Nigerian Journal of Pharmaceutical and Applied Science Research, 5(2):1-7, August 2016

WEBSITE: www.nijophasr.com *ISSN 1485-8059*

Anti-anemic, Anti-necrotic and Anti-fibrotic effect of *Vernonia amygdalina* post-treatment in Dimethylnitrosamine (DMN)-administered rats

Usunobun Usunomena and Okugbo OsarhiemeTinuade

Department of Basic Sciences (Biochemistry Unit), Faculty of Basic and Applied Sciences, Benson, Idahosa University, P.M.B. 1100, Benin City, Edo state, Nigeria..

ABSTRACT

This study aimed to assess the effect of ethanolic leaf extract of *Vernonia amygdalina* (VAE) post-treatment on biochemical indices in dimethylnitrosamine (DMN)-administered albino rats. A total of thirty (30) male rats shared into five (5) groups were used. Group 1 received distilled water only; group 2 received 200 mg/kg VAE only while group 3 received a single oral dose of 25 mg/kg DMN on the first day. Group 4 and 5 received single oral dose of 25 mg/kg DMN on the first day and were thereafter post-treated with 100 mg/kg and 200 mg/kg VAE respectively for one week. Results show that DMN administration caused significant (P<0.05) increases in aspartate aminotransferases (AST), alanine aminotransferases (ALT), total cholesterol(TC), triglyceride(TG), malondialdehyde (MDA), white blood cell(WBC), monocytes (MO), granulocyte (GR) and a significant (P<0.05) decrease in reduced glutathione(GSH), hemoglobin (Hb), red blood cells(RBC), platelets(PLT), and packed cell volume(PCV). However, VAE post-treatment resulted in a dose-dependent reversal of DMN-induced alterations of all the biochemical and hematological parameters determined towards normalcy. Histopathological studies showed that DMN administration caused severe hemorrhagic necrosis and fibrotic changes which corroborated the biochemical indices. The pathological damages were however mitigated after post-treatment with VAE in a dose-dependent manner. This study suggests that *Vernonia amygdalina* elicits ant-anemic and hepato-protective effect probably through its bioactive constituents.

Keywords: Dimethylnitrosamine, Fibrosis, Hematology, Necrosis, *Vernonia amygdalina*

INTRODUCTION

Vernonia amygdalina commonly called bitter leaf have a wide spectrum of medicinal applications including anti-fibrotic, anti-diabetic, anti-malarial and anti-helmitic (Usunobun *et al*., 2015; Usunobun and Okolie, 2016; Abosi and Raseroka, 2003). It has a rich content of phytochemicals including saponins and flavonoids (Usunobun and Okolie, 2015; 2016). Some scientists have opined that the flavonoids and its saponins are the active principles which confer antioxidant, anti-fibrotic and anti-tumor activities on the plant (Usunobun *et al*., 2015; Igile *et al*., 1994).

Dimethylnitrosamine (DMN), a semi-volatile organic chemical present at very low levels in certain foodstuffs especially those cooked, smoked, or cured is highly toxic, especially to the liver, and is a human carcinogen (Peto *et al*., 1991, Najim and Trussel, 2001, George *et al*., 2001). DMN, a potent hepatotoxin and mutagen is generated from the *in situ* reaction of dimethylamine (DMA) with monochloroamine in the disinfection process or the nitrosation of DMA by nitrite (Mitch and Sedlak, 2002; Gerecke and Sedlak, 2003). DMN exerts carcinogenic effects and induces hepatic necrosis through metabolic activation by CYP2E1 (Guengerich *et al*., 1991). The aim of this study was to ascertain the effect of *Vernonia amygdalina* post-treatment on transaminases, lipid profile and histology in rats pre-treated with dimethylnitrosamine.

MATERIALS AND METHODS

Collection, Identification and Preparation of plant materials

Fresh leaves of *Vernonia amygdalina* were purchased from a local market in Benin City, Edo state, Nigeria. The leaves were identified by a Botanist, Dr. C. O. Akoma in the Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, Benin city, Edo State. The *Vernonia amygdalina* leaves were separated from the stalk,

Corresponding author: Email: usunsquare@yahoo.com **Phone:**+2348034174871

washed and air-dried at room temperature (24°C) and then pulverized, crushed into fine powder and weighed.

