Science World Journal Vol 6 (No 3) 2011
www.scienceworldjournal.org
ISSN 1597-6343

Full Length Research Article

ACUTE AND SUBCHRONIC TOXICITY STUDIES OF KERNEL EXTRACT OF *Sclerocarya birrea* IN RATS.

*MUHAMMAD, S[1]., HASSAN, L. G[1]., DANGOGGO, S. M[1]., HASSAN, S. W[2]., UMAR, K. J[1]. & ALIYU, R. U[2].

[1]Department of Pure and Applied Chemistry,
[2]Department of Biochemistry,
Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.
*surajomabera@gmail.com

ABSTRACT

***Sclerocarya birrea* fruits are widely eaten in developing countries especially in rural areas and serves as nutrients supplements. However, they also contain phyto-toxin which may affect the normal functioning of the body. Acute toxicity was performed by a single oral administration at a dose of 3000 mg/kg body weight. Sub chronic evaluation was done by oral feeding of the rats with the seed kernel extract daily at doses of 1000, 2000, 3000 and 4000 mg/kg body weight for 28 days. The results of acute toxicity showed no mortality and general behavior changes. The lethal dosage (LD_{50}) was greater than 3000 mg/kg body weight. Rats fed with 1000 and 2000 mg/kg body weight of the extract showed increased body weights throughout the period of treatment but not significantly ($p<0.05$) different from the control group. Significant ($p<0.05$) reduction in the body weights were noticed in those administered with 3000 and 4000 mg/kg body weight at the 4th and all the weeks respectively. Significant ($p<0.05$) increased in serum total protein, albumin, bilirubin, transaminases, creatinine, urea, uric acid and electrolytes were observed in rats fed with 3000 to 4000 mg/kg body weight of the extract, suggesting liver and kidney toxicity. Therefore, the seed kernel extract of *S. birrea* may be relatively toxic at doses of 3000 and 4000 mg/kg body weight.**

Key words: *Sclerocarya birrea*, hepatorenal indices, toxicity, seed kernel.

INTRODUCTION

A significant proportion of indigenous fruits in West African sub region are seasonal forest products harvested for consumption on site or transported to other areas particularly urban centers for sale (Nnam & Njoku, 2005). The knowledge of the nutrients composition of some of these fruits enhances their use and increases their consumption which in turn improves the nutrient profile of the consuming populace (Nzeagwu & Onimawo, 2010). One of such tree is *Sclerocarya birrea* (*Anacardiaceae*) which its botanical description was reported by Moganedi *et al*., (2007), Hillman *et al*., (2008) and Ojewole *et al*., (2010). The tree bears pale yellow fruits (Plate 1) with a plain tough peel and fibrous juicy sweet-sour mucilaginous flesh (Hillman *et al*., 2008). The kernel of the fruits is widely eaten in developing countries not only during period of scarcity but during period of abundance; perhaps due to cultural acceptance (Ojewole *et al*., 2010).

Nutritional study of the plant's fruits revealed that the fruit juice contained 3.31% dry weight (DW) crude protein and 90.35% DW available carbohydrate (Hassan, *et al.,* 2010). Earlier studies showed that the seed kernel is edible and rich in oil (50 – 60%) and protein (28 – 36%) (Glew *et al*., 2004; Moganedi *et al*., 2007). On dry weight basis, *S. birrea* seed kernels contained appreciable amount of copper (24.8 µg/g), magnesium (4210 µg/g) and zinc (62.4 µg/g) (Glew *et al*., 2004).

Sclerocarya birrea tree was also reported to possess medicinal properties. Ojewole *et al*., (2010) reported that the stem bark aqueous extract is safe, and or non-toxic to mice and possess analgesic, anti-inflammatory and anti-diabetic properties while the polar extracts of the leaf and stem bark (inner bark) have antibacterial and antifungal activities. Other workers have reported on the antinutritional composition and toxicological properties of this plant (Hassan *et al*., 2010; Ojewole *et al*., 2010; Hassan *et al*., 2011).

PLATE 1. RIPE FRUITS OF *Sclerocarya birrea* (KOKWARO & GILLET, 1980).

