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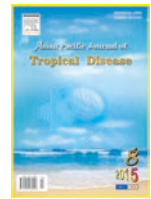
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### Alteration in biochemical indices following chronic administration of methanolic extract of Nigeria bee propolis in Wistar rats

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#### ABSTRACT

**Objective:** To determine the noxious attribute of methanol extract of Nigeria bee propolis at 300 and 600 mg/kg body weight in rats.

**Methods:** Healthy Wistar rats were indiscriminately assigned into three groups (A-C) of five rats each. The control rats (Group A) were orally given 0.5 mL of dimethyl sulfoxide daily for 21 days while rats in Groups B and C were administered the same 0.5 mL dimethyl sulfoxide containing 300 and 600 mg/kg body weight methanol extract of bee propolis respectively for 21 days.

**Results:** The bee propolis produced no significant ( $P > 0.05$ ) changes in the level of serum potassium ions, alkaline phosphatase and alanine aminotransferase activities whereas the level of total protein urea, creatinine and sodium ions decreased significantly ( $P < 0.05$ ). The activities of serum aspartate aminotransferase and the levels of serum potassium ions were raised significantly ( $P < 0.05$ ) during the 21 days of study.

**Conclusions:** The alteration in some of the biochemical indices of toxicity investigated, suggested a compromised functional integrity of the liver and kidney of the animals. Therefore, prolonged administration of the methanol extract of Nigeria bee propolis at 300 and 600 mg/kg body weight could be considered unsafe for therapeutic purpose and therefore should be taken with cautions when necessary.

## 1. Introduction

Propolis is a sticky honeybee resinous product with a wide array of biological application. It is produced by the honeybees to shut the cracks, and stabilize the temperature and moisture throughout the year in the hive. Phytochemistry study on bee propolis revealed varying composition ranging from pollens (5%), waxes (30%), plant resins (50%), essential and aromatic oils (10%), while other organic substances make up the remaining 5%. Bee propolis has been documented for its immunomodulatory, anti-inflammatory, bacteriostatic, hepatoprotective, anti-tumoral, anti-oxidative, hypotensive effects[1], bactericidal agent and for the treatments of atherosclerosis among many other uses[2]. Propolis has also

been reported to be used traditionally for the treatments of colds, rheumatism, heart disease, wounds, sprains, diabetes, ulcers[3] and dental caries[4]. Bee propolis is therefore, of high pharmaceutical claim and an interesting subject of study.

Since bee propolis has been used in traditional medicine since prehistoric times and it's now known to be a natural medicine with many beneficial effects, the present study attempts to study the implications of its administration on some serum biochemical parameters that play significant roles in determining health status of the animal. Many enzymes such as transaminase and phosphatase are detected in the serum as a result of their leakages from a damaged tissue into the body fluid[5]. Investigating the levels/activities of these biomarkers in serum during clinical diagnosis and toxicity studies will therefore, give valuable information on the functionality of the organs. Similarly, measurements of the levels/concentration of excretory metabolites such as creatine,  $K^+$ ,  $Na^+$ ,  $PO_4^{3-}$ , bicarbonate and urea can be used to evaluate the integrity of kidney[6]. Thus, these parameters were used to evaluate the toxicity and safety of bee propolis in this research.

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## 2. Materials and methods

### 2.1. Experimental animals

A total of 15 Wistar rats weighting ( $172.78 \pm 7.90$ ) g were procured from the Small Animal Breeding Division of Biochemistry Department, Federal University of Technology Minna. The rodents were conveniently accommodated under appropriate environmental conditions [12 h light and 12 h dark; temperature: ( $22 \pm 3$ ) °C; humidity: 40%–45%]. The rats were fed Bendel feeds and flour mills, (Edo State, Nigeria) and clean water *ad libitum*. The studies were carried out following the international accepted principles for laboratory animal use and care as described in the Canadian Council on Animals Care Guidelines and Protocol Review[7].

### 2.2. Collection of bee propolis

Propolis material was collected from an apiary in Akure, Ondo State, Nigeria. The identity of the propolis was authenticated by an entomologist in the Biological Sciences Department, Federal University of Technology, Minna, Nigeria. The propolis material was chopped into small pieces and air dried in the shade at room temperature for 2 weeks.

