



Hepatotoxic Effects of Ethanolic leaf Extract of *Breynia nivosa* (Snow bush) in Wistar rats

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ABSTRACT

The hepatotoxic effects of ethanolic leaf extract of *Breynia nivosa* in male albino Wistar rats were studied. The *Breynia nivosa* leaves were extracted using 70% ethanol. The phytochemicals were evaluated and found to contain – tannin, flavonoids, saponins, cardiac glycosides and cyanogenic glycosides. Twenty (20) adult male albino Wistar rats were randomly divided into four (4) groups – three tests of A, B, C and one control – and were administered with 400mg/kg, 200mg/kg and 100mg/kg of the ethanolic leaf extract of *Breynia nivosa* respectively for 21 days after two weeks of acclimatization. The animals were sacrificed under anaesthesia after the experiment and blood samples collected for determination of the antioxidant enzymes (superoxide dismutase and glutathione peroxidase), malondialdehyde, and liver enzyme: aspartate transaminase (AST), alkaline phosphatase (ALP), and alanine transaminase (ALT). The results showed that there was a significant decrease in superoxide dismutase level ($P < 0.05$). Changes in other markers (malondialdehyde and glutathione peroxidase) were however not statistically significant ($P > 0.05$) when compared with the control. The extract significantly increased ALT, AST, ALP levels ($p < 0.05$). Hence *Breynia nivosa* may be toxic and caution should be exercised in its consumption.

Original Research Article

Received: 14th Jun, 2021

Accepted: 5th Jul, 2021

Published: 30th Sept, 2021

Keywords:

Breynia nivosa
Glutathione peroxidase
Liver enzymes
Superoxide dismutase
Wistar rats

1. INTRODUCTION

Presently the almost forgone herbal derived medicinal products are now yet again gaining universal recognition and currently recommended for primary healthcare, but only a few plants have received thorough scientific investigation. Medicinal plants are natural products used since time immemorial for the treatment of various human diseases. In the world at large, the citizens and health practitioners are now starting to rely on herbal/plant derived medicinal substances as a substitute for scientifically proved therapies (Oyedemi, 2017).

The various compounds of this herbal medicine play a significant role against pathogenic bacteria and oxidative stress caused by abiotic stress in plant (Vogt, 2010). Presently one of the means used to ameliorate skin and oral infections as well as oxidative stress is the use of natural herbal products from plant origin, probably because of the perception

that long-term use of western medicine induces severe complications due to the non -therapeutic effect of drug (Amadi *et al.*, 2007).

*Breynia nivos*a is a shrub commonly known as "ice plant" or "snow bush" because of its beautiful snowy leaf appearance, especially during winter season. The leaves are round in shape with white, green and red coloration (Onyebule *et al.*, 2014). The plant is about 2m high and especially used for foliage, mainly domiciled in villages and towns of tropical Africa like South-eastern Nigeria. Its Leaves are simple, opposite with entire margin and ovate in shape. *B. nivos*a plant is locally called "ogwueze" by the Igbo tribe of the south-eastern Nigeria. *B. nivos*a has been proven to be among the many used herbal medicinal plants believed and/or proven to be used presently in managing many disease conditions such as malaria (Okokon, 2015).

Also studied was the effect of *B. nivos*a on the major liver enzymes – ALT, ALP and AST – to ascertain its toxic effect on liver.

This study was undertaken to ascertain the hepatotoxic effect of ethanolic leaf extract of *B. nivos*a in Wistar Rats.

2. MATERIALS AND METHOD

2.1 MATERIALS

Adult male Wistar rats, growers mash (feed), orogastric cannula, distilled water, hand gloves, weighing scale, metal plate and cup, 5ml syringe, wire, gauze, cage, dissection kits, saw dust, ketamine hydrochloride, *B. nivos*a (dried leaves).

2.2 PROCUREMENT OF EXPERIMENTAL ANIMALS

Twenty male adult Wistar rats (150g – 200g) were obtained from animal farm, the Animal House of the Physiology located at the Department of Human Physiology, Abia State University Uturu. The animals were bred in the experimental house of Department of Human Physiology, Faculty of Basic Medical Science Abia State University under standard conditions. They were acclimatized for two weeks, having free access to water and food. The animal feed was bought from a local market dealer in Amamptu, Uli, Anambra state

2.3 ETHICAL APPROVAL

Ethical approval for the research was sought and obtained from the Ethic committee of Abia State University Uturu.

