALT^{+}	AST$^{\#}$	ALP
A_C	0	9.15±1.72	23.00±1.63	14.24±1.60
B_C	500	12.66±2.15	13.00±2.12**	9.68±0.80
C_C	1000	9.31±0.67	20.80±1.91	13.79±0.80
D_C	1500	9.00±1.13	14.80±1.53*	11.04±1.13
E_C	2200	8.33±0.75	15.80±2.96	9.10±0.42
F_C	3000	16.32±2.89*	22.80±3.29	25.76±1.60

+Values differ significantly ($F = 3.207$, $p<0.05$), #Values differ significantly ($F = 3.295$, $p<0.05$), *Values differ significantly ($p<0.05$) from the respective control, **Value differs significantly ($p<0.01$) from the respective control

Table 4: Serum bilirubin levels of rats treated with acute oral doses of aqueous leaf extract of *A.. chevalieri*

		Parameters (mg dL^{-1})	
Groups	Doses (mg kg^{-1})	Total bilirubin	Direct bilirubin
A_C	0	5.49±0.33	4.20±0.20
B_C	500	5.55±0.12	4.20±0.11
C_C	1000	8.60±0.65	4.90±0.66
D_C	1500	5.55±0.11	2.01±0.07
E_C	2200	5.56±0.34	3.45±0.35
F_C	3000	5.11±0.38	4.92±0.20

The acute oral effect of the extract on serum proteins is present in Table 2. The results indicated no significant difference ($p>0.05$). When compared independently however, using LSD, globulin and A:G ratio of the animals in groups D_C and E_C were significantly ($p<0.05$) higher and lower, respectively than the control values.

The results of serum alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) of animals treated with acute doses of the crude aqueous extract of the plant material are presented in Table 3. The results indicated significant difference ($p<0.05$) for ALT and AST. Further analysis of the results indicated that the animals in group F_C had significantly ($p<0.05$) higher serum ALT while animal in group B_C ($p<0.01$) and group D_C ($p<0.05$) had significantly lower AST compared to the respective control values.

The results in Table 4 indicated no significant difference ($p>0.05$) in the levels of these parameters.

The results in Table 5 indicated no significant difference ($p>0.05$) in the serum electrolytes levels of animals on different doses of the extract. Separation of means however indicated that the rats on 2200 mg kg^{-1} body weight had significant ($p>0.05$) increase in serum sodium concentration compared to the control group.

The results in Table 6 indicated no significant difference ($p>0.05$). Urea concentration was however significantly higher ($p<0.05$) in rats treated with 3000 mg kg^{-1} body weight of the extract.

The effects of sub-chronic administration of the aqueous extract for 4 weeks on the weight of the animals are presented in Fig. 2. The results indicated no significant difference between the animals treated with the different doses of the extracts.

The results in Table 7 indicated a significant difference (<0.05) in the values obtained for globulin. The difference been inherent in the group treated with 150 mg kg^{-1} body weight.

The result indicated that the values obtained for AST ($p<0.05$) and ALP ($p<0.01$) differ significantly (Table 8) The animals on higher doses for four weeks had serum ALP significantly higher than the control values.

Table 5: Serum electrolytes levels of rats treated with acute oral doses of aqueous leaf extract of *A. chevalieri*

		Parameters (mmol L^{-1})		
Groups	Dose (mg kg^{-1})	HCO^{-}_3	Na^+	K^+
A_C	0	27.25±2.10	140.40±1.80	14.85±1.95
B_C	500	21.00±4.88	144.90±2.41	14.46±1.04
C_C	1000	25.75±1.35	148.42±2.16	13.34±0.55
D_C	1500	21.00±1.08	146.72±2.20	12.40±0.50
E_C	2200	23.00±0.84	151.84±4.07*	14.46±0.64
F_C	3000	22.60±3.59	144.00±3.79	15.00±2.77

*Differs significantly ($p>0.05$) from the control

Table 6: Some kidney function parameters of rats treated with acute oral doses of aqueous leaf extract of *A. chevalieri*

		Parameters		
Group	Dose (mg kg^{-1})	Uric acid (μmol L^{-1})	Urea (mmol L^{-1})	Creatinine (μmol L^{-1})
A_C	0	148.8±24.4	8.8±1.5	63.2±4.2
B_C	500	190.6±28.3	11.5±3.0	59.5±6.1
C_C	1000	166.2±24.5	9.1±1.2	61.2±5.4
D_C	1500	164.1±28.1	10.0±1.5	57.4±7.1
E_C	2200	135.4±27.5	9.9±1.1	65.3±3.9
F_C	3000	262.6±18.5	12.4±3.4*	79.5±5.2

