The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 14(2)

Histological and Biochemical Evaluation of the Liver Following Oral Administration of *Breynia Nivosa* on Adult Male Wistar Rats

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ABSTRACT

Toxicity from herbal plants has been underestimated due to the perception that drugs from plants are absolutely safe. *Breynia nivosa* is a herbal plant known for its use in the treatment of tooth infection, toothaches and headaches. However, severe organ injury has been described after ingestion of variety of different herbal preparations. This led to the study on the histopathological changes associated with oral toxicity of ethanolic extract of *B. nivosa* on the liver. Twenty Wistar rat weighing averagely 200-250 g were divided into four groups of five rats each (n=5). Group A served as the control group while groups (B, C, D) were the experimental groups which received 200 mg/kg, 400 mg/kg and 600 mg/kg/bw of ethanolic leaf extract of *Breynia nivosa* respectively through orogastric intubation for 28 days. 24 hours after the last administration, the rats were anesthetized using 40 mg/kg ketamine intraperitoneally. The liver was dissected and processed for paraffin section fsor histological studies. Blood samples were collected prior to harvesting the organs through cillary puncture for determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline Phosphatase (ALP). Biochemical results of the liver enzymes showed significant increase in serum ALT, AST and ALP of the treated groups (54.53±1.06), (78.73±3.98) and (192.54±3.60) compared to the control (26.66±1.06), (56.23±1.40) and (64.84±12.96) respectively. Histological result of the treated groups showed hepatic necrosis, congestion of hepatic sinusoids and loss of hepatic cells compared to the control group. These results showed that oral administration of ethanolic extract of *B. nivosa* can be toxic to the liver.

Keywords: *Breynia nivosa*, hepatic cells, central vein, hepatotoxicity

INTRODUCTION

The development of new drugs from herbal plants requires toxicological screening which is important for the extension of its therapeutic potential. According to the United State Food and Drug Administration (FDA), it is expedient to evaluate new molecules for its toxicity potential and pharmacological activity in animals. The toxic effects of chemicals, food substances, pharmaceuticals, etc. have attained great significance in the 21st century[1].

The use of herbal plants in developing countries is now on the increase, which is affirmative to a report by the World Health Organisation directives resulting in preclinical and clinical studies that provided the scientific foundation for the efficacy of many plants used in traditional medicine[2].

About 80% of rural populations are dependent on medicinal plants for their primary health care and this has contributed to an effective source for both traditional and modern medicine[3].Toxicity from phytochemical compounds of these herbal plants has been ignored because of the understanding that these plants are safe[4]. Determination of the efficiency and safety of herbal medicine is necessary as people use them for self-medication. There is paucity of information on the pharmacology and toxicology for most of these herbal remedies[5].

Breynia nivosa (*B. nivosa*) is a shrub which is commonly called "ice plant or snow bush" because of its beautiful variegated foliage leaf, especially in winter season. It has round leaves with mottled, multi-colored variegated with white, green and red coloration leaves[6, 7]. The plant is a shrub which is about 2 m high and primarily used for foliage, and mainly domicile in villages and towns of South-east Nigeria. Leaves are simple, opposite with entire margin and ovate in shape[8]. In South-east Nigeria, the plant is locally called "ogwueze" in Igbo language and the leaf extract is used in traditional medicine in the treatment of headaches, toothaches and tooth infections while the stem is usually used as chewing sticks[9].

The liver is the largest internal organ, positioned in the right upper quadrant of the abdomen. It performs a wide range of function, including synthesis of bile, glycogen storage and clotting factor production. However, it is necessary for metabolism of drugs and exogenous toxins. Hepatotoxicity is a prevalent pathology that involves various disorders including hepatitis, steatosis, cirrhosis, apoptosis, oxidative stress, fibrosis and hepatocellular carcinoma[10]. However, liver damage due to natural, phytochemical toxins or drugs is common but rarely recognized[11]. Hence, the present study is an attempt to investigate the effects of ethanolic extracts of *Breynia nivosa* on the liver of adult male Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Twenty Wistar rats weighing 150-180 g were obtained from Iyke Animal Farm located at Okofia Nnewi Anambra State, Nigeria. They were bred in the Experimental House of Faculty of Basic Medical Science Chukwuemeka Odumegwu Ojukwu University under standard environmental conditions of temperature and humidity (25 ± 0.5°C) and 12 hour light/dark cycle. They were acclimatized for two weeks, having free access to water and standard pellet diet ad libitum after obtaining approval from the Departmental animal ethics committee.

