

Full Length Research Paper

Investigation of some important phytochemical, nutritional properties and toxicological potentials of ethanol extracts of *Newbouldia laevis* leaf and stem

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The phytochemicals, nutritional and toxicological potentials of the ethanol extracts of the leaf and stem of *Newbouldia laevis* was investigated in this study. The percentage yields of *N. laevis* ethanol leaf and stem extracts were found to be 7.44 and 3.30% (w/w), respectively. The preliminary phytochemical screening showed that ethanol leaf and stem extracts contains alkaloids, flavonoids and tannins. The quantitative phytochemical analysis showed that the leaf and stem extracts contained respectively: alkaloids (14.74 ± 0.06 and 6.27 ± 0.0 mg/g), flavonoids (15.51 ± 0.04 and 5.18 ± 0.04 mg/g), cardiac glycosides (6.77 ± 0.02 mg/g), tannins (1.74 ± 0.11 mg/g), saponins (4.07 ± 0.06 mg/g), steroids (41.72 ± 0.02 mg/g) and terpenoids (8.67 ± 0.09 mg/g). The following amounts of vitamins and minerals were found in the leaf and stem-bark extracts, respectively; vitamin A (5.19 ± 0.00 and 3.01 ± 0.00 mg/100 g), vitamin C (2.35 ± 0.55 and 1.05 ± 0.08 mg/100 g) and vitamin E (9.33 ± 0.02 and 4.08 ± 0.11 mg/100 g); minerals: Mg (76.12 ± 0.04 and 54.25 ± 0.04 mg/100 g), Fe (16.84 ± 0.06 and 1.19 ± 0.03 mg/100 g) and Se (3.08 ± 0.03 and 0.29 ± 0.07 mg/100 g). The acute toxicity test of the ethanol leaf and stem extracts showed no toxicity up to 5000 mg/kg body weight.

Key words: *Newbouldia laevis*, phytochemical properties, vitamins, minerals, toxicity.

INTRODUCTION

A very large area of Nigeria ecological zones is populated with many plant species which have found their usefulness either directly or indirectly for humans (Oliver-Bever, 1986). The medicinal values of many of these plants cannot be over emphasized in the light of oral traditions and folklores from the distant past that have continued to extol the healing virtues of these plants and their extracts. One of such medicinal plant is *Newbouldia laevis*, whose medicinal values have stood the test of time. Plants contain active components such as anthraquinones, flavonoids, glycosides, saponins, tannins, etc which possess medical properties that are harnessed for the treatment of different diseases (Feher

and Schmidt, 2003). The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals in plants. These active substances that contain the healing property are known as the active principles and are found to differ from plant to plant (Galm and Shen, 2007). Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress.

Phytochemicals are naturally occurring and are believed to be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992; Ejele et al., 2012). The medicinal lie in

their component phytochemicals, which produce the definite physiological actions on human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds (Iwu, 2000). Some of these naturally occurring phytochemicals are anticarcinogenic and some others possess other beneficial properties, and are referred to as chemopreventers. One of the predominant mechanisms of their protective action is due to their antioxidant activity and the capacity to scavenge free radicals.

N. laevis (*Bignoniaceae*) is commonly known as African Border tree or boundary tree (Gbile and Adesina, 1986). It is called "Aduruku" in Hausa; "Ogirisi" in Igbo; "Ikhimi" in Edo and "Akoko" in Yoruba languages (Ogunlana and Ogunlana, 2008). It grows to a height of about 7.8 (up to 15 m), more usually a shrub of 2 to 3 m, many-stemmed forming clumps of gnarled branches. It is easily recognized by its short branches, coarsely toothed leaflets and purple and white flowers (Iwu, 1983). *N. laevis* is native to tropical Africa and grows from Guinea Savannas to dense forests, or moist and well-drained soils (Burkill, 1984). One remarkable thing about this plant is that it hardly dies hence it is used to indicate boundary marks among the Igbo people of South Eastern Nigeria (Gill, 1992). *N. laevis* have recently attracted research interest because it possesses antioxidant properties against a variety of physiologically relevant free radicals (Ajibolu et al., 2011). Therefore, there is need to continue the investigation of the mechanism by which plants and plant products protect and prevent tissues from damage by chemical compounds that generate reactive oxygen species in the system.

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves and stem of *N. laevis* were used for this study. The leaves and stem of *N. laevis* were collected within University of Nigeria, Nsukka and were identified in the Bioresources Development and Conservation Programme (BDGP) Research Centre, Nsukka, Enugu State. The fresh leaves and stem of *N. laevis* were washed with clean water to remove dirt and sand, drained and chopped. They were dried under shade for several days and then pulverized into fine powder.

