Acute and Sub-chronic Toxicity Studies on Methanol Stem Bark Extract of Frankincense Tree (*Boswellia dalzielii*) and Leaves Extract of Kenaf (*Hibiscus cannabinus*)

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**Author’s contribution**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

**Aim:** Frankincense tree (*Boswellia dalzielii*) and Kenaf (*H. cannabinus*) are plants abundantly found in north-western Nigeria. These plants are very popular among the locals as potent sources of ethno medicine. The present study investigates the oral acute toxicity potentials of methanolic stem bark extract of frankincense tree and Kenaf leaves, as well as sub-chronic toxicity potentials of the plants extracts on the kidney and liver of Albino rats.  
**Study Design:** Laboratory-experimental design was used for this study.  
**Place and Duration of Study:** This study was carried out between September 2019 and November 2019 at Biochemistry laboratory, Sokoto State University, Sokoto, Nigeria.  
**Methodology:** For the oral acute toxicity study, the revised “Up and Down” test (Limit Dose Test) was used to determine the LD$_{50}$ of the extracts. For sub-chronic toxicity study, twenty albino rats were used for each plant, and were divided into four groups of five animals each. Group I (control), Group II (received 200 mg extract/kg body weight), Group III (received 400 mg extract/kg body weight) and Group IV (received 800 mg extract/kg body weight). All administrations were given

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1. INTRODUCTION

Frankincense oil is prepared from aromatic hardened gum resins obtained by tapping Boswellia trees. One of the main components of frankincense oil is boswellic acid, a component known to have anti-neoplastic properties [1]. The dependence of human population on plants as medicine in the management of several illness is common among the developing countries because of its affordability and availability compared to modern medicine. This has resulted to increased reports of their suspected toxicity and adverse events. Such unwanted or adverse effects can be due to reactions occurring as a result of overdose, over duration, tolerance, dependence-addiction, hypersensitivity and allergic reactions as well as mid-term and long-term toxic reactions. It is such reactions that make toxicity evaluation necessary. Mechanisms of toxicity can be present in several ways. One of them is on target whereby, the toxicant binds to a targeted receptor unintended resulting to unfavourable reaction. Off target is another mechanism whereby the toxicant binds to unintended receptor resulting to unintended reaction [2]. Nontoxic compounds can be metabolized to toxic end products in the body organs, the toxicant metabolites can be excreted by the body, the accumulated toxicant or its products of metabolism can react with DNA resulting to DNA adducts which are mutagenic and can lead to cancer [3]. On the other hand, proteins adduct formed may cause abnormal immune response that can lead to cellular damage [4]. Moreover, toxic medicinal plants may impair the oxidative protective mechanisms leading to cell death through apoptosis or necrosis [5].

Studies of acute toxicity however tends to establish the dose-dependent unwanted (adverse) effect which may take place or the degree of safety of a pharmacological agent. The assessment of the lethal dose (LD₅₀) (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity. Apart from mortality, other biological effects and the time of onset, duration and degree of recovery on survived animals, are also important in acute toxicity evaluation.

A large number of modern medicines are produced from the natural sources. Out of them many preparations rely on the use of agents isolated from traditional medicines [6]. According to OECD guidelines, in order to ascertain the protection and effectiveness of a new drug, toxicological studies are extremely significant in animals like mice, rat, guinea pig, dog, rabbit, monkey etc [7]. Frankincense oil appears to distinguish cancerous from normal bladder cells and suppress cancer cell viability. Microarray and bioinformatics analysis proposed multiple pathways that can be activated by frankincense oil to induce bladder cancer cell death. Frankincense oil might represent an alternative intravesical agent for bladder cancer treatment [8].

Moreover, it is known that the consumption of medicinal plants without evaluating their efficacy and safety can result in unexpected or toxic effects that may affect the physiology of different organs in the human body. Liver and kidney are the first targets in toxicological evaluation because, they are involved in the metabolism and excretion of chemical compounds. Renal damage has been associated with the use of medicinal plants in the treatment of various diseases [9].