Extraction of the plant leaves

Ethanolic extract of the plant leaves was prepared by soaking 450g of the dry powdered plant leaves in 2.5 litres of absolute ethanol at room temperature for 48 hr (for thorough extraction). The extract was then filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The filtrate was thereafter concentrated using a rotary evaporator set at 40◦C to one-tenth its original volume and then finally freeze dried. The dried residue (crude extract) was then stored at 4ºC. Aliquot portions of the crude plant extract residue were weighed and dissolved in distilled water for use on each day of our experiments.

Experimental animals, DMN and extract administration

Adult male albino rats weighing 180-200 g were purchased from Anatomy Department, College of Basic Medical Sciences, University of Benin for the experiment. They were allowed to acclimatize for 7 days and maintained under standard photoperiodic conditions and kept in the animal house of the Department of Biochemistry, Faculty of Basic and Applied Sciences, Benson Idahosa University. The animals were provided standard pellet diet and water *ad libitum*. The DMN used in this work was synthesized according to the method of Vogel (1971). Administration of extract and DMN was orally using gavage and was done between 9.00 am - 10.00 am

A total of thirty male rats were assigned into one of the following groups: Group 1 (control) received distilled water; Group 2 received 200 mg/kg *Vernonia amygdalina* only for seven days. Group 3 received a single dose of DMN only (25 mg/kg) on the first day. Group 4 received a single dose of DMN (25 mg/kg) on day 1 and also *Vernonia amygdalina* (100 mg/kg) for seven days. Group 5 received a single dose of DMN (25 mg/kg) on day 1 and also *Vernonia amygdalina* (200 mg/kg) for seven days. 24hr after last administration, rats from each group were sacrificed by cervical dislocation. Blood samples were obtained through heart puncture via a syringe into a EDTA bottles for hematology or plain sterile bottles (for serum) which were allowed to stand for one hour to clot and thereafter centrifuged at 3000 rpm for 45 min at room temperature to obtain serum for biochemical analysis.

Following sacrifice, liver samples were quickly excised and rinsed with normal saline. A small portion of each liver sample was fixed in 10% phosphate-buffered formalin for histological examination while the remaining portions were stored at –20°C for biochemical analysis. 10% liver homogenate was prepared in physiological saline. The homogenate was centrifuged at 5000 g for 15 minutes and the clear supernatant obtained used for GSH and MDA analysis.

Biochemical parameters

Serum aspartate aminotransaminase (AST), alanine aminotransferase (ALT), triglyceride (TG) and total cholesterol (TC) were determined by using the RANDOX Kit according to the manufacturer's instructions. GSH was estimated colorimetrically by measuring the reduction of Ellman's reagent (5, 5'di-thio-bis-2-nitrobenzoic acid) at 412 nm as described by Ellman (1959). MDA was estimated in a colorimetric reaction with thiobarbituric acid (1979).

Histology

Liver sections fixed in formol-saline were processed for light microscopy at the Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Benin. The resultant slides were read and interpreted by a consultant pathologist.

Statistical analysis

Data obtained from this study were expressed as mean value ± standard deviation. Differences between means of control and tested groups were determined by One way ANOVA using Statistical Package for social scientist (SPSS). A probability level of less than 5% ($p<0.05$) was considered significant.

RESULTS

Effect of the ethanolic leaf extract of *Vernonia amygdalina* on serum AST, ALT, TC and TG in DMN-administered rats are shown in table 1. The DMN-administered rats showed a highly significant increase (P <0.05) in serum ALT, AST, TC and TG activities compared to normal control group. However, post-treatment of DMN-administered rats with *Vernonia amygdalina* ethanolic leaf extract resulted in a significant decrease in ALT, AST, TC and TG (P<0.05) compared to DMN-administered rats in a concentration-dependant manner with 200 mg/kg *Vernonia amygdalina* having a better significant decrease in all parameters.

Assessment of oxidative stress as shown in Table 1 revealed a significant (P<0.05) increase in MDA and decrease in GSH in DMN administered compared to controls whereas post-treatment with 100 mg/kg and 200 mg/kg *Vernonia amygdalina* for seven days significantly (P<0.05) decreased MDA and increased GSH compared to DMN alone administered rats.

The result shown in table 2 revealed a significant (P<0.05) decrease in RBC, Hb, PCV, PLT and LYM as well as a significant (P<0.05) increase in WBC, GR and MO in DMN administered rats while post-treatment with 100 mg/kg and 200 mg/kg *Vernonia amygdalina* significantly (P<0.05) attenuated these hematological parameters towards normalcy.