Extraction of plant materials

A quantity, 500 g of each of the powdered form of the leaves and stem of *N. laevis* were macerated in 1.5 L of ethanol for 48 h. The solution was filtered with Whatman no. 4 filter paper and the filtrate was concentrated to a semi-solid residue in an oven at 60°C.

Phytochemical screening

The phytochemical analysis of the leaves and stem of *N. laevis* were carried out according to the method of Harborne (1973) and Trease and Evans (2002) to identify its active constituents.

Quantitative phytochemical analysis

Alkaloid determination

The determination of alkaloid was as described by Harborne (1973). A portion (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to ¼ of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract till a precipitate was formed. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoids determination

This was determined according to the method of Harborne (1973). A quantity, 5 g of the sample was boiled in 50 ml of 2M HCl solution for 30 min under reflux. It was allowed to cool and then filtered through whatman No. 1 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The solution was filtered into a weighed crucible. The filtrate was heated to dryness in an oven at 60°C. The dried crucible was weighed again and the difference in the weight gave the quantity of flavonoid present in the sample.

Steroids determination

This was determined by the method described by Edeoga et al. (2005). A known weight of each sample was dispersed in 100 ml freshly distilled water and homogenized in a laboratory blender. The homogenate was filtered and the filtrate was eluted with normal ammonium hydroxide solution (pH 9). The eluate (2 ml) was put in test tube and mixed with 2 ml of chloroform. Ice-cold acetic anhydride (3 ml) was added to the mixture in the flask and 2 drops of conc. H₂SO₄ were cautiously added. Standard sterol solution was prepared and treated as described earlier. The absorbances of standard and prepared sample were measured in a spectrophotometer at 420 nm.

Determination of vitamin contents

The vitamin assay was performed with the method of Pearson (1976).

Vitamin A: A quantity of 1.0 g of ground sample was macerated with 20 ml of petroleum ether. This was decanted into a test tube and then evaporated to dryness. 0.2 ml of chloroform-acetic anhydride (1:1, v/v) was added to the residue. 2 ml of TCA-chloroform in like (1:1 v/v) was added to the resulting solution and absorbance was measured at 620 nm. Vitamin A standard was prepared in like manner and the absorbance taken at 620 nm. The concentration of vitamin A in the sample was extrapolated from the standard curve.

Vitamin E: A quantity of 1 g of the sample was macerated with 20 ml of ethanol and then filtered. 0.2% ferric chloride in ethanol and 1 ml of 0.5% α-α-dipyridine to 1 ml of the filtrate. This was diluted to 5 ml with distilled water. Absorbance was taken at 520 nm. The standard solutions were prepared similarly and the concentration of vitamin E extrapolated from the standard curve.

Vitamin C: A quantity of 1 g of sample was macerated with 20 ml of 0.4% oxalic acid. This was filtered and to 1 ml of the filtrate was added 9 ml of Indolephenol reagent. The standard solution of vita-

vitamin C was prepared similarly and the absorbances of the standard solution and the sample were read at 520 nm. The concentration of vitamin C was extrapolated from the standard curve of vitamin C.

Determination of mineral contents of the leaves and stem of *N. laevis*

The method of AOAC (1970) was used. 2 g of sample was weighed into a crucible and ashed into a furnace at 550°C for 6 h. The ash was cooled, 6N HCl was added and boiled for 10 min, while covering the crucible with a watch glass. After boiling the sample, it was allowed to cool and filtered into 100 ml volumetric flask. The crucible was washed with distilled water and the washings added to the ash filtrate. The ash filtrate was then made up to 100 ml with distilled water. An aliquot of the filtrate was aspirated into the atomic absorption spectrophotometer and the absorbance values corresponding to different minerals recorded. The percentage of the elements in the samples was calculated from the absorbance values of the samples and standard solutions.

Determination of magnesium

A precipitate formed in the previous test was removed by filtration and made strongly alkaline with ammonia. A volume of 1 cm³ of 10% sodium phosphate solution was added. The formation of a crystalline precipitate indicated the presence of magnesium.

Determination of ferric iron

Several cubic centimeters of the solution was acidified with hydrochloric acid, and 1 cm³ of 10% ammonium thiocyanate was added. The formation of a red colour indicated the presence of ferric iron. If negative, take a second portion and a few drops of hydrogen peroxide and warm. This will oxidize any ferrous iron (iron II) to ferric, which can be detected as aforementioned.