2.4 PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of *B. nivos*a were collected from Prof. Akpuaka's compound at Abagana, Awka, Anambra state, Nigeria. It was identified and authenticated by a botanist at the Department of Botany, Nnamdi Azikiwe University Awka.

2.5 PHYTOCHEMICAL ANALYSIS

In the phytochemical analysis of the powdered *B. nivos*a leaves, tests for alkaloids, flavonoids, cardiac glycosides, tannins, cyanogenic glycosides, Anthracine glycosides, saponins, were carried out using standard methods reported by Ezekwesili and Ogbunugafor (2015).

2.6 PREPARATION OF THE EXTRACT

*B. nivos*a leaves were shade-dried at room temperature for two weeks. The leaves were then ground to powdered form using electric blender. Exactly 200g of the dried powdered form was then soaked in 70% ethanol for 48 hours. The mixture was then filtered and evaporated to dryness using water bath at 40°C. The extract (0.02g, 0.04g and 0.08g respectively) was then diluted with appropriate millilitre of distilled water to get 100mg/kg, 200mg/kg and 400mg/kg using the formula below.

Dose (ml) = (required dose (mg/kg) × weight of animal (kg)) / stock (mg)

The prepared extract was stored in a refrigerator at 4°C until time for use.

2.7 EXPERIMENTAL DESIGN AND PROTOCOL

The rats were divided into four groups (control, A, B and C) of five animals each. The first group served as the control and was given food and water *ad libitum* throughout the period of the research, while groups A, B and C were the experimental groups and were administered with 400mg/kg, 200mg/kg and 100mg/kg of ethanolic leaf extract of *B. nivosa* extract respectively for 21 days through oral administration using orogastric cannula. The dosage was based on the outcome of the toxicity test (LD50) (Lork, 1983).

2.8 COLLECTION OF BLOOD SAMPLES

Twenty-four (24) hours after the administration of the last dose, the animals were anaesthetised under light ether. Blood samples were collected through ocular puncture and were taken to Laboratory for determination of glutathione peroxidase, superoxide dismutase and malondialdehyde, alkaline phosphatase, alanine transaminase, aspartate transaminase.

SOD was assayed by colorimetric method of Misra and Fredovich (1972). MDA level was determined by the colorimetric method of Gutteridge and Wilkins (1982).

The activity of glutathione peroxidase was determined by the method of Rotruk *et al.* (1973). AST and ALT were determined using the spectrophotometric method of Bergermeyer *et al.* (1979). ALP was assayed using the spectrophotometric method of Schlebusch *et al.* (1974).

2.9 STATISTICAL ANALYSIS

The results were subjected to statistical analysis using SPSS (version 25.0) and P value less than 0.05 (P<0.05) was regarded as significant.

3. RESULTS AND DISCUSSION

The result of the phytochemical analysis revealed the following: alkaloids (+++), flavonoids (+++), glycosides (++), tannins (++), saponins (+), and starch (++). Alkaloids and flavonoids were found in large quantities. There was a moderate amount of glycosides, tannins, and starch. Small quantities of saponins were detected. The findings are in agreement with that reported by Onyegbule *et al.* (2014). The observed effects of this plant in this research may be attributed to the phytochemicals it contains (Trease and Evans, 1983). In the following section, the results of liver enzymes – aspartate transaminase, alanine transaminase and alkaline phosphatase were discussed as the statistical analysis shows that the liver enzymes were significantly increased (P<0.05). This shows that *B. nivosa* possesses toxic effect on the liver of albino Wistar rats.

The result of malondialdehyde (MDA) showed there was increase in the statistical means of the test groups when compared with the control group. However, these increases were not statistically significant (P>0.05) (Fig. 1).

