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Impact of *Newbouldia laevis* Root Extract on Liver Enzymes in Rats

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Abstract
This twenty-eight-day experimental study aimed to investigate the impact of *Newbouldia laevis* root extract on liver enzymes in rats. Sixteen male Wistar rats were randomly divided into four groups, each consisting of four rats. The first group, designated as the control, received only feed and water. Meanwhile, groups two, three, and four were administered the plant extract at doses of 200, 400, and 600 mg/kg, respectively. To assess the potential effects of the extract on aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels in rat serum were analyzed using the standard method. The AST level was significantly ($p<0.05$) lower in the group that received 200 mg/kg of extract than in the group that received 600 mg/kg of extract. Additionally, after 28 days, the control group's AST levels were still considerably ($p<0.05$) lower than those of the groups that received the extract. The ALT results at 14 and 28 days following the administration of the extracts did not significantly differ across the groups when compared to each other. Following 28 days of treatment, the 400 mg/kg extract group had much higher ALP levels ($p<0.05$), but there was no significant difference ($p>0.05$) between any of the other groups. The findings contribute valuable insights into the possible biochemical effect of *N. laevis* extract.
Keywords: Newbouldia laevis, ethanol extracts, rats, and liver enzymes

Introduction

Traditional medicine relies heavily on medicinal plants, and the indigenous knowledge needed to use herbal medicine effectively varies among cultures and tribes (Dassekpo *et al*., 2020).The increased awareness of the need for humans to rely on the natural resources around them and to use traditional medical knowledge as a source of novel pharmaceuticals has increased research on medicinal plants (Okagu *et al*., 2022a). *Newbouldia laevis* has been utilized in the treatment of various diseases for an extended period, even though traditional medicine practitioners might not be familiar with the specific bioactive components of the plant they commonly recommend (Ukwubile *et al*., 2023). Existing scientific literature provides evidence that these traditional prescriptions may demonstrate effectiveness in treating numerous diseases (Dassekpo *et al*., 2020). The leaves of *N. laevis* contain bioactive compounds that are advantageous for humans and animals (Ayoola *et al*., 2016). *Newbouldia*, a plant genus within the Bignoniaceae family, is indigenous to Africa. This genus is monotypic, encompassing the single species, *N. laevis*, commonly referred to as the boundary tree (Blench, 2015). The plant displays glossy, dark green leaves and produces prominent, attractive purple flowers at its terminal ends. Frequently cultivated for ornamental purposes, it readily reproduces through cuttings. It is commonly used as a recognizable living fence and boundary tree across its range, which includes Senegal, Sierra Leone, Nimba Mountain (Guinea/Liberia), Ivory Coast, Ghana, Nigeria, Gabon, and Zaïre (Maciej Serda *et al*., 2013).

N. laevis has been extensively investigated and widely used for different medicinal purposes owing to its bioactive components (Sofowora, 1996; Umeyor *et al*.,

2016; Ushie *et al*., 2022; Usman & Osuji, 2007). This makes it possible for this herbal remedy to function like pharmaceuticals and to have pharmacological advantages. It is used as a vermifuge in treating a variety of conditions, including piles, rheumatic swellings, elephantiasis, diarrhea, syphilis, constipation, and roundworms. Additionally, it has been reported to be helpful for chest discomfort, epilepsy, earaches, aching feet, and convulsions in youngsters (Akunyili, 2000). The leaf, stem, and fruits have been used as a stomachache remedy, wound treatment, and febrifuge(Usman & Osuji, 2007). Also, *N. laevis* (Bignoniaceae) has been used to treat central nervous system (CNS) illnesses such as psychosis, sleeplessness, convulsions, and related anxiety and depression, according to ethnobotanical surveys (Murtala & Akindele, 2020). Moreover, it has been reported that the plant's leaf and root extracts have dose-dependent analgesic, anti-inflammatory, and anti-convulsant properties in rats, supporting the plant's application in Nigerian traditional medicine to treat these conditions (Ukwubile *et al*., 2023). The impact of an aqueous-ethanol extract from the bark of *N. laevis*, along with its n-hexane, ethyl acetate, and aqueous fractions, was investigated with ethanol-induced acute stomach ulceration (Okagu *et al*., 2022). The study indicated that pre-administration of the extract and its solvent fractions substantially suppressed ulceration in rats. Furthermore, to speed up parturition and make it easier for the placenta to exit the body after delivery, traditional healers, especially in Southeastern Nigeria, frequently use *N. laevis* to aid in childbirth and preserve the developing embryo (Ebafor & Sanni, 2009). The findings of Bafor *et al*. revealed that the aqueous and ethanol extracts of *N. laevis*'s leaves both directly stimulate and increase the frequency of spontaneous uterine contractions.