Acute toxicity test of the ethanol extracts of the leaves and stem of *Newbouldia laevis*

The method of Lorke (1983) was used for the acute toxicity test of the leaves and stem-bark of *N. laevis*. Thirty six (36) albino mice were utilized in this study. The test involved two stages. In stage one, the animals were grouped into three groups of three rats each and were given 10, 100 and 1000 mg/kg body weight of the extracts respectively and in the second stage, 1600, 2900 and 5000 mg/kg body weight of the extracts were administered to the animals. The administration of the extracts was done orally. The median lethal dose (LD₅₀) was calculated from the second phase.

Statistical analysis

Data were mean of three replicates ± SD. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. One way analysis of variance was adopted for comparison, and the results were subjected to post hoc test using least square deviation (LSD). The data were expressed as mean ± standard deviation. P < 0.05 was considered significant.

RESULTS

Table 1 shows that both leaves and stem ethanol extracts

Table 1. Preliminary phytochemical screening of ethanol extracts of *N. laevis* leaves and stem.

Variable	Leaves	Stem
Alkaloids	++	+
Flavonoids	+++	+
Steroids	+	-
Saponins	+	-
Glycosides	++	-
Terpenoids	+	-
Tannins	+	+

+ slightly present, +++ highly present, ++ moderately present, - absent.

of *N. laevis* contain alkaloids, flavonoids, tannins, steroids, terpenoids, cardiac glycosides and saponins were not detected in the ethanolic stem extract but were present in the leaves extract. There were slight differences in the alkaloids and flavonoids contents in the leaves and stem extracts. Table 2 shows that the leaves extract contains higher amounts of phytochemicals to the stem extract. Table 3 shows that both leaves and stem extracts contain vitamins A, C and E but in different quantities. The mineral content of the leaf and stem were respectively found to be 76.12 ± 0.04 and 54.25 ± 0.04 mg/100 g for magnesium; 16.84 ± 0.06 and 1.19 ± 0.03 mg/100 g for iron; 3.08 ± 0.03 and 0.29 ± 0.07 mg/100 g for selenium (Table 4). The acute toxicity test of ethanol extracts of *N. laevis* leaves and stem showed no death up to 5000 mg/kg body weight (Table 5).

Effects of ethanol extract of *N. laevis* leaves and stem on organ histology

Histopathology explanation

Microscopic examination of the liver and kidney sections of the control rat showed normal morphological structure of the central vein and kupffer cells respectively as shown in Figure 1. On the other hand, microscopic investigation of the liver and kidney section of diabetic untreated rat demonstrated various areas of hepatocyte degeneration of the liver and mild congestion of the glomerulus of the kidney (Figure 2). Investigation of the liver and kidney sections of diabetic treated with glibenclamide (standard drug) revealed normal histological structure of the tissues as shown in Figure 3. Treatment with the leaves extract in the dose of 200 mg/kg body weight showed partial restoration of normal histological structure of the liver and kidney with few disturbances in the liver and kidney cells arrangements (Figure 4). Treatment with the leaves extract in the dose of 400 mg/kg body weight showed complete restoration of normal histological structure of the liver and kidney with no disturbance in the cell arrangements (Figure 5) when compared to group 2

Table 2. Quantitative phytochemical constituents of ethanol extracts of *N. laevis* leaves and stem.

Phytochemical constituent (mg/g)	Leaves (mean±SD)	Stem (mean±SD)
Alkaloids	14.74 ± 0.06	6.27 ± 0.02
Flavonoids	15.51 ± 0.04	5.18 ± 0.04
Steroids	41.72 ± 0.02	-
Saponins	4.07 ± 0.06	-
Tannins	1.74 ± 0.11	0.21±0.01
Cardiac glycosides	6.77 ± 0.02	-
Terpenoids	8.67 ± 0.09	-

Table 3. Vitamin constituents of ethanol extracts of *N. laevis* leaves and stem.

Vitamin constituent (mg/100 g)	Leave (mean±SD)	Stem (mean±SD)
A	5.19 ± 0.00	3.01 ± 0.00
C	2.35 ± 0.55	1.05 ± 0.08
E	9.33 ± 0.02	4.08 ± 0.11

Table 4. Mineral constituents of ethanol extracts of *N. laevis* leaves and stem.

