

Original Research Article

Assessment of median lethal dose and target organ toxicity of oral sub-chronic administration of *Newbouldialaavis* in rat and micemodel of toxicity.

Comment [U1]: Add and mice

Abstract

The acute and sub-chronic toxicities of the leaf extract of *N. laevis*, an ethnomedicinal herb use in the management of diabetes mellitus was investigated. For the acute toxicity study, 10 – 5000 mg/kg of the extract were administered orally to mice and obvious signs of toxic symptoms and mortality monitored for 24 h post extract administration. In the sub-chronic study, 302 and 604 mg/kg of the extract were orally administered daily for 90 days. Body weight changes as well as haematological and biochemical parameters were determined periodically. Qualitative phytochemistry was also conducted. Presence of flavonoids, saponins, tannins, reducing sugar, steroids, terpenoids, alkaloids and glycosides phytocompounds in the extract were detected. The oral LD₅₀ was estimated to be above 5,000 mg/kg in mice. Ninety days oral administration of ethanol extract of *N. laevis* produced a significant ($P < 0.05$) reduction in body weight at 604 mg/kg on the 31st day and at both 302 and 604 mg/kg on the 61st and 91st days compared to 5% Tween 20 vehicle control group. For liver function enzymes, the extract at both doses (302 and 604 mg/kg) produced significant ($P < 0.05$) reduction in serum ALT enzyme activity at the 91st day with non-significant reduction in other liver function enzymes compared to vehicle control group. Non-significant changes were also recorded for haematological and kidney function markers. The results from this study provide evidence for safety profile of the ethanol leaves extract of *N. laevis* thus supportive of its validity in the use for treatment of chronic diseases like diabetes.

Keywords: *Newbouldialaavis*; Toxicity; liver enzymes; kidney integrity; hematology

Introduction

The use of herbal medicines and phytonutrients continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings (Ekor, 2013). This past decade has obviously witnessed a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries (Ekor, 2013), with complementary and alternative medicines (CAMs) now becoming mainstream in the UK and the rest of Europe, as well as in North America and Australia (Kemppainen *et al.*, 2018). It is estimated that up to four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare (Ekor, 2013).

The recent resurgence of public interest in herbal remedies has been attributed to several factors some of which include various claims on the efficacy or effectiveness of plant medicines (Welzet

al., 2018), preference of consumers for natural therapies and a greater interest in alternative medicines (Welzet *al.*, 2018;), erroneous belief that herbal products are superior to manufactured products (Ekor, 2013; Ezekwesili-Ofilian Okaka 2019), dissatisfaction with the results from orthodox pharmaceuticals and the belief that herbal medicines might be effective in the treatment of certain diseases where conventional therapies and medicines have proven to be ineffective or inadequate (Ekor, 2013; Welzet *al.*, 2018), high cost and side effects of most modern drugs (Ekor, 2013), patients' belief that their physicians have not properly identified the problem; hence the feeling that herbal remedies are another option (Ekor, 2013; Bennadi, 2014, Welzet *al.*, 2018) and a movement toward self-medication - lack of time to see a physician, confidentiality, "it worked for my friend or relative syndrome".

As the global use of herbal medicinal products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized (Ekor, 2013). Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored (Ekor, 2013; Welzet *al.*, 2018). This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational use more difficult.

Newbouldialaevs (Bignoniaceae) is a small tree about 7 – 8 m tall (Useful Tropical Plants, 2022). It is known as Ogilisi or Ogirisi in the Igbo culture of Nigeria while the Hausas call it Aduruku and the Yorubas, Akoko. In Ghana, it is referred to as Sesemasa, but known as Kinkin in Mali (Useful Tropical Plants, 2022). The roots and leaves are used in the treatment of dysentery, malaria, elephantiasis, migraines and seizures (Bothonet *al.*, 2014). The bark and twigs are used to treat pelvic pain in women, peptic ulcer disease, ear ache, skin ulcers, epilepsy, hemorrhoids and constipation, while the flowers are known for their anti-inflammatory activities (Baforand Sanni, 2009). The leaves are soaked in ethanol for the treatment of diabetes and sickle cell disease (Kolawoleet *al.*, 2013).

We have previously conducted extensive studies on the ethanol leaf extract and fractions of *N. laevis* which include establishment of the median antidiabetic effective dose (Mbagwuet *al.*, 2021); establishment of improved effect on glucose and fat homeostasis in a type-2 diabetes mice model (Mbagwuet *al.*, 2020); demonstration that inhibition of oxidative stress and gastric emptying are part of its mechanisms of antidiabetic activity (Mbagwuet *al.*, 2021) and the isolation of two new caffeic acid glycosides (Newboulasides A and B) with α -amylase inhibitory activity (Mbagwuet *al.*, 2022). Since diabetes requires prolonged time for management and treatment which might be associated with accumulated drug toxicity, this study evaluated the sub-chronic toxicity of the ethanol leaf extract of *N. laevis* in animal model of toxicity.

Materials and method

Plant material

Leaves of *Newbouldialaevs* were collected in April 2013 from Oba, in Udenu Local Government Area of Enugu State. The plant material was authenticated by a Taxonomist, Of the Bioresearch Development and Conservation Project (BDGP), Nsukka. The Voucher specimen was deposited in the herbarium of the Faculty of Pharmaceutical Science, NnamdiAzikiweUniversity,

Agulu Campus. Fresh whole leaves weighing 4.3 kg were cleaned and shade-dried for 14 days. A total of 2.5 kg of dry sample was obtained, which was later pulverized with a mechanical grinder (Gx160 Delmar 5.5HP, Honda Motor CO., LTD, Japan) and later used for solvent extraction.

