

## Acute and Subchronic Toxicity Studies of *Sclerocarya birrea* Peels Extract in Rats

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### Abstract

*Sclerocarya birrea* fruits are widely eaten in developing countries especially in rural areas where they serve as nutritional supplements. However, they may also contain phyto-toxins which may affect the normal functioning of the body. Acute toxicity study was performed by a single oral administration of a dose of 3000 mg/kg body weight. Sub chronic toxicity evaluation was conducted by oral feeding of the rats with the peels extract daily at doses of 1000, 2000, 3000 and 4000 mg/kg body weight for 28 days. The results of acute toxicity show no mortality and general behaviour changes. The lethal dosage (LD<sub>50</sub>) was greater than 3000 mg/kg body weight. Rats fed with 1000, 2000 and 3000mg/kg body weight of the extract have higher body weights throughout the period of treatment but not significantly ( $p>0.05$ ) different from the control group. Rats fed with 4000mg/kg body weight have significantly ( $p<0.05$ ) lower body weight throughout the period of treatment. Significantly ( $p<0.05$ ) higher serum total proteins, albumin, bilirubin, transaminases, creatinine, urea, uric acid and electrolytes were recorded in rats fed with 3000 to 4000 mg/kg body weight of the extract, suggesting liver and kidney toxicities. Therefore, the peels extract of *S. birrea* may be relatively toxic at doses of 3000 and 4000 mg/kg body weight. Further studies are required for isolation and characterization of the toxic compounds.

**Keywords:** *Sclerocarya birrea*; Hepatorenal indices; Toxicity; Peels; Albino rats.

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## 1. Introduction

A significant proportion of indigenous fruits in the West African sub region are seasonal forest products harvested for consumption on site or transported to other areas particularly urban centers for sale [1]. 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 a good proportion of the populace [2]. One of such tree is *Sclerocarya birrea* (*Anacardiaceae*) whose botanical description was reported by Moganedi *et al.* [3], Hillman *et al.* [4] and Ojewole *et al.* [5]. The tree bears pale yellow fruits (Plate 1) with a plain tough peel and fibrous juicy sweet-sour mucilaginous flesh [4]. The kernel of the fruits is widely eaten in developing countries not only during period of food scarcity but also during period of abundance, perhaps due to cultural acceptance [5].

Glew *et al.* [7] and Moganedi *et al.* [8] reported that *S. birrea* seed kernel is edible and rich in oil (50 – 60%) and protein (28 – 36%). On dry weight basis, *S. birrea* seed kernels contained appreciable amounts of copper (24.8 µg/g), magnesium (4210 µg/g) and zinc (62.4 µg/g). The fruit juice (on dry weight) was reported to contain 3.31% crude protein and 90.35% available carbohydrate [9].

*Sclerocarya birrea* tree possess medicinal properties. Ojewole *et al.* [5] reported that *Sclerocarya birrea* stem bark aqueous extract is safe, and or non-toxic to mice and possess analgesic, anti-inflammatory and anti-diabetic properties [5] noted that polar extracts of *Sclerocarya birrea* leaf and stem bark (inner bark) have antibacterial and antifungal activities. The fruit also contains important phytochemicals such as tannins, saponins, flavonoids and glycosides [5]. Antinutritional composition and toxicological studies of *S. birrea* fruit juice have also been reported [9,10].

Even though wild plants are important sources of nutrients and phytochemicals that play a role in protecting against conditions such as cardiovascular disease and cancer, they also contain other compounds that cause hepatic/tubular necrosis [11]. To our knowledge, the toxicity study of the peels of *S. birrea* in Northern Nigeria is scanty. Therefore, this paper reports the evaluation of the safety of peels extract of the fruits by acute and sub chronic oral administration in rats.



Plate 1: Ripe fruits of *Sclerocarya birrea* [6]

## 2. Materials and Methods

### 2.1. Sampling and Sample Treatment

Two kilogrammes (2 kg) of matured and ripe *Sclerocarya birrea* fruits were collected in June, 2010 from More area, Kware local government area of Sokoto State, Nigeria. Five trees were randomly selected and the fruits were collected from different branches and representative sample taken using alternate shovel method as described by

[12]. The juice, peels and seeds were separated by squeezing ripe fruits. The peels were air dried and pulverized to fine powder using pestle and mortar, sieved to pass through 80-mesh sieve and stored in air tight paper bags inside a desiccator. The dried powder was used to prepare the extracts.

## 2.2. Preparation of the Extracts

Fifty grammes (50g) 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 and then reconstituted with distilled water and used for toxicity studies.

$$\% \text{ Extract} = \frac{\text{Weight of extract}}{\text{Sample weight}} \times 100$$

## 2.3. Toxicological Studies

### 2.3.1. Animals

Albino rats (males and females) weighing 165 to 300g were purchased from the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept at the animal house of the department in a wire mesh cages. They were 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 ethical guidelines reported by Zimmerman [13] and in accordance with U. S. guidelines as contained in the National Institute of Health guide for the care and use of laboratory animals [14].

