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HEPATOCURATIVE EFFECT OF METHANOL EXTRACT OF NEWBOULDIA LAEVIS LEAVES IN ALLOXAN-INDUCED LIVER DAMAGE IN ALBINO RATS

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Abstract

The aim of the study is to evaluate the effect (Hepatocurative and antibacterial potential) of Newboulda laevis leaves in alloxan-induced liver damage in Wister Albino Rats. The leaves of Newbouldia laevis was bought from a local market in Wukari, Taraba State. The leaves of Newbouldia laevis was chop and then pulverized into fine powder. The methanol crude extracts andthirty-two albino Wistar rats weighing 90-220 g was used for this research. Liver toxicity was induced in male rats by an intraperitoneal single dose of 150 mg/kg body weight. samples were subjected to biochemical estimation of liver function. The result showed a significant ($p \leq 0.05$) increase in serum AST level for group II (60.6 ± 1.12) which was administered high dose of Alloxan and serves as the negative control group, when compared to the normal control group I (35.09 ± 2.07) which only received feed and water. From the result described in table 4.0 above, it showed a significant ($p \leq 0.05$) increase in ALT level for group II (58.11 ± 0.42) negative control when compared with group I (20.00 ± 0.41) normal control. Group III (29.98 ± 0.12) which received standard drug

showed a significant decrease in ALT which is a sign that there is some level of repair on the liver cells. Group IV (36.22 ± 0.s70), group V (30.00 ± 0.27) and group VI (29.99 ± 0.12) which received 100, 200 and 400 mg/kg body weight of Newbouldia laevis leaves extract respectively when compared with the negative control group II, showed a significant decrease in ALT level. There was a significant ($p \leq 0.05$) increase in ALP level for group II (255.80 ± 1.12) which received Alloxan when compared with the normal control group I (80.21 ± 0.44) providing evidence to support the increase in ALT and AST level in group II, suggestive of liver injury. The treatment groups III, VI, V and VI showed significant ($p < 0.05$) difference, when compared to the negative control group. No significant ($p < 0.05$) difference between group V and VII. It was deduced that the total protein for group II (10.95 ± 0.37) significantly ($p < 0.05$) decreased when compared to the normal control group I (14.13 ± 0.55). This is because low levels of total protein in the blood can occur because of impaired function of the liver. Administration of Newbouldia laevis leaves extract significantly ($p < 0.05$) elevated total protein levels for group IV (10.99 ± 0.41), V (12.76 ± 0.41) and VI (12.98 ± 0.58). Albumin being the most abundant protein in the blood, equally followed same pattern as total protein, with Albumin level of (10.83 ± 0.10), (6.13 ± 0.05), (9.82 ± 0.18) (6.75 ± 0.13), (7.99 ± 0.10) and (9.60 ± 0.13) for group I, II, III, IV, V and V respectively. Thus, emphasizing the potential of the extract as a source agent for medication for ailment and diseases.

Keywords: Hepatocurative, Methanol Extract, Newbouldia Laevis, Leaves, Alloxan, Liver, Albino Rats

INTRODUCTION

The liver is a silent powerhouse, silently performing hundreds of vital tasks to keep us healthy. It filters toxins from our blood, produces essential proteins, and helps us digest food (Lewis et al., 2010). But like any hard-working organ, the liver is vulnerable to damage. Liver damage, also known as hepatic injury, is a broad term encompassing various conditions that impair the liver's function (Khuro et al., 2004). It can be a silent condition, with no noticeable symptoms in the early stages, making it all the more crucial to be aware of the potential causes and risk factors (Berto et al., 2012).

Historically medicinal plants have been provided a good source of inspiration for novel therapeutic drugs which has made a large contribution to health and well-being of humans. It has been used over the years to as curative agents against many infections and have been

exploited in the traditional medicine with their curative potentials well documented (Ibrahim et al., 2011). Most plants are capable of synthesizing some chemicals compounds that's are able to perform physiological action in the body. These chemicals compounds are known as phytochemicals. Plant product was used by man for health care delivery for centuries. The use of plant extract as medicine is wide spread throughout the world. While, plant disease remedies are as old as human history, it was estimated that about 80% of useful bioactive plant derived pharmaceuticals are used around the world as was discovered by University academicians, researcher from the field of traditional herbal medicine (Diba et al., 2013). Newbouldia laevis is a tropical plant belonging to the family of Bignoniaceae. It is among the most useful plants in Africa and grows up to 10 m height with a cauliflorous habit (Akerele et al., 2011).

It is an ever-greenish plant with a height of approximately 7–8 m high in the west Africa and up to 20 m in Nigeria. The plant has a characteristics shiny dark green leaves with large purple flowers (Bafor and Sanni, 2009). Different African countries have different names for Newbouldia laevis e.g Togo call it lifui, Ghana call it sesemasa, Hausa call it Aduruku, Igbo call it ogilisi or ògírìsì, Senegal call it gimgid, the Gambia call it kallihi, Yoruba call it Akoko, Guinea call it canhom, Urhobo call it Ogiriki, Sierra Leone call it Sherbro, Mali call it kinkin, Edo state call it íkhímì, Tiv call it Kontor, while the Ibibio call it itömö (Burkill, 1985). Newbouldia laevis is usually grown as an ornamental tree and planted by cuttings. It is a very popular plant in the African continent and is highly valuable due to its numerous immense benefits to human race (Egba et al., 2014). Some parts of Nigeria commonly regard this tree as the tree of fertility or the tree of life. Newbouldia laevis has been traditionally used in African herbal medicine for various medicinal purposes. Different parts of the plant, including the leaves, bark, and roots, are used to treat a range of health conditions such as malaria, gastrointestinal disorders, respiratory issues, and skin ailments (Bafor and Sanni, 2009).

Newbouldia laevis (Bignoniaceae) is commonly known as smooth Newbouldia or boundary tree. It is called 'Aduruku' in Hausa; 'Ogirisi' in Igbo; 'Ikhimi' in Edo and 'Akoko' in Yoruba languages (Hutchinson and Dalziel, 1963). It grows to a height of about 7 - 8 (up to 15) meters, more usually a shrub of 2-3 metres, many – stemmed forming clumps of gnarled branches (Arbonnier, 2004). Newbouldia laevis is widely used in African folk medicine for the treatment of malaria and fever, stomach-ache, coughs, sexually transmitted diseases, tooth ache, breast cancer, and constipation (Eyong et al., 2015). The

antimicrobial potential of methanol extract of the leaf has been reported in literature while the antinflammatory and antimalarial activities of the root extract have also been documented (Usman and Osuji, 2007).