Animals

Adult Swiss albino rats (250 – 300 g) and mice (25 – 30 g) used for the study were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University, Agulu Campus. The animals were fed with pelletized feed (Vital Feeds, Nigeria) and had access to water *ad libitum*. Housing of the animals was done in standard cages in the Animal House of the Department of Pharmacology and Toxicology. The animals were allowed to acclimatize for 5 days before they were used for the study. All animal experiments were conducted in compliance with NIH Guide for the Care and Use of Laboratory Animals (Pub. No. 85 -23 Revised 1985).

Extraction

A 2.5 kg quantity of pulverized leaves of *N. laevis* was cold macerated in 10 L of aqueous ethanol (70%) for 72 h with intermittent shaking. The resulting solution was filtered, and the filtrate was pre-concentrated *in vacuo* using a rotary evaporator at 40°C and thereafter, dried to a constant weight using an open water bath at the same 40°C to obtain the ethanol extract.

Phytochemical analysis

The phytochemical analysis of the leaf extract and fractions were carried out using standard methods (Odohet *et al.*, 2019).

Acute toxicity study of the ethanol leaf extract of *N. laevis*

The acute toxicity test was carried out using modified Lorke's method (Lorke, 1983). In the first phase of the study, 3 groups of 3 mice each were orally given 10, 100 and 1000 mg/kg body weight respectively of the extract. The animals were observed for 24 hours post administration for signs of toxicity and mortality. The second phase comprised of 4 mice that were orally administered 2000, 3000, 4000 and 5000 mg/kg body weight of the extract respectively. The animals were again observed for signs of toxicity and mortality for 24 hours.

Sub chronic toxicity study

Comment [U2]: Insert modified

Forty-five(45) healthy albino rats randomized into 3 groups of 15 animals each were used. Group A was administered 302 mg/kg (ED₅₀) of *N. laevis* ethanol leaf extract. Group B were given 604 mg/kg (Double ED₅₀) while group C served as control and received only 5ml/kg of 5% Tween 20 for 90 days. The animals were weighed every 7 days. On the 31st, 61st and 91st days of the study, 5 rats from each group were anaesthetized using diethyl ether and blood samples collected through the retro-orbital plexus and used for the estimation of hematological parameters - Haemoglobin concentration (Hb), Packed cell volume (PVC), Red blood cell (RBC) count and white blood cell (WBC) count (Diallo *et al.*, 2010). For the estimation of the serum enzyme levels, the blood samples were allowed to coagulate for 30 minutes and the clear serum was separated by centrifuging at 2500 rpm for 10 minutes and was then used for analysis of biochemical hepatic markers- Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), and kidney function markers - Blood Urea Nitrogen (BUN) and Creatinine (Bigoniya *et al.*, 2015).

Statistical analysis

The results were analyzed using SPSS version 16 and presented as mean \pm SEM. Significance between control and extract treated group were determined using one way ANOVA and multiple post hoc comparison done using Tukey HSD . A p value of less than 0.05 was considered significant.

Results

Extraction and phytochemical analysis

The percentage yield of the ethanol extract was 15.56%. Phytochemical analysis revealed the presence of flavonoids, saponins, tannins, reducing sugar, steroids, terpenoids, alkaloids and glycosides in the extract (Table 1).

Table 1: Yield and phytochemical analysis of the extract

| Phytochemicals | Extract |
|----------------|--------------------|
| Flavonoids | + |
| Saponins | + |
| Tannins | + |
| Reducing sugar | + |
| Steroids | + |
| Terpenoids | + |
| Alkaloids | + |
| Glycosides | + |
| Yield (%) | 15.56 ^a |

+ Present, - Absent. a - calculated from 2.5kg pulverized leaves.

Acute toxicity

Single oral dose administration of the extract revealed no obvious signs of toxicity or mortality up to 5,000 mg/kg body weight. Reduced physical activity was observed at the second phase of

the study at the doses above 3,000 mg/kg. However, normal activity was restored one hour post-administration of the extract. The oral LD₅₀ was therefore above 5,000 mg/kg in mice.

Result of subchronic toxicity effect of *N.laevis* extract

Ninety days oral administration of ethanol extract of *N. laevis* produced a significant ($P < 0.05$) reduction in body weight at 604 mg/kg on the 31st day and at both 302 and 604 mg/kg on the 61st and 91st days compared to 5% Tween 20 vehicle control group (Figure 1). Haematological result revealed non-significant increase in packed cell volume (PCV), white blood cell count (WBC) and red blood cell count (RBC) compared to vehicle control at both 302 and 604 mg/kg of the extract (Figure 2).

For liver function enzymes, the extract at both doses (302 and 604 mg/kg) produced significant ($P < 0.05$) reduction in serum ALT enzyme activity at the 91st day with non-significant reduction in other liver function enzymes compared to vehicle control group (Figure 3).

Kidney function indices as measured by serum creatinine and blood urea nitrogen (BUN) showed dose dependent non-significant reductions in serum creatinine at both 302 and 604 mg/kg when compared to vehicle control value. For BUN, there was an initial serum increase at 31st and 61st days at both 302 and 604 mg/kg treated groups with subsequent reduction on the 91st day. These changes were not significant when compared with 5% Tween 20 vehicle control group (Figure 4).