Existing research has primarily centred on assessing the medicinal properties of the plants, yet regrettably, insufficient emphasis has been placed on investigating the plants' toxicity. Therefore, this study was initiated to explore the impact of *N. laevis* on the liver enzymes of albino rats. The liver serves as the primary organ for metabolism, secretion, and the elimination of substances. It plays a crucial role in preserving, executing, and regulating homeostasis within the body (Tahmasebi *et al*., 2018). Medications have the potential to induce a range of liver injuries, spanning from mild disruptions in function, such as elevated serum aminotransferase activity, to severe organ damage, including hepatocellular necrosis or intrahepatic cholestasis (Chatterjee *et al*., 2006).

Materials and methods

Materials

Collection and identification of plant materials

Fresh roots of *N. laevis* were gathered from Izzi in the Abakaliki local government of Ebonyi state, and Mr Emmanuel Nwankwo, a plant taxonomist from the Applied Biology Department at Ebonyi State University, Abakaliki, was responsible for identifying the samples.

Experimental animal

Twenty male Wistar rats, weighing between 160 and 180 g, were procured from the animal house of the Department of Veterinary Medicine at the University of Nigeria, Nsukka. These rats were acclimatized for seven days and were provided with unrestricted access to food and clean water.

Methods

Extraction of plant materials

The plant's root samples were initially washed with distilled water, then air-dried at room temperature away from sunlight, and subsequently pulverized using a grinding machine. The extraction process involved cold maceration at room temperature, where 200 g of the powdered plant material was soaked in 2 L of ethanol (Swem *et al*., 2020). The mixture underwent intermittent vigorous shaking and was later filtered after 48 hours using Whiteman filter paper size 1. The obtained filtrate was concentrated under vacuum conditions using a rotary evaporator.

Experimental design

Sixteen male Wistar rats were randomly divided into four groups, each consisting of four rats. The first group, designated as the control, received only feed and water. Meanwhile, groups two, three, and four were administered the plant extract at doses of 200, 400, and 600 mg/kg, respectively.

Liver function assessment

Following the initial 14 days, venous punctures were used to obtain blood samples, which were then placed in a sterile specimen bottle for analysis. For the final analysis, the same procedure was carried out after twenty-eight (28) days. The collected blood samples were subsequently used for the quantitative determination of liver function enzymes.

Determination of the AST and ALT activity

The activity of AST and ALT were determined by the method of Reitman and Frankel as outlined in the Randox kit used (Reitman & Frankel, 1957).

Determination of the ALP activity

Utilizing the Randox kit, the Babson *et al*. protocol was used to measure the activity of alkaline phosphatase (Babson *et al*., 1966).

Statistical analysis

The means ± standard deviation was used to express the data. Using the Statistical Package for the Social Sciences (SPSS) version 20, comparisons between the control and treated groups were carried out at the same time point using a one-way analysis of variance (ANOVA). The least significant differences were identified at $p < 0.05$.