Table 1: Effect of *Vernonia amygdalina* post- treatment on AST, ALT, TC, TG, MDA and GSH in rats administered Dimethylnitrosamine (DMN).

Treatment groups	ALT (U/L)	AST (U/L)	TC (mg/dl)	TG (mg/dl)	MDA ($x10^{-7}$)	GSH (mM)
Control	15.35± 0.58^a	19.01±2.00^a	90.90±5.73^a	74.63±6.89^a	4.25±0.07^a	3.70±0.02^a
VAE alone (200mg/kg)	12.33± 0.58^a	17.00±2.00^b	85.15±6.08^a	84.73±5.03^a	4.35±1.34^a	3.45±0.03^a
DMN 25mg /kg	65.15± 5.05^b	89.07± 3.23^b	169.27± 4.10^b	198.94±8.23^b	16.85±1.52^b	1.53±0.11^b
VAE (100mg/kg) + DMN (25mg/kg)	34.17± 2.32^c	49.67±3.19^b	129.41±3.94^c	134.28±5.95^c	8.10±0.10^c	2.88±0.12^c
VAE (200 mg/k + DMN (25mg/kg)	27.27± 2.80^d	44.12±2.56^b	105.24±4.88^d	111.04±5.61^d	6.60±0.81^d	3.01±0.21^c

Values are expressed as mean ± SEM (n=5). VAE = *Vernonia amygdalina*, DMN = Dimethylnitrosamine, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, TC = Total cholesterol, TG = Triglyceride, MDA = Malondialdehyde, GSH = Reduced Glutathione

Values in same column with different superscript are significantly different (p<0.05).

Table 2: Effect of *Vernonia amygdalina* post- treatment on Hematological parameters in rats administered Dimethylnitrosamine (DMN)

Treatment groups	WBC ($x10^3$/ɥL)	RBC ($x10^6$/ɥL)	Hb (g/dl)	PCV (%)	PLT ($x10^3$/ɥL)	LY (%)	GR (%)
Control	8.30±0.53^a	7.54±0.27^a	13.46±0.96^a	44.30±2.28^a	571±10.10^a	53.05±3.34^a	35.20±2.03^a
VAE alone (200mg/kg)	8.75±5.44^a	7.44±0.32^a	11.05±0.21^b	45.75±2.49^a	665±15.60^b	48.70±2.98^a	41.55±2.47^b
DMN (25mg /kg)	18.00±2.94^b	5.01±0.24^b	9.70±0.42^c	35.80±1.84^b	134±10.90^c	30.50±2.48^b	49.45±3.26^c
VAE (100 mg/kg) + DMN 25mg/kg	13.40±1.52^c	6.33±0.40^c	12.70±0.91^a	41.96±1.73^a	203±5.20^d	39.90±2.65^c	36.70±3.14^a
VAE (200mg/kg) +DMN(25mg/kg)	12.20±2.82^c	6.62±0.32^c	11.90±0.98^a	42.60±1.99^a	215±10.80^d	40.70±1.41^c	38.20±1.56^a

Values are expressed as mean ± SEM (n=5). VAE = *Vernonia amygdalina*, DMN = Dimethylnitrosamine, WBC = White blood cell, RBC = Red blood cell, Hb = Hemoglobin, PCV = Packed cell volume, PlT = Platelet, LY = Lymphocyte, GR = Granulocyte

Values in same column with different superscript are significantly different (p<0.05).

Histopathological studies showed that DMN caused severe liver damage including sinusoidal dilation, haemorhagic centrilobular necrosis, collagen accumulation, vacuolization, inflammatory cell infiltration (Plate 3) when compared with control liver (Plate 1). The above changes were reduced in the liver of rats treated with *Vernonia amygdalina* (Plate 4 and 5). The histological pattern was normal in rats treated with *Vernonia amygdalina* alone as shown below.