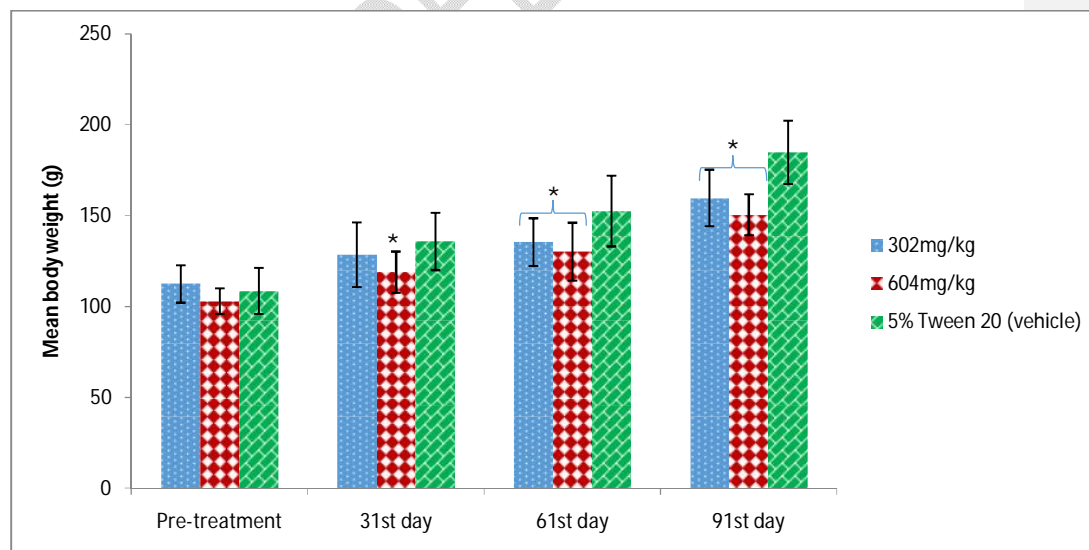
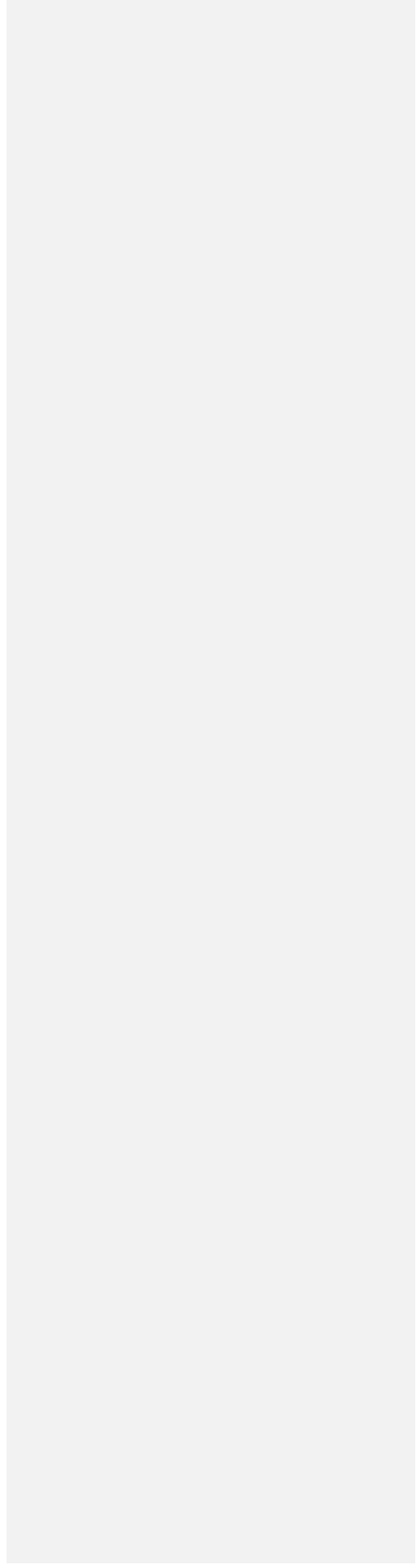