### 2.3. Chemical reagents and assay kits

The enzyme assay kits for transaminase [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], phosphatase [alkaline phosphatase (ALP)] and kits for total protein, potassium ion, bicarbonate, chlorides ion, sodium ion, creatinine and urea were procured from United Kingdom Laboratories Ltd. (Randox). Other analytical grade reagents used for this study were prepared in deionized/distilled water.

### 2.4. Preparation of propolis extract

Two hundreds grams of propolis pellets were percolated in 1 600 mL of absolute methanol and subsequently allowed to stand in the shade for 48 h before filtration, using filter paper (Whatman No. 1). The extract concentrate was stored in air-tight vials in the refrigerator at 4 °C, until needed for bio-assay.

### 2.5. Rat grouping and experimental protocol

Fifteen Wistar rats were divided into three groups of 5 rats each. The control rats (Group A) were given 0.5 mL of dimethyl sulfoxide. The extract treated rats (Groups B and C) were given equal volume of dimethyl sulfoxide containing 300 and 600 mg/kg body weight of methanol extracts of bee propolis respectively once daily for 21 days.

### 2.6. Collection of blood and preparation of serum

Collection of blood and preparation of serum was performed as described previously[8]. The animals were anaesthetized in ether vapour. After loosing their consciousness, the jugular veins were revealed by clearing the fur and skin area of the neck. The

veins were sharply cut with sterile blade and the bloods were collected into clean tube containing no anticoagulant. The bloods were allowed to clot at room temperature. The clotted blood was centrifuged for 15 min at 33.5 r/min and the cleared serum was pipette into a clean well labeled serum bottles. The serums were kept frozen and used within 12 h of preparation.

### 2.7. Determination of biochemical parameters

According to the method described by Tietz *et al.*[9], the activities of ALP were determined. The activities of transaminase (AST and ALT) were determined as described by Reitman and Frankel[10]. The level of serum total proteins was estimated using biuret in accordance with the method described by Burtis *et al.*[11]. The level of serum creatinine and urea were assayed as described by Blass *et al.*[12], and Veniamin and Vakirtzi-Lemonias[13], respectively, while the levels of  $\text{Na}^+$ ,  $\text{K}^+$ , bicarbonate and chloride ion were assayed by flame photometry[9].

### 2.8. Statistical analysis

Significant differences between the groups were determined using analysis of variance (ANOVA), while post test analysis was conducted with Duncan's multiple comparison tests. Values were calculated for each analysis as mean  $\pm$  SEM. Values were regarded as statistically significant when  $P < 0.05$ [14].

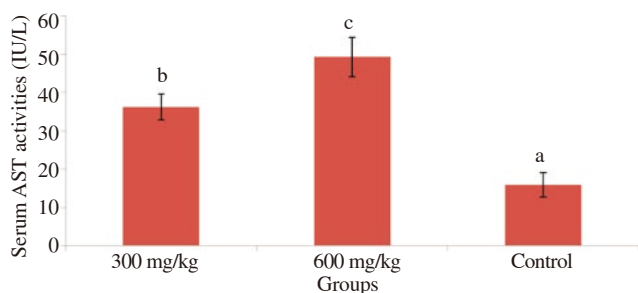
## 3. Results

### 3.1. Biomarker enzymes and total protein

The effects of 21 days daily administration of methanol extract of Nigerian bee propolis on some biomarker enzymes and total proteins in serum of rats are shown in Figures 1-4. Twenty one days administration (300 and 600 mg/kg body weight) of bee propolis produced a significant ( $P < 0.05$ ) dose dependent increase in serum AST activities (Figure 1), but did not produce any significant alteration ( $P > 0.05$ ) for serum ALT (Figure 2) and ALP (Figure 4) activities when compared with the corresponding activities from the control rats. The 21 days administration of the extract also resulted in significant dose dependent reduction in serum total protein of the rats ( $P < 0.05$ ) (Figure 3).

