JMBR: A Peer-review Journal of Biomedical Sciences
December 2015, Vol. 14 No. 2 pp 31-39

AMELIORATIVE EFFECT OF AQUEOUS LEAF EXTRACT OF BITTER LEAF (Vernonia amygdalina) ON RIFAMPICIN INDUCED RENAL TOXICITY IN ADULT WISTAR RATS

EHIMIGBAI, Agbonluai Richard .O[1]; ANEKE, Amuche Stella[2]

ABSTRACT

The aim of this study was to determine the ameliorative effect of aqueous extract of Vernonia amygdalina leaf on rifampicin induced kidney toxicity on adult wistar rats. A total of 40 (forty) adult wistar rats weighing between 190 g to 240 g were divided into 4 groups of ten rats per group. Group A rats were placed on normal diet only while Group B rats received 250 mg/ kg body weight / day (BWT/D) of rifampicin via orogastric tube. Group C rats received 250mg / kg BWT/D of V. amygdalina leaf via orogastric tube. Group D rats received 250 mg/ kg BWT/D of rifampicin and 250mg / kg BWT/D of V. amygdalina leaf via orogastric tube and all the dosage were given for 30 days. The result revealed that group B showed marked increase in the activity of the serum urea, creatinine, catalase and superoxide dismutase along with reduction in level of malonyl dehydrogenase. While the other groups showed normal value of serum catalase, superioxide dismutase, malonyl dehydrogenase, urea and creatinine. Also group B showed severe asymmetric vascular media hypertrophy, intimal erosion and patchy tubular cell cloudy swelling; while group D revealed mild asymmetric media hypertrophy and focal tubular cell cloudy swelling. It can be concluded that the extract was able to ameliorate the rifampicin toxicity when co-administered together.

INTRODUCTION

Medicinal plants contain potentially valuable chemicals that serve as source for the manufacturing of modern medicines.[1] Therefore, the knowledge about medicinal plants and their uses offer a vital input to both human and livestock health care delivery in the country.[2] Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is a semisynthetic component derived from streptomyces species that is usually used as a first line drug for the treatment of tuberculosis globally.[3] A lot of cases of acute renal failure along with elevated bilirubin and urea level following rifampicin treatment have been reported.[4,5]

In Nigeria, Vernonia amygdalina is commonly called Ewuro in Yoruba dialect. It is a widely used tropical shrub, 1-3m in height, with petiole leaf measuring about 6mm in diameter, which is ellipsoid in shape.[6] Naturally, V. amygdalina is characterized by a bitter taste when eating or chewed, but this bitter taste can be removed when the leaf

KEYWORDS: *Vernonia amygdalina leaf, Rifampicin, Kidney toxicity, superioxide dismutase, Urea and Creatinine.*

EHIMIGBAI, Agbonluai Richard .O[1]; ANEKE, Amuche Stella[2]
[1]Lecturer, Department Of Anatomy, Faculty Of Basic Medical Science, College Of Medical Science, University Of Benin, Benin City, Edo State, Nigeria. +2348060780281.
Email address -*ehirichard22@ yahoo.com*
[2]Research Assistant, Department Of Anatomy, Faculty Of Basic Medical Science, College Of Medical Science, University Of Benin, Benin City, Edo State, Nigeria.

****Correspondence***

Ehimigbai, Agbonluai Richard .O
Lecturer
Department Of Anatomy, Faculty Of Basic Medical Science, College Of Medical Science, University Of Benin, Benin City, Edo State, Nigeria. ***Phone no-****+2348060780281*
Email Address -*ehirichard22@ yahoo.com*

is boiled with water or soaked and washed in a lot of water. Bitter leaf soup is very popular among the Owan people in the south- southern part of Nigeria.

Futhermore, the aqueous leaf extract exhibited protective activity because of its antioxidant property which is attributed to its terpenoid content available in the leaves.[7] While its glycosides, saponins and tannins content were discovered to contribute to the purgative strength of the plant via astringence, direct stimulation and surface emulsification of these components.[8] The ash of V. amygdalina contained a lot of nitrogen, magnesium, phosphorus, calcium, sodium and potassium.[9] Research has shown that V. amygdalina extracts are of pharmacological importance as an antimalarial, antibacterial, anticancer and a hypoglycaemic agent.[10,11,12,13] This plant has also been recommended for use as chewing stick to preserve oral health by removing cariogenic micro-organisms.[14]

The aim of the experiment was to determine the ameliorative effect of aqueous extract of V. amygdalina leaf on rifampicin induced kidney toxicity on adult wistar rats.