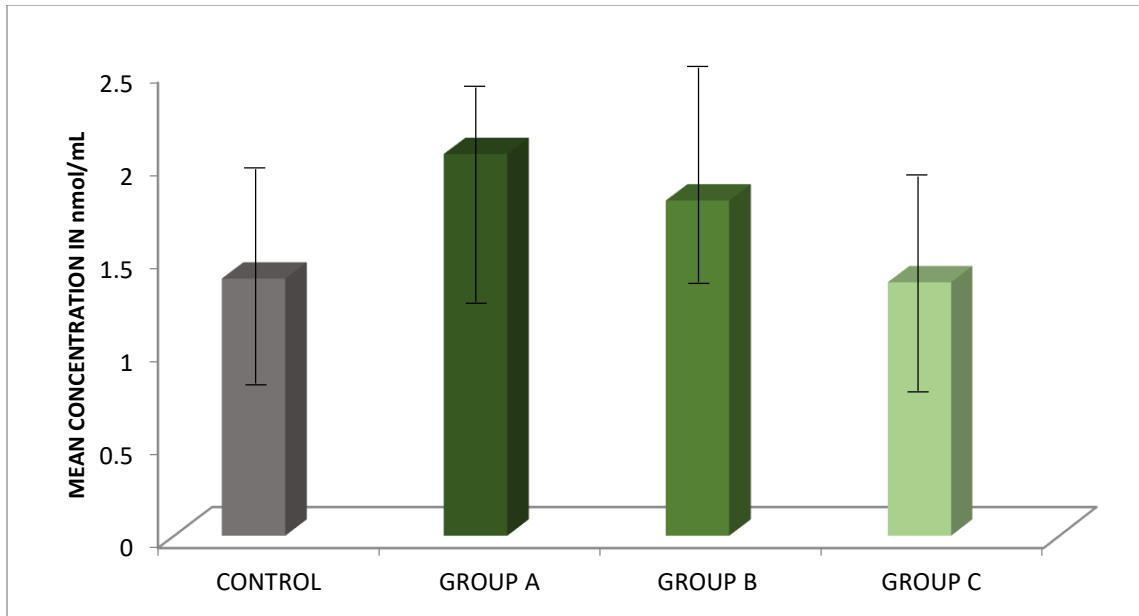


Figure 1: Effect of ethanolic leaf extract of *B. nivosa* on malondialdehyde (MDA)

The increase observed in the concentration of MDA may be as a result of the phytochemical cyanogenic glycosides moderately present in the plant leaves. On hydrolysis of cyanogenic glycosides cyanide which is highly toxic is formed leading to several maladies like increase in lipid peroxidation.

Malondialdehyde is a marker for oxidative stress. The increase in malondialdehyde (MDA) level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals. Hence its slight increase though not significant may be as result of increased oxidative stress probably resulting from the administration of the extract. It may be possible that the mechanism of hepatotoxicity by ethanol extract of *B. nivosa* is due to its lipid peroxidation effect.

The result of glutathione peroxidase showed that there was no significant change ($p > 0.05$) in the parameters between the control group and the test groups (groups A, B and C) despite the different dosing. However there was slight decrease in the statistical means although it was not significant (Fig. 2). This may be as a result of the presence of the phytochemical cyanogenic glycosides which has a non-competitive inhibitory effect on the enzyme.

