Assessment of median lethal dose and target organ toxicity of oral sub-chronic administration of *Newbouldia laevis* in rat and mice model of toxicity

Abstract

The acute and sub-chronic toxicities of the leaf extract of *Newbouldia 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 LD_{50} 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: *Newbouldia laevis;* Toxicity; Liver enzymes; kidney integrity; Hematology

1.0 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" [1]. "This past decade has obviously witnessed a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries [2], 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" [3]. "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" [4].

"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, preference of consumers for natural therapies and a greater interest in alternative medicines, erroneous belief that herbal products are superior to manufactured products [5], 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, high cost and side effects of most modern drugs, patients' belief that their physicians have not properly identified the problem; hence the feeling that herbal remedies are another option" [6] and a movement toward self-medication - lack of time to see a physician, confidentiality, "it worked for my friend or relative syndrome" [7].

"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" [4]. "Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored" [8]. 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.

Newbouldia laevis (Bignoniaceae) is a small tree about 7 – 8 m tall. 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. The roots and leaves are used in the treatment of dysentery, malaria, elephantiasis, migraines and seizures [9]. 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 [10]. The leaves are soaked in ethanol for the treatment of diabetes and sickle cell disease [11].

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 [12]; establishment of improved effect on glucose and fat homeostasis in a type-2 diabesity mice model [13]; demonstration that inhibition of oxidative stress and gastric emptying are part of it mechanisms of antidiabetic activity [12] and the isolation of two new caffeic acid glycosides (Newboulasides A and B) with α-amylase inhibitory activity [14]. 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.

2.0 MATERIALS AND METHOD

2.1 Plant Material

"Leaves of *Newbouldia laevis* 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 (BDCP), Nsukka. The Voucher specimen was deposited in the herbarium of the Faculty of Pharmaceutical Science, Nnamdi Azikiwe University, Agulu Campus. Fresh whole leaves weighing 4.3kg were cleaned and dried under shade for 14 days". [13] A total of 2.5kg 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.

2.2 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 5days 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" [13] (Pub. No. 85 -23 Revised 1985).

2.3 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 using the method described by Abubakar and Haque [15]. 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°Cto obtain the ethanol extract.

2.4 Phytochemical Analysis

"The phytochemical analysis of the leaf extract and fractions were carried out using standard methods" [16].

2.5 Acute Toxicity Study

"The acute toxicity test was carried out using modified Lorke's method" [17]. "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". [13]

2.6 Sub-chronic Toxicity Study

Forty-five (45) healthy albino rats randomized into 3 groups of 15 animals each were used. Group A was administered 302 mg/kg (ED_{50}) of *N. laevis* ethanol leaf extract. Group B were given 604 mg/kg (Double ED_{50}) 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 [18]. "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" [19].

2.7 Statistical Analysis

The results were analyzed using SPSS version 16 and presented as mean ± 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.

3.0 RESULTS

3.1 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 [13]

Phytocompounds	Extract
Flavonoids	+
Saponins	+
Tannins	+
Reducing sugar	+
Steroids	+
Terpenoids	+
Alkaloids	+
Glycosides	+
Yield (%)	15.56[a]

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

3.2 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 LD_{50} was therefore above 5,000 mg/kg in mice.