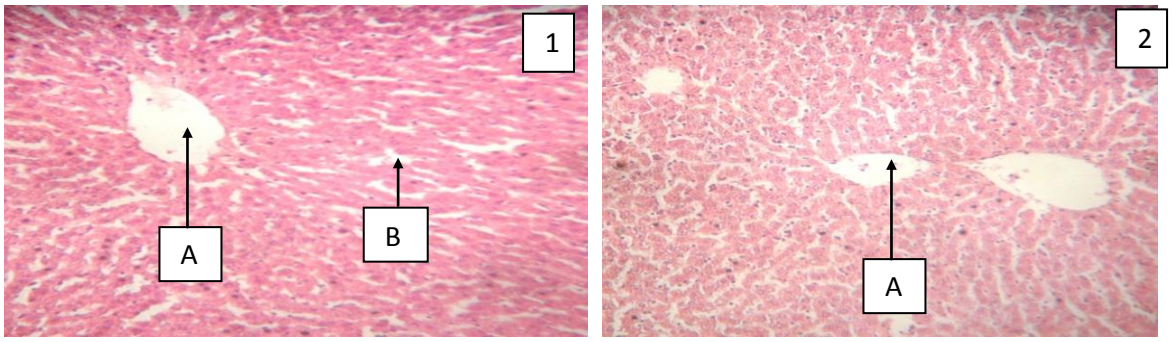

Plate 1: Liver of control rats composed of portal triad, Hepatocytes and sinusoid (H & E x 10). It shows of liver parenchyma with central vein (A) and radiating column of hepatocytes (B). Portal tracts appear normal.
Plate 2: Liver of rats treated with *Vernonia amygdalina* alone (200 mg/kg) showing preserved histological details of the liver (A) with fairly prominent features.

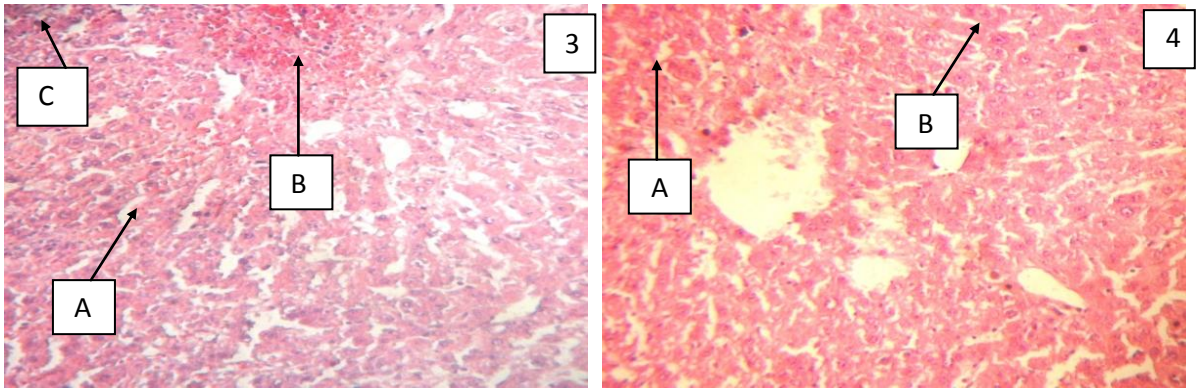

Plate 3: Liver of rats treated with DMN alone (25 mg/kg) showing visible distortion in the liver architecture, noticeable neutrophilic infiltrates (A) and prominent congestion in the central vein with evident necrotic (B), accumulation of collagen fibers (C) and fibrotic changes.
Plate 4: Liver of rats treated with 25 mg/kg DMN and *Vernonia amygdalina* (100 mg/kg) showing slight distortion in the liver architecture (A) with visible hepatocyte (B) and fairly distinct nucleus as well as few neutrophilic infiltrates (B), congestion around the central vein and slight evident of fibrotic regeneration.

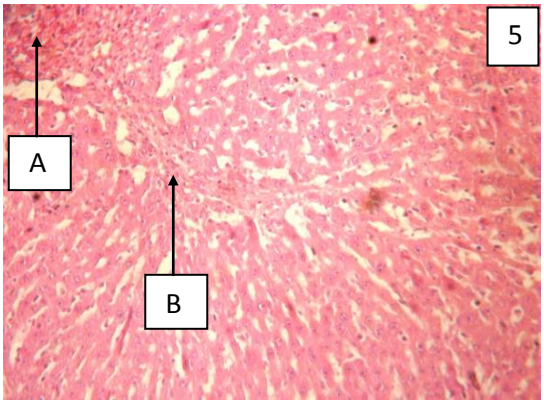

Plate 5: Liver of rats treated with 25 mg/kg DMN and *Vernonia amygdalina* (200 mg/kg) showing Preserved histological details of the liver with fairly prominent features, slight distortion in the liver architecture (A) with visible hepatocyte and fairly distinct nucleus as well as few neutrophilic infiltrates, congestion around the central vein (B) but absence of fibrotic changes.