Scientific reports on the phytochemical constituents of the plant revealed the presence of alkaloids and phenylpropanoids in the root (Germann et al., 2006), flavonoids, and tannins in the leaf (Usman and Osuji, 2007). Wound infection is detrimental to wound healing which is a complex process that can be delayed by many potential factors (Edwards and Harding, 2004). A variety of disorders commonly affect the eye and vary in severity from mild but annoying allergic conjunctivitis to sight threatening infections (Anton et al., 1996). As it has been asserted that medicinal plants constitute a continuous source of new compounds with the potential to act against multi-resistant bacteria (Al-Bayati et al., 2008).

Figure 1: Showing the new boulda leavis leaves with its blossom flower.

Statement of Problems

In Nigeria, a silent storm rages beneath the surface of daily life. It's a storm that cripples' lives, snatches families, and casts a long shadow of illness across the nation (Unekwe and Ekweozor, 2018). This storm is liver damage, a complex problem with global tentacles that grips Nigeria with a particular intensity (World Health Organization, 2023). The roots of this storm lie in multiple, tangled causes. Viral infections like hepatitis B and C run rampant, often transmitted unknowingly from mother to child or through contaminated needles. Unregulated herbal remedies, promising quick fixes but harboring hidden toxins, can wreak havoc on the delicate liver tissue. The burden of poverty and limited access to

clean water and sanitation fuels the fire, creating breeding grounds for infections that further endanger the vital organ (World Health Organization, 2023).

The consequences are devastating. Cirrhosis, liver fibrosis, and cancer become grim realities for many Nigerians, stealing decades of life and inflicting untold suffering. The healthcare system, already strained by resource limitations, buckles under the immense pressure. Liver transplants, a beacon of hope in developed nations, remain a distant dream for most Nigerians, their cost an insurmountable barrier (Nwokedi and Obiano, 2015). But liver damage in Nigeria is not an isolated crisis. It reflects a global challenge, a stark reminder of the interconnectedness of human health. The viruses that afflict Nigeria also plague other continents. The poverty that fuels its spread echoes in marginalized communities worldwide. Addressing this issue demands a global response, a concerted effort to share knowledge, resources, and solutions.

Significance of the Study

Studying the effects of Newbouldia laevis leaves extracts on alloxan-induced liver damage represents a significant opportunity in utilizing readily available plant resources for healthcare holds the potential for both environmental and economic benefits. Developing treatments based on Newbouldia laevis could contribute to a more sustainable healthcare system while empowering local communities with knowledge about valuable medicinal resources.

Trado-Medical Uses

The trado-medical uses of N. laevis widely depend on the ethnic location of the plant and the part of the plant used. The Togolese and Nigerians use the leaves prepared as a decoction alone or in combination with other plants and administered orally for the treatment of malaria and fever (Yusuf et al., 2016). Also, the Tiv people of North-Central Nigeria prepare a polyherbal decoction of the leaves mixed with leaves of Crossopteryx febrifugg and Morinda lucida and taken orally to treat malaria (Tor-anyiin et al., 2003). Inhabitants of Omo Forest Reserve in Western Nigeria boil the leaves in combination with leaves of Mangifera indica and M. lucida and taken orally to treat malaria and fever.28 Similarly, in Southern Nigeria, the Ikwere people use the leaves, stem bark and roots for treating migraine, skin infections, fever, malaria, stomach ache, epilepsy and conjunctivitis (Nwauzoma et al, 2013. Furthermore, the Ijebu people of Southwest Nigeria use the leaves decoction in combination with Momordica charantia, Vernonia amygdalina and Ocimum

gratissimum leaves to treat measles, while the stem bark boiled with sugar cane juice is used in the treatment of dysmenorrhea by the Ibibio of Southern Nigeria (Sonibare et al., 2009). In other reports, Ghanaians, Cameroonians and Nigerians use the bark, roots and leaves for the treatment toothache, stomachache, diarrhea, dysentery, malaria, fever, breast cancer, sexually transmitted diseases (STDs), anemia, ulcer, arthritis, rheumatism, hemorrhoids, constipation cardiovascular diseases, diabetes, cough, elephantiasis and urinogenital tract infection (Aladesanmi et al., 1994).

Thus, Alloxan is the chemical used for induced liver damage in albino rats. Its also known as known as 5,5-dihydroxyl pyrimi-dine-2,4,6-trione is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analog (Lenzen, 2008). The compound has the molecular formulae, C4H2N2O4 and a relative molecular mass of 142.06. Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving diabetes. Among the known diabetogenic agents which include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load and anti-insulin serum; alloxan and streptozotocin (STZ) are the most widely used in diabetes studies (Lenzen, 2008). Junod et al. (2017) suggested that the mechanisms by which Alloxan monohydrate brings about its diabetic state are selective damage of pancreatic insulin secreting beta cells, which causes cells to become less active.