Even though wild plants are important sources of nutrients and phytocompounds that play a role in protection against conditions such as cardiovascular disease and cancer, they also contain other compounds that may lead to hepatic/tubular necrosis (Caswell, 2009). To our knowledge, information on the toxicity of the seed kernel of *S. birrea* in Northern Nigeria is scanty. Therefore, this paper reports the evaluation of the safety of seed kernel extract of the plant by acute and sub chronic oral administration in rats.

MATERIALS AND METHODS

Sampling and sample treatment: Two kilogrammes (2 kg) of matured and ripe *Sclerocarya birrea* fruits were collected in June, 2010 from More village of Kware local government area, Sokoto State, Nigeria. Five trees were randomly selected and the fruits were collected from different branches of the trees, as described by Hassan & Umar (2004). Representative sample was taken using alternate shovel method (Alan, 1996). The juice, peels and seeds were separated by squeezing ripe fruits. The seeds were air dried and the kernel removed manually using hammer, pulverized to fine powder using pestle and mortar, sieved to pass through 80-meish sieve and stored in air tight paper bags inside a desiccator. The dried powder was used to prepare the extracts.

Preparation of the extracts: Fifty grammes (50 g) of the powdered sample were extracted with distilled water for 24 hours and filtered. The filtrate was evaporated to dryness using an oven (Gallenkamp, England) at 50 °C to a constant weight. The percentage extract was calculated using equation 1.0 and then reconstituted with distilled water and used for toxicity studies.

$$\% \text{ Extract} = \frac{\text{Weight of extract}}{\text{Sample weight}} \qquad \ldots 1.0$$

Toxicological studies

Animals: Albino rats (males and females) weighing 165 t0 300 g were purchased from the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria kept at the animal house of the department in a wire mesh cages fed with grower's feed and tap water *ad libitum* for two weeks to acclimatize before starting the experiment. Animal treatment and handling were done according to the standard ethical guidelines (Zimmerman, 1983; NIH Publication no. 18 - 23).

Administration of the extracts

Acute toxicity studies (Determination of LD_{50}): A $1cm^3$ aqueous extract of the sample (3000mg/kg body weight) was administered to 5 groups of one rat each (one after the other at a grace observation period of 24 hrs) in a single oral dose using a feeding needle. Another (control group) received distilled water. Observation for toxic symptoms was made and recorded systematically at 1, 2, 4 and 6 hrs after administration. Finally, the number of survivors was noted after 48hrs for each animal. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD, 2001).

Sub-acute toxicity studies: A total of thirty albino rats were divided into five groups of six each. The animals in groups 2, 3, 4 and 5 were orally administered with ($1cm^3$ of 1000, 2000, 3000 and 4000mg/kg body weight) of the extract once daily for 28 days respectively. Animals in group 1 served as the control group (i. e. 0.00mg/kg) and received only drinking water by the same route. The body weights of all the animals before and within 28 days (weekly) of treatment were recorded.

Blood sample and Clinical chemistry: The animals were sacrificed 24 hours after the last treatment after which blood samples were collected, allowed to clot and then centrifuged at 3000rpm for 10 minutes to obtain sera. The biochemical parameters, serum total protein (TP) and total albumin (TA) were determined by the method of Cheesbrough (1991). Total bilirubin (TB) was analyzed (Randox kit) using the method reported by Hassan *et al*., (2005). Serum alanine amino transferase (ALT) and aspatate amino transferase (AST) were done using Randox assay kit by standard method of Reitman & Frankel (1957). Alkaline phosphatase (ALP) was estimated by the Randox (colorimetric) method of Rec (1972). Serum electrolyte and creatinine (colorimetric with deproteinization) were performed by the methods of Henry (1974). Urea (diacetylmonoxime) was analyzed using method of Wybenga *et al*., (1971) and uric acid estimated by the method of Morin & Prox (1973).

RESULTS

Acute Toxicity: There was neither sign of toxity nor death of the experimental rats during the 48 hr of observation after oral administration of the aqueous extract from the fruits of *S. birrea* at a single dose of 3000 mg/kg. Further evaluation of toxicity carried out by observing body weight gain did not reveal significant difference ($p>0.05$) in 1000 and 2000mg/kg body weight extracts compared with their control group, but significant ($p<0.05$) reduction of the weights were noticed in rats administered with 3000 mg/kg body weight at 4th week when compared with the control group. For 4000 mg/kg body weight rats, significant ($p<0.05$) reduction of the weights were noticed from 1st to 4th week (Table 1).