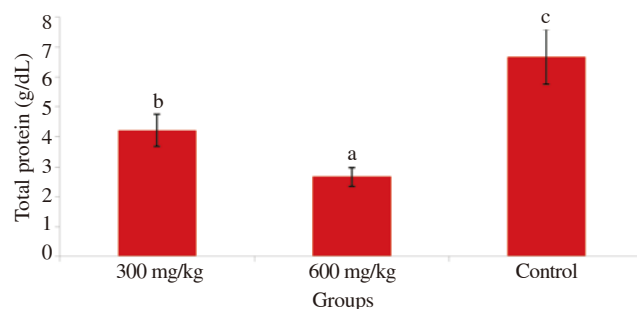
### 3.2. Serum electrolyte, urea and creatinine

Table 1 illustrates the changes in the urea, electrolyte and creatinine following 21 days administration of methanol extract of Nigerian bee propolis. The concentration of serum urea, creatinine and sodium ions in rats given bee propolis extract for 21 days decreased significantly ( $P < 0.05$ ), however 300 mg/kg of the extract did not alter the concentration of creatinine when compared with the normal value. The serum concentration of potassium ions was significantly raised in extract administered rat than the normal value. However, the serum concentration of bicarbonate and chlorides ion was not adversely effected by the 21 days of extract administration except a decrease in chloride that was observed at 300 mg/kg.



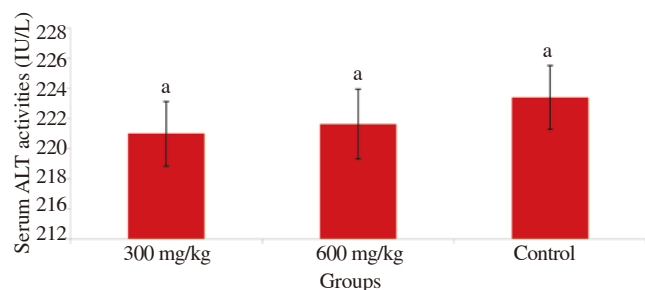
**Figure 1.** Effect of chronic administration of methanol extract of Nigerian bee propolis on serum AST activities.

Values are expressed as mean  $\pm$  SEM. Each mean is an average of five replicates ( $n = 5$ ). Columns carrying different superscripts differ significantly at  $P < 0.05$ .



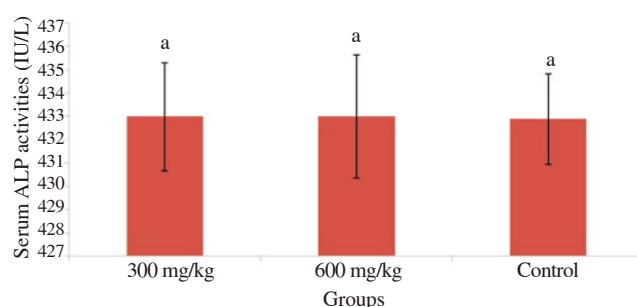
**Figure 3.** Effect of 21 days/daily administration of methanol extract of Nigerian bee propolis on serum total proteins.

Values are expressed as Mean  $\pm$  SEM. Each mean is an average of five replicates ( $n = 5$ ). Columns carrying different superscripts differ significantly at  $P < 0.05$ .



**Figure 2.** Effect of 21 days/daily administration of methanol extract of Nigerian bee propolis on serum ALT activities.

Values are expressed as mean  $\pm$  SEM. Each mean is an average of five replicates ( $n = 5$ ).



**Figure 4.** Effect of 21 days/daily administration of methanol extract of Nigerian bee propolis on ALP serum activities.

Values are expressed as mean  $\pm$  SEM. Each mean is an average of five replicates ( $n = 5$ ).

**Table 1**

Creatinine, urea and electrolyte in serum of rats administered methanol extract of Nigerian bee propolis.

Groups	Urea (mmol/L)	Creatinine ( $\mu$ mol/L)	Sodium (MEq/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
300 mg/kg	27.10 $\pm$ 2.01 <sup>a</sup>	0.51 $\pm$ 0.04 <sup>b</sup>	138.33 $\pm$ 0.10 <sup>b</sup>	7.06 $\pm$ 0.61 <sup>b</sup>	63.30 $\pm$ 2.21 <sup>a</sup>	42.33 $\pm$ 1.09 <sup>b</sup>
600 mg/kg	28.10 $\pm$ 1.90 <sup>a</sup>	0.43 $\pm$ 0.09 <sup>a</sup>	144.33 $\pm$ 4.19 <sup>ab</sup>	6.33 $\pm$ 0.19 <sup>ab</sup>	86.60 $\pm$ 2.35 <sup>b</sup>	42.31 $\pm$ 1.05 <sup>a</sup>
Control group	39.55 $\pm$ 3.01 <sup>b</sup>	0.54 $\pm$ 0.02 <sup>b</sup>	150.09 $\pm$ 4.12 <sup>b</sup>	5.50 $\pm$ 0.97 <sup>a</sup>	89.25 $\pm$ 3.54 <sup>b</sup>	48.50 $\pm$ 2.91 <sup>a</sup>

Values are mean  $\pm$  SEM of 4 determinations. Columns carrying different superscripts differ significantly at  $P < 0.05$ .