Results and Discussion

Results

Effect of N. leavis on serum AST values

The AST levels, 14 days post-administration, were 38.67±3.2, 48±2, 49±5.3, and 50.33±2.3 U/L for the control group and the groups that received 200, 400, and 600 mg/kg of ethanol extracts of *N. leavis*, respectively (as depicted in Fig. 1). After 14 days, there were no significant differences ($p>0.05$) in AST values among the groups that received the extracts, while the control group exhibited significantly lower ($p<0.05$) AST levels compared to the extract-administered groups. At a different time, point, 28 days post-administration, the AST values were 41±2.7, 47±4.5, 52.67±3.5, and 56.33±4 U/L for the control group, and the groups administered 200, 400, and 600 mg/kg of ethanol extracts of *N. leavis*. It was observed that after 28 days, no significant differences ($p>0.05$) in AST values were observed between the groups administered 200 and 400 mg/kg of extracts. Still, the group administered 200 mg/kg of extract showed a significantly lower ($p<0.05$) AST level compared to the group administered 600 mg/kg of extract. Notably, the control group continued to display significantly lower ($p<0.05$) AST levels compared to the extract-administered groups after 28 days.

Effect of N. leavis on serum ALT values

Fourteen days after the ethanol extracts of *N. leavis* were administered, the ALT levels were 37±3.6, 38.67±1.52, 39.33±1.15 and 39.67±2.1 U/L in the control group and the groups that received 200, 400, and 600 mg/kg of the extracts, respectively. Twenty-eight days later, the ALT values in the control group groups were 38.33±1.5, 38.70±1.2, 39±2.7, and 40±1.2 U/L (Fig. 2). When the groups were compared to one another, there were no significant differences in the ALT values at 14 or 28 days after the extracts were administered.

Effect of N. leavis on serum ALP values

The ALP levels, 14 days post-administration, were 22±1.1, 29.49±2.5, 27.32±1.3, and 26.54±2 U/L in the control group and the groups receiving 200, 400, and 600 mg/kg of ethanol extracts of *N. leavis*, respectively (Fig. 3). At 14 days post-administration, the control group exhibited significantly lower ($p < 0.05$) ALP levels compared to the other groups, while there was no significant difference ($p > 0.05$) between the ALT values of the extract-administered groups. Meanwhile, 28 days post-administration, the AST values were 28.379±1.5, 29.49±1.8, 32.89±2.5, and 31.22±1.3 U/L in the control group and the groups (as illustrated in Fig. 3). Twenty-eight days post-administration, the group administered 400 mg/kg of extract displayed significantly higher ($p < 0.05$) ALP levels, whereas every other group showed no significant difference ($p > 0.05$) when compared to each other.

Discussion

Liver diseases represent significant global health challenges, presenting substantial difficulties for numerous countries, particularly those in the developing regions of the world (Osigwe *et al*., 2017). The structural integrity of the liver and damage can be evaluated using the enzyme biomarkers AST, ALT, and ALP, which can help in the clinical determination of liver toxicity diseases (Eluu *et al*., 2018; S. C. Eluu *et al*., 2019; McGill, 2016; Ozer *et al*., 2008). The effects of ethanol extracts of *N leavis* on AST, ALT, and ALP levels are illustrated in Fig. 1, 2, and 3. The study explored the implications of ethanol extracts of *N. leavis* on AST, ALT, and ALP levels in rat models, addressing both short-term (14 days) and longer-term (28 days) effects. The findings give unique insights into the dynamics of AST levels among the control group and those administered varied doses of *N. leavis* extracts. The serum aminotransferase activities are elevated in all cases of liver diseases (Adeyemi & Olayaki, 2017; Owumi & Dim, 2019). Under normal circumstances, the activity of these enzymes in the bloodstream is minimal. However, in the event of liver cell necrosis, they are released into the systemic circulation, leading to an elevation in their activities in the blood (Eluu *et al*., 2024; Owumi & Dim, 2019). This study revealed that there were no biochemical alterations brought about by *N. leavis* extracts (Fig. 1, 2 and 3). The values of AST, ALT, and ALP recorded in this study are within the established reference ranges for AST, ALT, and ALP are 50 to 150 IU/L, 10 to 40 IU/L, and 30 to 130 IU/L, respectively (Hasan *et al*., 2018; Sharp & Villano, 2013). This plant's phytochemical components may be responsible for the observed effects of ethanol extracts of *N. leavis* on liver biomarkers. These findings are consistent with earlier research showing the extract and fractions' potential to lower these enzymes' serum activity in liver damage induced by CCL4 (Mbagwu *et al*., 2021).