Mineral constituent (mg/100 g)	Leaves (mean±SD)	Stem (mean±SD)
Mg	76.12 ± 0.04	54.25 ± 0.04
Fe	16.84 ± 0.06	1.19 ± 0.03
Se	3.08 ± 0.03	0.29 ± 0.07

Table 5. Phases I and II of the acute toxicity (LD₅₀) test of *N. laevis*.

Parameter	Dosage (mg/kg body weight)	Mortality (leaves stem)
Phase I		
Group 1	10	0/3 0/3
Group 2	100	0/3 0/3
Group 3	1000	0/3 0/3
Phase II		
Group 1	1600	0/3 0/3
Group 2	2900	0/3 0/3
Group 3	5000	0/3 0/3

(diabetic untreated rat).

Moreover, the treatment with the stem extract in doses of 200 and 400 mg/kg body weights showed normal histological structure of the portal areas of the liver and the glomerular tufts of the kidney (Figures 6 and 7).

DISCUSSION

Preliminary and quantitative phytochemical constituents of the leaves and stem were investigated as well as their vitamin and mineral contents and toxicological potentials. The preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids and tannins in both the leaf and stem extracts. Steroids, saponins, glycosides and terpenoids were only present in the leaves extract. The presence of tannins, terpenoids, flavonoids, steroids and cardiac glycosides in the leaf extract was in line with the reports of Usman and Osuji (2007) and Azando et al. (2011). Several available literature reports are discordant on the phytochemical composition of the plant. Usman and Osuji (2007) did not detect the presence of alkaloids and saponins in their study while Dandjesso et al. (2012) reported the absence of alkaloids, flavonoids, saponins and steroids on the leaf extract. Ejele et al. (2012) reported in their work the absence of flavonoids and steroids while Akerlele et al. (2011) reported the presence of saponins and steroids in the stem extract but was not detected in this study. Differences in these reports could be due to environmental factors, time of collection and handling. The quantitative phytochemical analysis in this study indicates higher concentrations of phytochemicals on the leaf extract compared to the stem as shown in Tables 1 and 2. Phytochemicals are secondary plants metabolites responsible for many observed bioactivity of plant extracts. They are known to possess antioxidant, anti-inflammatory, antibacterial, immunomodulatory and antisickling activities (Egba et al., 2012). The presence of those metabolites no doubt is indication of the potential medicinal usefulness of the plant extracts.

The result of this work shows that the two extracts possess some biologically active compounds which could serve as potential sources of drugs. Saponin has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Price et al., 1987). Presence of tannins as shown in the result suggests the ability of this plant to play a major role as antidiarrhoeic and antihaemorrhagic agent (Asquith and Butler, 1986). Flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic and antiviral antineoplastic activity (Alan and Miller, 1996). Many of