Preparation and Ethanolic Leaf Extraction of *Breynia Nivosa*

Leaves of *Breynia nivosa* were dried in the shade for two weeks. The dried plants' materials were reduced to powder using mortar and pistle. The powdered material was soaked in 50% ethanol in a soxhlet extractor for 48 hr at 60°C. After extraction, the mixture was filtered and evaporated to dryness at 60°C using a rotary evaporator. The percentage yield was stored at 4°C in the refrigerator for future usage. The extract was dissolved in distilled water to give the final concentration of 200 mg/kg, 400 mg/kg, 600 mg/kg bw.

Subacute toxicity study

Twenty male adult Wistar rats (200-250 g) were divided into four groups of five animals each and were housed under the same conditions. Group A served as the normal control and received food and water *ad libtum* while groups B, C and D served as the experimental groups. The ethanolic extract of *B. nivosa* was administrated for 28 days at doses of 200, 400 and 600 mg/kg body weight to groups B, C and D respectively. Toxic manifestations and mortality were monitored daily till the end of the experimental period. 24 hours after the last administration, the rats were anesthetized using 40 mg/kg ketamine intraperitoneally. The liver was dissected and processed for paraffin section for histological studies. Blood samples were collected prior to harvesting the organs through cillary puncture for determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline Phosphatase (ALP).

Statistical Analysis

Data were analyzed and experimental values were expressed as the Mean ± SEM and P value was calculated using two-way analysis of variance (ANOVA) followed by *post hoc* multiple comparisons by using SPSS software version 21. $P \leq 0.05$ was considered statistically significant.

RESULTS

Effects of *Breynia nivosa* on liver enzymes

The effects of the extracts from leaves of *BreyniaNivosa* on the liver enzymes (AST, ALP & ALT) are presented in Table 1. The results indicate a significant increase in AST of 200mg/kg, 400mg/kg and 600 mg/kg treated groups (64.96±0.010), (73.13±2.66) and (78.73±3.98) compared with their control (56.23±1.40) respectively. There was significant increase in ALT of the treated animals compared with their control. Table 4.2 shows the effect of different doses of ethanolic leaf extract of *Breynia nivosa* at $p \leq 0.05$ was significant at (0.000). Table 4.3 compares different variables of extract dosage administered at different groups. The turkey HSD showed $p \leq 0.05$ was significant when comparing the control to the treated groups.

Table 1: AST, ALT, levels of rats treated with oral administration of ethanolic extract of *Breynia nivosa*

Groups	Dose (mg/kg)	ALT (u/L)	AST (u/L)	ALP (IU/L)
A (Control)	Nil	26.66±1.06	56.23±1.40	64.84±12.96
B	200	40.00±1.51	64.96±0.10	186.06±4.77
C	400	47.06±1.41	73.13±2.66	174.94±8.98
D	600	54.53±1.06	78.73±3.98	192.54±3.60

Values are expressed as Mean±SEM. Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)

The table above (Table 1) showed the descriptive statistics for different liver enzymes. The total mean and standard error of Mean of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with treatment groups of 200 mg/kg,400 mg/kg and 600 mg/kg dose of *B. nivosa* are given as (69.59 ±17.88), (23.06± 4.23) and (23.68±3.91) respectively.