TABLE 1. WEIGHT OF RATS (G) AS AFFECTED BY DOSES OF *Sclerocarya birrea* KERNEL (EXTRACTS) AFTER FOUR WEEKS OF ADMINISTRATION.

Dose (mg/kg)	Initial weight	1st week	2nd week	3rd week	4th week
0.00 (control)	166.82±1.48	167.81±1.03	167.91±1.77	168.16±1.92	168.97±1.06
1000	193.28±0.93	193.74±0.71	194.06±0.91	195.00±0.67	195.74±0.74
2000	203.53±3.11	204.02±3.44	204.50±3.46	205.32±2.87	205.73±2.61
3000	208.88±1.13	209.57±1.47	209.80±1.33	208.74±0.57	208.43±0.51*
4000	206.21±2.75	206.04±2.60*	205.93±2.56*	205.66±2.51*	205.29±2.61*

Values are mean ± standard deviation.

*= Significantly different from the control ($P < 0.05$) using one way analysis of variance.

Subchronic toxicity: As shown in Table 2, the rats treatment group with the extract at the dose of 3000 and 4000 mg/kg/day had the liver function indices (TB, ALP, AST and ALT**)** significantly ($p<0.05$) higher than the control while no significant difference ($p>0.05$) was observed at lower doses. It was also observed that TP and TA were significantly lower only for rats given upto 4000 mg/kg/day of the extract. For Kidney function indices (creatinine, urea, uric acid, sodium and potassium) in rats administered with *S. birrea* kernel extracts,) no significant difference ($p>0.05$) was observed in the group administered with 1000 and 2000 mg/kg/day when compared to the control, while those administered with 3000 and 4000 mg/kg/day of the extract have significant ($p<0.05$) increase in the kidney function parameters (Table 3).

TABLE 2. LIVER FUNCTION INDICES IN RATS ADMINISTERED WITH *S. birrea* KERNEL (EXTRACTS).

Dose (mg/Kg)	TP (g/dl)	TA (g/dl)	TB (g/dl)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
0.00	6.49±3.78	4.53±0.47	4.98±2.10	106.63±1.94	28.37±3.18	10.28±3.21
1000	6.35±1.00	4.52±1.11	5.16±2.14	332.43±2.53	32.30±2.41	13.53±0.39
2000	6.15±2.00	4.11±0.33	5.24±3.20	403.54±2.42	35.62±0.45	21.85±1.84
3000	5.58±1.60	3.61±0.40	5.86±1.8*	412.58±2.26*	45.52±0.53*	28.54±0.11*
4000	5.31±1.00*	3.55±1.20*	6.78±0.15*	422.28±2.39*	59.05±0.78*	30.80±0.62*

Values are mean ± standard deviation.

*= Significantly different from the control ($P < 0.05$) using one way analysis of variance.

TABLE 3. KIDNEY FUNCTION INDICES IN RATS ADMINISTERED WITH *S. birrea* KERNEL (EXTRACTS).

Dose (mg/kg)	Creatinine (µmole/l)	Urea (Mmole/l)	Uric acid (µmole/l)	Sodium (ppm)	Potassium (ppm)
0.00(Control)	82.49±0.74	9.86±1.80	200.99±0.86	32.07±0.25	8.91±1.83
1000	85.19±0.89	10.36±0.36	204.29±0.73	31.92±0.89	9.41±0.51
2000	92.00±1.12	11.56±0.43	209.39±0.46	31.01±1.04	9.95±0.86
3000	96.31±0.76*	12.64±0.45*	212.79±0.81*	28.76±0.16*	10.24±2.11*
4000	102.64±2.09*	14.15±1.84*	219.47±1.17*	27.11±0.10*	10.89±1.71*

Values are mean ± standard deviation.
*= Significantly different from the control (P < 0.05) using one way analysis of variance.