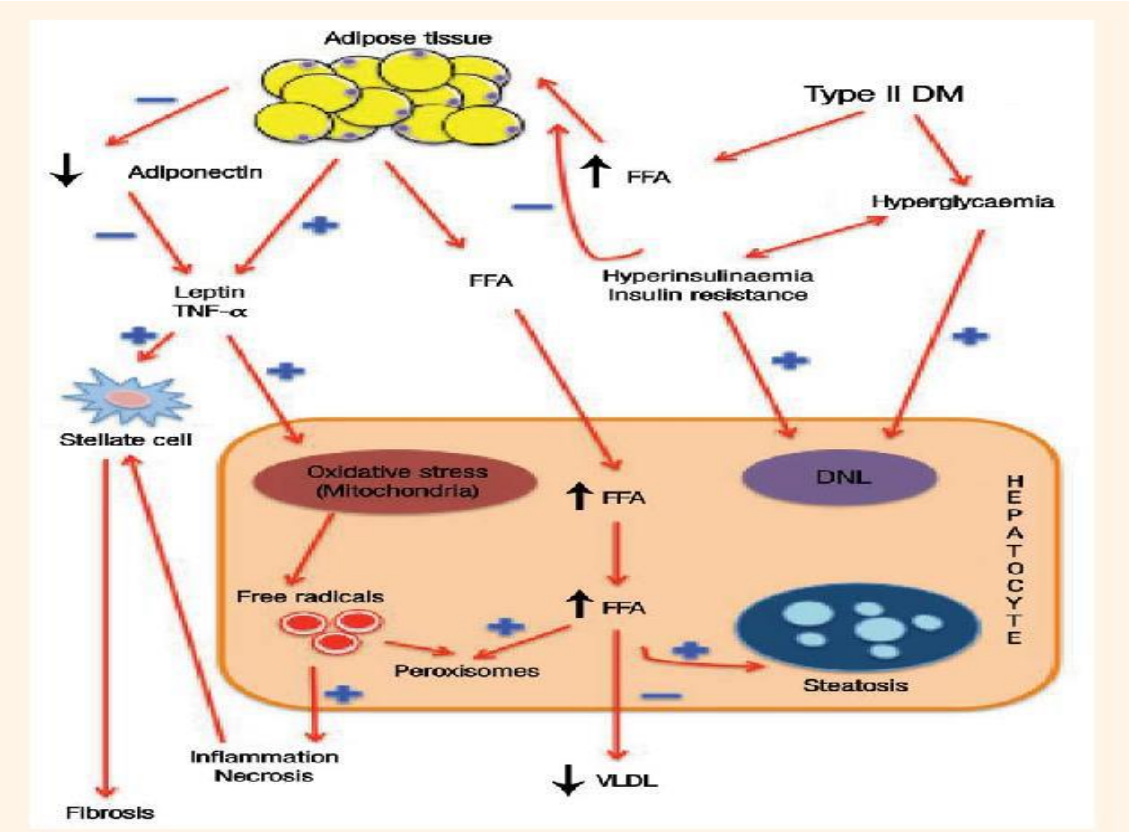


Figure 2: Alloxan Mechanism of Inducing Liver Damage

Alloxan Mechanism of Inducing Liver Damage

Alloxan, a diabetogenic compound, wreaks havoc on the liver through a complex interplay of mechanisms, ultimately leading to cell death and the characteristic features of diabetic liver disease. Understanding this intricate dance of destruction is crucial for developing effective therapeutic strategies. One of the primary culprits is alloxan's potent oxidative stress. It disrupts the delicate balance of reactive oxygen species (ROS) and antioxidants within hepatocytes, tipping the scales towards a pro-oxidant environment. This oxidative onslaught damages cellular components like proteins, lipids, and DNA, culminating in mitochondrial dysfunction and energy depletion (Szkudelski, 2012).

Furthermore, alloxan depletes intracellular glutathione (GSH), a key antioxidant molecule. This depletion weakens the liver's defence against ROS, exacerbating the oxidative stress and paving the way for further cellular damage (Wu et al., 2018). Alloxan's nefarious effects extend beyond mere oxidative mayhem. It disrupts calcium homeostasis, leading to a dangerous rise in intracellular calcium levels. This calcium overload activates proteases and

phospholipases, enzymes that dismantle cellular structures and trigger apoptosis, the programmed cell death pathway (Cárdenas, 2011). Adding to the mayhem, alloxan impairs insulin signalling, hindering the liver's ability to regulate glucose metabolism. This metabolic dysregulation disrupts energy homeostasis and contributes to further cellular stress (Kang, 2012). The final act of this tragic play involves inflammation. Alloxan triggers the release of pro-inflammatory cytokines, attracting immune cells that infiltrate the liver and exacerbate tissue damage. This inflammatory response further amplifies the oxidative stress and cell death cascade (Li, 2014).

Diagram below show the mechanism of liver damage in diabetes mellitus. Insulin resistance causes peripheral adipocytes to undergo lipolysis. Free fatty acids are then released to the bloodstream and eventually accumulate in the liver. At the same time, adipocytokines release tumour necrosis factor-α and leptin, worsening the hepatocyte damage by increasing oxidative stress in the mitochondria. The combined action of mitochondrial oxidative stress, hyperinsulinemia and hyperglycaemia produce free radicals which in turn induce inflammation and cellular necrosis. Tissue inflammation stimulates the hepatic stellate cells to produce collagen, leading to fibrosis, cirrhosis and, finally, hepatocellular carcinomas (Mohamed et al., 2016).

METARIALS AND METHODS

Materials

Wooden mortar and pestle, Rat cages, Digital analytical weighing balance (Ohaus: PA-1000), Beakers, Whatman number 1 filter paper, Conical flask, Spatula, Measuring cylinder, Separating funnel, Aluminum foil, Sample bottles, Plastic funnels, Masking tape, Thermostatic water cabinet (Model:HH-W420), Canula attached to a graduated syringe, Dissection kits, Desiccator, Rotary evaporator, Refrigerator Centrifuge and Spectrophotometer.

Sample Collection

The leaves of *Newbouldia laevis* was bought from a local market in Wukari, Taraba State.

Extract preparation

The leaves of *Newbouldia laevis* was chop into pieces, air-dried for four days and then pulverized into fine powder. About 250 g of the powdered bark extracted with 2 L of

ethanol using maceration method for 72 hrs. The methanol was concentrated in a rotary evaporator after filteration. The extract obtained was kept in an air-tight container in the refrigerator till required. This gave the ethanol extract.

Fractionation of crude methanol extract of*. N. laevis* stem bark

50 ml of crude methanol extract was measured using a measuring cylinder into a separating funnel; the methanol fraction settled at the bottom of the separating funnel. The methanol fraction was collected into a beaker and was concentrated using rotary evaporator under reduced pressure and concentrates transferred into air-tight container and preserved in the refrigerator at 4ºC prior to administration. 24

Liver Damage Induction

Normal saline and 2g sodium chloride were dissolved into water. Liver toxicity was induced in male rats by an intraperitoneal single dose of 150 mg/kg body weight (Abouzed *et al.,* 2018). The animals were given the alloxan sample according to their body weight.

Animals and Experimental Protocol

32 albino Wistar rats weighing 90-220 g was used for this research. Animals was handled following the guidelines of the National Institutes of Health (NIH) for laboratory animal welfare, and the experimental protocol which was approved by the Local Ethics Committee and Animals Research (AU14-190323-2-7). The rats were housed in cages and maintained at a temperature of 22±2°C, relative humidity of 40-60%, with a 12 h/12 h light/dark cycle and open access to pellet diet and water *ad libitum*. After a couple of weeks of adaptation, rats were randomly assigned to four groups with six animals each as follows: group I "control normal group" rats did not receive any drugs, group II "negative group "received only alloxan prepared mixture. Group III "positive "received treatment with alloxan and as treated with a known drug. Group IV to group VI received treatment of alloxan and *Newbouldia laevis* aqueous extract (1 g/kg orally for 30 days) (Soren *et al.,* 2016). Towards the end of the experimental time, rats were anesthetized using chloroform then euthanized, and the blood were taken for further analysis.

Blood Samples

Blood specimens were singly gathered from rat aorta in nonheparinized glass tubes and left for 15 min. at 25°C to clot before being centrifuged at 3000 × g for 15 min. Sera were retained at -80°C until used.