* Values differ significantly ($p<0.05$) from the control

Table 7: Serum proteins levels of rats treated sub-chronic oral doses of aqueous leaf extract of *A. chevalieri*

		Parameters			
Group	Dose (mg kg^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})#	A:G ratio
S_C	0	5.63±0.46	4.88±0.42	1.17±0.58	3.35±1.45
T_C	150	6.83±0.38*	3.53±0.52*	3.30±0.25*	1.10±0.23*
V_C	300	6.25±0.39	4.98±0.41	1.28±0.32	3.97±1.00
X_C	500	6.38±0.31	4.43±0.24	1.95±0.15	2.30±0.20
Y_C	1000	6.85±0.15	4.63±0.44	2.23±0.40	2.40±0.63
Z_C	1500	6.43±0.24	3.98±0.35	2.45±0.44	1.88±0.48

Values differ significantly ($F = 4.042$, $p<0.05$), *Values differ significantly ($p<0.05$) from the respective control

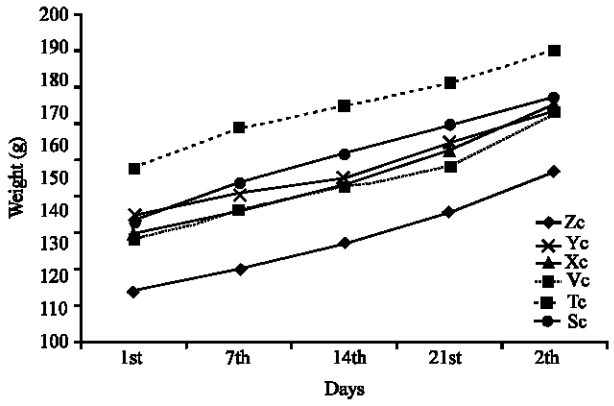

Fig. 2: Weight changes of rats treated with oral sub-chronic doses of aqueous leaf extract of *A. Chevalieri*

Table 8: Liver enzymes levels of rats treated sub-chronic oral doses of aqueous leaf extract of *A. chevalieri*

		Parameters (U L^{-1})		
Groups	Dose (mg kg^{-1})	ALT	AST#	ALP##
S_C	0	9.15±1.72	23.00±1.63	14.50±1.6
T_C	150	17.07±1.41	19.50±3.60	57.74±21.13
V_C	300	14.00±0.77	53.00±6.00**	126.98±32.71*
X_C	500	18.80±2.60	34.33±3.33	149.05±22.34**
Y_C	1000	22.00±2.95	26.30±2.40	117.30±23.43*
Z_C	1500	16.00±5.66	22.50±4.55	111.80±12.55*

#Values differ significantly (F =3.325; p<0.05), ##Values differ significantly (F= 4.99; p<0.01), * values differ significantly (p<0.05) from the control value, **Values differ significantly (p<0.01) from the control value

Table 9: Serum bilirubin levels of rats treated sub-chronic oral doses of aqueous leaf extract of *A. chevalieri*

		Parameters (mg dL^{-1})	
Groups	Dose (mg kg^{-1})	Total bilirubin	Direct bilirubin
S_C	0	5.49±0.33	4.20±0.20
T_C	150	3.70±1.11	1.62±0.34
V_C	300	4.40±1.20	2.46±0.27
X_C	500	3.68±1.82	2.46±0.74
Y_C	1000	2.47±1.07	1.64±0.49
Z_C	1500	5.60±2.60	3.08±1.20

Table 10: Serum electrolytes levels of rats treated with sub-chronic oral doses of aqueous leaf extract of *A.chevalieri*

		Parameters (mmol L^{-1})		
Groups	Dose (mg kg^{-1})	HCO^-_3	Na^+	K^+
S_C	0	27.25±2.10	140.50±3.600	14.85±1.95
T_C	150	17.67±2.72	166.87±12.42	19.85±0.87
V_C	300	23.67±1.33	174.18±12.75	16.83±0.75
X_C	500	18.00±1.15	171.25±3.110	16.83±0.75
Y_C	1000	21.50±0.50	162.48±7.810	16.83±0.75
Z_C	1500	23.25±3.96	159.22±10.96	16.83±0.75

Table 11: Some kidney function parameters of rats treated with sub-chronic oral doses of aqueous leaf extract of *A. chevalieri*