Conclusion

Overall, the study contributes to our understanding of the potential impact of *N. laevis* extracts on liver function, highlighting their potential therapeutic implications for liver health. The outcome of the study implies that the plant did not cause any significant alteration.

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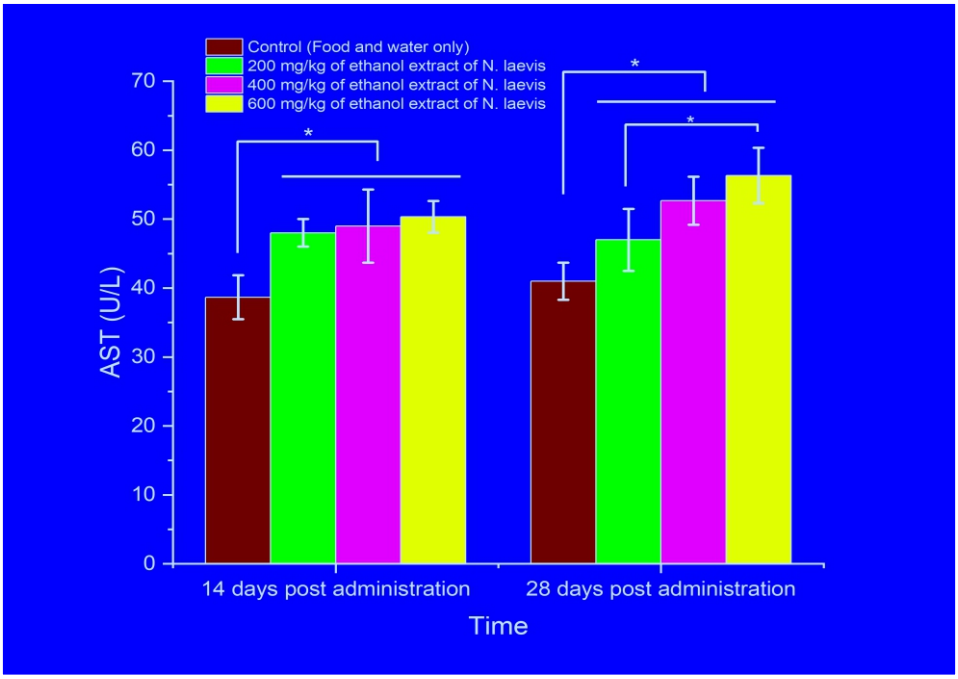

***Figure 1**: Effects of* **N. leavis** *on serum AST levels. The values are presented as mean ± standard deviation, with a sample size (n) of 4. The asterisk (*) denotes significant differences at p < 0.05.*