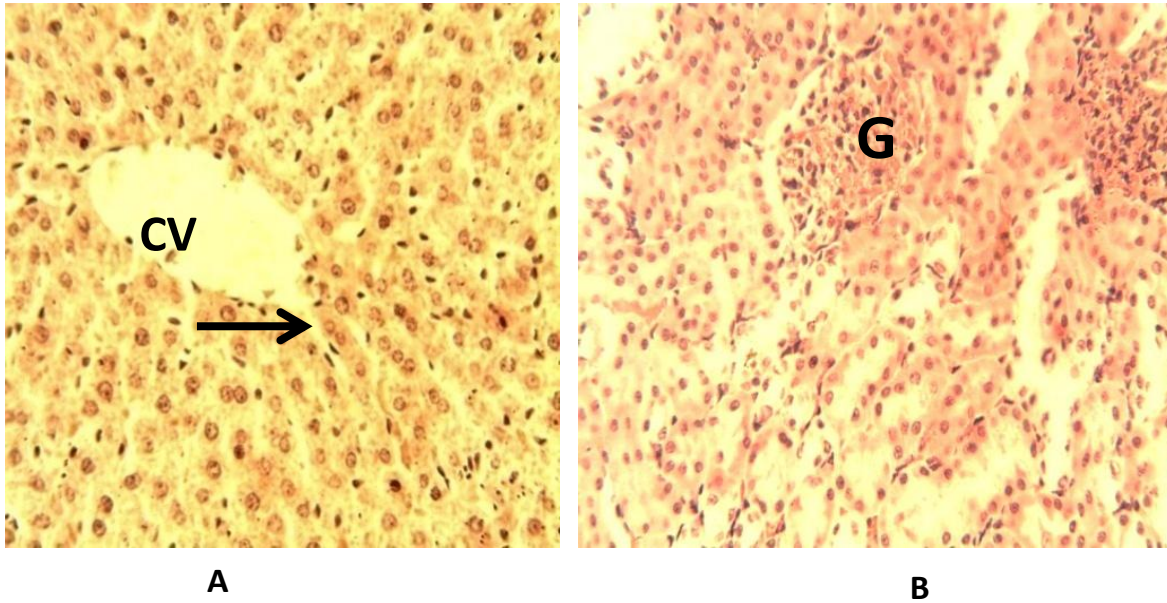


Figure 1. Photomicrograph of sections of organs from control rats showing, A) Normal liver with its central vein (CV) and Kupffer cells along the sinusoids (arrow), B) Kidney showing normal glomerulus (G) and renal tubules H&E x400.

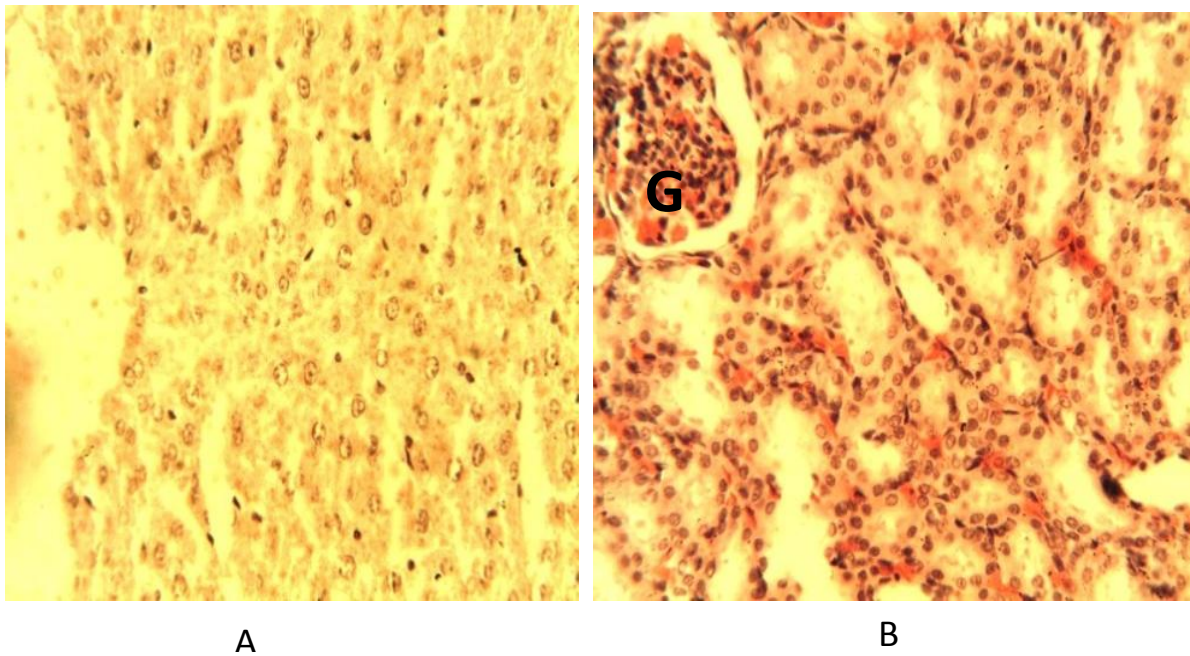


Figure 2. Histologic sections of organs from untreated diabetic rats. A) Liver with focal areas of hepatocyte degeneration, B) kidney having mild congestion of the glomerulus (G) H&E x400.

these alleged effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chelators (Nakayama et al., 1993). Steroidal compounds are of importance in pharmaceuticals because of their relationship with compounds used as sex hormones (Okwu, 2001). The terpenoids have also been shown to decrease blood sugar level in animal studies (Kuzuyama

and Seto, 2003). Glycosides may be crucial in the transduction of intracellular signals mediated by neurotransmitters, hormones, and neuromodulators receptors (Neer, 1995), activated by certain biological enzymes through hydrolysis, resulting in the separation of the sugar portion. When activated, these molecules can act on different intracellular targets (glycoside-linked signal trans-

