

Original article:



Antihepatotoxic efficacy of *Vernonia amygdalina* ethanolic leaf extract on Dimethylnitrosamine (DMN)-induced liver damage in rats

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Abstract

Introduction: Dimethylnitrosamine (DMN) is a potent hepatotoxin, carcinogen and mutagen whose toxicity is mediated by its reactive metabolites and not by the parent compound. The antihepatotoxic efficacy of ethanolic leaf extract of *Vernonia amygdalina* against DMN-induced liver damage in rats thus, needs to be investigated.

Methodology: Rats were given DMN, at a single dose of 12 g/kg body weight orally on day 8 after pretreatment with 400mg/kg *Vernonia amygdalina* for 7 days and thereafter investigated for toxicity and hepatoprotection 48 hours later.

Result: DMN produced liver damage in rats as manifested by the significant rise ($p < 0.05$) in serum levels of alanine aminotransferase (ALT), total cholesterol (TC) and decrease in serum total protein (TP), globulin, albumin, white blood cells (WBCs), red blood cells (RBCs), packed cell volume (PCV), platelets and hemoglobin (HB) levels compared to control. However, Pretreatment of rats with ethanolic leaf extract of *Vernonia amygdalina* (400 mg/kg body weight) once daily for seven days to DMN treated rats significantly attenuated ($p < 0.05$) the toxicity by DMN.

Conclusion: Ethanolic leaf extracts of *Vernonia amygdalina* having antihepatotoxic properties minimized the deleterious effects generated by the hepatotoxin, DMN, thereby suggesting its use as a potent antihepatotoxic agent.

Keywords: Dimethylnitrosamine, *Vernonia amygdalina*, Antihepatotoxic, Hematological parameters

Introduction

Liver, the key organ of metabolism and excretion is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Thus, disorders associated with this organ are numerous and varied. While a curative agent has not yet been found in modern medicine, the currently usage of corticosteroids and immunosuppressive agents only brought about symptomatic relief [1]. Dimethylnitrosamine (DMN) is a potent hepatotoxin, carcinogen and mutagen (2) which exerts carcinogenic effects, cause fibrosis and induces hepatic necrosis in experimental animals through metabolic activation by CYP2E1 (2-4).

Occupational exposure to DMN may happen in a large number of places including industries such as tanneries, pesticide manufacturing plants, rubber and tire manufacturing plants, alkylamine manufacture/use industries, fish processing industries, foundries, and dye manufacturing plants (5). Under certain conditions, DMN may be found in outdoor air, surface waters (rivers and lakes, for example), and soil (5).

Vernonia amygdalina belongs to the family of *Asteraceae*. It is commonly called “Bitter leaf” in English language, `oriwo` in Esan language, “Shuwaka” in Hausa language, “Onugbu” in Igbo language and “Ewuro” in Yoruba language.

Medicinally, the leaves are widely used for fevers and are known as quinine substitute⁽⁶⁾. It is used to prepare cough medicine in Ghana⁽⁷⁾ and the root infusion is taken in Nigeria as an antihelminthic as well as for enteritis and rheumatism⁽⁸⁾. *Vernonia amygdalina* have been proved in human medicine to possess potent anti-malarial and anti-helminthic properties⁽⁹⁾ as well as anti-tumorigenic properties⁽¹⁰⁾. Strong antioxidant activities have been reported for flavonoids from *Vernonia amygdalina* and, its saponins have been reported to elicit anti-tumoral activities in leukemia cells⁽¹¹⁾. Peptides from *Vernonia amygdalina* are known to be potent inhibitor of mitogen-activated proteins kinases, which are crucial for breast tumor growth and also represents a key regulatory point for the tumour⁽¹²⁾. The present study was conducted to investigate the potential antihepatotoxic efficacy of *Vernonia amygdalina* ethanolic leaf extract on Dimethylnitrosamine-induced liver injury in rats.