		Parameters		
Groups	Dose (mg kg^{-1})	Uric acid (μmol L^{-1})+++	Urea (μmol L^{-1})	Creatinine (μmol L^{-1})
S_C	0	148.80±24.4	8.8±1.50	63.20±4.20
T_C	150	520.38±12.15***	15.8±0.50	69.50±6.46
V_C	300	520.63±18.7***	10.8±1.70	68.02±4.14
X_C	500	340.00±34.7**	11.1±1.90	59.94±3.17
Y_C	1000	361.30±27.3**	13.3±2.70	73.36±3.80
Z_C	1500	520.60±17.41***	9.1±0.73	69.93±8.34

+++Values differ significantly (p<0.001), **Values differ significantly from control (p<0.01), ***Values differ significantly from control (p<0.001)

Serum bilirubins of the animals treated with sub-chronic doses of the extract have presented in no significant difference (p>0.05) (Table 9).

Serum electrolytes of the sub-chronic toxicity tests have no significant difference (p>0.05) (Table 10).

There were no significant differences (p>0.05) as a result of treat treatment with different doses on serum urea and creatinine (Table 11). Uric acid levels of the animals treated with different doses however differ significantly (p<0.001).

DISCUSSION

The major hindrance to the use of traditional herbal preparations is the lack of scientific and clinical data in support of better understanding of the efficacy and safety of the drugs. This is due

largely to the negligence of the evaluation of the toxicity and adverse drug reactions of herbal medicines, as they are considered natural and thus, erroneously, safe. Some plant extracts could be inherently dangerous, containing naturally occurring toxins, which may be cytotoxic or carcinogenic (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 these 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). In the current study therefore, liver and kidney functions parameters of animals treated with both acute and sub-chronic doses of the crude aqueous preparation of *A. chevalieri* were assessed. One of the toxicological indices used for the assessment of the safety of drugs is lethal dose 50% (LD_{50}) the amount of acute dose of the drug required to kill half of the test population. In the current study, the 72 h LD_{50} for the oral administration of the crude extract was calculated according to the Arithmetic method of Karber (Dede and Dogara, 2004) to be >3000 mg kg^{-1} body weight of rat. Based on Hodge and Sterner scale (CCOHS, 2005), a test drug administered orally is considered extremely toxic at≤ 1 mg kg^{-1}, highly toxic at 1-50 mg kg^{-1}, moderately toxic at 50-500 mg kg^{-1}, slightly toxic at 500-5000 mg kg^{-1}; practically non toxic at 5000-15,000 mg kg^{-1} and relatively harmless at≥ 15,00 mg kg^{-1}. In the current study, the crude extract could be declared practically non-toxic or at worst slightly toxic. At the therapeutic dose of the drug however, which is just about 4% of the approximate LD_{50} (Saidu *et al*., 2007), the drug may be safe. Various LD_{50} values have been reported for different substances. The oral (rat) LD_{50} of ethanol extract of *Vitex leucoxylon* leaf (>3000 mg kg^{-1}), cold water infusion extract of the same plant (1050 mg kg^{-1}), ethanolic extracts of *Ailanthus excelsa* (1000 mg kg^{-1}), *Toddalia asiatica* (350 mg kg^{-1}) and *Araucaria bidwilli* (250 mg kg^{-1}) have been reported (Dahanukar *et al*., 2000). The LD_{50} of *Boerhavia diffusa* has been reported to be >2000 mg kg^{-1} body weight in both mice and rats (Orisakwe *et al*., 2003).

The results of the weight changes (Fig. 1 and 2) in the current study indicated no significant ($p > 0.05$) effects in both acute and sub-chronic toxicity tests. This may be an indication that the drug does not affect the feed utilisation ratio of the animals. In all the groups and both tests models, the animals fed well (results could not be consistently recorded and not shown). In a similar study, combinations of some medicinal plants (Tripala) used in traditional Thai medicine for adjusting patients element in summer have been reported at sub-acute doses to lower the body weight of Wister rats and food intake, the effect they attributed to high content of tannin in the extract (Chavalittumrong *et al*., 1996). The body weight of animals treated with sub-chronic doses of aqueous extract of *Boerhavia diffusa* was reported to increase progressively (Orisakwe *et al*., 2003).