Table 1: Schedule for administration of *Vernonia amygdalina* leaf extract and Rifampicin in adult rats

Group	Dose / Kg body weight	Number of Days / Route
Control group (A)	Normal rat diet	30 days / orally
Rifampicin treated group (B)	250mg / Kg Rifampicin[5]	30 days / orally
Vernonia amygdalina treated group (C)	250mg/Kg of *V. amygdalina* leaf extract	30 days / orally
Combination group (D)	250mg/Kg V. *amygdalina* leaf extract plus 250mg / Kg Rifampicin	30 days / orally

The experimental wistar rats at the end of 30 days were sacrificed by cervical dislocation. The kidneys were excised and harvested using a ventral midline abdominal incision and quickly preserved in 10% formol-saline. Blood samples were collected via the abdominal aorta and put inside the plain bottles, while serum and red blood cells were separated using bucket centrifuge machine with model number centrifuge 80 – 3/ Alpin Medical Instrument, England for analysis.

The already labelled kidney tissues were dehydrated in alcohol, cleared in xylene and impregnated with molten paraffin wax. The tissue blocks were sectioned

using a rotary microtome of Leica brand with model number RM2125RTS from Leica Biosystems Nussloch Gmbh, China at 5 micron thickness, dewaxed in xylene and rehydrated in descending order before staining with haematoxylin and eosin and then observed microscopically.[15]

Biochemical assays: Serum was used to analysed the biochemical parameters.

Urea Assay: The activities of urea was determined by the method of Fawcet and Scott, 1960.[16]

Add 10μl of distilled water, standard solution and the serum to blank, standard and sample respectively. Then add 0.1ml of sodium nitroprusside and urease to the test tubes. Mix the content and incubate at 37^0C for 12minutes. To each test tubes add 2.50ml of phenol solution and sodium hypochlorite solution. Mix and incubate at 37^0 C for another 12minutes. Read the Absorbance of sample and standard against blank at 546nm.

Creatinne Assay: The activities of creatinine was measured by the method of Bartels et al, 1972.[17] 1.0ml of a mixture of 0.32mol/l of sodium hydroxide and 35mmol/l picric acid was added to 0.1ml of the sample. Also, 1.0ml of the standard solution was added to 1.0ml of the sample in another test tube. The combination was equilibrated and absorbance A1 of the standard and sample was read after 40seconds at 490nm. After 120secs later absorbance A2 of the standard and sample was taken.

Malonyl dehydrogenase (MDA) Assay: The serum MDA activities was determined by the method of Beuge and Aust.[18]

A total of 380 mg of thiobarbituric acid (TBA) was added to 2ml of 0.25N (HCL) before 17g of Trichloroacetic acid (TCA) was then added to the mixture making altogether a volume of 100ml. The solution was heated at 100^0 C in a water bath to dissolve the thiobarbituric acid. Thereafter, 1ml of serum was added to 2ml of the mixture and mixed very well. The solution was heated in a water bath for 17min, before the precipitant was removed through centrifugation method.

Sample absorbance was estimated at 540nm against blank. MDA was expressed at nmol / ml.

Catalase (CAT) Assay: The CAT activities was determined by the method of Cohen et al, 1970.[19] The spectrophotometric standard for catalase is made up of 1.0ml of 6M H_2SO_4 , 5.5ml of 0.05M phosphate buffer (P^H 7.4) and 7.0ml of 0.01M $KMnO_4$ as a component. The mixture is quickly equilibrated by inversion. The Absorbance is read at 480nm 40-60seconds against the distilled water.