Methodology

Preparation and extraction of the plant leaves

Vernonia amygdalina leaf samples were gotten from Benin city, Edo state, Nigeria and authenticated by a botanist at the Department of Basic sciences, Benson Idahosa University. Fresh matured leaves of *Vernonia amygdalina* were separated from stalk, washed and air-dried for at room temperature (24°C) and then pulverized, crushed into fine powder using a manual blender and weighed. Ethanolic (Absolute) extracts of the plant were prepared by soaking 500g of the dry powered plant material in 1.2litres of absolute ethanol and then kept at room temperature for 48hours (for thorough extraction). At the end of

the 48hours, the extracts were filtered first using a Whatmann filter paper No.42 (125mm) and then with cotton wool. The *Vernonia amygdalina* ethanolic extracts were concentrated using a rotary evaporator with water bath set at 40°C to one-tenth its original volume and then finally with water bath at 37°C. The dried residue (crude extract) was then stored at 4°C. Aliquot portion of the crude plant extract residue was weighed and dissolve in distilled for use on each day of experiment.

Apparatus

During the course of the experiment, the following apparatus were used to achieve the desired results. They includes Rotary evaporator, water bath, measuring cylinder, beakers, filter paper, cotton wool, funnel, weighing balance, spectrophotometer, cuvette, centrifuge, test tubes, stopwatch, syringes, spatula.

Chemicals

Absolute ethanol, Dimethylnitrosamine (DMN), Sodium hydroxide Solution. DMN used in this work was synthesized in a fume chamber at the Department of Biochemistry, University of Ibadan, Oyo state, Nigeria, according to the method of Vogel⁽¹³⁾.

Experimental animals

Twenty six (26) male albino wistar rats that weighed between 155 – 205g gotten from Edo state were used for the experiment. The animals approved by Benson Idahosa University Ethical Committee in accordance with "Principles of Laboratory Animal Care" were kept in Biochemistry Departments' animal house. The condition of the animal house was of standard and appropriate cleaning was ensured.

Administration of plant extract and toxicant

A total of twenty six male wistar rats were specifically assigned into one of the following groups:

Groups	Number of rats	Treatment	Body weight
1	5	Control (water)	155g-160g
2	8	DMN alone (12mg/kg)	190g-205g
3	6	<i>Vernonia amygdalina</i> alone (400mg/kg)	160g-170g
4	7	<i>Vernonia amygdalina</i> (400mg/kg) +DMN (12mg/kg)	170g-180g

- Control rats received normal
- Rats in group 3 and 4 were administered 400mg/kg of *Vernonia amygdalina* for 7 days
- Rats in group 2 and 4 received a single oral dose of 12mg/kg DMN on day 8. DMN was prepared using physiological saline.

Blood collection

At day 10 of experiment, rats from each group were weighed and sacrificed by cervical dislocation. Blood samples were obtained through heart puncture for hematological and liver function analysis. Blood for hematology was collected using EDTA bottles. Blood samples for liver function analysis were taken into centrifuge tube with rubber caps, labeled and centrifuged at 3000 rpm for 15 min.

Biochemical analysis

Serum AST was determined by the method of Reitman and Frankel (¹⁴). Serum total protein and albumin were analyzed using the biuret and bromocresol green methods, respectively. In both cases, commercially available test kits, products of Randox laboratories, U.K. were used and with the manufacturers instructions strictly adhered to. The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration. Serum total cholesterol was measured using commercial enzyme assay kits (Randox laboratories, U.K). Hematological parameters like haemoglobin (Hb), Packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC) and

platelet were monitored using Hemavet 850 (CDC technologies, oxford, CT) at the University of Benin teaching hospital.

Statistical Analysis

Results are reported as mean \pm standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA). The value of $p < 0.05$ was considered as statistically significant.

Observations and results

Administration of single dose of 12 mg/kg body weight of DMN to wistar rats produced significant changes in the biochemical parameters when compared to the control group. ALT and total cholesterol were significantly elevated ($P < 0.05$), while the serum total protein, globulin and serum albumin levels were reduced in the DMN-treated rats as compared to the control group (Tables 1). However pretreatment of the rats with 400mg/kg *Vernonia amygdalina* ethanolic leaf extract produced a significant reduction in the levels of ALT and total cholesterol as well as significant increase in the levels of total protein, globulin and albumin compared to values in the DMN alone treated group.

DMN treatment also significantly decreased WBCs, platelet, hemoglobin, RBCs, and PCV in DMN alone treated group compared to other groups (Table 2).