DISCUSSION

Dimethylnitrosamine (DMN) is a potent hepatotoxin, carcinogen and mutagen whose toxicity is mediated by its reactive metabolites and not by the parent compound (George *et al.*, 2001). The results of this study show that DMN treatment could have affected the lipid metabolism of liver cholesterol levels. This is evidenced from the present observations that, DMN caused a significant ($P< 0.05$) increase in the levels of total cholesterol. It can be assumed that hypercholesterolemia in DMN intoxicated rats resulted from damage of hepatic parenchymal cells that led to disturbance of lipid metabolism in liver (Havel 1986). The elevated level of serum cholesterol in DMN alone group may be attributed to increase in the concentration of acetyl CoA arising probably from enhanced β-oxidation of fatty acid (Rang *et al.*, 1995). However, rats post-treated with *Vernonia amygdalina* ethanol leaf extract showed a significant ($p< 0.05$) decline in cholesterol values compared to DMN intoxicated alone rats. The mechanism of lipid lowering effects of *Vernonia amygdalina* extract might be attributed to an inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferease *in vitro*. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver (Matsuda, 1994).

Liver function enzymes such as AST and ALT are commonly used in clinical practice to screen for liver disease, monitor the progression of a known disease and determine the effects of potentially hepatotoxic drugs (Harris, 2005). In this study, hepatotoxicity was evidenced by a significant increase ($P<0.05$) in activities of serum AST and ALT in the group treated with DMN compared with controls. The increase in the serum enzyme levels may be due to their increased leakage from damaged and necrotic hepatocytes as a result of DMN toxicity. The increased levels of AST and ALT in serum are indicative of cellular liver leakage and loss of functional integrity in the cell membrane of the liver (Drotmann and Lowhorn, 1978). However, treatment with 100 mg/kg and 200 mg/kg b.w of ethanolic leaf extract of *Vernonia amygdalina* to rats decreased the DMN induced elevated ALT and AST. This suggests the maintenance of structural integrity of the hepatocytic cell membrane or regeneration of damage liver cells by *Vernonia amygdalina*. Post-treatment with various dose levels of *Vernonia amygdalina* mediated a reduction in the levels of these enzymes towards the normal value indicating a stabilization of plasma membrane as well as repair of hepatic tissue damage caused by DMN. This effect is in agreement with the common view that serum levels of transaminase return to normal following healing of liver parenchyma and regeneration of hepatocytes (Thabrew *et al.*, 1987).

The decrease in HB concentration in DMN-pretreated rats signifies that the rats ability to provide sufficient oxygen to tissues is restricted considerably and will result in decrease of physical activity. The decrease in PCV in DMN administered rats can be attributable to reduction in RBC caused by either destruction or reduction in size of RBC. The decrease in RBC in DMN administerd rats may be attributed to hemolysis as a result of haemorrhages and reduced erythropoiesis. The low platelet concentration in DMN-administerd rats may be due to decreased production or increased destruction. The decrease in PCV, Hb, RBC and platelets can be said to be due to toxicity of DMN on blood forming organs suggesting anemic condition of the DMN-administerd rats. *Vernonia amygdalina* at 100 mg/kg and 200 mg/kg attenuated the concentrations of Hb, RBC, PCV and platelets towards normalcy suggesting anti-anemic potentials of *Vernonia amygdalina* which can be attributable to bioactive agents such as flavonoids present in *Vernonia amygdalina* (Usunobun and Okolie, 2015; 2016).

The concentration of malondiadehyde (MDA) may reflect the degree of oxidative stress. In this study, the significant increase in MDA and decrease in GSH

in DMN-administered indicates an increased level of free radicals which may attack the polyunsaturated fatty acids in cell membrane. However, 100 mg/kg and 200 mg/kg *Vernonia amygdalina* significantly decreased MDA and increased GSH indicating that the plant scavenge free radicals and reduce oxidative stress. The ability of *Vernonia amygdalina* to reduce oxidative stress may be attributed to reported presence of phytochemicals such as flavonoids, saponins and alkaloids in *Vernonaia amygdalina* leaves (Usunobun and Okolie, 2015; 2016)

Histopathological examination of the liver of animals treated with DMN alone reveals massive loss of cellular boundary indicating hepatotoxicity, severe hemorrhagic necrosis and fibrotic changes, whereas liver of animal treated with 100 and 200 mg/kg of *Vernonia amygdalina* showed moderate, marked and mild reduction in the observed changes in the DMN treated group.