Figure 1: Effect of ethanol extract of *N. laevis* on body weight

UNDER PEER REVIEW



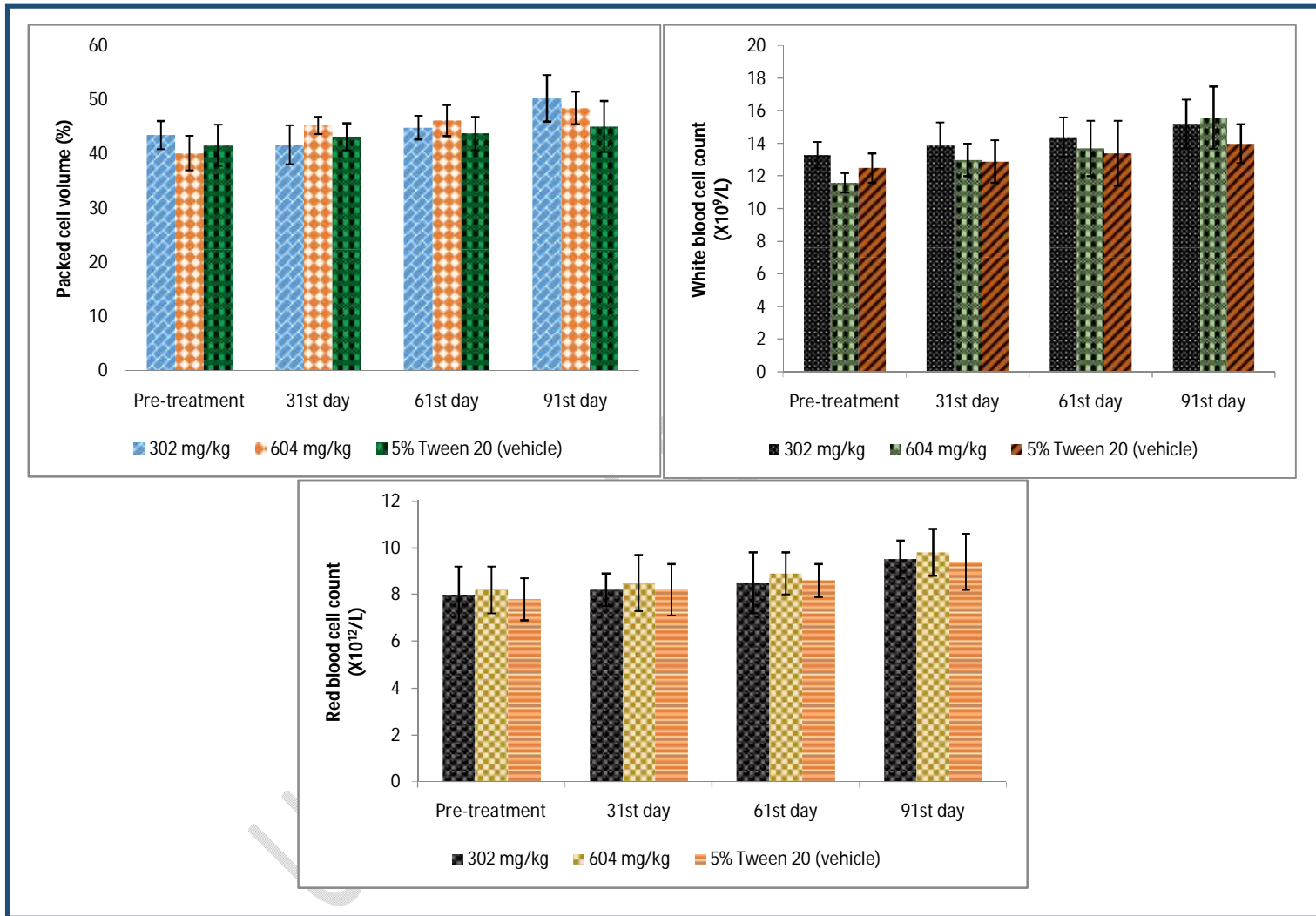


Figure 2: Effect of ethanol extract of *N. laevis* on hematological parameters