However, *Vernonia amygdalina* pretreatment significantly mitigated the induced changes in hematological parameters

Table 4.1: Effect of *Vernonia amygdalina* ethanolic extract on serum Total cholesterol (TC), Alanine transaminase (ALT), Total protein (TP), Globulin and Albumin (ALB) in DMN-induced toxicity

Treatment groups	TC (mg/dl)	ALT (U/l)	TP (g/dl)	ALB (g/dl)	Globulin (g/dl)
Control group	95.40±9.20 ^a	18.70±3.80 ^a	8.0±0.3 ^a	3.6 ± 0.2 ^a	4.4±0.1
DMN alone group(12mg/kg)	154.60±13.20 ^b	69.00±1.70 ^b	4.8 ± 0.2 ^b	2.3 ± 0.1 ^b	2.5±0.1
<i>Vernonia amygdalina</i> group (400mg/kg)	103.10±8.90 ^a	18.70±1.90 ^a	8.2±0.3 ^a	3.5±0.1 ^a	4.7±0.2
<i>Vernonia amygdalina</i> (400mg/kg) + DMN (12mg/kg)	109.20±27.50 ^a	39.50±7.20 ^c	6.1±0.3 ^c	2.8±0.2 ^c	3.3±0.1

Values are expressed as mean ± SD, n= 5, DMN=Dimethylnitrosamine; ALT=Alanine aminotransferase; TC=Total cholesterol; TP=Total protein; ALB=Albumin. Data are statistically significant at P<0.05 compared to normal control group. Values with the same superscript in each column (a, b, c, d) are not significantly different (p<0.05).

Table 2: Effect of *Vernonia amygdalina* ethanolic leaf extract on Hematological Parameters in DMN-induced toxicity

Parameters	Group 1 (control)	Group 2 (12mg/kg DMN)	Group 3 (400mg/kg <i>V. amygdalina</i> only)	Group 4 (400mg/kg <i>V. amygdalina</i> + 12mg/kg DMN)
WBC (x10 ³ /µl)	12.30± 0.00 ^a	2.75± 0.60 ^b	10.65±0.60 ^c	8.25±0.30 ^d
RBC (x10 ⁶ /µl)	9.91 ±1.60 ^a	7.47± 0.80 ^b	9.81±1.40 ^a	9.80±0.30 ^a
Hemoglobin (g/dl)	15.80 ± 1.00 ^a	10.80 ± 0.40 ^b	15.40±0.70 ^a	15.10±0.10 ^a
Platelet (x 10 ³ /µl)	318.00 ± 23.00 ^a	83.00 ± 10.70 ^b	404.50±4.50 ^c	124.00±9.00 ^d
Hematocrit (%)	46.10 ± 4.50 ^a	33.75 ± 1.10 ^b	44.75±1.00 ^a	42.40±2.40 ^a

Values are given as mean ± standard deviation, n= 5, DMN=Dimethylnitrosamine, WBC=White blood cell; RBC=Red blood cell . Values with the same superscript in each row (a, b, c, d) are not significantly different (p<0.05)

Discussion

The analysis of serum biochemical parameters provides important information about visceral organ damage in rabbits, particularly for the liver and the kidneys (15-16). Level of ALT, a marker enzyme of

liver injury increased significantly in the DMN alone group after DMN administration (p<0.05) compared to normal control group (Table 4.1). The increase in activities of ALT, a liver marker enzyme in the serum of DMN alone induced rats indicates damage to

hepatic cells. Increase in serum level of AST and ALT have been attributed to damaged structural integrity of the liver. This is because they are cytoplasmic in their location and are released into circulation after cellular damage⁽¹⁷⁾. Pretreatment with *Vernonia amygdalina* ethanolic leaf extracts caused significant decrease in the activities of ALT. Thus the extract protected the hepatocytes from DMN-induced injuries. The stabilization of transaminases denotes the renewal of the normal hepatic activity. The abnormal high level of serum ALT in this study is a consequence of DMN-induced liver dysfunction. However pretreatment with *Vernonia amygdalina* ethanolic leaf extract reduced the enhanced level of serum ALT and thus seem to offer a protection and maintains the functional integrity of hepatic cells. This observation is consistent with earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice⁽¹⁸⁾.