Superoxide Dismutase (SOD) Assay: The SOD level in the serum was determined by the method of Misra and Fridovich, 1972.[20] 0.4ml of the dilute supernatant of the sample was added to 5ml of 0.05M carbonate at buffer pH of 10.2 which was equilibrated in a spectrophotometer for 3 to 4 minute. The reaction was on track by the application of 0.6ml of newly prepared 0.3mM epinephrine as substrate to the buffer-supernatant mixture which was mixed quickly by inversion. The reference cuvette contained 5ml of buffer, 0.6ml of epinephrine and 0.4ml of distilled water. The increase in absorbance at 480nm arises due to the adrenochrome being formed which was observed every 30 seconds for 2 minutes.

Statistical analysis: Data were expressed as the mean ± SEM. The data were analyzed by analysis of variance (ANOVA) followed by least square difference using the Statistical Package for the Social Sciences (S.P.S.S. 17). The level of significance was set at $P<0.001$.

RESULTS

Table 2: Antioxidant Enzyme Parameters for All Groups

		GROUP A	GROUP B	GROUP C	GROUP D	P-VALUE
CAT (umol/L)	Mean(SD)	153.86(4.30)	264.29(9.76)	161.00(3.16)	167.14(7.5)	<0.001
SOD (ng/mL)	Mean(SD)	78.29(2.87)	119.29(6.08)	83.71(2.69)	89.00(2.08)	<0.001
MDA (umol/L)	Mean(SD)	11.57(2.23)	2.13(0.51)	12.00(2.16)	8.14(0.90)	<0.001

Table 3: Renal Function Parameters For All Groups

		GROUP A	GROUP B	GROUP C	GROUP D	P-VALUE
UREA (mg/dl)	Mean(SD)	23.43(2.99)	58.86(8.93)	24.00(2.58)	25.14(3.02)	<0.001
CREATININE (mg/dl)	Mean(SD)	0.81(0.01)	2.00(0.63)	0.83(0.03)	0.83(0.02)	<0.001

The mean concentration of urea and creatinine were elevated in rats that were given only rifampicin (58.86 ± 8.93 and 264.29 ± 9.76 respectively) when compared with other groups.

The concentration of SOD and CAT in rats treated with Rifampicin were significantly elevated (119.29 ± 6.08 and 264.29 ± 9.76 respectively) while MDA concentration were significantly reduced (2.13 ± 0.59).

Comparison of mean urea, creatinine, SOD, CAT and MDA levels of treated rats in all groups B, C, and D with controls were found to be statistically significant ($P<0.001$).

The mean SOD and CAT concentrations showed significant differences ($P<0.001$) in groups treated with V. amygdalina leaf extract, rifampicin and co-administration of rifampicin and V. amygdalina compared with control.