Assessment of Liver Function Tests

Serum was separated from the clotted blood by centrifugation Refrigerated centrifuge at 4000 rpm at 4°C for 5 minutes. Serum samples were immediately subjected to biochemical estimation of liver function: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma-glutamyl transferase (GGT), Total protein (T.P) and Albumin (ALB) using uv/visible Spectrophotometer.

Assessment of Aspartate Aminotransferase (AST) Activity

AST activity was determined by the method described by (Reitman and Frankel, 1957) using Randox reagent kit.

Principle:

L-Aspartate and α-Ketoglutarate react in the presence of AST in the sample to yield oxaloacetate and L-glutamate.

α – oxoglutarate + L– aspartate GOT L- glutamate + oxaloacetate.

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4- dinitrophenylhydrazine

Reagent mixture for determination of GGT

Sample 100 µL

Mixed and incubated for 1 minute at 37ºC. The change in absorbance per minute (ΔOD/min) during 3 minutes was read.

Calculation Gamma GT activity (U/L) = (OD/min) x 1158

Determination of Total Protein (T.P)

The total protein concentration was determined using the method described by (Weichselbaum, 1946) using Randox reagent kit.

Principle:

Cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex.

Reagent mixture for determination of Total protein

	Reagent Blank	Standard	Sample
Distilled Water	20 µL	-	-
Standard (CAL)	-	20 µL	20 µL
Serum	-	-	20 µL
R1	1000 µL	1000 µL	1000 µL

Serum sample was mixed and incubated for exactly 30mins at +20 to +250C, after which the absorbance of the sample and that standard was read against the reagent blank at 546nm.

Calculation;Total protein Conc. = 19 x A sample (g/dL).

Determination of Albumin (ALB)

The serum albumin concentration was determined by the method described by (Doumasa, et al., 1971) using Agappe kit.

Principle: The reaction between albumin from serum and the dye bromocresol-green produces a change in colour that is proportional to the albumin concentration.

Reagent mixture for determination of Albumin (ALB)

	Blank	Standard	Sample
Reagent	1000 µL	1000 µL	1000 µL
Standard			
Sample	-	10 µL	-

Mixed and incubated for 1 minute. The absorbance of standard and sample against the reagent blank were measured

Calculation; **Calculation**

$$\text{Albumin Conc. (g/dL)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 3$$

Statistical Analysis

Statistical analysis was carried out with the use of one-way analysis of variance (ANOVA) and furthers with Duncan multiple comparisons using Statistical Package for Social

Sciences (SPSS), version 23. The result means were compared for significance at $p<0.05$ and the group results presented as mean ± standard deviation (n = 5).

RESULTS

Result obtained from the experiment, showing the effect of extract on the liver by assessing the concentration of liver enzymes in the blood was described in this chapter.

Table 1: Effect of *Newbouldia laevis* leaves methanol crude extract on hepatic marker enzymes

GROUPS	ALT (U/I)	AST (U/I)	ALP (U/I)
Normal Control	20.00 ± 0.41[a]	35.09 ± 2.07[a]	80.21 ± 0.44[a]
Negative Control	58.11 ± 0.42[d]	60.60 ± 1.12[d]	255.80 ± 1.12[d]
Positive Control	29.98 ± 0.12[b]	38.00 ± 2.18[b]	80.69 ± 1.49[a]
100 mg/kg	36.22 ± 0.70[c]	50.00 ± 2.11[c]	94.17 ± 1.68[c]
200 mg/kg	30.00 ± 0.27[b]	48.11 ± 1.17[c]	84.59 ± 0.68[b]
400 mg/kg	29.99 ± 0.12[b]	40.23 ± 1.32[b]	84.86 ± 0.95[b]

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

Key: ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase

Table 2: Effect of Newbouldia laevis leaves methanol crude extract on hepatic marker serum proteins

GROUPS	TB (µmol/L)	DB (µmol/L)	TP (g/dL)	ALB (g/dL)
Normal Control	4.58 ± 0.27[a]	3.29 ± 0.12[a]	14.13 ± 0.55[c]	10.83 ± 0.10[d]
Negative Control	15.62 ± 0.09[d]	8.41 ± 0.10[d]	10.95 ± 0.37[a]	6.13 ± 0.05[a]
Positive Control	8.10 ± 0.17[c]	4.42 ± 0.12[b]	14.42 ± 0.26[c]	9.82 ± 0.18[c]

100 mg/kg	6.06 ± 0.13 [b]	5.10 ± 0.06 [c]	10.99 ± 0.41 [a]	6.25 ± 0.13 [a]
200 mg/kg	6.00 ± 0.18 [b]	3.75 ± 0.16 [a]	12.76 ± 0.41 [b]	7.99 ± 0.10 [b]
400 mg/kg	5.86 ± 0.18 [b]	4.57 ± 0.13 [b]	12.98 ± 0.58 [b]	9.60 ± 0.13 [c]

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

Key: TB: total bilirubin, DB: direct bilirubin, TP: total protein, ALB: albumin

Effect of Newbouldia laevis leaves Extract on Total Protein Activity

The result for Total Protein (TP) activity as presented on table 4.2, showed a significant ($p<0.05$) difference in TP level for group II (10.95 ± 0.37 [a]), group IV, (10.99 ± 0.41 [a]), group V (12.76 ± 0.41 [b]), group VI (12.98 ± 0.58 [b]), when compared to the normal control group I (14.13 ± 0.55 [c]), but no significant ($p<0.05$) difference was observed between group III (14.42 ± 0.26 [c]), and group I. There is also a significant ($p<0.05$) difference across the groups (I, III, V, and VI) when compared with the negative control group (II). No significant ($p<0.05$) difference was observed between group IV and group II.

Effect of *Newbouldia laevis* leaves Extract on ALBUMIN Activity

Result for Albumin (AB) as presented on table 2, showed a significant ($p<0.05$) difference in AB level for group II (6.13 ± 0.05 [a]), group III (9.82 ± 0.18 [c]), IV (6.25 ± 0.13 [a]), group V (7.99 ± 0.10 [b]) and group VI (9.60 ± 0.13 [c]), when compared to the normal control group I (10.83 ± 0.10 [d]) but no significant ($p<0.05$) difference was observed between group II and group IV, group III and group VI.