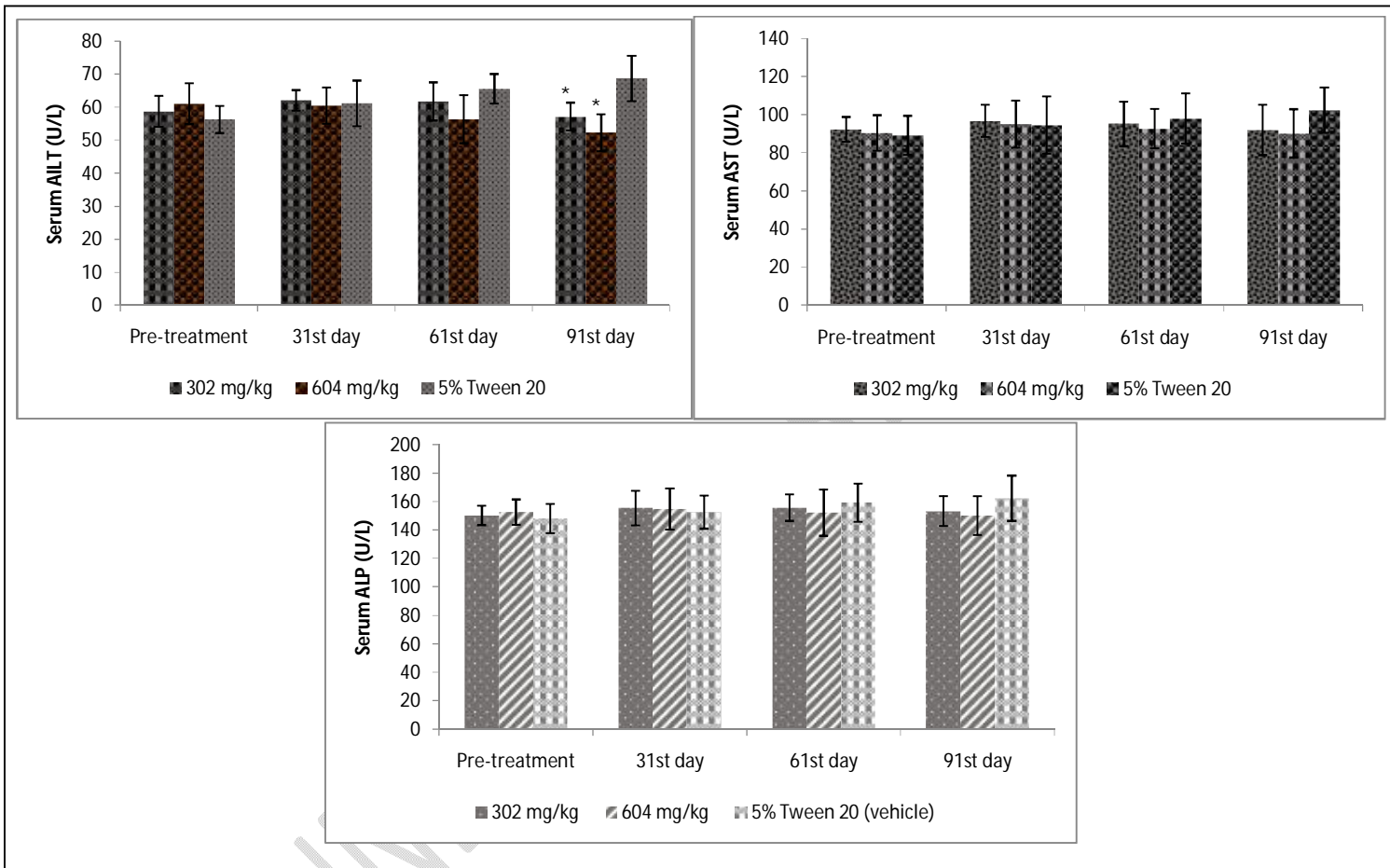


Figure 3: Effect of ethanol extract of *N. laevis* serum liver enzymes

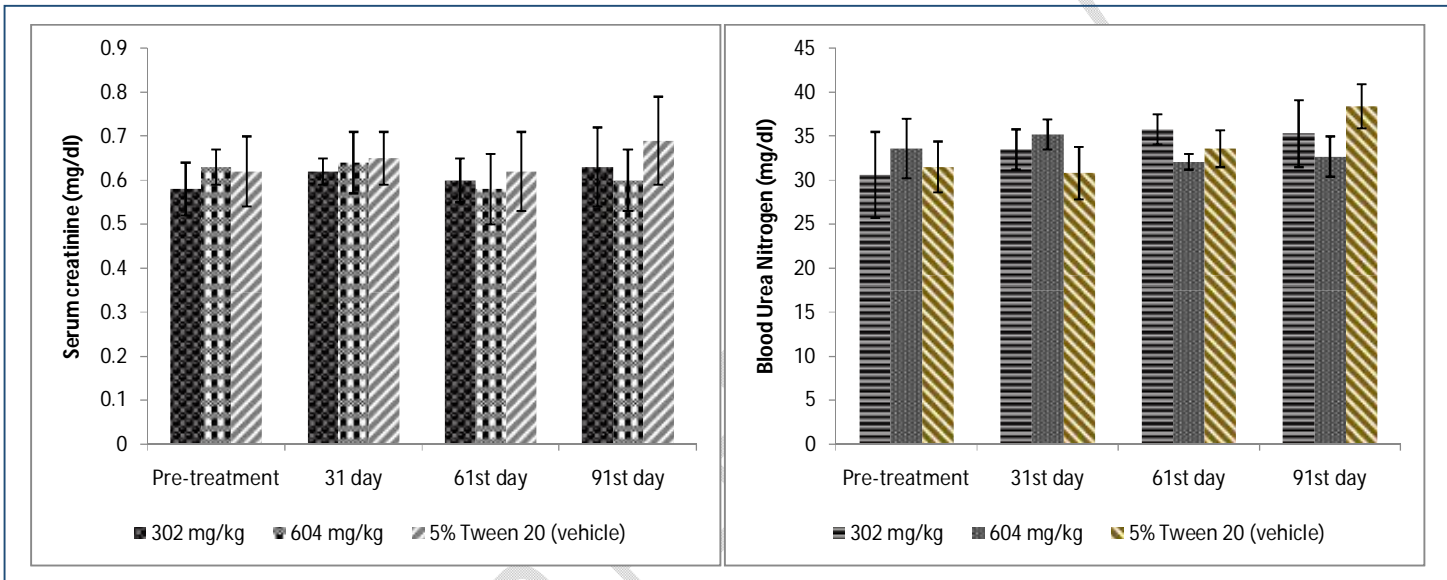
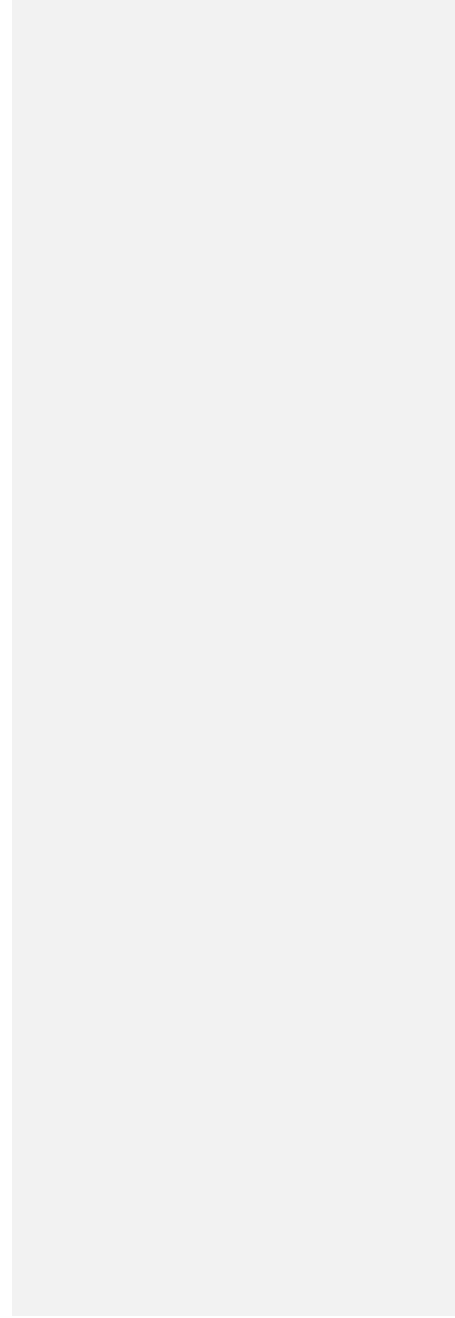