The liver plays a key role in many metabolic processes of not only itself but of other tissues as well. This fact demonstrates the biochemical altruistic nature of this organ. Severe hepatic injury, as a result of the metabolism of some of the toxic phytochemicals found in medicinal plants and failure of the metabolic products to be eliminated by the liver (Geidam *et al*., 2004), may be associated with marked distortion of these functions. Albumin is the most abundant of the plasma proteins with the physiological role of maintenance of osmotic pressure, transportation of both endogenous and exogenous substances and serving as protein reserve. The ability of the liver to synthesize albumin is diminished if the synthetic function of the organ is affected (Whitby *et al*., 1989). Rapid reduction in serum albumin is therefore apparent in hepatitis and liver cirrhosis. The assay of serum total protein alone may not portray the true picture of the metabolic state of the individual, since the concentration of the individual proteins do not rise or fall in parallel with one another (Whitby *et al*., 1989). Increased plasma total protein concentration may be due to dehydration, to increased plasma immunoglobulin concentration due to infection. Plasma concentration of proteins may decrease as a result of over hydration, impaired protein synthesis due to malnutrition, malabsorption, liver disease, hypogammaglobulinaemia or increased protein loss due to renal, gastrointestinal and skin disorders. In the current study the serum protein profiles were not significantly different between the animals

on different doses of the extract for both toxicity tests. These results demonstrate the fact that the synthetic function of the liver of the animal exposed to oral acute and sub-chronic doses is not affected. Additionally, there is no sign of infection as neither the globulin levels nor the A:G ratio of the animals treated with both the acute and sub-chronic doses were significantly ($p>0.05$) affected.

Bilirubin is a useful index of the excretory function of the liver, in addition to its being a useful tool in the assessment of haemolytic anaemia. In the current work the plant extract did not caused any significant changes in the bilirubin levels of the animals treated with either acute or sub-chronic doses of the leaf extract of *A. chevalieri*. Consequently, it may be stated that the excretory function of the liver in rat is not affected significantly as a result of the administration of oral acute or sub-chronic doses of the extract.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (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. The activity of AST is located in the microsomal and mitochondrial portions of the liver cells as well as in the skin, skeletal and cardiac muscles, pancrease and kidney. ALT measurements are 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). A normal ALT in the presence of elevated activities of AST and lactate dehydrogenase rules out the hepatic origin of the enzyme. A marked elevation of ALT, however, in the presence of mild to moderate elevation of AST is suggestive of either hepatic disease or hepatic disease combined with other conditions (Tilkian *et al*., 1979). In the current study acute oral doses of the extract caused significant changes ($p<0.05$) in both AST and ALT. While the increase in ALT in these animals was dose dependent, as the values obtained for the animals on the highest dose of the extract was significantly ($p<0.05$) higher than the value obtained for the control, the observed decrease in AST was not dose dependent. It may be infer, therefore, that these changes may not be due to the effect of the administered extract but rather due to some other intrinsic factors. In the sub-chronic toxicity test, similar result patterns were observed. ALT was normal with significant changes ($p<0.05$) in the AST. The observed changes in AST were due to significant increase ($p<0.01$) in the value of the animals treated with 300 mg kg^{-1} body weight compared to the control group. Those animals treated with higher doses however, did not show any significant difference ($p>0.05$) with the control. This is also an indication that these changes may not necessarily be related to the extract. Singh and Devkota (2003) also reported that aqueous extract of *Piper methylsticum* did not affect significantly the serum levels of ALT, AST, ALP and lactate dehydrogenase activities. In a similar study, Effraim *et al*. (2000) demonstrated that animals treated with aqueous extract of *Ocinum gratissium*, used for the treatment of rheumatism and paralysis did not affect the activities of ALT, AST and ALP.

The activity of Alkaline phosphatase (ALP) 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 and osteoblastic bone diseases (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). In the present study, the alkaline phosphatase activities of animals treated with acute oral doses of the extract were not significantly different ($p>0.05$). When treated sub-chronically however, the enzyme activity of the animals on different doses differs significantly ($p<0.01$). This observation, vis-à-vis other clinical pictures ruled out the possibility of liver obstruction. It could be possible however that the significant increases observed in the activity of this enzyme may not be unconnected to the fact that some female members of the groups conceived (as each group both sexes were housed in the same cage) during the 28 th day experimental period (results not shown), while others even gave birth to normal litters. The serum ALP activity is elevated during pregnancy, as a

result of the production of the enzyme by the placenta and possibly as a result of increased maternal osteoblastic activity (Tilkian *et al*., 1979; Whitby *et al*., 1989). The placenta ALP isozyme is distinguishable from the liver's, preferably by electrophoresis or by virtue of its heat stability and inhibition by phenylalanine (Wolf, 1978).

Kidney function is affected by a number of factors, which may ultimately result in its failure. Causes of kidney failure include destruction of the tubules in the kidney by drugs, including phytochemicals. As a result, the two main functions of the kidney: the glomerular filtration and tubular re-absorption and secretion may be affected.