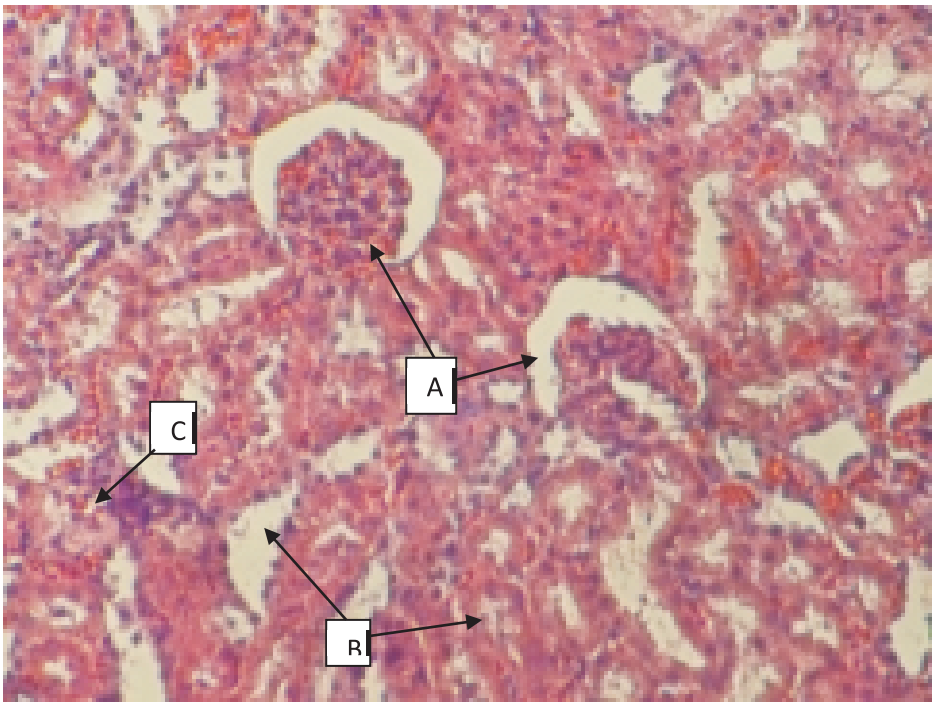

Plate.1 Control: Rat kidney composed of glomeruli A, tubules B and interstitial space C (H&E x 100)

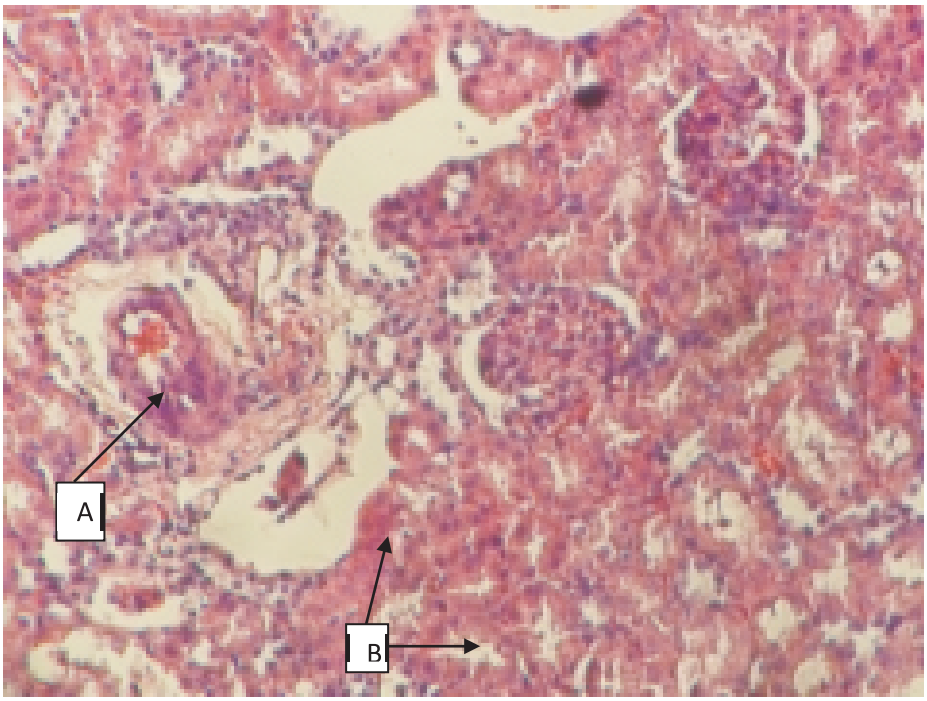

Plate.2: Rat kidney given rifampicin only showing asymmetric vascular media hypertrophy, intimal erosion A and patchy tubular cell cloudy swelling (necrosis) B (H&E x 100)