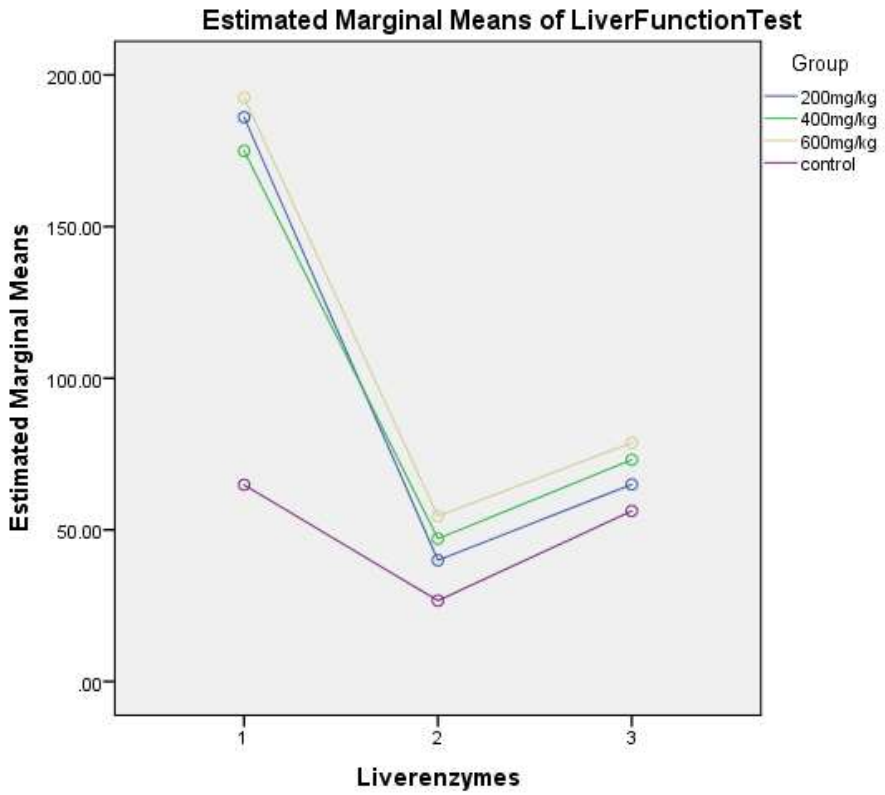

Fig 1: Anova graph for liver enzyme

In Figure 1, the lines showing the control group and the 600 mg/kg treatment groups were not parallel to each other. This means that there is a significant interaction (change) in the groups tested for different dosage toxicity for *B. nivosa* while the treatment groups of 200 mg/kg and 400mg/kg are parallel to each other, this signifies that the toxicity measure of *B. nivosa* between the groups of 200 mg/kg and 400 mg/kg was not significant.

Histological Examination of the Liver

Histological study of the liver using Hematoxylin and Eosin staining method revealed cords of hepatocytes well preserved, well demarcated sinusoids, well preserved central vein, no fatty changes with no area of necrosis (Figure 2). Liver section treated with 200 mg/kg of ethanolic leaf extract of *Breynia nivosa* revealed congestion of central vein with distorted cords of hepatocytes (Figure 3). Sections of the liver

treated with 400 mg/kg of ethanolic leaf extract of *Breynia nivosa* revealed mild infiltration of hepatic cells. In a few plate 3 hepatocytes, a small, pycnotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm were observed with dilatation of central vein (Figure 4). Sections of liver treated with 600 mg/kg of ethanolic leaf extract of *Breynia nivosa* revealed necrosis of hepatic cells with severe congestion of central vein. The cytoplasm of some hepatocytes was light, necrotic changes were evident; enlarged and contained vacuoles (Figure 5).