Since the plasma concentration of creatinine is relatively constant under normal circumstances, unless Glomerulus Filtration Rate (GFR) changes, as a result of defective renal function, plasma concentration of creatinine is a good index of measuring GFR. This is by virtue of the inverse relationship between the serum creatinine concentration and GFR (Whitby *et al*., 1989). In the current work, the serum creatinine concentrations of the animals treated with both acute and sub-chronic oral doses of the extract were not significantly different ($p>0.05$). This may be an indication that the extract contained no substance that may compromise this vital function of the kidney.

This fact is supported by the observation that the serum urea concentrations of the animals were also not significantly different ($p>0.05$). Though plasma urea concentration is less reliable than the creatinine as an index of GFR, by virtue of the fact that it diffuses back into the renal tubular cells and its plasma concentration is dependent on the state of the liver function and protein intake and oxidation (Tilkian *et al*., 1979), estimation of the two complement each other in evaluating this function of the kidney.

One of the commonest causes of hyperuricaemia is gout, in which there are either tophi or acute arthritis. Mild asymptomatic idiopathic hyperuricaemia has also been associated with hyperlipidaemia and coronary heart disease (Tilkian *et al*., 1979). Hyperuricaemia as a result of chronic renal failure can be ascertained by correlating uric acid level with urea and creatinine. The general trend in the result of the acute oral toxicity test in the current studies suggests that the drug has no effect on the serum level of uric acid. When treated with the extract sub-chronically however, the drug exerts significant effect ($p<0.001$) on the serum level of this metabolite. The effect seems to be dose dependent and therefore may be related to the administered substance. It is almost certain, the problem is not renal, but may be due to some other factors. This assertion is informed from the fact that serum urea and creatinine levels, which are also expected to be raise if the problem were renal, are normal. It may suffice at this juncture therefore, to project that the hyperuricaemia observed in these animals may be due to its overproduction as a result of activation of PRPP-amidotransferase or xanthine oxidase (Whitby *et al*., 1989) or some other factors.

The levels of electrolytes in the blood are the outcome of fine regulatory mechanism of ionic charges +and the osmotic balance. This homeostasis is achieved by an interplay involving the kidney, the lungs and endocrine system (Tilkian *et al*., 1979). Sodium is the major cation of the extracellular fluid where it regulates acid-base equilibrium and protects the body against excessive fluid loss. Hypernatraemia though rare, may occur in dehydration and steroid hormone administration. Hyponatraemia, on the other hand is more common and may be due to chronic sodium losing nephropathy, loss of gastrointestinal secretion through diarrhoea or vomiting, loss from skin as a result of burns, loss from kidneys through the use of diuretics and metabolic loss through starvation or diabetic ketoacidosis (Tilkian *et al*., 1979). Potassium is the major intracellular cation with similar role to those of sodium. Hyperkalaemia is usually encountered frequently in renal failure, improper use of K^+ sparing diuretics, hypoaldosteronism, insulin deficiency associated hyperglycaemia, Addison's disease and massive tissue destruction (Eccles, 1993; Tilkian *et al*., 1979). Excessive renal loss of potassium is associated with diuresis, renal loss as a result of potassium losing nephropathy or as a result of renal tubular acidosis. Other causes of hypokalaemia include excessive loss without adequate replacement as in chronic diarrhoea, malabsorption syndrome, perspiration and chronic fever, chronic

stress, poor dietary habit, Cushing's syndrome, hyperaldosteronism, liver disease with ascites, use of some drugs such as indomethacin, phenylbutazone and steroid hormone (Eccles, 1993; Tilkian *et al*., 1979; Whitby *et al*., 1989). Plasma bicarbonate ion concentration is increased in respiratory acidosis and metabolic alkalosis but decreased in respiratory alkalosis and metabolic acidosis ((Eccles, 1993; Tilkian *et al*., 1979, Whitby *et al*., 1989; Holmes, 1993). In the current research the serum electrolytes of the animals treated with both acute and sub-chronic doses of the extract were not significantly different ($p>0.05$). This is an indication that the extract may not have any significant effects on the water, electrolyte and acid-base balance. Normal serum levels of electrolytes of animals treated with extract of *M. balsamina* have also been reported (Geidam *et al*., 2004).

CONCLUSION

In view of the serum biochemical parameters of the animals treated with both the acute and sub-chronic doses of the oral aqueous leaf extract of *A. chevalieri*, it may be apparent to suggest that the plant extract may be safe, especially at the therapeutic dose which is far lower than the tested doses. The little or nor toxicity observed in this studies may not be unconnected with the fact that the secondary plant metabolites that may likely cause toxicity effects may be absent from the extract or if present may only be present in very minute non-toxic levels.

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