### 2.3.2. Acute Toxicity Studies (Determination of LD<sub>50</sub>)

A 1cm<sup>3</sup> 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. The toxicological effect was assessed on the basis of mortality, which was expressed as LD<sub>50</sub> and calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development [15].

### 2.3.3. Sub-acute Toxicity Studies

A total of thirty albino rats were divided into five groups of six rats each. Animal in groups 2, 3, 4 and 5 were orally administered 1cm<sup>3</sup> 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.

### 2.3.4. Blood Sample and Clinical Chemistry

The animals were sacrificed 24 hours after the last treatment, blood samples were collected allowed to clot and then centrifuged at 3000rpm for 10 min to obtain sera. The biochemical parameters, serum total protein (TP) and total albumin (TA) were determined by the method of Cheesbrough [16]. Total bilirubin (TB) was analyzed (Randox kit) using the method reported by Hassan *et al.* [17]. Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were done using Randox assay kit by standard method of Reitman and Frankel [18]. Alkaline phosphatase (ALP) was estimated by the Randox (colorimetric) method of Rec [19]. Serum electrolyte and

creatinine (colorimetric with deproteinization) were performed by the methods of Henry [20]. Urea was analyzed using method of Wybenga *et al.* [21]. Uric acid was estimated using the method of Morin and Prox [22].

## 2.4. Statistical analysis

The data obtained was statistically analyzed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the results were reported as mean  $\pm$  standard deviation of the values. Significant difference between the means was determined using LSD at 5% level.

## 3. Results And Discussion

### 3.1. Results

#### 3.1.1. The Percentage Yield

The percentage yield of the extract was 20g/100g of the peels which is an indication that the peels contain some important nutritional or medicinal phytochemicals.

#### 3.1.2. Body Weight

Rats fed 1000 and 2000 mg/kg body weight of the extracts show higher body weights throughout the period of treatment but not significantly ( $p > 0.05$ ) different from the control group. Significantly ( $p < 0.05$ ) lower body weights were recorded in rats administered 4000mg/kg body throughout the period of treatment compared with the control group of the animals (Table 1).

Table 1: Effects of Sub-Chronic administration of Doses of *Sclerocarya birrea* Peels (extracts) on body weights of Experimental Rats

Dose(mg/Kg Body weight )	Initial weight	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
0.00(control)	283.53 $\pm$ 3.98	287.60 $\pm$ 1.28	296.99 $\pm$ 1.63	300.31 $\pm$ 0.17	300.46 $\pm$ 1.30
1000	222.93 $\pm$ 2.10	231.63 $\pm$ 2.79	252.32 $\pm$ 1.27	263.15 $\pm$ 1.32	284.80 $\pm$ 1.69
2000	261.57 $\pm$ 0.69	263.25 $\pm$ 1.31	271.16 $\pm$ 1.02	272.37 $\pm$ 1.04	276.12 $\pm$ 0.90
3000	211.40 $\pm$ 3.44	212.83 $\pm$ 1.85	215.03 $\pm$ 1.24	217.92 $\pm$ 3.26	218.80 $\pm$ 3.80
4000	285.04 $\pm$ 3.72	283.18 $\pm$ 2.69*	281.92 $\pm$ 1.88*	280.27 $\pm$ 1.38*	279.15 $\pm$ 0.84*

Values are mean  $\pm$  standard deviation

\*= Significantly different from the control ( $P < 0.05$ ) using one way analysis of variance (6)

#### 3.1.3. Acute Toxicity ( $LD_{50}$ )

Acute toxicity at 3000mg/kg body weight of the peels extract produced no mortality after 48 hrs of observation, indicating that the mean ( $LD_{50}$ ) of the extract is greater than 3000mg/kg body weight.

#### 3.1.4. Sub-acute Toxicity

There was a significant ( $p < 0.05$ ) changes in the liver function indices (Table 2) and kidney function indices (Table 3) at higher doses of the extract compared with the control.