3.3 Result of Sub-chronic 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).

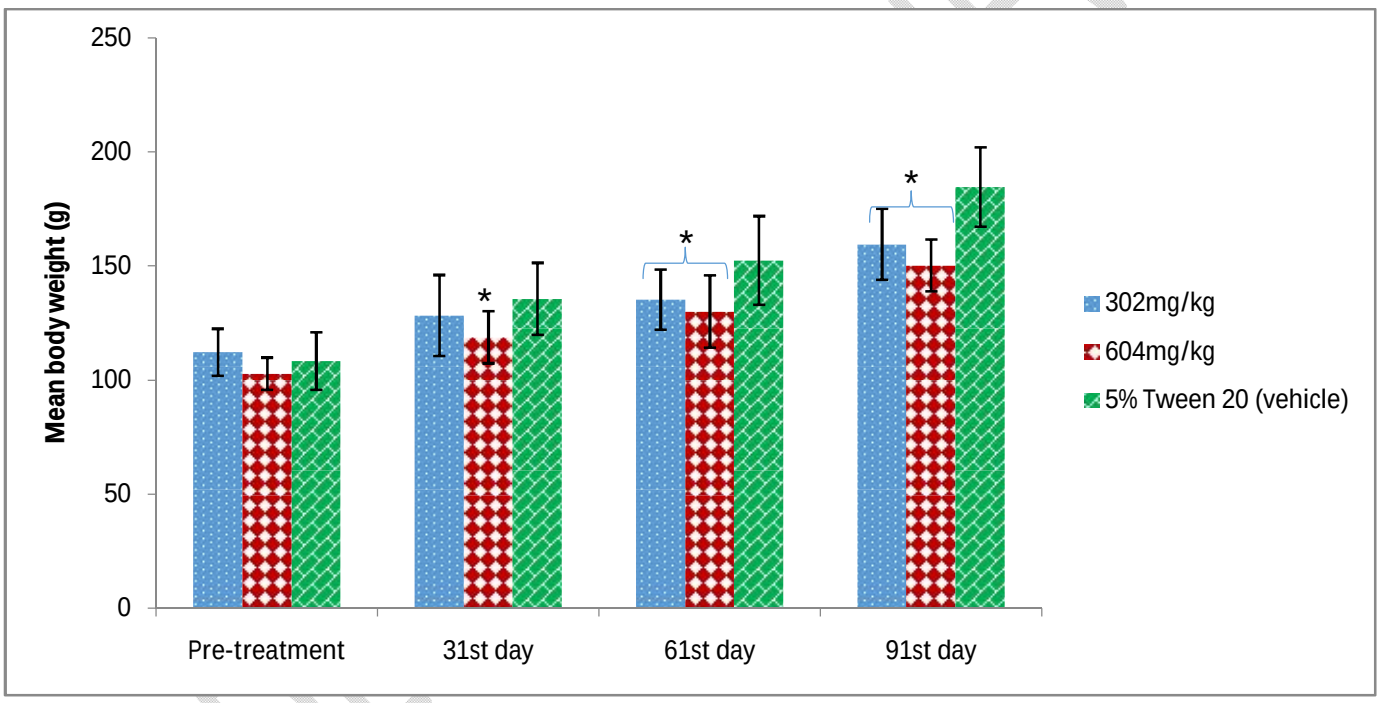

Figure 1: Effect of ethanol extract of *N. laevis* on body weight

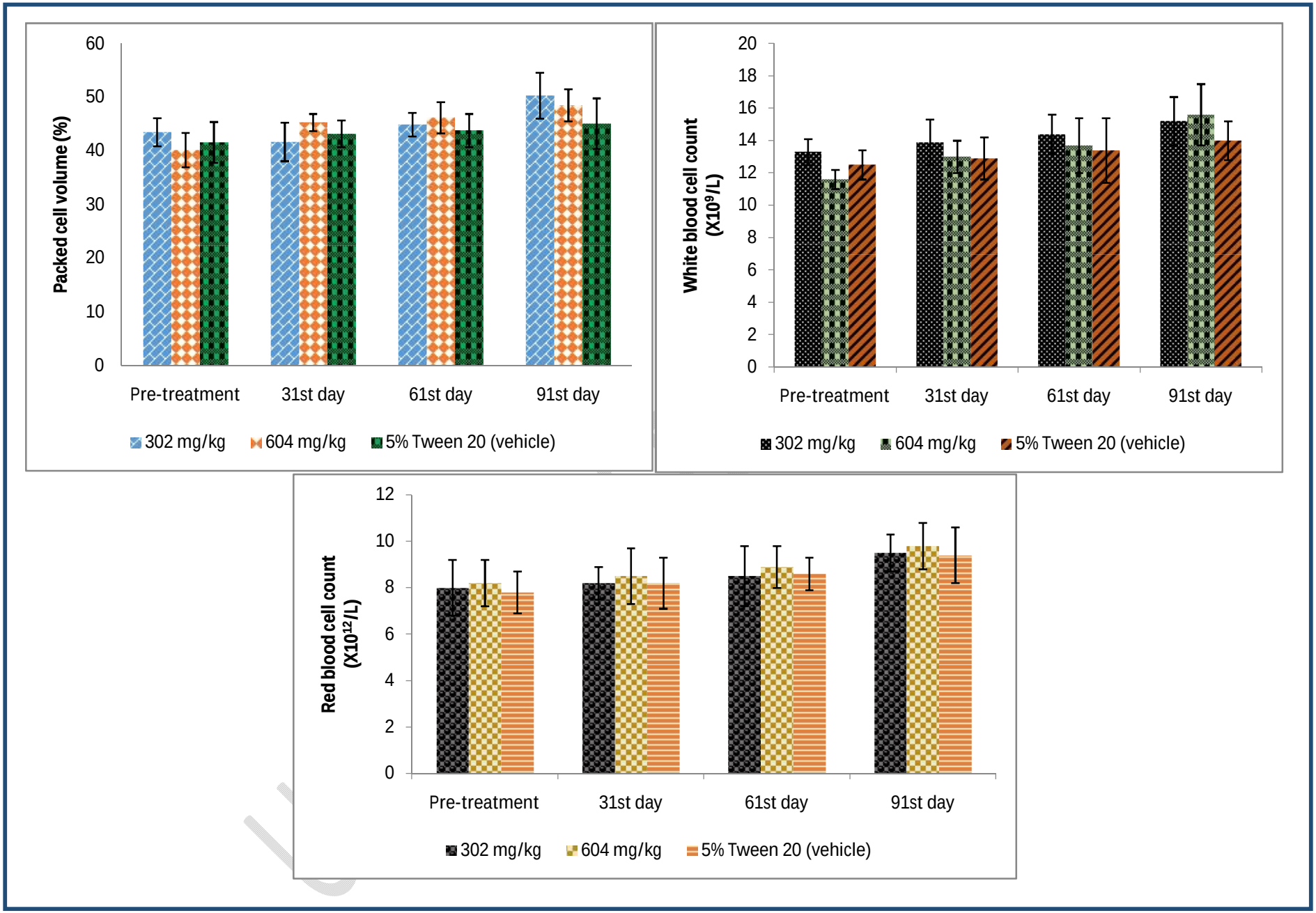

Figure 2: Effect of ethanol extract of *N. laevis* on hematological parameters

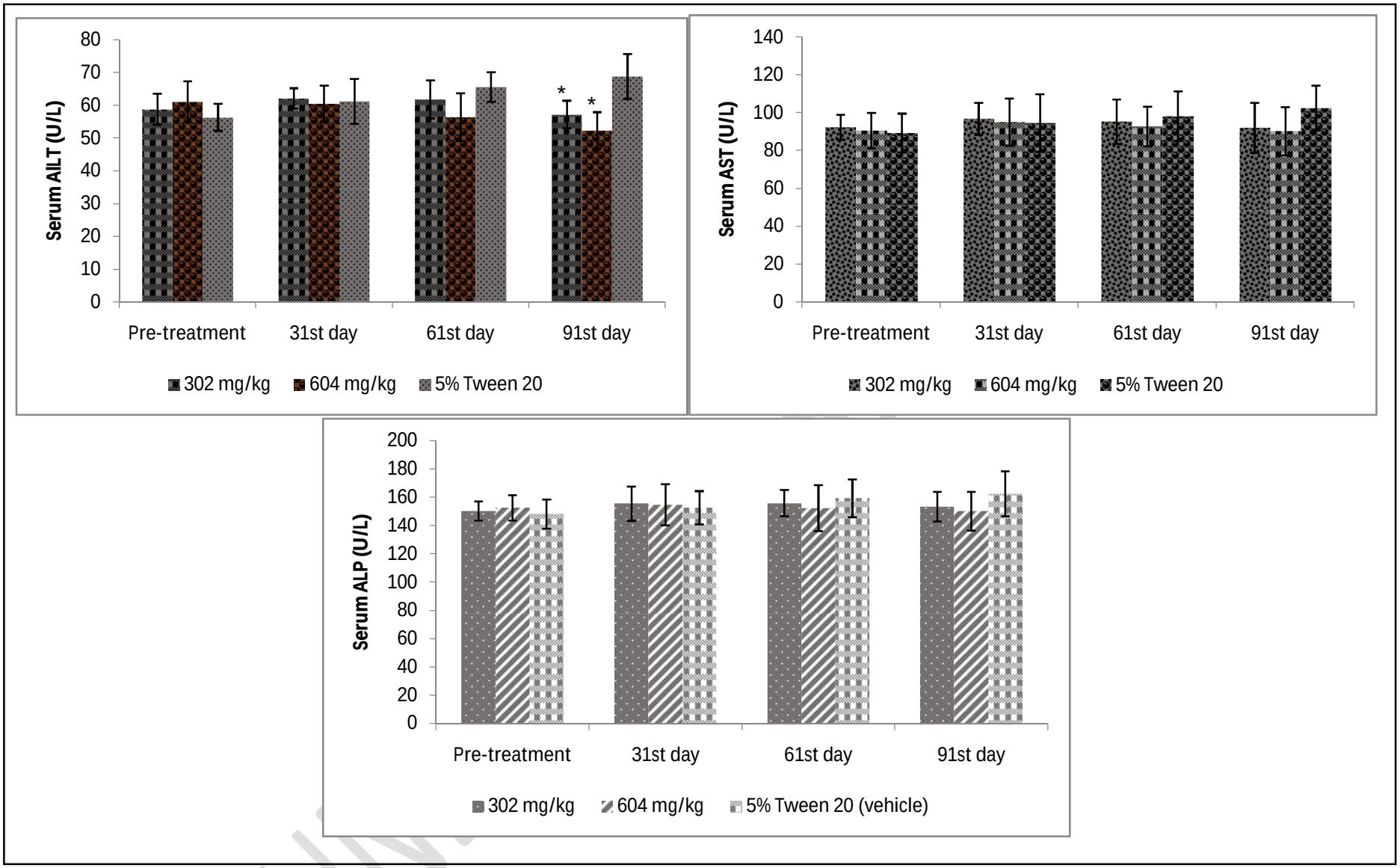

Figure 3: Effect of ethanol extract of *N. laevis* on serum liver enzymes

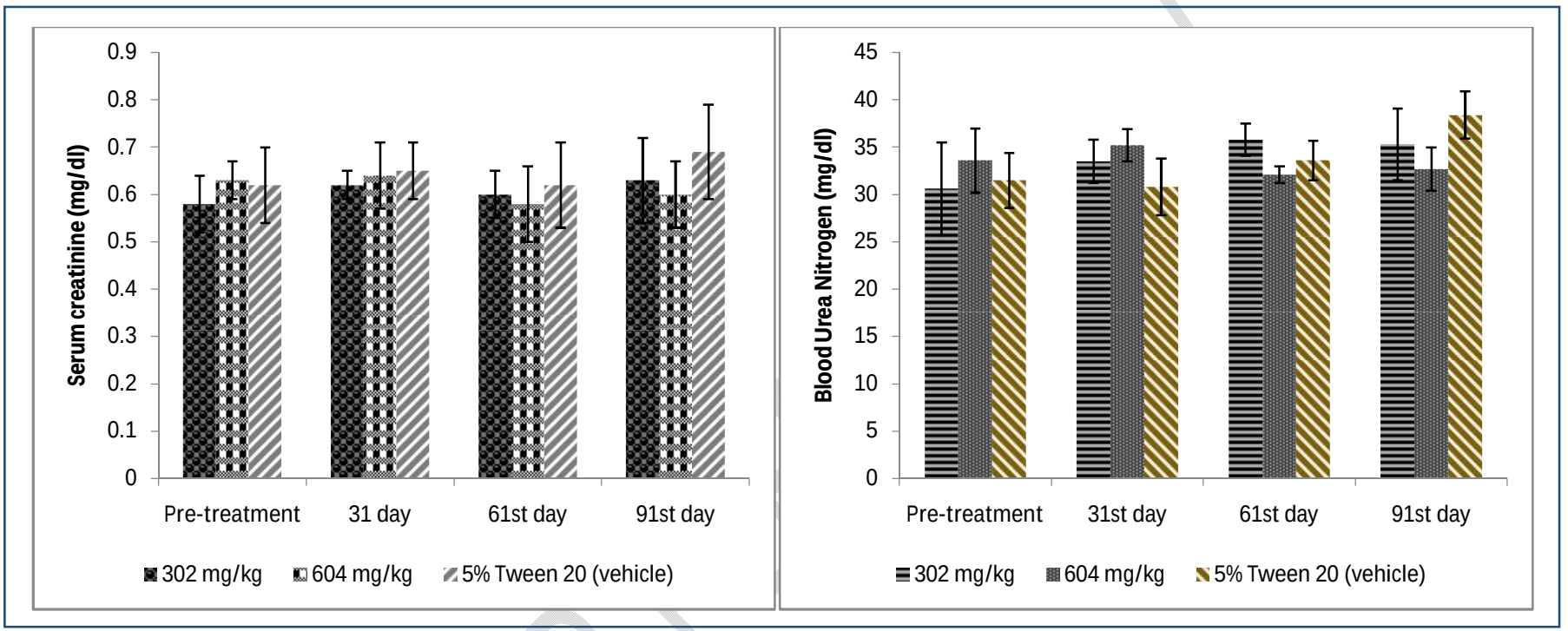

Figure 4: Effect of ethanol extract of *N. laevis* on kidney function parameters

4.0 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 [20]. 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 [21]. Although there may be challenges in extrapolation of animal findings to humans, experimental animals still present numerous advantages in human experimental toxicity protocol [22]. 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 [23]. All substances 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 [24]. 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 (ED_{50}) of *N. laevis* as well as double of this dose.