Figure 4: Effect of ethanol extract of *N. laevis* on kidney function parameters

UNDER

UNDER PEER REVIEW



Discussion

Toxicity testing is an important aspect of drug development knowing that even herbal medicines have potential side-effects which could vary from being mild, moderate, and severe, to life-threatening (Ekor, 2013; Jitäreanu et al., 2023). Granted that the objective of most toxicity testing is to evaluate the potential risk to humans, most of the testing is carried out on experimental animals because of the difficulty in obtaining human data due to ethical reasons (Erhirhie et al., 2018). Although there may be challenges in extrapolation of animal findings to humans, experimental animals still present numerous advantages in human experimental toxicity protocol (Norman, 2019). This study evaluated the acute and sub-chronic effect of *N. laevis* in rat model of toxicity.

Toxicity is a relative event that depends not only on the toxic properties of the substance tested but the dose administered (Vandenberg and Blumberg, 2018). All substance can be toxic at particular dose what matters most in therapeutic agents is the potentials for adverse effect at effective dose administered for treatment of disease conditions (Jaishankar et al., 2014). This concept shaped our choice of dose selection in this study. We tested the sub-chronic toxicity potentials of the anti-diabetic therapeutic effective dose (ED₅₀) of *N. laevis* as well as double of this dose.

Comment [U3]: substances

The acute oral toxicity of the ethanol leaf extract of *N. laevis* estimated to be above 5,000 mg/kg body weight in mice is in line with the report by Owolabi et al (2011) who documented an oral LD₅₀ value of 6 g/kg body weight in Wistar albino rats using the ethanol leaf extract of the plant. Kolawole et al. (2013) carried out the toxicological assessment of the ethanolic extract of the leaves of *Newbouldialaavis* in albino mice and reported an oral LD₅₀ value of 5400 mg/kg body weight. According to expert recommendations, a chemical with a large LD₅₀ (>5,000 mg/kg) is practically non-toxic and may not likely cause any toxicity on short-term exposure (Erhirhie et al., 2018). With this recommendation in view, the ethanol leaf extract of *N. laevis* may be non-toxic on acute oral exposure.

Significant reduction in mean body weight after chronic administration of the extract could be as a result of the anti-nutritional effects of the saponins and tannins that are present in the leaf extract (Aganga and Tshwenyane, 2003). The body weight reduction may be an added advantage in the management of diabetes where a simple weight reduction can improve insulin sensitivity and enhance glucose metabolism (Mackenzie and Elliott, 2014).

The liver represents an organ with a high susceptibility to toxic effects of drugs due to its fundamental anatomy, high metabolic potential and ability to clear xenobiotics from the blood (0). Liver damage is often reflected by biochemical abnormalities like rise in level of enzymes normally found predominantly in the liver (Gu and Manautou, 2012). Serum changes in these enzymes concentration reflect hepatocyte integrity (Lala et al., 2022). Injury to the liver whether acute or chronic results in an increase in serum concentrations of aspartate (AST) and alanine aminotransferases (ALT) (Giannini et al., 2005). Both aminotransferases are highly concentrated in the liver (Giannini et al., 2005). AST is also diffusely represented in the heart, skeletal muscle, kidney, brain and red blood cells while ALT has low concentration in skeletal muscle and kidney (Ndrepapa, 2021). Increase in ALT serum level is therefore more specific for liver damage (Giannini et al., 2005). Thus, the decreased ALT levels reported in this present study may signify

Comment [U4]: insert a reference

a hepatoprotective benefit of the ethanol leaf extract. Antioxidant activity have been shown in several studies as one of the possible mechanisms of hepatoprotection (Harish and Shivanandappa, 2006; Kinoshita et al., 2007; Huang et al., 2010). Anaduaka *et al.* (2013) evaluated the effects of the extracts of *N. laevis* leaves and stem on liver marker enzymes and antioxidant enzymes in rat models. They reported an increased activities of superoxide dismutase, catalase and glutathione levels in the diabetic rats after treatment. High levels of alkaline phosphatase (ALP) and alanine aminotransferase (ALT), which are typical of oxidative stress conditions, were differentially ameliorated after treatment with the ethanol extracts of *N. laevis* leaves and stem in a dose-dependent manner. Our previous study on the leaves extract also reveals strong antioxidant potentials both in vitro and in vivo (Mbagwue *et al.*, 2021). Non-hepatotoxic effect after chronic administration of the extract may have been resulted from it antioxidant mediated hepatoprotective effect.