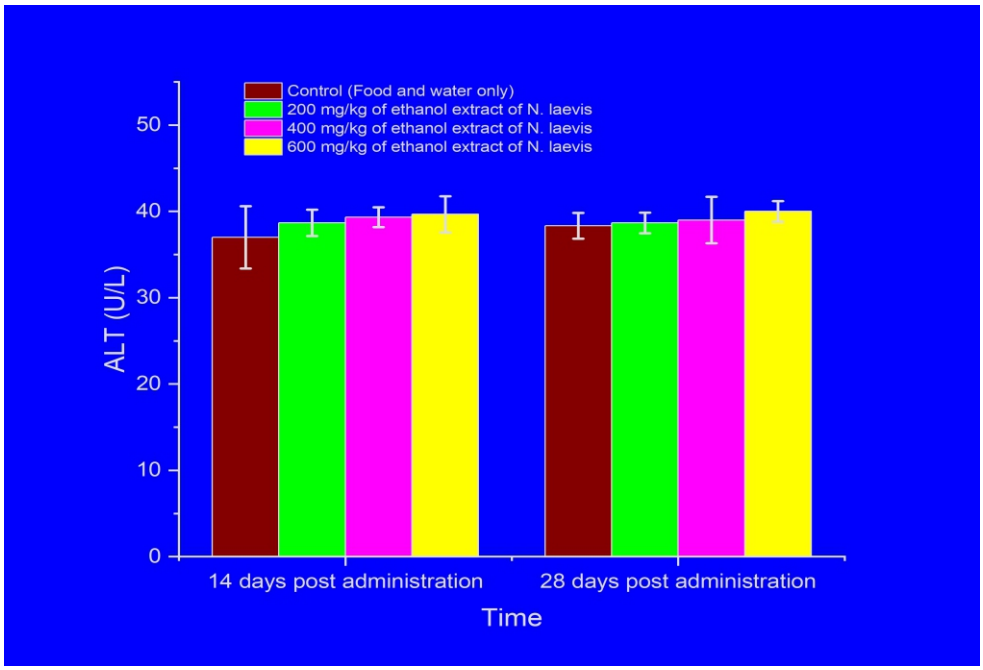

***Figure 2**: Effects of* N. leavis *on serum ALT levels. The values are presented as mean ± standard deviation, with a sample size (n) of 4. The asterisk (*) denotes significant differences at p < 0.05.*

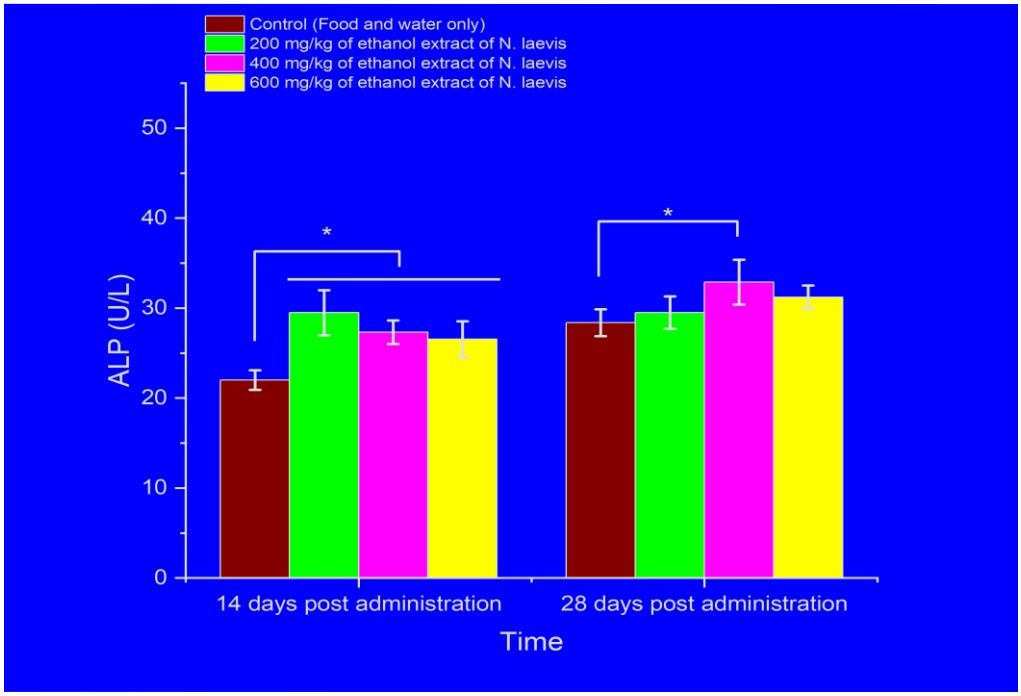

*Figure 3**: Effects of* **N. leavis** *on serum ALT levels. The values are presented as mean ± standard deviation, with a sample size (n) of 4. The asterisk (*) denotes significant differences at p < 0.05.***