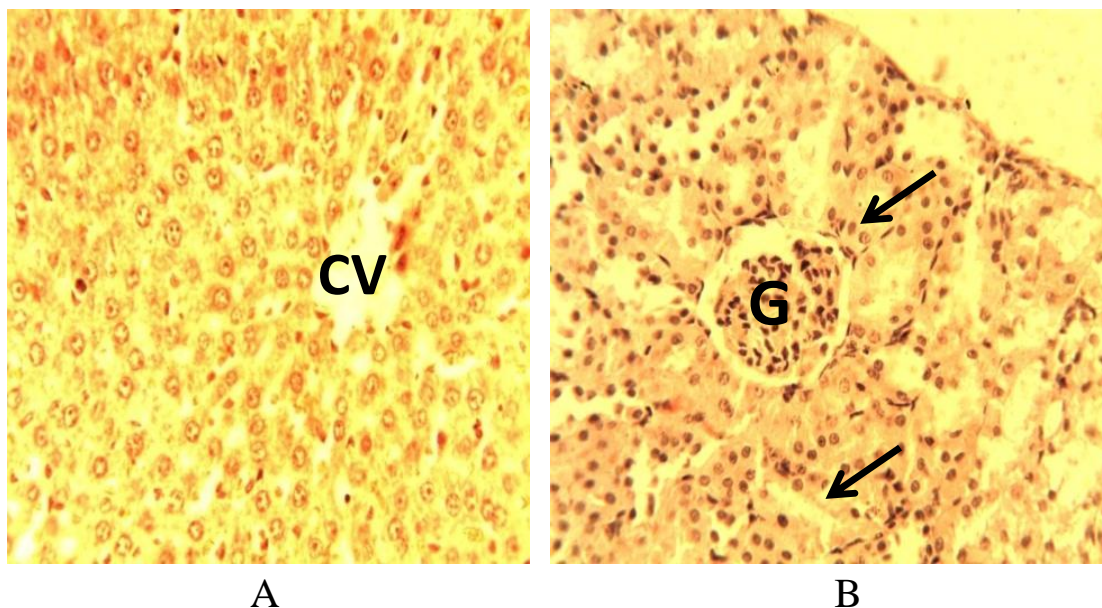


Figure 3. Photomicrograph of sections of organs from rats treated with glibenclamide showing A) Liver with the central vein (CV), and B) The kidney with no observable histologic change [the glomerulus (G) and renal tubules 'arrow']. H&E $\times 400$.

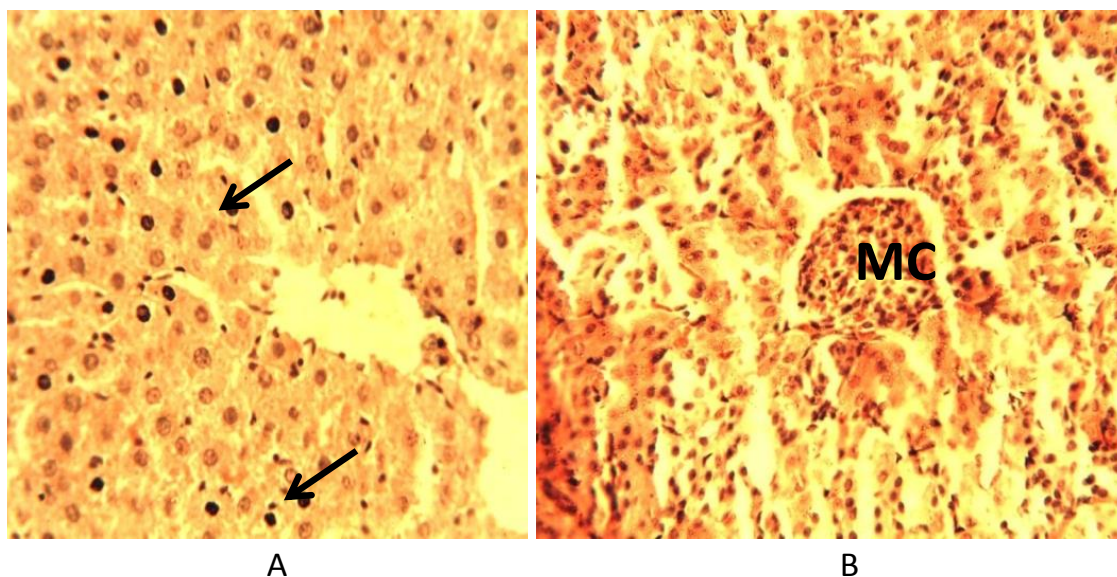


Figure 4. Histologic sections of organs from rats treated with 200 mg/kg of ethanol leaf extract showing A) Liver with apoptotic cells (arrow) and B) kidney with mild congestion (MC) of the glomerulus. H&E $\times 400$.

duction proteins) (Dwivedi, 2006).