The kidney is the main site for excretion of many drugs and their metabolites which may be toxic (Lea-Henry *et al.*, 2018). This characteristic exposes the kidney to a variety of potential toxicants. Urea and creatinine are nitrogenous end products of metabolism. The rationale for their use to assess renal function is that plasma/serum levels of both reflect glomerular filtration rate (GFR) which defines kidney function (Goundenet *et al.*, 2022).

Another important target organ to drug toxicity is the haematological system. The proliferative nature of this system and its constant circulation carrying drugs and their metabolites from one site to another in the body makes them particularly sensitive target of drug toxicity (Guengerich, 2011). The haematological system is also susceptible to secondary effects of toxic agents that affect the supply of nutrients such as iron; the clearance of toxins and metabolites, such as urea or the production of vital growth factors such as erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF) (Bath and Sprigg, 2007). The consequences of direct or indirect damage to blood cells and their precursors are potentially life-threatening and include but not limited to hypoxia, hemorrhage and infection (Yoshida *et al.*, 2019). Lack of haematotoxicity and renal toxicity after 90 days oral administration of *N. laevis* are additional indications of likely safety of this plant extract.

Comment [U5]: make

Conclusion

The results from this study provide evidence for safety profile of the ethanol leaves extract of *N. laevis* thus supportive of its validity in the use for treatment of chronic diseases like diabetes.

References

- Ekor, M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4: 177. doi: 10.3389/fphar.2013.00177.
- Kemppainen, L. M., Kemppainen, T. T., Reippainen, J. A., Salmenniemi, S. T. and Vuolanto, P. H. (2018). Use of complementary and alternative medicine in Europe: Health-related and sociodemographic determinants. *Scandinavian Journal of Public Health*, 46(4): 448–455. doi: 10.1177/1403494817733869.
- Welz, A. N., Emberger-Klein, A. and Menrad, K. (2018). Why people use herbal medicine: insights from a focus-group study in Germany. *BMC Complementary and Alternative Medicine*. 18: 92. <https://doi.org/10.1186/s12906-018-2160-6>.
- Ezekwesili-Ofili, J. O. and Okaka, A. N. (2019). Herbal Medicines in African Traditional Medicine. <https://www.intechopen.com/chapters/64851>. Accessed on 21/04/2023.
- Bennadi, D. (2014). Self-medication: A current challenge. *Journal of Basic Clinical Pharmacy*, 5(1): 19–23. doi: 10.4103/0976-0105.128253.
- Useful Tropical Plants (2022). *Newbouldialaavis*. <https://tropical.theferns.info/viewtropical.php?id=Newbouldia+laevis>. Accessed 22/04/2023.
- Bothon, F., moustapha, M., Bogninous, G., Dossa, P., Yehouenou, B., Medoatinsa, S., Noudogbessi, J., Avlessi, F. and Sohounhloue D. (2014). Chemical characterization and Biological activities of *Newbouldialaavis* and *pterocarpussantalinoide*s leaves. *Bulletin of environment, pharmacology and life sciences*, 3(11): 9-15.
- Bafor, E. and Sanni, U. (2009). Uterine contractile effects of the aqueous and ethanol leaf extracts of *NewbouldiaLaevis* (Bignoniaceae) *in vitro*. *Indian Journal of Pharmaceutical Sciences*, 71(2): 124–127. doi: 10.4103/0250-474X.54274.
- Kolawole, O., Akanyi, M., Awe, O. and Akiibinu, M. (2013). Ethanolic extract of leaves of *Newbouldialaavis* attenuates Glycosylation of hemoglobin and lipid peroxidation in diabetic rats. *American Journal of Pharmacology and Toxicology*, 8, 179 – 186.
- Mbagwu, I. S., Akah, P. A., Ajaghaku, A. A., Ugwu, O. C., and Ajaghaku, D. L. (2021). Inhibition of oxidative stress and gastric emptying as additional mechanisms of antidiabetic activity of *Newbouldialaavis*. *Phytomedicine Plus*, 1(2021): 100023. <https://doi.org/10.1016/j.phyplu.2021.100023>.
- Mbagwu, I. S., Akah, P. A. and Ajaghaku, D. L. (2020). *Newbouldialaavis* improved glucose and fat homeostasis in a TYPE-2 diabetes mice model. *Journal of Ethnopharmacology*, 251: 112555. doi: 10.1016/j.jep.2020.112555.

Mbagwu, I. S., Akah, P. A., Ajaghaku, D. L., Ike, J. C. and Okoye, F. B. (2022). Newboulasides A and B, two new caffeic acid glycosides from *Newbouldialaevis* with α -amylase inhibitory activity. *Natural Product Research*, 36(3): 726-734. doi: 10.1080/14786419.2020.1799362.

Jităreanu, A., Trifan, A., Vieriu, M., Caba, I., Mărțu, I. and Agoroaei, L. (2023). Current Trends in Toxicity Assessment of Herbal Medicines: A Narrative Review. *Processes*, 11(1): 83. <https://doi.org/10.3390/pr11010083>.

Erhirhie, E. O., Ihekwereme, C. P. and Ilodigwe, E. E. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology*, 11(1): 5–12. doi: 10.2478/intox-2018-0001.

Norman, G. A. (2019). Limitations of Animal Studies for Predicting Toxicity in Clinical Trials. *JACC: Basic to Translational Science*, 4(7): 845–854. doi: 10.1016/j.jacbts.2019.10.008.