Table 2: Liver Function Indices in Rats Administered Doses of *Sclerocarya birrea* Peels (extracts)

Dose (mg/Kg)	TP (g/dl)	ALB (g/dl)	TB (g/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
0.00	6.24 $\pm$ 2.63	5.31 $\pm$ 1.00	5.29 $\pm$ 1.15	15.94 $\pm$ 3.14	9.88 $\pm$ 2.08	98.48 $\pm$ 2.08
1000	6.01 $\pm$ 3.21	5.28 $\pm$ 1.53	6.12 $\pm$ 0.11	16.25 $\pm$ 2.80	10.51 $\pm$ 1.52	99.45 $\pm$ 1.52
2000	5.84 $\pm$ 3.51	5.23 $\pm$ 2.08	6.32 $\pm$ 2.51	17.07 $\pm$ 0.32	12.27 $\pm$ 3.01	104.58 $\pm$ 3.01
3000	5.64 $\pm$ 2.10	5.20 $\pm$ 1.15	6.45 $\pm$ 1.00*	17.42 $\pm$ 0.13	13.57 $\pm$ 1.00*	108.22 $\pm$ 1.10*
4000	5.53 $\pm$ 2.00	5.04 $\pm$ 2.00	6.81 $\pm$ 1.20*	18.73 $\pm$ 1.80*	14.18 $\pm$ 3.10*	110.25 $\pm$ 3.10*

Values are mean  $\pm$  standard deviation. TP=Total protein. ALB=Albumin. TB=Total bilirubin. ALP=Alkaline phosphatase. ALT=Alanine aminotransferase. AST=Aspartate aminotransferase. \*= Significantly different from the control ( $P < 0.05$ ) using one way analysis of variance (6)

Table3: Kidney Function Indices in Rats Administered Doses of with *Sclerocarya birrea* Peels (extracts).

Dose (mg/Kg)	Creatinine ( $\mu\text{mol/L}$ )	Urea ( $\text{mmol/L}$ )	Uric acid ( $\mu\text{mol/L}$ )	Sodium (ppm)	Potassium (ppm)
0.00(Control)	121.50 $\pm$ 0.60	2.42 $\pm$ 1.81	204.76 $\pm$ 2.97	30.16 $\pm$ 0.12	1.49 $\pm$ 0.21
1000	124.19 $\pm$ 1.87	2.67 $\pm$ 1.01	214.43 $\pm$ 3.99	28.35 $\pm$ 0.15	1.99 $\pm$ 1.00
2000	140.70 $\pm$ 0.82	3.80 $\pm$ 0.51	274.76 $\pm$ 5.49	27.17 $\pm$ 0.29	2.25 $\pm$ 1.20
3000	170.83 $\pm$ 0.76*	4.36 $\pm$ 2.11*	305.12 $\pm$ 5.04*	24.33 $\pm$ 0.19*	3.18 $\pm$ 2.00*
4000	198.58 $\pm$ 0.64*	5.55 $\pm$ 2.20*	422.30 $\pm$ 2.78*	18.24 $\pm$ 4.04*	4.21 $\pm$ 2.41*

Values are mean  $\pm$  standard deviation. \* = Significantly different from the control ( $P < 0.05$ ) using one way analysis of variance (6)

### 3.2. Discussion

#### 3.2.1. Body Weight

The reduction in weight could be due to reduced fluid and water intake, which may be secondary to a feeling of fullness and loss of appetite after administration of the extract [23]. The reduction in the body weight could be due to high tannins content which might hinder protein bioavailability [24].

#### 3.2.2. Acute Toxicity ( $LD_{50}$ )

The mean lethal dose ( $LD_{50}$ ) 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 [17]. The very high value of the  $LD_{50}$  indicated that the extract of the peel is practically non-toxic.

#### 3.2.3. Sub-acute Toxicity

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 [25]. 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 inhibit protein synthesis in the rats which may be due to high tannin contents which was reported present [5], 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 [26]. The water solubility of bilirubin allows 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. The significant ( $p < 0.05$ ) increase in the total bilirubin of rats fed with 3000 and 4000mg/kg body weight is an indication that the extract interfere with the metabolism of bilirubin in the liver [26].

ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum[27]. It is often employed to assess the integrity of plasma membrane and endoplasmic reticulum [28]. The significant ( $p < 0.05$ ) increase in the serum ALP may be attributed to renal or intestinal damage, biliary track damage and inflammation [26]. The increase could be attributed to enzyme activation by the phytochemical constituents of the peels specifically saponins which are known to have hypocholesterolemic activities which may aid in lessening the metabolic burden in the liver [28].

The ALT and AST are liver specific enzyme markers of necrotic injury and cholestasis [29]. The significant increase at high doses could be due to damage to the hepatic cell or heart attack [30] and may have been induced by some phytochemicals of the peels extract.

Serum urea, uric acid, creatinine and electrolytes are markers of damage to renal function [31]. The significant increase ( $p < 0.05$ ) in serum urea may be due to increased protein catabolism or renal dysfunction [32]. 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 [17]. The observed changes in biochemical indices of renal function may have been induced by the phytochemical constituents of the peels extract.

#### 4. Conclusion

The results revealed that the peels extract may have effect on liver and kidney functions at high doses and should be used cautiously. Studies on structural elucidation of the active components and mechanism (s) of toxicity of the extract are therefore recommended.

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