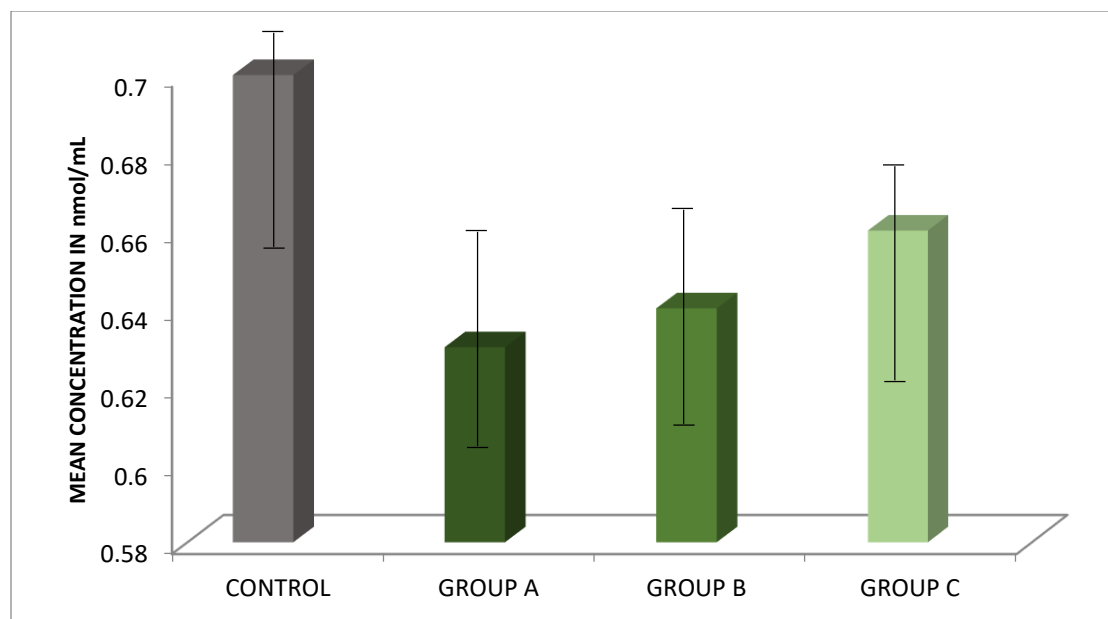


Figure 2: Effect of ethanolic leaf extract of *B. nivosa* on glutathione peroxidase (GPx)

There was a significant decrease in superoxide dismutase level (Fig. 3). This could be as a result of the cyanogenic glycosides in the *B. nivosa*. The physiological importance of superoxide dismutase (SOD) is illustrated by the severe pathologies evident in experimental animal genetically engineered to lack this enzyme. For example, mice lacking SOD develop a wide range of diseases, including hepatocellular carcinoma (Elchuri *et al.*, 2005).

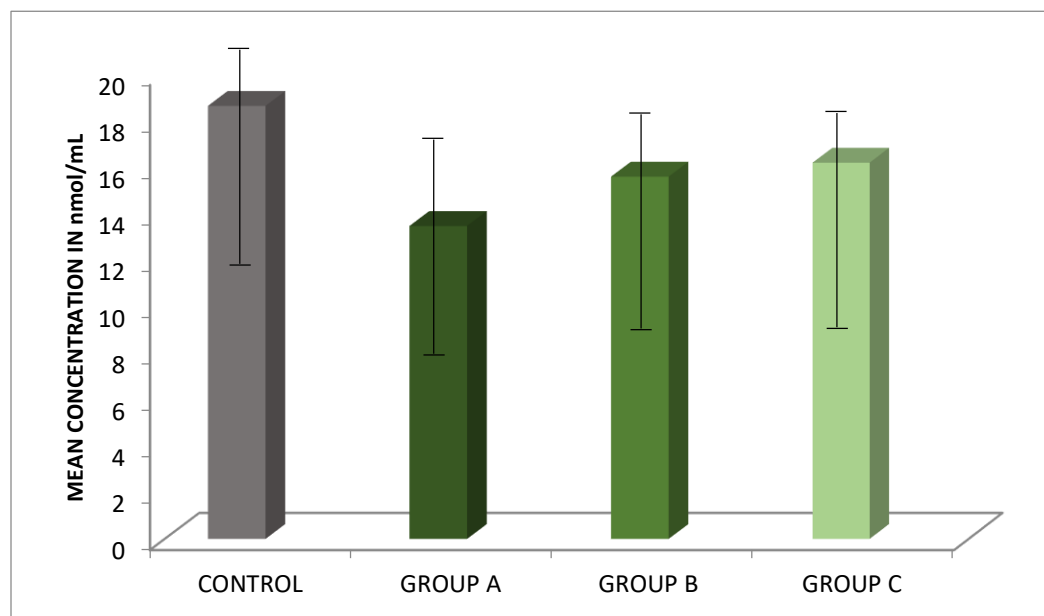


Figure 3: Effects of ethanolic leaf extract of *B. nivosa* on superoxide dismutase (SOD)

The above findings on superoxide dismutase, even that of glutathione peroxidase and malondialdehyde could be the reason for the result of the serum analysis of liver enzymes, which its status may be used to ascertain the state or condition of the liver.

However, it is of great importance for one to note that elevation of serum blood level of alanine transaminase or aspartate transaminase or alkaline phosphatase does not depict any specific hepatic anomaly rather it only indicates injury to the liver that could cause malfunctioning of the liver functions.

It was found that there was significant increase in the means of all the test groups when compared with the mean of the control at $p < 0.05$. This probably is as a result of the reduced antioxidant and slightly elevated malondialdehyde, exposing the liver to the negative actions of free radicals caused probably by the deteriorating effect of cyanide toxin produced as a result of hydrolysis of cyanogenic glycosides present in the plant.