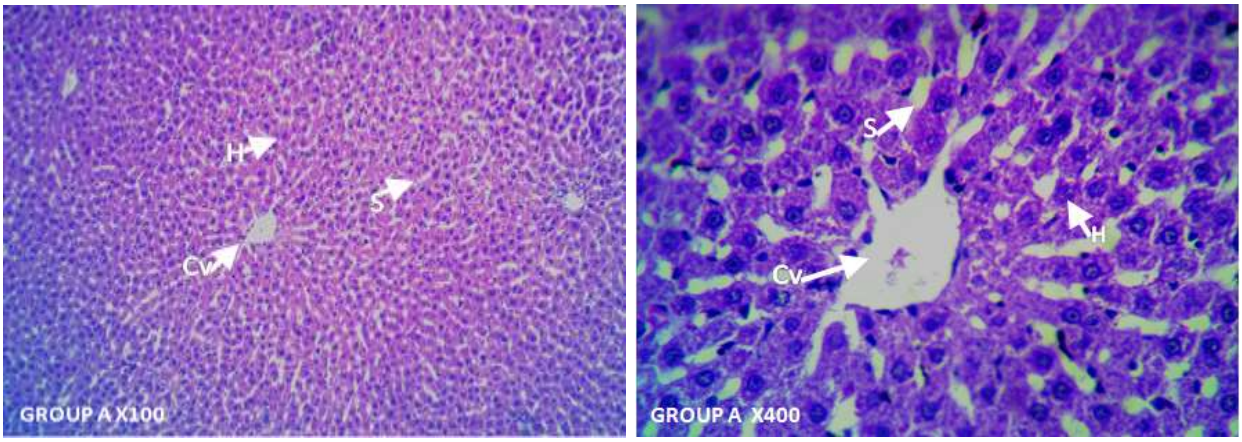

Figure 2: Representative photomicrographs of sections of the liver of control rats not treated with the leaf extract of *Breynia nivosa* showing cords of hepatocytes well preserved (H), cytoplasm not vacuolated, sinusoids well demarcated (S), well preserved central vein (Cv), no area of necrosis, no fatty change, and no fatty degeneration.

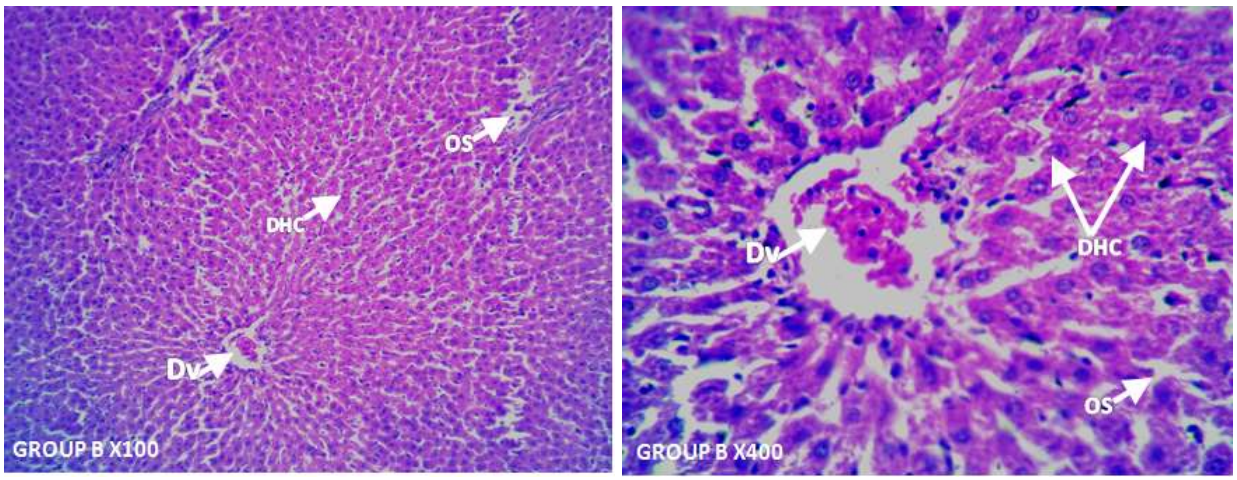

Figure 3: Representative photomicrographs of sections of the liver treated with 200 mg/kg of ethanolic leaf extract of *Breynia nivosa* showing distinct congestion of central vein (Dv), distorted cords of hepatocytes (DHC), obliterated sinusoids (OS) with fatty appearance.