The result shows appreciable vitamin A, C and E content in the plant extracts. Vitamin C and E are potent natural antioxidants that scavenge free radicals and ameliorate their deleterious effects. The presence of these vitamins in the extracts suggests their possible role in curbing the incidence of oxidative stress in humans and animals. Calcium is a major factor sustaining strong

bones and plays a vital role in muscle contraction and relaxation, blood clotting, synaptic transmission and absorption of vitamin B₁₂. Potassium and magnesium are known to decrease blood pressure. Potassium plays a role in controlling skeletal muscle contraction and nerve impulse transmission. Patients with soft bone problems are usually placed on high calcium and potassium vegetables meals (Mensah et al., 2008). The acute toxicity (LD₅₀)

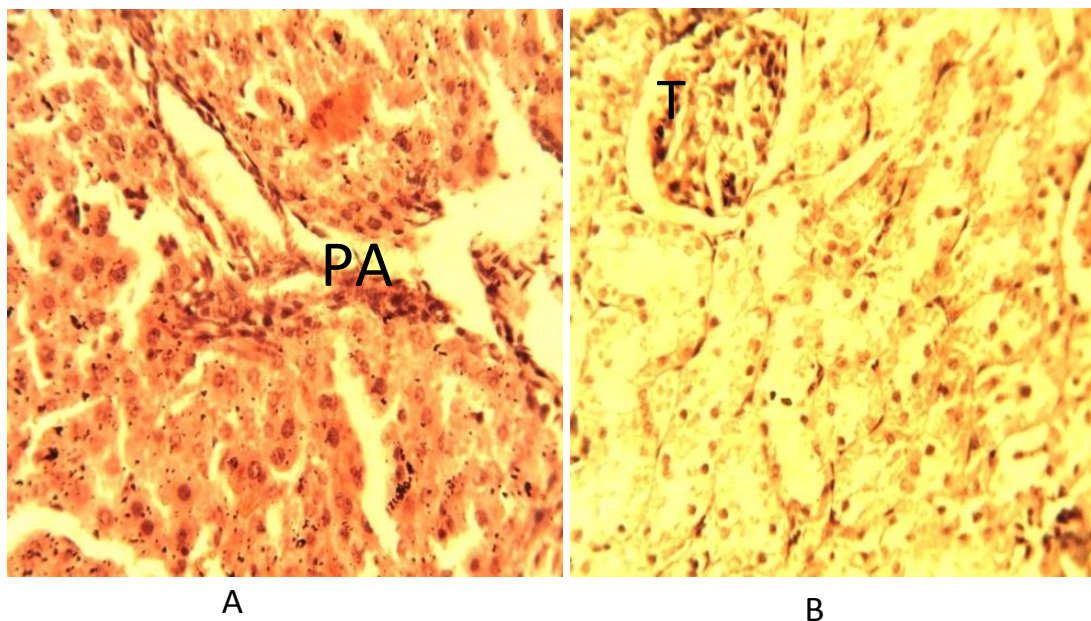


Figure 5. Photomicrograph of sections of organs from rats treated with 400 mg/kg ethanol leaves extract. A) Liver showing the portal area (PA) with no remarkable histologic change and B) Kidney with normal tubules and glomerular tufts (T). H&E $\times 400$.