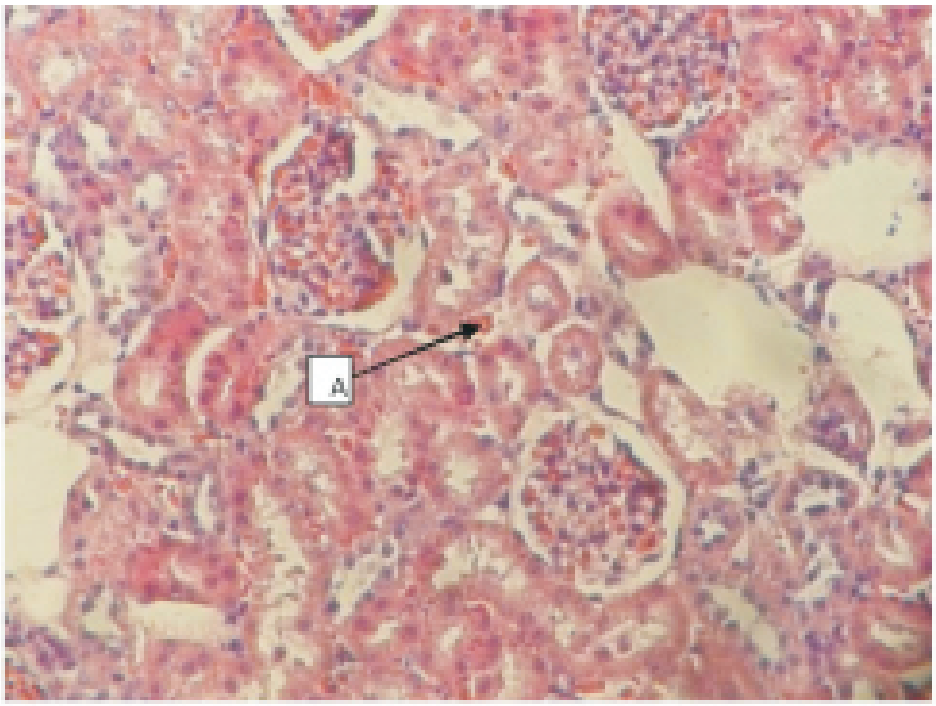

PLATE 3: Rat kidney given Bitter leaf only showing mild interstitial congestion A (H&EX100)

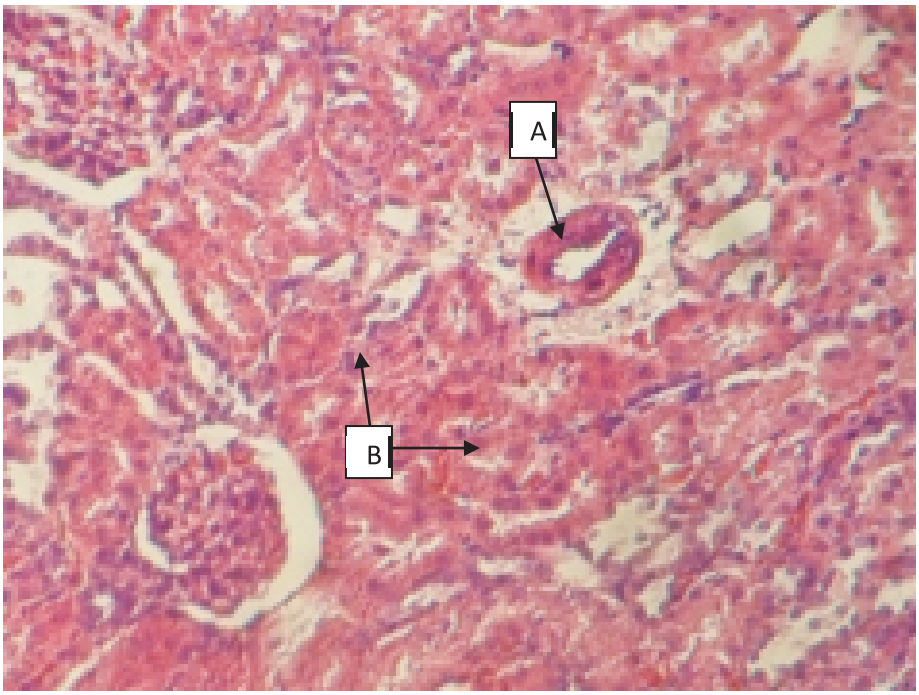

Plate. 4: Rat kidney given rifampicin and bitter leaf extract showing mild asymmetric media hypertrophy A, focal tubular cell cloudy swelling (necrosis) B (H&E x 100)

Plate 1 is the control group where the rat was placed on normal livestock feed and water ad labium and the micrograph shows normal glomerulus, tubules and interstitial spaces.