It was also found that aspartate amino transaminase was also significantly elevated in all the test groups when compared with the control at $p < 0.05$ (Fig. 4). AST also being an important marker enzyme used to determine the statuses of the liver, its increase may be as a result of damage to various organ apart from the liver like the brain, heart, kidney, that is the reason why other liver test like ALT are taken with the AST to ascertain that the damage is coming from the liver. On this note it could be said that because ALT which is also a good liver marker is significantly elevated (Fig. 5) that the cause of the elevation is as a result of hepatic injury. Furthermore, there was significant elevation of the test results when compared with that of the control at $p < 0.05$.

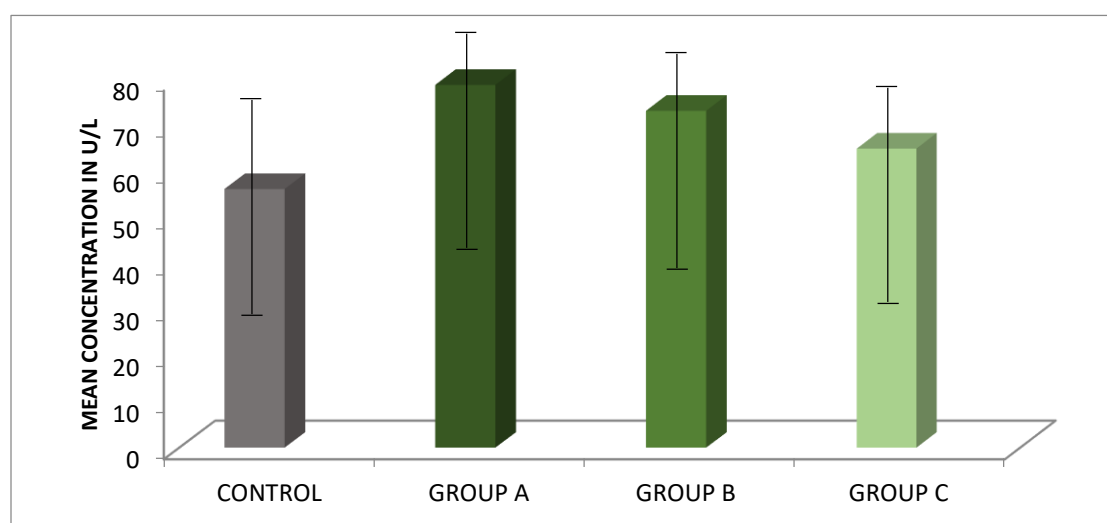


Figure 4: Effect of ethanolic leaf extract of *B. nivosa* on serum AST level in albino Wistar rats

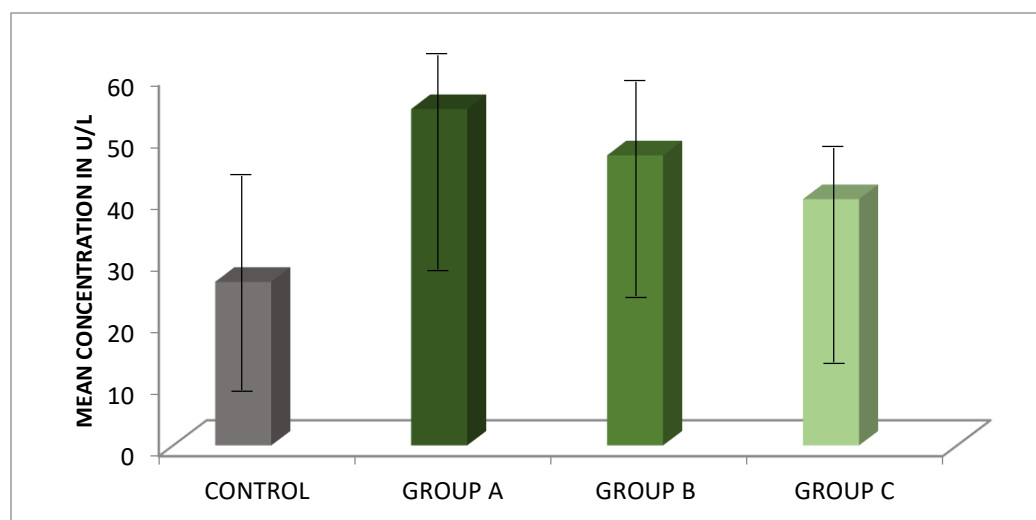


Figure 5: Effect of ethanolic leaf extract of *B. nivosa* on serum ALT level in albino Wistar rats

Alkaline phosphatase (ALP) levels were significantly elevated in tests groups compared with the control at $p < 0.05$ (Fig. 6). It must be noted that increased levels of ALP may be seen in cirrhosis, hepatitis, and congestive cardiac failure (Rosalki and Mcintyre, 1999).