Effect of *Newbouldia laevis* leaves Extract on Total Bilirubin Level

Result for Total Bilirubin (TB) as presented on table 4.2, showed a significant ($p<0.05$) difference in TB level for group II (15.62 ± 0.09 [d]), group III (8.10 ± 0.17 [c]), IV (6.06 ± 0.13 [b]), group V (6.00

± 0.18 [b]) and group VI (5.86 ± 0.18 [b]), when compared to the normal control group I (4.58 ± 0.27[a]). A significant ($p<0.05$) difference was observed for all groups, when compared to the negative control group II. But no significant ($p<0.05$) difference was observed between group IV, V and VI.

Effect of leaves Extract on Total Bilirubin Level

Result for Direct Bilirubin (DB) as presented on table 2, showed a significant ($p<0.05$) difference in DB level for group II (8.41 ± 0.10^{d}), group III (4.42 ± 0.12^{b}), IV (5.10 ± 0.06^{c}), and group VI (4.57 ± 0.13^{b}), except for group V which showed no significant difference when they are compared to the normal control group I (3.29 ± 0.12^{a}). Significant ($p<0.05$) difference was observed for all groups, when compared to the negative control group II, but no significant difference was observed between group III and group VI.

4Effect of *Newbouldia laevis* leaves Extract on Total Bilirubin Level

From table 3, significant antibacterial activity was observed at all concentrations compared to the control, as denoted by '*'.

ALT

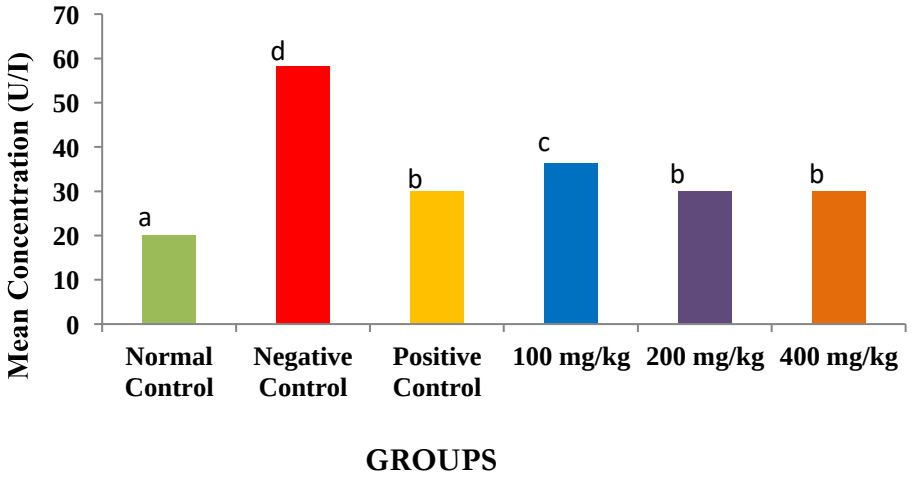
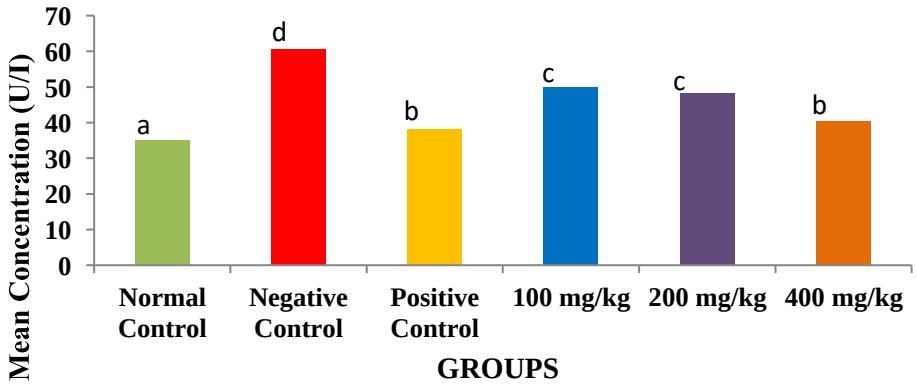