This study tends to identify the level of toxicity of Frankincence tree (Boswellia dalzielii) and Kenaf leaves, given the recent observation towards using these plants by the herbalist to treat illnesses, such as infection [10], wound healing and many others. kenaf leaves was applied for treatment of Guinea worms and the stem bark has been used for anaemia treatment in Africa [11]. In ayurvedic medicine, the kenaf

orally for 28 days. Liver and kidney markers were determined using standard methods.

**Result:** The oral acute toxicity test of the plant extracts at 3000 mg/kg body weight showed no mortality for 24 hours and subsequent 14 days of administration. LD₅₀ for both plants is therefore greater than 3000 mg/kg. The result shows no significant differences (p > 0.05) on liver and kidney function biomarkers investigated when Group II, III and IV are compared with control.

**Conclusion:** This suggests that Frankincense stem bark and kenaf leaves extracts may be safe in rats at doses less than or equal 3000 mg/kg.

**Keywords:** Frankincense tree; kenaf; toxicity; kidney and liver.
leaves are used for bilious, blood, diabetes, coughs and throat disorder [11,12]. It is considered as an important fiber crop and exploited for its fibrous stem with numerous industrial applications (Paper and pulp, fabrics, textiles, biocomposites, insulation mats, absorption material, animal bedding e.t.c). Kenaf has been reported to exhibit various properties associated with anodynes, aperitifs, aphrodisiacs, anti-inflammatory medications and antioxidants for leaf and seed, and It has also been related to weight gain anemia and fatigue [12]. Therefore, there is need to provide scientific bases to justify the safety status of these medicinal plants.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The stem bark of Boswellia dalzielii was collected from Zuru local government area of Kebbi State, Nigeria, while kenaf leaf was collected in Modurawa, one of the villages in Wamako local government area of Sokoto state, Nigeria. The stem bark and the leaves were washed with clean water to remove dust particles, and were air dried under shade.

2.2 Preparation of Extracts

Pestle and mortar was used for grinding the plant to powder form. 70 g of powdered Boswellia dalzielii stem bark and 30 g powdered kenaf leaves were weighed using a weighing balance and transferred into two different 1000 ml beakers. 800 ml of 70% methanol was added to the beaker containing stem bark and 400 ml of the same methanol was added into the beaker containing kenaf powder and left for 72 hours after which the extracts were filtered using whatman filter paper. The filtrates were dried using a hot air oven at reduced temperature (45°C) to produce the extracts which were then used for the analysis.

2.3 Experimental Animals

Twenty five (25) Wister albino rats 8 to 12 weeks old were used for this study for each plant extract. Five (5) rats for acute toxicity study and twenty (20) rats for sub-chronic toxicity study. The animals were housed in wired cages made by the department of vetenary science, Usman Danfodiyo University Sokoto, city campus Sokoto, Nigeria. The animals were kept at the biochemistry department laboratory under room temperature (25°C) and relative humidity of between 30% to 50% during the experiment [13].

2.4 Acute Toxicity Study

The limit test dose up and down procedure of organization for economic and cultural Development [13] was employed. 3000mg/kg body weight of each plant extract was administered to 5 rats each in a single dose and were observed every fifteen minutes for the first one hour and thereafter, every 1 hour for 3 hours and up to 48 hours for toxicity signs (such as weakness, loss of appetite, difficulty in movement) and mortality. Surviving animals were observed for two week.

2.5 Sub-Chronic Toxicity Study

For each plant extract, twenty (20) healthy albino rats of different sexes were divided into four groups, containing five (5) rats as follows:

- Group I : (Control); receives 2 ml of normal saline
- Group II : receives 200 mg/kg body weight of the extract per day
- Group III : receives 400 mg/kg body weight of the extract per day
- Group IV : receives 800 mg/kg body weight body weight of the extract per day

The experiment was conducted within a period of 28 days as per OECD guidelines [14] and the extracts were administered orally. On the 29th day the experimental animals were sacrificed under chloroform anesthesia and blood samples were taken by cardiac puncture. The samples were spun using centrifuge to obtain serum. The serum samples obtained were kept at 0 °C for biochemical test.