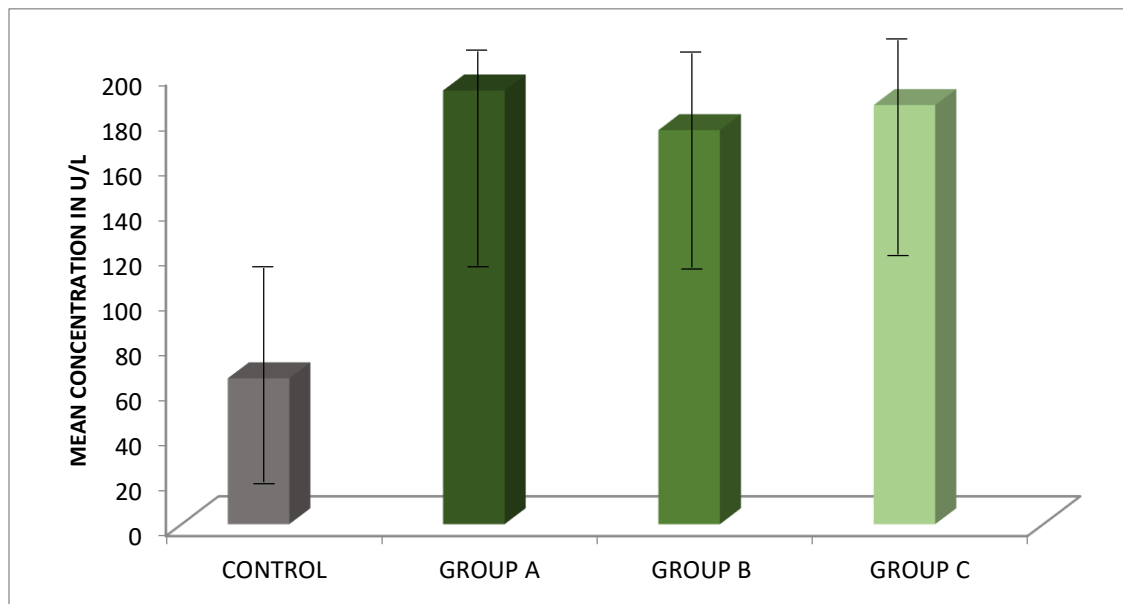


Figure 6: Effect of ethanolic leaf extract of *B. nivosa* on serum ALP level in albino Wistar rats

This shows that at different doses, administration of *B. nivosa* presents with liver toxicity. This can further confirm that *B. nivosa*, decreasing the antioxidant – superoxide dismutase – exposed the liver to toxic effect of reactive oxygen species or to oxidative stress. *It was found that mice lacking SOD1 developed a wide range of pathologies, including hepatocellular carcinoma (Elchuri et al., 2005).*

4. CONCLUSION

The findings from this research revealed the hepatotoxic effect of *B. nivosa* ethanolic leaf extract in albino Wistar rat. Ethanolic leaf extract of *B. nivosa* also showed in this study to reduce the level of superoxide dismutase and glutathione peroxidase which are body's marker antioxidants while it increased the level of malondiadehyde, proving that the plant may possess peroxidation effect in albino Wistar rats. Considering the beneficial effects and the use of this plant in ethnomedicine, especially in tooth infections (Amadi *et al.*, 2007), it should be used with caution. Therefore, the use of snow bush (*B. nivosa*) should be in moderation.

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How to cite this article

Enwelum, H.O., Nwankwo, A.A. and Nwozor, C.M. (2021). Hepatotoxic Effects of Ethanolic leaf Extract of *Breynia nivosa* (Snow bush) in Wistar rats. *Tropical Journal of Applied Natural Sciences*, 3(2): 1-8. <https://doi.org/10.25240/tjans.v3i2.1>

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