Figure 1: Bar chart showing alanine transaminase concentration

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

AST



Figures 2: Bar chart showing aspartate transaminase concentration

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

ALP

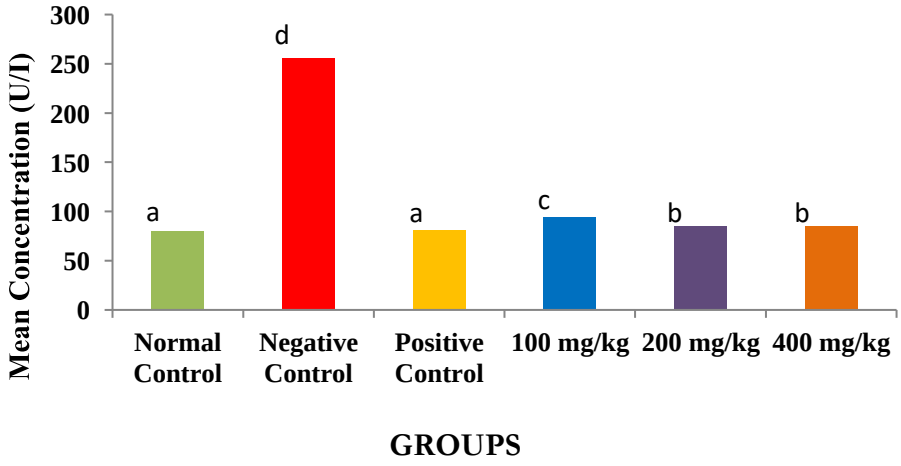


Figure 3: Bar chart showing alkaline phosphatase concentration

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

TB

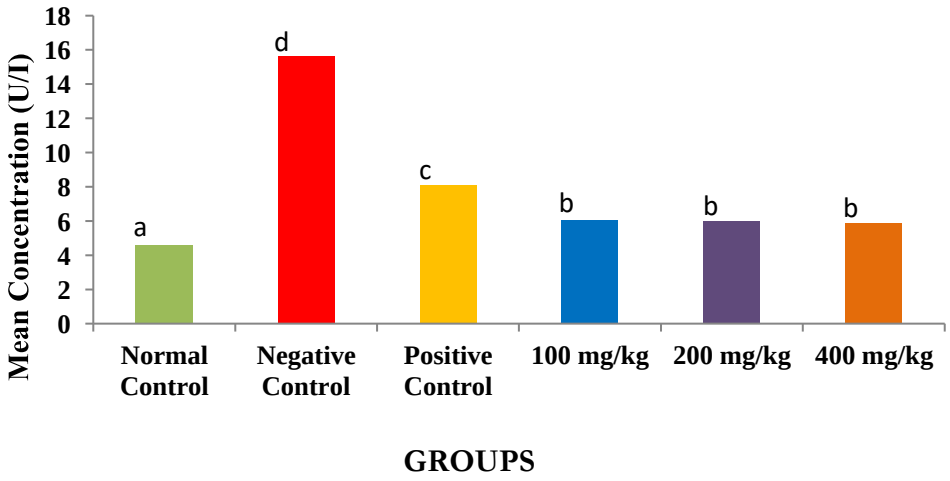


Figure 4: Bar chart showing total bilirubin concentration

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

DB

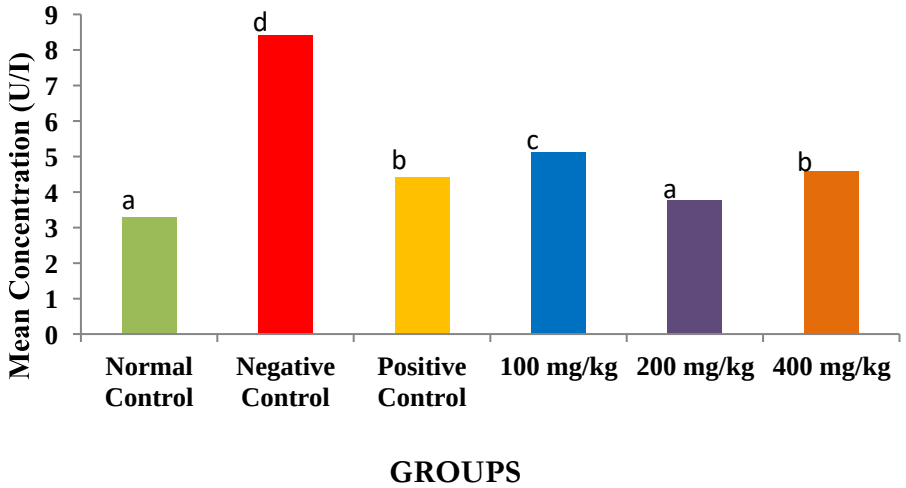


Figure 5: Bar chart showing direct bilirubin concentration

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

TP

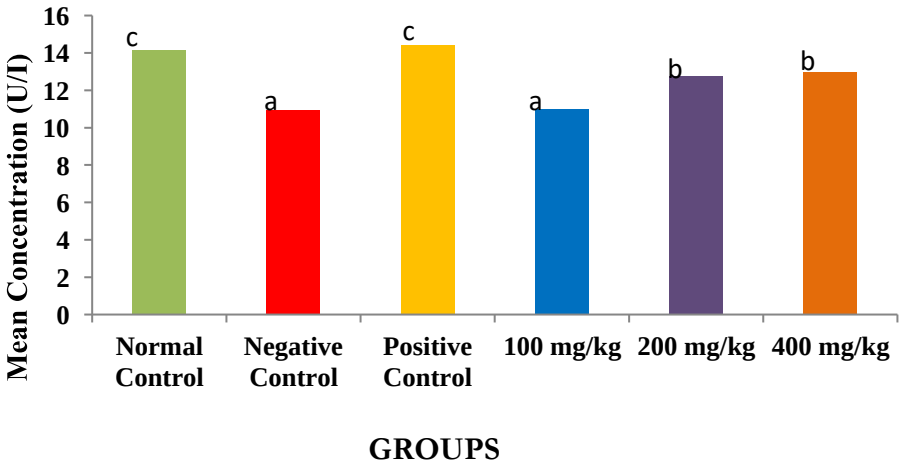


Figure 6: Bar chart showing total protein concentration

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript across group indicates a significant ($p<0.05$) difference.

ALB

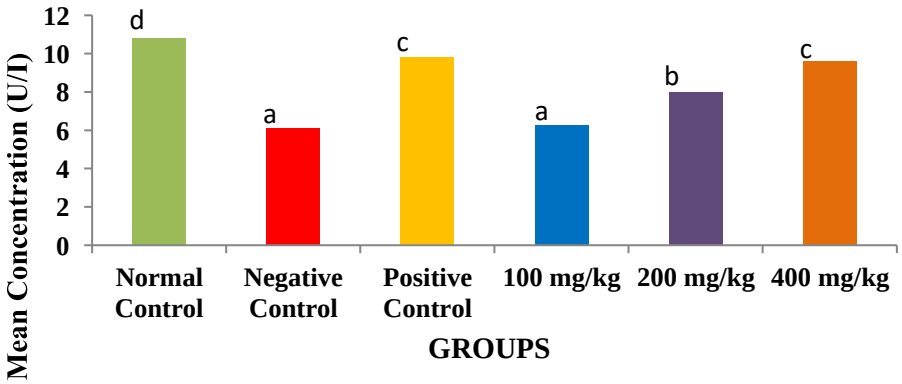


Figure 7: Bar chart showing albumin concentration

Values are presented as mean ± S.E.M for four rats in each group. Values with different superscript acrosss group indicates a significant ($p<0.05$) difference.