Vandenberg, L. N. and Blumberg, B. (2018). *Comprehensive Toxicology*. <https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/measures-of-toxicity>. Accessed on 22/04/2023.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. and Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2): 60–72. doi: 10.2478/intox-2014-0009.

Owolabi, O., Amaechina, F. and Okoro, M. (2011). Effect of ethanol leaf extract of *Newbouldialaevis* on blood glucose levels in diabetic rats. *Tropical Journal of Pharmaceutical Research*, 10(30): 249 – 254.

Aganga, A. and Tshwenyane, S. (2003). Feeding values and anti-nutritive factors of forage tree legumes. *Pakistan Journal of Nutrition*, 2(3): 170-177.

Mackenzre, R. and Elliott, B. (2014). Akt/PKB activation and insulin signaling: a novel insulin signaling pathway in the treatment of type 2 Diabetes. *Diabetes, metabolic syndrome and obesity: Targets and therapy*, 7, 55-64.

Gu, X. and Manautou, J. E. (2012). Molecular mechanisms underlying chemical liver injury. *Expert Reviews in Molecular Medicine*, 14: e4. doi: 10.1017/S1462399411002110.

Lala, V., Zubair, M. and Minter, D. A. (2022). *Liver Function Tests*. <https://www.ncbi.nlm.nih.gov/books/NBK482489/>. Accessed on 22/04/2023.

Edoardo G. Giannini, Roberto Testa, and Vincenzo Savarino. (2005). Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal*, 172(3): 367–379. doi: 10.1503/cmaj.1040752.

- Ndrepepa, G. (2021). Aspartate aminotransferase and cardiovascular disease—a narrative review. *Journal of Laboratory and Precision Medicine*, 6: 6. <http://dx.doi.org/10.21037/jlpm-20-93>.
- Harish, R. and Shivavandappa, T. (2006). Antioxidant activity and hepatoprotective potential of *Phyllanthusniruri*. *Journal of food chemistry*, 95(2): 180 – 185. DOI: 10.1016/j.foodchem.2004.11.049
- Kinoshita, S., Inoue, Y., Nakama, S., Ichiba, T. and Aniya, Y. (2007). Antioxidant and hepatoprotective actions of medicinal herb, *Terminalia catappa* L. from Okinawa Island and its tannin corilagin. *Phytomedicine*, 4(11): 755-762. DOI: 10.1016/j.phymed.2006.12.012.
- Huang, B., Ban, X., He, J., Tong, J., Tian, J. and Wang, Y. (2010). Hepatoprotective and antioxidant activity of the ethanolic extract of edible lotus (*NelumbonuciferaGaerta*) leaves. *Journal of food chemistry*, 120(3): 873 – 878. DOI: 10.1016/j.foodchem.2009.11.020.
- Lea-Henry, T. N., Carland, J. E., Stocker, S. L., Sevastos, J., Roberts, D. M. (2018). Clinical Pharmacokinetics in Kidney Disease. *Clinical Journal of the American Society of Nephrology*, 13(7): 1085-1095. DOI: 10.2215/CJN.00340118.
- Gounden, V., Bhatt; H. and Jialal, I. (2022). Renal Function Tests. <https://www.ncbi.nlm.nih.gov/books/NBK507821/>. Accessed on 22/04/2023.
- Guengerich, F. P. (2011). Mechanisms of Drug Toxicity and Relevance to Pharmaceutical Development. *Drug Metabolism and Pharmacokinetics*, 26(1): 3–14.doi: 10.2133/dmpk.dmpk-10-rv-062.
- Bath, P. M. and Sprigg, N. (2007). Colony stimulating factors (including erythropoietin, granulocyte colony stimulating factor and analogues) for stroke. *Cochrane Database of Systematic Reviews*, (2): CD005207.doi: 10.1002/14651858.CD005207.pub3.
- Yoshida, T., Prudent, M. and D'Alessandro, A. (2019). Red blood cell storage lesion: causes and potential clinical consequences. *Blood Transfusion*, 17(1): 27–52. doi: 10.2450/2019.0217-18.
- Anaduaka, E., Ogugua, V., Egba, S. and Apeh, V. (2013). Comparative antidiabetic effects of ethanol extracts of *Newbouldialeavis* leaves and stem on serum lipid profile and lipid peroxidation status in alloxan induced diabetic rats. *World Journal of Pharmaceutical Sciences*, 2, 833 – 845.
- National Research Council (US) Institute for Laboratory Animal Research (NIH 1996). Washington (DC): National Academics Press (US).
- Odoh, U. E., Obi, P. E., Ezea, C. C., Anwuchaepe, A. U. (2019). *Phytochemical methods in plant analysis*. 1st Ed. Pascal Communications, Nsukka, Enugu, Nigeria. 47 p.
- Lorkes, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, Vol. 53, pp. 275-289.

Diallo, A., Eklugadegkeku, K., Agbonon, A., Aklikokou, K., Creepy, E. and Gbeassor, M. (2010). Acute and Sub chronic (28-Day) oral toxicity Studies of hydroalcoholic extract of *Lannaekerstingii* Engl. And *K. Krause* (Anacardiaceae) stem bark. *Journal of Pharmacology and Toxicology*, 5(7): 343 – 349.

Bigoniya, P., Sahu, T. and Tiwari, V. (2015). Hematological and biochemical effects of Sub chronic artesunate exposure in rats. *Toxicology Reports*, 2, 280 – 288.

UNDER PEER REVIEW