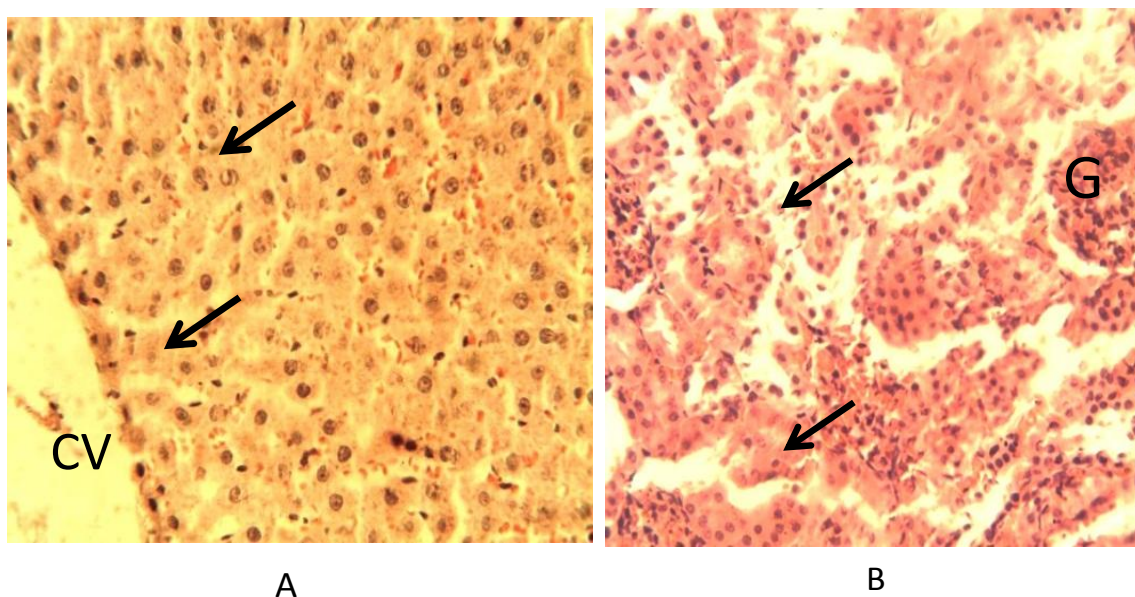


Figure 6. Photomicrograph of sections of organs from rats treated with 200 mg/kg of ethanol stem extract. A) Liver showing the central vein (CV) and normal plates of hepatocytes (arrows), B) kidney showing mild congestion of the glomerulus (G) and interstitium (arrows). H&E $\times 400$.

(LD₅₀) test of the ethanol extracts of *N. laevis* leaf and stem shows that the plant extracts were not toxic up to 5000 mg/kg body weight. This indicates that the leaves and stem extracts are safe for human and animal consumption and compliments earlier studies (Owolabi et al., 2011). This observation is supported by the histopathological examination which showed clear restoration

of diabetes induced pathological changes in tissue sections. The plants studied here can be seen as a potential source of useful drugs. Further studies are going on in this plant in order to identify, characterize and elucidate the structure of the bioactive compounds. There is equally need to study the toxicological effect of the plant with prolonged usage.

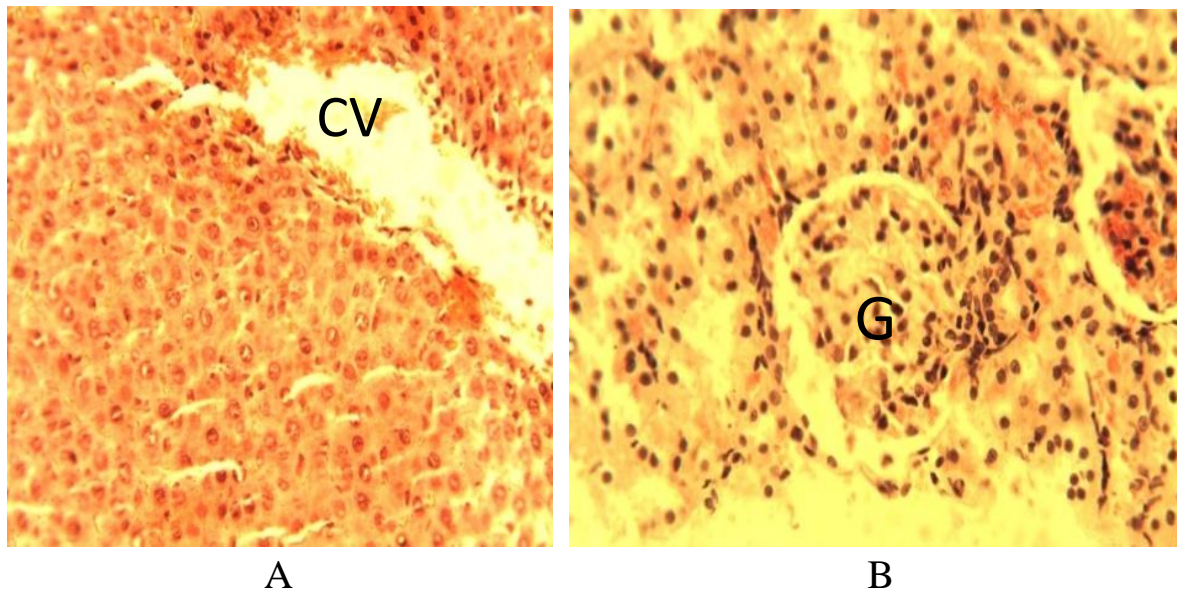


Figure 7. Histologic sections of organs from rats treated with 400 mg/kg ethanol stem extract. A) Liver showing central vein (CV) and normal hepatocytes, B) kidney showing mild hypercellularity of the glomerulus (G). H&E x400.

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