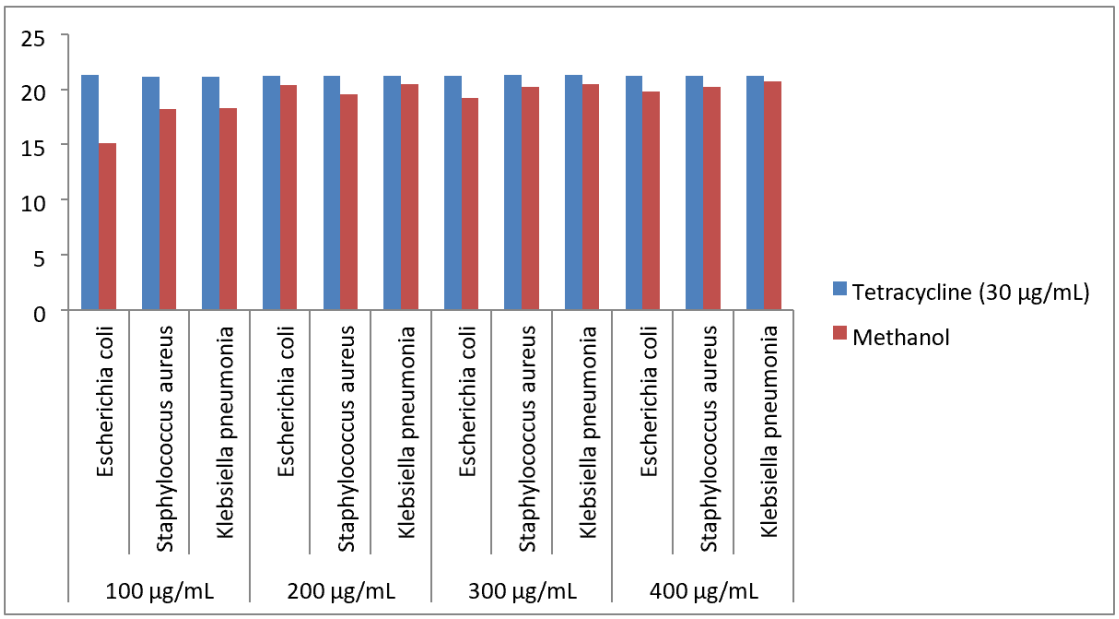


Figure 8: Bar chart showing inhibition zones

DISCUSSION

The liver is a silent powerhouse, silently performing hundreds of vital tasks to keep us healthy. It filters toxins from our blood, produces essential proteins, and helps us digest food (Lewis et al., 2010). But like any hard-working organ, the liver is vulnerable to damage (Hepatic injury) (Khuro et al., 2004). Alloxan, a diabetogenic compound, wreaks havoc on the liver through a complex interplay of mechanisms, ultimately leading to cell death (Szkudelski, 2012). Liver damage leads to increase of the serum marker enzymes which are released from the liver in to blood and low concentration of the serum markers in the liver.

The result of the investigation, as represented in table 1.0, shows a significant ($p \leq 0.05$) increase in serum AST level for group II (60.6 ± 1.12) which was administered high dose of Alloxan and serves as the negative control group, when compared to the normal control group I (35.09 ± 2.07) which only received feed and water. A significant decrease in the treatment groups that received the standard drug and Newbouldia laevis leaves extract was noted, when compared with the serum level of the negative control group. This establish two facts; that there was a probable liver injury induced by Alloxan leading to increase serum AST level, and that the extract was able to reduce the injury on the liver leading to a decrease liver enzyme. But because AST is localized in the heart, brain, skeletal muscle, and

liver tissue, we cannot be certain of the liver injury, so we look at the enzyme ALT which is predominant in the liver.

From the result described in table 1.0 above, it showed a significant ($p \leq 0.05$) increase in ALT level for group II (58.11 ± 0.42) negative control when compared with group I (20.00 ± 0.41) normal control. This confirms and supports the result obtained for AST level indicating liver injury caused by Alloxan biotransformation taking into consideration that ALT is primarily expressed in the liver. When liver cells are damaged, they release ALT into the bloodstream. High levels of ALT in your blood may be a sign of a liver injury or disease (MedlinePlus 2022). Group III (29.98 ± 0.12) which received standard drug showed a significant decrease in ALT which is a sign that there is some level of repair on the liver cells. Group IV (36.22 ± 0.70), group V (30.00 ± 0.27) and group VI (29.99 ± 0.12) which received 100, 200 and 400 mg/kg body weight of Newbouldia laevis leaves extract respectively when compared with the negative control group II, showed a significant decrease in ALT level.

It was noted that the standard drug was more effective when compared to 100mg/kg body weight of the extract, but no significant ($p \leq 0.05$) difference was observed between the standard group and group V and VI meaning a high dose does the circumventing the standard drug does in reducing the damage on the liver. This is in line with study conducted by (Sulaiman 2021), on "In vitro Antioxidant and In vivo Hepatocurative and Nephrocurative Activities of Aqueous Leaf Extract of Newbouldia laevis in Albino Rats" which shows that Newbouldia laevis significantly ($p<0.05$) reduced levels of serum biomarkers of hepatotoxicity: ALT and AST.

From table 1: it can be seen also, that there was a significant ($p \leq 0.05$) increase in ALP level for group II (255.80 ± 1.12) which received Alloxan when compared with the normal control group I (80.21 ± 0.44) providing evidence to support the increase in ALT and AST level in group II, suggestive of liver injury. The treatment groups III, VI, V and VI showed significant ($p<0.05$) difference, when compared to the negative control group. No significant ($p<0.05$) difference between group V and VII. ALP is secreted in other organs such as the bile or the bones, not just in the liver. So, findings for ALP is only a support to the findings on AST and ALT, as increased ALP can be due to a bone disease.

In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract (Kalakonda et al.,

2022). Damage to hepatocellular structures causes increases in the level of both conjugated (direct bilirubin) and unconjugated (total bilirubin) in circulation (Tripathi and Jialal 2023). With the observed significance increase in Total and direct bilirubin (table 4.2), for group II (15.62 ± 0.09) and (8.41 ± 0.10) respectively when compared with the control group I (4.58 ± 0.27) and (3.29 ± 0.12) for total and direct bilirubin respectively is suggestive of damage to hepatocellular structures.

This is in concordance with an experiment carried out by Lucchesi et al. (2015), in which he noted elevated bilirubin parameter in hepatic injury. The administration of standard drug and extract significantly reduced the liver bilirubin level to almost normal. No significant difference was noted between the different doses of the extract.

From table 2, it was deduced that the total protein for group II (10.95 ± 0.37) significantly ($p<0.05$) decreased when compared to the normal control group I (14.13 ± 0.55). This is because low levels of total protein in the blood can occur because of impaired function of the liver (Liver Function Tests - Mayo Clinic, 2023). Administration of *Newbouldia laevis* leaves extract significantly ($p<0.05$) elevated total protein levels for group IV (10.99 ± 0.41), V (12.76 ± 0.41) and VI (12.98 ± 0.58). Albumin being the most abundant protein in the blood (Campos et al., 2024), equally followed same pattern as total protein, with Albumin level of (10.83 ± 0.10), (6.13± 0.05), (9.82 ± 0.18) (6.75 ± 0.13), (7.99 ± 0.10) and (9.60 ± 0.13) for group I, II, III, IV, V and V respectively.

CONCLUSION

The liver, a crucial organ, plays a silent yet vital role in maintaining overall health. It performs numerous functions, such as detoxifying the blood, producing essential proteins, and aiding in digestion. However, the liver is susceptible to damage, particularly from external factors like hepatotoxic compounds such as Alloxan.

The administration of Alloxan, a diabetogenic compound, induced liver injury as evidenced by the significant increase in serum AST, ALT, ALP, and bilirubin levels in the negative control group (Group II). These elevated levels of liver enzymes and bilirubin indicate hepatocellular damage and dysfunction. The subsequent decrease in total protein levels further supports the impairment of liver function in the negative control group.

Notably, the experimental groups treated with the standard drug and *Newbouldia laevis* leaves extract showed a significant ameliorative effect on the Alloxan-induced liver injury. The decrease in serum AST, ALT, ALP, and bilirubin levels, coupled with the increase in total protein levels, suggests a potential hepatoprotective role of the extract. This protective effect was more pronounced with higher doses of the extract, with no significant difference observed between the standard drug and the highest dose of the extract.