Plate 2 shows the histology of the kidney in which the rats in the group were treated orally with 250mg / kg of rifampicin daily for 30 days and it showed severe asymmetric vascular media hypertrophy, intimal erosion and patchy tubular cell cloudy swelling. Plate 3 shows the histology of the kidney that was treated via orogastric tube for 30 days with 250mg / kg of V. amygdalina leaf and it revealed mild interstitial congestion. Plate 4 shows the histology of the kidney that was treated via orogastric tube for 30 days with 250mg / kg of rifampicin and 250mg / kg of V. amygdalina leaf and it showed mild asymmetric media hypertrophy and focal tubular cell cloudy swelling.

DISCUSSION

Herbal leaves are widely accepted to be a blessing to mankind in the third world countries.

Therefore Vernonia amygdalina may keenly contribute in the clearance of harmful (to the kidney) and carcinogenic xenobiotics by the induction of phase 2 enzymes.[21] Physiologically, both urea and creatinine are desecrate products that are naturally excreted from the blood through the filtration process of the renal blood. The considerable elevation in the urea level in the serum of rifampicin given rats (Table 1) is in accordance with the studies of Yanardag et al.[4] and Tasduq et al.[5]

Inclusively, there was normalcy in both the urea and creatinine values in the control group, V. amygdalina treated group and the combination of Vernonia amygdalina and Rifampicin treated group (Table 1) which therefore signify the non-toxic and reno- protective nature of Vernonia amygdalina leaf. These findings were corroborated by previous work done on the extract[22]. Amole et al. discovered that the microscopic examination of kidney tissue has showed no histological abnormalities when compared with control slides after administration of aqueous extract of Vernonia amygdalina leaf via orogastric route.

In the present study,The mean catalase and superoxide dismutase were highest while that of malonaldehyde was lowest in rifampicin treated group (Table 1). These outcome may be due to the destructive activity of oxidative stress which are stalled by endogenous antioxidant enzymes.[23] The mean catalase, superoxide dismutase and MDA were within normal range in the Vernonia amygdalina treated group, Vernonia amygdalina and rifampicin treated group and control group (Table 1) because of the antioxidant strength of Vernonia amygdalina in protecting cellular architectures from free radicals scavenging activities from drugs like rifampicin. This study is consistent with previous work done .[24] This non- toxic effects may have arises from the donation of electron to the free radicals by the flavonoid content of the Vernonia amygdalina.

Histologically, the group that were given rifampicin only showed severe asymmetric vascular media hypertrophy, intimal erosion and patchy tubular cell cloudy swelling (necrosis) of the renal tissue. The mechanism of toxicity of rifampicin may be explained by earlier work done. [25] Grunfeld et al. reported that the renal biopsy of the rat treated with rifampicin showed severe, focal or diffuse

tubular necrosis with mild interstitial changes. These report is in accordance with our findings (Fig.2).

Futhermore, the group that was treated with the extract of bitter leaf and the control group showed normal microscopic architecture of the kidney, while the group that was given both rifampicin and bitter leaf extract showed mild asymmetric media hypertrophy with focal cloudy swelling of the tubule (Fig.4). This mild protective effect of bitter leaf may be due to the flavonoid content of the extract as documented.[24] They opined that the flavanoids and its saponins are the active principles which confer antioxidant activities on the Vernonia amygdalina plant. The protective effect of Vernonia amygdalina leaf may also come from its free radical scavenging activity or by hampering the generation of reactive oxygen species thereby demonstrating its antioxidant capability.

CONCLUSION

The present work showed that aqueous extract of Vernonia amygdalina leaf as an ameliorative effect on rifampicin induced kidney toxicity as evidenced histologically and biochemically. The extract donated its electrons to free radicals thereby preventing the free radicals from being toxic.

It is recommended that aqueous extract of Vernonia amygdalina can act as an adjunct in chronic treatment of patient with rifampicin.

REFERENCES

1. Okigbo R, Anuagasi C, Amadi J. Advances in selected medicinal and aromatic plants indigenous to Africa. J. Med. Plants Res. 2009; 2: 086-095.

2. Endashaw B. Study on actual situation of medicinal plants in Ethiopia. JAICAF. 2007; 2: 1-9.

3. Eminzade S, Uras F, Izzettin FV. Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. Nutr. Metab. 2008; 5: 18.

4. Covic A, Goldsmith DJ, Segall L, Stoicescu C, Lungu S, Vovovat C, Covic M. Rifampicin-induced acute renal failure : a series of 60 patients. Nehprol. Dial. Transplant.1998; 13(4): 924-929.