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., [25] who documented an oral LD_{50} value of 6 g/kg body weight in Wister albino rats using the ethanol leaf extract of the plant. Kolawole et al., [11] carried out the toxicological assessment of the ethanolic extract of the leaves of *Newbouldia laevis* in albino mice and reported an oral LD_{50} value of 5400 mg/kg body weight. According to expert recommendations, a chemical with a large LD_{50} (>5,000 mg/kg) is practically non-toxic and may not likely cause any toxicity on short-term exposure [21]. With this recommendation in view, the ethanol leaf extract of *N. leavis* 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 [26]. 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 [27].

“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” [28]. Liver damage is often reflected by biochemical abnormalities like rise in level of enzymes normally found predominantly in the liver [29]. Serum changes in these enzymes concentration reflect hepatocyte integrity [30]. “Injury to the liver whether acute or chronic results in an increase in serum concentrations of aspartate (AST) and alanine aminotransferases (ALT)” [31]. “Both aminotransferases are highly concentrated in the liver” [31]. “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” [32]. “Increase in ALT serum level is therefore more specific for liver damage” [31]. Thus, the decreased ALT levels reported in this present study may signify a hepatoprotective benefit of the ethanol leaf extract. Antioxidant activity have been shown in several studies as one of the possible mechanisms of hepatoprotection [33 – 36] 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" [12]. 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 [37]. 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 [38].

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 make them particularly sensitive target of drug toxicity [39]. "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)" [40]. 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 [41]. Lack of haematotoxicity and renal toxicity after 90 days oral administration of *N. laevis* are additional indications of likely safety of this plant extract.

Conclusion

The results from this study provide evidence for safety profile of the ethanol leaves extract of *N. laevis*. The median lethal dose was above the limit dose of 5000 mg/kg while no adverse effects were recorded on major target organs of drug toxicity like the liver, kidney and hematological system. These evidences provide support for the validity of ethanol extract of *N. laevis* in the use for treatment of chronic diseases like diabetes.

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