DISCUSSION

The percentage yield: The percentage yield of the extract was 6.67g/100g of the kernel which is an indication that the kernel could contain some important nutritional or medicinal phytocompounds.

Acute toxicity (LD50): Acute toxicity test at 3000mg/kg body weight of the kernel extracts produced no mortality after 48 hrs of observation which indicates that the mean lethal dose (LD50) of the extract is greater than 3000mg/kg body weight. Generally, acute toxicity did not produce any grossly negative behavioural changes such as excitement, restlessness, convulsions or coma in the rats, instead reduced reaction to noise was observed suggesting that, the extract may have depressant effect on the central nervous system (Hassan et al., 2005).

Sub-acute toxicity: The result of liver function indices was presented in Table 2. There was a significant (p<0.05) decrease in the serum total protein and albumin of the rats administered dose of 4000mg/kg body weight. Albumin is synthesized by the liver and as such, it represents a major synthetic protein and is a marker of the ability of the liver to synthesize proteins (Johnston, 1999). The decrease in the serum total protein and albumin indicates that the synthetic function of the liver has been affected though malnutrition can cause decrease in albumin (hypo albuminemia) without associated liver disease. A significant (p<0.05) decrease in the serum proteins and albumin clearly shows that the extract may inhibits protein synthesis in the rats although the values are still within the normal range (5.6 to 7.6 g/dl) as reported by The Rat Fan Club (2010).

Bilirubin is a major break down product of haemoglobin (Oboh, 2005). The water solubility of bilirubin allows the bilirubin to be excreted in the bile; the bile is then used to digest food. As the liver becomes irritated, the total bilirubin may rise. As presented in Table 2, there was a significant (p<0.05) increase in the total bilirubin in the serum of rats fed with 3000 and 4000mg/kg body weight which is an indication that the extract interfere with the metabolism of bilirubin in the liver (Oboh, 2005).

ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum (Wright & Plummer, 1974). The significant (p<0.05) increase in the serum ALP could be due to renal or intestinal damage, biliary track damage and inflammation (Oboh, 2005).The increase could be attributed to enzyme activation by the phytochemical constituents of the kernel.

The ALT and AST are liver specific enzyme markers of necrotic injury and cholestasis (Speech & Liehr, 1983). The significant increase could be due to damage to the hepatic cell or heart attack (Hearly *et al.,* 1995) and may have been induced by some phytocompounds of the kernel extract.

Serum urea, uric acid, creatinine and electrolytes are markers of damage to renal function (Harold *et al.,* 1980). The significant (p<0.05) decrease in sodium and increase in potassium in the group treated with 3000 and 4000mg/kg body weight are also signs of renal failure (Hassan et al., 2005). The changes in biochemical indices of renal function may have been induced by the phytochemical constituents of the kernel extract.

Conclusion: The results revealed that the seed kernel extract may have effect on liver and kidney functions at high doses and should be used cautiously. The mechanism (s) of toxicity of the extract is still being investigated.

REFERENCES

Alan, W. (1996). *Soil and the environment: An introduction,* Cambridge University press.

Caswell, H. (2009). *The role of fruit juice in the diet. An over view.* British Nutrition Foundation. High Holborn House, London, UK, pp 40-45.

Cheesbrough, M. (1991). *Medical laboratory manual for tropical countries.* Vol. 11. 2nd edition, ELSB. Cambridge. 508-511pp.

Glew, R. S., VanderJagt, D. J., Huang, Y. S. & Chuang, L. T. (2004). Nutritional analysis of the edible pit of *Sclerocarya birrea* in the Republic of *Niger. Journal of Food Composition and Analysis.17*: 99-111.

Harold, V., Alan, H. G. & Maurice, B. (1980). *Practical Clinical Biochemistry.* William Heinemann, London. Pp 10-15.

Hassan, L. G. & Umar, K. J. (2004). Proximate and mineral composition of seeds and pulp of African locust bean (*Parkia biglobosa* L.). *Nigerian Journal of Basic and Applied Sciences. 13:*15-27.

Hassan, S. W., Umar, R. A., Ebbo, A. A. & Matazu. I. K. (2005). Phytochemical, Antibacterial, and Toxicity study of *Parkinsonia aculeate* L. (Fabaceae) . *Nigerian Journal of Biochemistry and Molecular Biology. 20(2)*:89-96.