5. Tasduq SA, Kaiser P, Sharma SC, Johri RK. Potentiation of isoniazid-induced liver toxicity by rifampicin in a combinational therapy of antitubercular drugs (rifampicin, isoniazid and pyrazinamide) in Wistar rats. A toxicity profile study. Hepatol. Res.2007; 37: 845–853.

6. Igile GO, Olesyek W, Burda S, Jurzysta N. Nutritional assessment of Vernonia amygdalina leaves in growing mice. J. Agric. Food Chemistry.1995; 43: 2126-2166.

7. Babalola OO, Anetor JI, Adeniyi FA. Amelioration of carbon tetrachloride-induced hepatotoxicity by terpenoid extract from leaves of Vernonia amydgalina. Afr. J. Med. Sci. 2001; 30: 91-93.

8. Awe SO, Makinde JM, Olajide OA. Cathartic effect of the leaf extract of Vernonia amygdalina. Fitoterapia. 1999; 70: 161–165.

9. Enikuomehin OA, Ikotun T, Ekpo EJA. Evaluation of ash from some tropical plants of Nigeria for the control of Sclerotium rolfsii Sacc. on wheat (Triticum aestivum L.). Mycopathoologia. 1998; 142:81-87.

10. Masaba SC. The antimalarial activity of Vernonia amygdalina Del (Compositae). Trans. Roy. Soc. Trop. Med. Hyg.2000; 94: 694-695.

11. Cos P, Hermans N, Bruyne TD, Apers S, Sindambiwe JB, Berghe DV, Pieters L, Vlietinkck AJ. Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. J. Ethnopharmacol. 2002; 79: 155-163.

12. Izevbigie EB, Bryant JL, Walker A. A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth. Exp. Biol. Med. 2004; 229: 163-169.

13. Taiwo IA, Odeigah PGC, Ogunkanmi LA. The glycaemic effects of Vernonia amygdalina and Vernonia tenoreana with tolbutamide in rats and the implications for the treatment of diabetes mellitus. J. Sci. Res. Dev. 2009; 11: 122-130.

14. Etkin NL. Local knowledge of biotic diversity and its conservation in rural Hausa land, Northern Nigeria. Economic Botany.2002; 56:73-88.

15. Drury RAB, Wallington EA and Cameron RC. Histological techniques: 4th ed., Oxford university press NY. USA. 1976; 279-280.

16. Fawcett JK and Scott JE. A rapid and precise method for the determination of urea. J Clin Pathol. 1960; 13(2): 156-159.

17. Bartels H, Bohmer M, Heierli C. Serum creatinine determination without protein precipitation. Clinica Chemica Acta. 1972; 37: 193-197.

18. Buege JA, Aust SD. The thiobarbuturic acid assay. Methods Enzymol 1978; 52:306-307

19. Cohen D, Dembiec D and Marcus J Measurement of catalase activity in tissue extracts. Annals Biochemistry. 1970; 34:30-38.

20. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170 -3175.

21. Howard CB, Stevens J, Izevbigie EB, Walker A, Mcdaniel O. Time and dose-dependent modulation of phase 1 and phase 2 gene expression in response to treatment of MCF-7 cells with a natural anti-cancer agent. CellMolBiol. 2003; 49(7):1057 – 1065.

22. Amole O, Izegbu M, Onakoya A, Dada M. Toxicity studies of the aqueous extract of Vernonia amygdalina. J. Biomed Res. 2006; 17: 39-40.

23. Winterbourn CC. Superoxide as an intracellular sink. Free Radical Bio Med. 1993;14: 85–90.

24. Igile GO, Oleszek w, Jurzysta M, Burda S, Fafunso M, Fasanmade AA. Flavonoids from Vernonia amygdalina and their antioxidant activities. J AGR FOOD CHEM. 1994; 42: 2445 – 2448.

25. Grunfeld JP, Kleinknecht D, Droz D. Acute interstitial nephritis. In: Diseases of the Kidney. Little Brown, Boston.1993; pp.1331 –1353.