The present *in vivo* study have further demonstrated the hepatoprotective potential of ethanolic leaf extract of *Vernonia amygdalina* against DMN-induced liver damage and it is possible that a probable mechanism of hepatoprotection is the antioxidant activity as the plant have been reported to be rich in flavonoids, Vitamin C, saponins, alkaloids and tannins. It is therefore not out of place to assume that the hepatoprotective effect observed with the ethanolic leaf extract of *Vernonia amygdalina* may be due to any of these constituents or a combination of them.

REFERENCES

Abosi AO, Raseroka BH. (2003). *In vivo* antimalarial activity of *Vernonia amygdalina*. *British Journal of Biomedical Science* 60: 89-91.

Drotmann RB, Lowghorn GT. (1987). Serum enzymes as indicators of chemical induced Liver damage. *Drug Chemistry and Toxicology* 1: 163 – 171.

Ellman, GL. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82:70–77.

George J, Rao KR, Stern R, Chandrakasan G. (2001). Dimethylnitrosamine-induced liver injury in rats: the early deposition of collagen. *Toxicology* 156: 129–138.

Gerecke AC, Sedlak D. (2003). Precursors of *N*-nitrosodimethylamine in natural water. *Environmental Science and Technology* 37(7): 1331-1336.

Guengerich, FP, Kim DH, Iwasaki M. (1991). Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chemical Research and Toxicology* 4(2): 168-179.

Havel RJ. (1986). Functional activities of hepatic lipoproteins receptors. *Annual Review Physiology* 48: 119– 134.

Harris EH. (2005). Elevated liver function test in type 2 diabetes. *Clinical Diabetes* 23(3): 115-119.

Igile GO, Oleszek W, Jurzysta M, Burda S, Fafunso M, Fasanmade AA. (1994). Flavonoids from *Vernonia amygdalina* and their antioxidant activities. *Journal of Agriculture and Food Chemistry* 42: 2445 – 2448.

Matsuda K (1994). ACAT inhibitors as antiatherosclerotic agents: compounds and mechanisms. *Medical Research and Review* 14: 271–305.

Mitch WA, Sedlak D. (2002). Formation of *N*-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environmental Science and Technology* 36(4): 588-595.

Najim I, Trussel RR. (2001). NDMA formation in water and wastewater. *Journal Of American Water Works Association* 93(2):92-99

Ohkawa H, Ohishi N, Yogi K, (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Annals of Biochemistry* 95: 351–358.

Peto R, Gray R, Brantom, P, Grasso P. (1991). Dose and Time Relationships for Tumor Induction in the Liver and Esophagus of 4080 Inbred Rats by Chronic Ingestion of N-Nitrosodiethylamine or N-Nitrosodimethylamine. *Cancer Research* 51 (23 Part 2): 6452–6469.

Rang HP, Dale MM, Ritter JM (1995*). Pharmacology*. 3rd edn. Churchill Livingstone, New York

Thabrew MI, Joice PD, Rajatissa WA. (1987). Comparative study of the efficacy of *Paetta indica*

and *Osbeckia octandra* in the treatment of liver dsysfunction. *Planta Medica* 53: 239 – 241.

Usunobun U, Okolie NP, Eze IG. (2015). Inhibitory Effect of *Vernonia amygdalina* on Dimethylnitrosamine (DMN)-induced Liver Fibrosis in Rats. *International Journal of Clinical Pharmacology and Toxicology* 4(4): 179-184.

Usunobun U, Okolie PN (2016). Dimethylnitrosamine (DMN)-induced fibrotic rats: Effect of *Vernonia amygdalina* on extracellular matrix and hepatic/lysosomal integrity. *International Journal of Pharmacology and Toxicology* 4 (1): 7-11

Usunobun U, Okolie NP. (2015). Phytochemical, trace and mineral composition of *Vernonia amygdalina* leaves. *International Journal of Biological and Pharmaceutical Research* 6(5): 393-399.

Usunobun U and Okolie PN (2016). Phytochemical analysis and proximate composition of *Vernonia amygdalina*. *International Journal of Scientific World* 4(1): 11-14.

Vogel AI. (1971). A textbook of practical organic Chemistry including qualitative organic analysis. Longman group limited, London. 426.