Hassan, L. G., Dangoggo, S. M., Hassan, S. W., Muhammad, S. & Umar, K. J. (2010). Nutritional and Antinutritional Composition of *Sclerocarya birrea* fruit juice. *Nigerian Journal of Basic and Applied Sciences 18(2)*:222-228.

Hassan, L. G., Dangoggo, S. M., Hassan, S. W. Muhammad, S. & Umar, K. J. (2011). Serum biochemical response of rats fed with *Sclerocarya birrea* juice. *African Journal of Food Science 5(4):* 208-212.

Hearly, K., Sambaiah, A. & Cole, N. (1995). Spices as beneficial hypo-cholesterolemic food adjuncts: a review. *Food Reviews International 20*:187-220.

Henry, R. J. (1974). *Determination of Serum Creatinine. Clinical Chemistry Principles and techniques.* 2nd Edition, Harper Row, pp 525.

Hillman, Z., Mizrahi, Y & Beit-Yannai, E. (2008). Evaluation of valuable nutrients in selected genotypes of marula (*Sclerocarya birrea* sp caffra). *Sciencetia Horticulturae 117:* 321-328.

Kokwaro, J. O. & Gillett, J. B. (1980). Notes on the Anacardiaceae of East Africa. *Kew Bulletin 34(4)*: 745-756.

Moganedi, K. L. M., Colpaert, N., Breyne, P., Sibara, M. M. & Goyvaerts E. M. A. (2007). Determination of genetic stability and grafted marula trees using AFLP markers. *Sciencetia Horticulturae 111:* 293-299.

Morin, L. G. & Prox, J. (1973). Uric acid. *American Journal of Clinical Pathology 60:* 691-694.

Nnam, N. M. & Njoku, I. E. (2005). Production and Evaluation of Nutrient and Sensory properties of juices made from citrus fruits. *Nigerian Journal of Biochemistry and Molecular Biology 26:* 62-66.

Nzeagwu, O. C. & Onimawo, I. A. (2010). Nutrient compositon and sensory properties of juice made from Pitanga Cherry (*Eugenia uniflora* L) fruits. *African Journal of Food Agriculture Nutrition and Development 10:* 2379-2393.

Oboh, G. (2005). Hepatoprotective property of ethanolic and aqueous extracts of *Telfairia occidentalis* (Fluted Pumpkin) leaves against garlic-induced oxidative stress. *Journal of Medicinal Food. 8(4):* 560-563.

OECD. (2001). Guidelines for testing of chemicals. Acute oral toxicities up and down procedure. 425: 1-26. Retrieved on 23th February, 2011 from www.oecd.org/dataoecd/17/51/1948378.pdf.

Ojewole, O. A., Tariro, M., Witness, D. H., Chiwororo, A. & Owira, M. (2010). *Sclerocarya birrea* (A. Rich): A Review of its phytochemistry, pharmacology and toxicology and its ethnomedicinal uses. *Journal of Ethnopharmacology 24:* 633-639.

Rec, GSCC. (1972). Serum alkaline phosphatase. *Journal of Clinical Chemistry and Clinical Biology 10*: 182.

Reitman, N. R. & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic. *American Journal of Clinical Pathology 28(1)*: 56-63.

Speech, H. J. & Liehr, H. (1983). Of what value are SGOT/SGPT, GGT/AP and IgA ratios in the differential diagnosis of advanced liver diseases? *Z. Gastroenterol. 21*: 89-96.

The Rat Fan Club (2010). Normal Laboratory values. Retrieved on 23th February, 2011 from www.ratfanclub.org/values.html.

Wright, P. J & Plummer, D. T. (1974). The use of urinary enzyme measurement to detect renal changes caused by nephrotoxic compounds. *Biochemistry and Pharmacology. 12*: 65.

Wybenga, D. R. D., Glorgio, J. & Pileggy, V. J. (1971). Determination of serum urea by Diacetyl monoxime method. *Journal of Clinical Chemistry 17*: 891-895.

Zimmerman, M. (1983). Ethical guidelines for investigation of experimental pain in conscious animal. *Pain 16(2)*: 109-110.