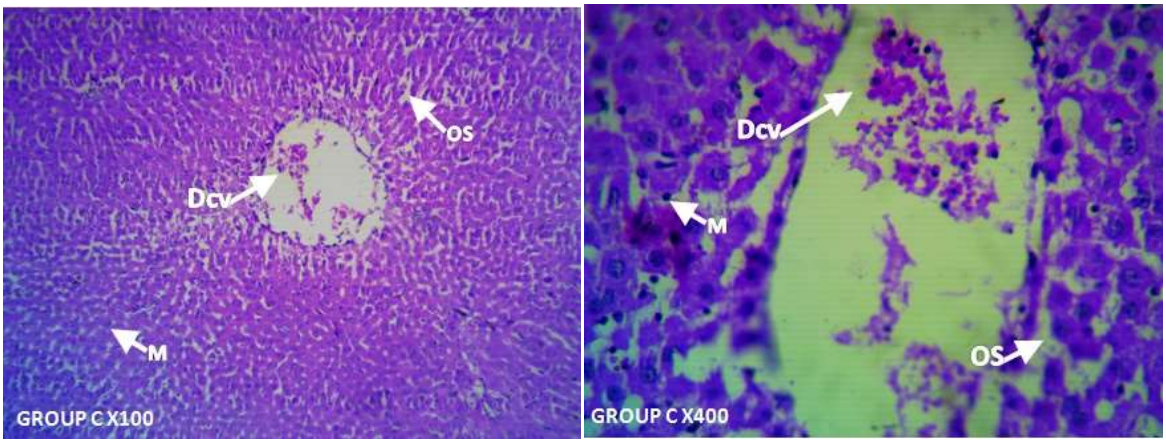

Figure 4: Representative photomicrographs of sections of the liver treated with 400 mg/kg of ethanolic leaf extract of *Breynia nivosa* showing mild hepatic cell infiltration, severe obliterated sinusoids (OS), pyknotic cellular nucleus with condensed chromatin (M), lack of nucleolus and strongly acidophilic cytoplasm, with congested dilatation of central vein (Dcv).

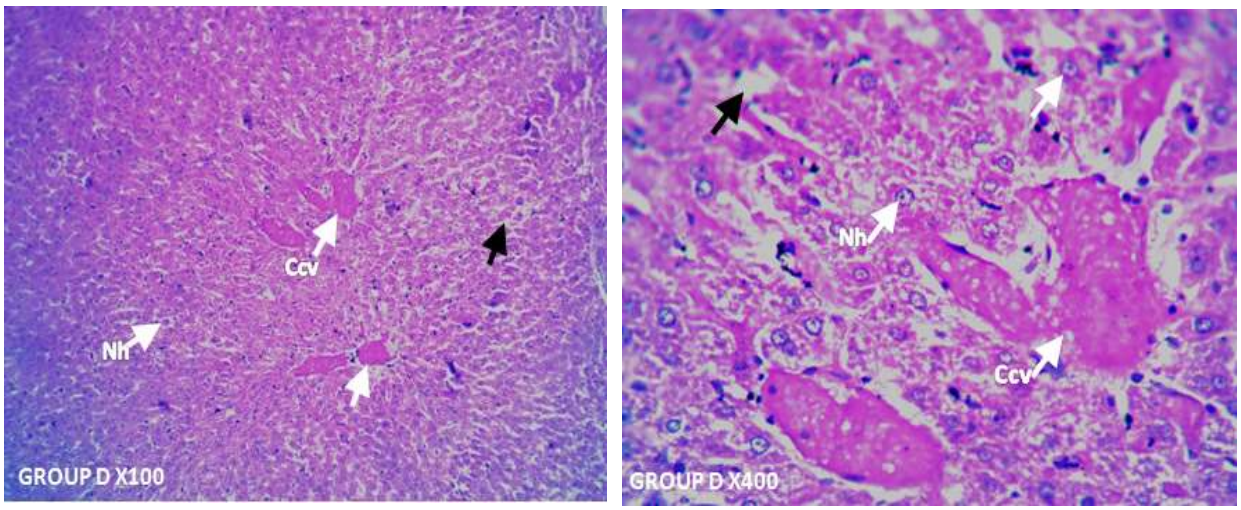

Figure 5: Representative photomicrographs of sections of the liver treated with 600 mg/kg of ethanolic leaf extract of *Breynia nivosa* showing severe congestion of central vein (Ccv), necrosis of hepatic cells (Nh) enlarged and contained vacuoles with distorted sinusoid (black arrows).

DISCUSSION

Evaluation of Serum Liver Enzymes

The liver is an organ involved in many metabolic functions and is prone to xenobiotic injury because of its central role in xenobiotic metabolism[12]. Hepatotoxic drugs cause damage to the liver[13, 14]. Liver function tests conducted through blood assays give information about the state of the liver, describing its functionality (albumin), cellular integrity (transaminases) and its link with the biliary tract (alkaline phosphatase)[15].

The aminotransferases are the most frequently utilized and specific indicator of hepatocellular necrosis[16]. Serum glutamic pyruvic transaminase or Alanine aminotransferase (SGPT/ALT) is the enzyme produced within the cells of the liver and the serum levels of this enzyme increases after liver damage due to increased membrane permeability or liver cell necrosis and cytosol leakage into the serum[17]. The results of the present study showed that ALT level was increased in the rats treated with ethanolic leaf extract of *B. nivosa.*

There are several reports that documented increase in ALT and AST levels in almost all liver diseases. The highest elevations occur in severe viral hepatitis; drug

or toxin induced hepatic necrosis and circulatory shock. Although enzyme levels may reflect the extent of hepatocellular necrosis they do not correlate with eventual outcome[18, 19].