2.6 Biochemical Tests

2.6.1 Liver function tests

- Serum AST activity was measured by method described by Reitman and Frankel [15].
- Serum ALT activity was measured by method described by Reitman and Frankel [15].
- Serum Abumin level was measured using Bromocresol Green Method described by Spencer and Price [16].
- Serum Total protein level was measured using Biuret Method described by Doumas [17].
- Serum total bilirubin level was measured using method described by Jendrassik and Grof [18].
2.6.2 Kidney function tests

Serum urea level was investigated using Urease-Berthelot colorimetric method of Fawcett and Scott [19].

Serum bicarbonate was estimated using a titrimetric method [20].

Serum Creatinine level was measured by Jafee’s method and serum Uric acid level was measured using method described by Trinder [21].

2.7 Statistical Analysis

Data obtained from estimations was expressed as mean ± standard error of mean, and was analyzed statistically using one way analysis of variance, ANOVA, using Graph pad instat software (version 5 San Diego, USA). Results were considered statistically significant at p<0.05.

3. RESULTS AND DISCUSSION

The study was designed to investigate the safety status of stem bark extract of Frankincense tree and leaves extract of Kenaf. From the test carried out, the methalonic extracts of the two plants showed no sign of toxic symptoms or mortality at 3000 mg/kg body weight of the extracts (Table 1). This result is similar to the Finding of [22] where aqueous root extract of P. thonningiiischum was used, in which result indicated that the LD<sub>50</sub> is greater than 3000mg/kg of the Extract.

The Result in Tables 2 and 3 shows no significant difference (p>0.05) between the mean values of liver function biomarkers of group II, III and IV treated with the methanolic stem bark extract of Frankincense tree and leaves extract of Kenaf respectively when compared to the control group.

The Result in Table 4 shows no significant difference (p>0.05) between the mean values of kidney function biomarkers of group II, III and IV treated with the methanolic stem bark extract of Frankincense tree when compared to the control group.

The Result in Table 5 shows no significant difference (p>0.05) between the mean values of kidney function biomarkers of group II, III and IV treated with the methanolic leaves extract of Kenaf when compared to the control group with the exception of serum creatinine. There is significant difference (p<0.05) in the mean value of serum creatinine of group IV when compared to control.

This result is in agreement with the finding of [23] in which the methanolic leaf extract of Cassia singuena F.(Fresen) was investigated in Wister rats. There was is no significant difference between the mean values of the treated groups compared with the control group.

Table 1. Record of mortality in the oral acute toxicity study on methanolic stem bark extract of Frankincense tree and leaves extract of Kenaf

| Plant extract                | Dose (mg/kg) | Mortality |
|-----------------------------|-------------|-----------|
| Frankincense tree (B. dazielli) | 3000       | 0/5       |
| Kenaf (H. cannabinus)       | 3000       | 0/5       |
| Number of mortality in the group= 0, Number of rats in the group is =5 |

Table 2. Serum levels of liver function biomarkers of rats treated with stem bark methanolic extract of Frankincense tree (B. dazielli)

| Analytes                  | Groups |
|---------------------------|--------|
|                           | I      | II     | III    | IV     |
| AST(IU/L)                 | 24.75±0.59 | 38.75±1.82 | 34.75±2.1 | 43.50±1.28 |
| ALT(IU/L)                 | 22.75±0.79 | 28.00±0.33 | 21.75±0.01 | 27.00±0.72 |
| Total protein (g/dl)      | 5.65±0.45 | 4.85±1.00 | 6.45±0.49 | 6.17±0.06 |
| Albumin (g/dl)            | 3.27±0.17 | 3.25±0.16 | 2.87±0.06 | 3.52±0.50 |
| Total bilirubin (mg/dl)   | 0.65±0.12 | 0.70±0.08 | 0.69±0.08 | 0.66±0.66 |
| Direct bilirubin (mg/dl)  | 0.18±0.05 | 0.19±0.03 | 0.25±0.00 | 0.18±0.02 |