#### 4. Discussion

Evaluation of biochemical indices in serum and organs of animals has become the most valuable tools for assessing the integrity and functionality of organs as well as risk assessment, pathological condition and general health status of the body. Defeat in the activities of these biomarkers in serum and body tissues are to a sensible degree the detrimental effect of a drug or extract/compound under investigation[15]. Alkaline phosphatase has been widely use as biomarker enzyme for assessing the integrity of endoplasmic reticulum and plasma membrane. The observed non-significant difference from the control values in the activities of ALP after 21 days of propolis extract administration suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane has not been comprised. It also indicates that the extract did not inhibit or activate the activities of the enzyme molecule *in situ*[16].

Transaminase enzymes especially the AST and ALT are considered to be biomarkers of compromised liver activities and to a certain level can offer a quantitative measured of the extent of hepatocellular damage[17], the ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST[18]. Consequently, in the present work marked increase in AST activities observed after treatment with propolis may have occurred as a result

of metabolism of the extract by the liver and hepatic tissue turnover, as response by the body system towards overcoming stress induced by the test substance[19]. Such increase AST activities will negatively influence the metabolism of macromolecules, thus affecting adenosine triphosphate generation. The serum ALT is cytosolic in origin the non-significant change in the serum ALT activities following 21 days of the bee propoli could be translated to no outflow of this enzyme from the liver into the extracellular fluid and that the bee propolis has not compromised the integrity of liver with respect to ALT activities. Probably the methanol extract of bee propolis demonstrated selective toxicity on transaminase since only the activity of AST was altered in the rat's serum.

Kidney function parameters are valuable tools for assessing the integrity of various parts of the kidney[20]. The level of creatinine, electrolytes, urea, and serum total protein could also provide significant information regarding the influence of a drug/compound/extract on the glomerular and tubular region of the kidney[21]. The observed decrease in the level of serum total proteins indicates an impaired functional activity of the liver arising from the administration of the extract. The extract of propolis might have interfered with the balance in the order of synthesis and utilization of total protein, from the system of the rats. Such decrease could results into hydration with consequent effects on cellular homeostasis and

health status of the rats.

The noteworthy decrease in urea concentration observed in rats treated with bee propolis for 21 days could be attributed to renal dysfunction leading to excessive urea excretion from the kidney, or destruction of the urea cycle leading to a decrease urea production[22], while the increase in the level of creatinine observed at 600 mg/kg may be accredited to compromise functional integrity of the kidney. The bee propolis must have either altered the metabolism of creatinine in favored of increased synthesis or the tubular excretory function of the kidney has been impaired[23].

The significant decrease in the level of Na<sup>+</sup> concentrations following 21 days administration of the methanol extract of bee propolis could result from extreme loss of Na<sup>+</sup> pool body fluids, or increase production of mineralocorticoids such as of aldosterone which stimulate Na<sup>+</sup>/H<sup>+</sup> exchanger with consequent increase Na<sup>+</sup> reabsorption in the kidney tubules[21]. The hypokalaemia effect of the extracts could also be linked with the hypernatraemia observed in this study. These effects imply that normal functioning of kidney tubules as regard to these electrolytes has been altered.

The insignificant effect of bee propolis on the concentration of serum bicarbonate suggests that the propolis did not cause any adverse effect on excretion of this electrolyte by the kidney. Among all the biomarkers of kidney function investigated in this study, it is unclear why the methanol extract of bee propolis did not alter the level of only bicarbonate, it may, however be explained by selective toxicity attribute of natural product.

The alteration in some of the biochemical indices of toxicity investigated suggested a compromised functional integrity of the liver and kidney of the animals. Therefore, prolonged administration the methanol extract of Nigeria bee propolis at 300 and 600 mg/kg body weight could not be considered safe dose for therapeutic purpose and therefore should be taken with cautions when necessary.

### Conflict of interest statement

We declare that we have no conflict of interest.

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