In the present study, the moderate increase of ALT may be an indication of acute hepatitis, acute billiary tract obstruction, liver tumor and cirrhosis of the liver caused by the administration of ethanolic leaf extract of *B. nivosa*. This is in line with the findings of the present study which showed increase in serum ALT and AST of the treated rats compared to the control. Large increases in mitochondria AST occur in serum after extensive tissue necrosis. Because of this, assay of mitochondria AST have been advocated in myocardial infarction. Mitochondria AST are also increased in chronic liver disease[20].

Serum ALP originates mostly from the liver and bones. Increase in serum ALP level is usually a characteristic finding in cholestatic liver disease[21]. In the present study, there was significant increase in the ALP of animals treated with ethanolic leaf extract of *B. nivosa* compared to the control. The cause of the significant increase in ALP level of the treatment groups is not known but it may be inferred that doses of *B. nivosa* may be adversely affecting liver function. This might be the result of rise in ALP level of the treated rats. Regardless of the cause of the acute hepatic failure a low ratio of alkaline phosphatase to bilirubin is associated with a poor prognosis[22].

However, several documents have reported decrease in the level of serum alkaline phosphatase due to liver damage may be associated with pernicious anemia, zinc deficiency and hypophosphatasia[23].

Histopathological Findings on the Liver

Herbal medicines are widely perceived by the public as being natural, healthful and free from side effects or any potential risks due to their natural origins and are often considered as food supplements and not drugs. Medicinal herbs are usually self-prescribed by the consumers and there is a lack of control and review in terms of dose, manner, and frequency of administration. The chemicals in medicinal herbs may be natural to the plant, but they are not natural to the human body. Plants are known to be composed of several phytochemicals which may work synergistically, additively or antagonistically[24].

The usefulness or toxic effects of the natural medicinal products typically result from combinations of various phytochemicals present in the plant[25, 26, 27]. Hence, despite their therapeutic potentials, there is a need to assess the toxicity of all medicinal plants in order to ascertain their safety. In this study, we investigated the toxicity of the ethanolic extract of *B. nivosa* on the liver using histopathological parameters.

The functional studies in toxicology should be coupled with appropriate histological studies. Morphological studies are however, useful for the anatomical localization of action of toxins[28].The administration of leaf extract of *B. nivosa* resulted in histopathological changes in the liver manifested by sporadic hepatic necrosis, pyknotic cellular nucleus and vacuolations of some hepatocytes, cellular infiltration, congestion of central vein, hepatocytes with fatty appearance (Figure 3) and dilatation and congestion of hepatic sinusoids. Hemorrhage of central vein, hepatic necrosis and loss of hepatic architecture were seen in group treated with 600 mg/kg of ethanolic leaf extract of *B. nivosa*.

Hepatic injuries such as necrosis and fulminant hepatic failure (FHF), which are often produced on exposure of the tissue to toxins, virus or many chemical agents[29], constitute a major health hazard. Liver necrosis and FHF are the two important clinical conditions of liver. Necrosis is a complex process, characterized by the simultaneous activation of multiple deregulated pathways that culminate in the loss of cell membrane integrity causing leakage of cellular constituents[30].

Although no toxicity study has been conducted on the leaf extract of *B. nivosa,* other constituent herbs and plants that have been worked on has shown to have synergetic effects and altered the physiological and pathological state of the animals. Morphological observation, together with functional test showed that leaf extracts of *B. nivosa* administered led to liver injury. Thus, it may be inferred that high doses of *B. nivosa* may be adversely affecting the liver. The toxic effect of ethanolic leaf extract of *B. nivosa* on the liver may be due to anyone or more of the phytochemical present in the extract.

CONCLUSION

From the result of this study, it is indicative that consumption of high doses of leaf extract of *B. nivosa* could jeopardize the physiological state of liver when consumed over a long period. There is a significant increase in ALT, AST and ALP, suggestive of hepatotoxicity.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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