Legend: Values are expressed as Mean ± SEM. There is no significant difference between the mean values of the groups in a row (p>0.05)
Table 3. Serum levels of liver function biomarkers of rats treated with methanolic extract of kenaf leaves (H. cannabinus)

| Analytes             | Groups |
|----------------------|--------|
|                      | I      | II      | III     | IV      |
| AST(IU/L)            | 15.75±4.23 | 38.25±19.21 | 39.75±7.27 | 41.25±8.12 |
| ALT(IU/L)            | 17.00±1.92 | 20.50±2.84  | 23.00±3.08  | 23.75±4.97  |
| Total protein(g/dl)  | 3.85±0.65  | 4.48±0.43   | 5.45±1.34   | 5.85±1.17   |
| Albumin (g/dl)       | 3.05±0.25   | 3.55±0.13   | 3.58±0.40   | 3.93±0.65   |
| Total bilirubin(mg/dl)| 0.35±0.07 | 0.55±0.16   | 0.64±0.03   | 0.70±0.06   |
| Direct bilirubin(mg/dl)| 0.17±0.02 | 0.21±0.06   | 0.25±0.07   | 0.33±0.05   |

Legend: Values are expressed as Mean ± SEM. There is no significant difference between the mean values of the groups in a row (p>0.05).

Table 4. Serum levels of kidney function biomarkers in rats treated with stem bark methanolic extract of Frankincense tree (B. dazielli)

| Analytes              | Groups |
|-----------------------|--------|
|                      | I      | II      | III     | IV      |
| Urea (mg/dl)         | 34.75±0.59 | 29.25±0.59 | 35.50±1.72 | 31.00±0.79 |
| Creatinine (mg/dl)   | 7.00±0.57  | 6.25±0.47  | 6.50±2.60  | 5.75±0.47  |
| Uric acid (mg/dl)    | 4.45 ± 1.02 | 4.15 ± 1.62 | 3.02±0.54  | 4.35±2.32  |
| Bicarbonate (mEq/l)  | 23.5 ± 0.95 | 22.5 ± 0.50 | 21.5 ± 0.64 | 23.75±0.75 |

Legend: Values are expressed as Mean ± SEM. There is no significant difference between the mean values of the groups in a row (p>0.05).

Table 5. Serum levels of kidney function biomarkers in rats treated with methanolic extract of kenaf leaves (H. cannabinus)

| Analytes             | Groups |
|----------------------|--------|
|                      | I      | II      | III     | IV      |
| Urea (mg/dl)         | 33.50±4.09 | 36.25±4.80 | 37.25±5.34 | 44.75±4.95 |
| Creatinine (mg/dl)   | 7.00±0.57  | 6.75±0.48a  | 8.00±0.71  | 9.50±6.45b  |
| Uric acid (mg/dl)    | 1.60 ± 0.45 | 1.80 ± 0.50 | 2.00±0.75  | 2.30±0.47   |
| Bicarbonate (mEq/l)  | 24.00 ± 0.86 | 24.00 ± 1.08 | 23.5 ± 1.04 | 24.75±1.12 |

Legend: Values are expressed as Mean ± SEM. Means with different superscript letters in a row are significantly different (p<0.05).

4. CONCLUSION

The methanolic stem bark extract of Frankincense tree (B. dazielli) and leaves extract of kenaf (H. cannabinus) have LD₉₀ greater than 3000 mg/kg and do not significantly elevate the liver and kidney functions biomarkers investigated in albino rats at the tested doses. Therefore, these plants extracts may be safe at doses less than or equal to 3000 mg/kg.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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