REFERENCES

Lewis Hc, Wichmann O & Duizer E. (2010). Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review. *Epidemiol Infect,;* 138(2): 145-166.

Khuro MS, Kamili S & Yattoo GN. Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area. J Gastroenterol Hepatol, 2004; 19(7): 778.

Berto A, Martelli F Grierson S & Banks M. (2012). Hepatitis E virus in pork food chain, UK, 2009-2010. Emerg Infect Dis.,; 18(8): 1358-1360.

Diba Cheng, B. Liang, and Y. Li (2013). "Antihyperglycemic effect of Ginkgo biloba extract in streptozotocin-induced diabetes in rats," *BioMed Research International,* vol. 2013, 7 pages, 2013

Akerele, J. O., Ayinde, B. A., and Ngiagah, J. (2011), Phytochemical and Antibacterial Evaluations of the Stem Bark of Newbouldia laevis against Isolates from Infected Wounds and Eyes, *Tropical Journal of Pharmaceutical Research.* 10 (2): 211-217.

Bafor, E. and Sanni, U. (2009), Uterine contractile effects of the aqueous and ethanol leaf extracts of Newbouldia Laevis (Bignoniaceae) in vitro, *Indian Journal of Pharm Sci.* 71(2), 124– 126.

Burkill H. (1997). The Useful Plants of West Tropical Africa. 2nd ed. Vol. 4 (Families M-R), Kew: Royal Botanic Gardens; 1997.

Egba, S. I., Sunday, G. I. and Anaduaka, E. G. (2014), The effect of oral administration of aqueous extract of Newbouldia laevis leaves on fertility hormones of male albino rats, *Journal of Pharmacy and Biological Sciences,*9:3, pp. 61-62

Hutchinson J, Dalziel JM. (1963). Flora of West Tropical Africa vol II. London: Crown Agents for Oversea Government and Administration, pp 435-436

Arbonnier M. Trees, (2004). Shrubs and Lianas of West African Dry Zones. CIRAD, Margraf Publishers, GMBH MNHN, Cote d'Ivoire,; p 194.

Eyong OK, Krohn K, Hussain H, Folefoc NG, Nkengfack AE, Schulz B, Hu Q. (2005). Newbouldiaquinone and newbouldiamide: A napthoquinone-anthraquinone couple and a new ceramide from Newbouldia laevis. *Chem. Pharm Bull;* 53: 616-619.

Usman H, Osuji JC. (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of Newbouldia laevis. Afr J Trad Compl & Alt Med; 4(4): 476-480.

Germann K, Kaloga M, Ferreira D, Marais JP, Kolodziej H. (2006). Newbouldioside A-C Phenylethanoid Glycosides from the stembark of Newbouldia leavis. *Phytochem;* 67(8): 805–811.

Anton CD, Andries GG, Hermien G. (1996). Common eye disorders. In: Eric Herfindal T, Dick Gourlry R, editors. Textbook of Therapeutics: Drug and Disease Management, 6th edition, Williams and Wilkins, USA,; p 937

Unekwe, P. C., & Ekweozor, C. C. (2018). The role of herbal remedies in liver damage in Nigeria: A review. *International Journal of Medical Toxicology and Pharmaceutical Science,* 8(2), 82-87. https://www.mdpi.com/2305-6304/6/2/24

World Health Organization. (2023, December 12). Chronic liver disease. https://www.who.int/data/gho/indicator-metadata-registry/imr-details/1179

Nwokedi, E. I., & Obiano, N. O. (2015). Management of liver disease in Nigeria. *Journal of Gastroenterology and Hepatology Research,* 4(3), 37.

Yusuf LM, Ahmed A, Lawal U, Abubakar IU. (2016). Ethnobotanical survey of indigenous plants used in the treatment of malaria in Dutsin-Ma metropolitan area of Katsina State. *Katsina J Nat Applied Sci.* 5(1):183-89

Tor-anyiin TA, Sha'ato R, Oluma HOA. (2003). Ethnobotanical Survey of anti-malarial medicinal plants amongst the Tiv People of Nigeria. *J Herbs Spices Med Plants.*10(3):61-74. doi:10.1300/ J044v10n03_07

Nwauzoma AB, Dappa MS. (2013). Ethnobotanical studies of Port Harcourt Metropolis, Nigeria. *Int Sch Res Notices.*829424

Sonibare MA, Moody JO, Adesanya EO. Use of medicinal plants for the treatment of measles in Nigeria. *J Ethnopharmacol.* 2009; 122:268-72. doi: 10.1016/j.jep.2009.01.004 Steven K. Herrine, MD. The merck manual home health handbook, August 2012.

Aladesanmi AS, Rene N, Fontaine C, Pays M. (1994). Pyrazole alkaloids from Newbouldza laevis. *Phytochem.;*35(4):1053-5

Szkudelski T (2001). "The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas". *Physiol Res.* 50 (6): 537–46.

Szkudelski T (2001). "The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas". *Physiol Res.* 50 (6): 537–46.

Wu, Y., Sun, J., & Lin, C. (2018). Alloxan-induced liver injury: The role of glutathione depletion and Nrf2 signaling. *Redox Biology,* 14, 196-202.

Cárdenas, C. (2011). Alloxan-induced liver injury in mice: Role of calcium overload and mitochondrial dysfunction. *Molecular and Cellular Biochemistry,* 355(1-2), 19-28.

Kang, H. W. (2012). Alloxan-induced liver injury in mice: Impairment of insulin signaling and the PI3K/Akt pathway. *Toxicology Letters,* 210(3), 296-302.

Li, M. (2014). Alloxan-induced liver injury in mice: Role of oxidative stress and inflammatory response. *Experimental and Molecular Medicine,* 46(8), e87.

Mohamed, Jamaludin & Nafizah, Nazratun & Abd Hamid, Zariyantey & Budin, Siti. (2016). Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation. *Sultan Qaboos University Medical Journal.* 16. e132-141. 10.18295/squmj.2016.16.02.002.

Sulaiman, Mohammed & Jada, Suleiman & Elizabeth, Augustine & Modibbo, Abubakar. (2021). In vitro Antioxidant and In vivo Hepatocurative and Nephrocurative Activities of Aqueous Leaf Extract of *Newbouldia laevis* in Albino Rats. *Sumerianz Journal of Biotechnology*. 2(3), 241-253.

Kalakonda A, Jenkins BA, John S. Physiology, Bilirubin (2022). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470290/