Haematological parameters generally provide information on inflammation, necrosis, various infections of visceral organs and the presence of stress factors^(15, 16, 19). In the present study, DMN decreased white blood cells (WBCs), Platelets, Red blood cells (RBCs), packed cell volume (PCV), and Hemoglobin (Hb) in DMN alone treated group compared to control and extract pretreated groups (Table 2). However, pretreatment with 400mg/kg *Vernonia amygdalina* significantly enhanced the WBCs, platelets, PCV, RBCs and Hemoglobin (Table 2). This indicates that *Vernonia amygdalina* improves immunity function and decrease inflammation. WBCs or leukocytes play the main role in immune responses⁽²⁰⁾. These cells carry out the many tasks required to protect the body against disease-causing microbes and abnormal cells. Some

types of leukocytes patrol the circulation, seeking foreign invaders and diseased, damaged, or dead cells. These WBCs provide a general or non-specific level of immune protection^(21 - 22). The decreased value of leukocytes depicted by DMN alone group (Table 2) shows an infectious process that has overwhelmed the immune system. The decrease in WBCs may be due to impairment in the rate of entrance of the hematological parameter into the blood from the bone marrow and an enhanced rate of removal from circulation. However, the group pretreated with *Vernonia amygdalina* before DMN intoxication significantly increased WBCs, thus the immune system is boosted when compared to the DMN alone group. Reduction in platelets count in experimental animals has been reported to indicate adverse effect on the oxygen-carrying capacity of the blood as well as thrombopoietin^(23 - 24). Platelets play a very important role in the coagulation of blood to help stop bleeding. Greatly increased levels of platelets (>450) pose a great risk of forming blood clots associated with heart attacks and strokes. Severe decrease in platelet levels pose a danger of hemorrhaging if the body tissue is cut. There was a severe decreased level of platelets in DMN alone group (Table 2) compared to other groups. One of the reasons for such decrease of thrombocyte count could be either decreased hematopoietic activity after DMN exposure⁽²⁵⁾ or serious damage to bone marrow, i.e. its parent cells -mononuclealthromboplasts⁽²⁶⁾ or due to bleeding. PCV also known as hematocrit when increased beyond normal indicates a hyperactive spleen, infection, dehydration or lung pathologies while a decreased hematocrit indicates inadequate RBC production or increased loss or breakdown of cells. A statistically significant fall in values of the erythrocyte count and haematocrit was recorded in

DMN alone group (Table 2) compared to extracts pretreated groups. A possible reason for the decrease of erythrocyte count and haematocrit values in DMN alone group is either bleeding⁽²⁷⁾ or significant reduction in or complete cessation of erythrocyte production⁽²⁸⁾, or a combination of these two causes. Hemoglobin, a substance formed with iron, carries oxygen through the blood. An increased level may be due to an overactive spleen or dehydration while a decreased level is due to iron-deficiency anemia. Thus the decreased hemoglobin in DMN alone group could be due to iron-deficiency anemia. This decrease in the hematological parameters may be due to many factors such as inhibition of protein synthesis as evidenced by lower serum albumin^[29], decrease of the total iron binding capacity^[30].

Protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify toxicants, which enter into the animal body^[31]. The liver not only synthesizes the proteins for its needs but produces numerous export proteins. Among these proteins, serum albumin is the most important. Serum albumin level is an important predictive marker in advanced liver diseases. Liver diseases often results in the alteration of both structure and function of albumin: hypoalbuminemia due to reduced synthesis, oxidative modification in its structure and binding of bilirubin to its active sites etc. In addition to the above roles, another significant functional property of albumins is its catalytic activity toward a broad range of molecules like esters, amides, and phosphates etc^[32 - 33]. In this research, DMN induced liver damage in rats which is indicated by the decrease in levels of serum albumin, globulin, and total protein. However, pretreatment of rats with *Vernonia amygdalina* ethanolic leaf extract effectively protected the rats against DMN-induced

hepatotoxicity as evidenced by the increase in contents of globulin, total protein and albumin. The decrease in serum total protein observed in DMN alone treated group (Table 4.1) may be associated with the decrease in the number of hepatocytes which in turn may have led to the decreased hepatic capacity to synthesize protein⁽³⁴⁾ but the restoration of the level of serum total protein, globulin and albumin upon pretreatment of *Vernonia amygdalina* ethanolic leaf extract is a reflection of the hepatoprotective nature of this plant. The decrease in serum total protein in DMN alone group agrees with George⁽³⁵⁾, that decrease in serum protein in hepatotoxicity states simply indicates the presence of para proteins or decreased antibody production. The total protein, and albumin level may decrease due to liver dysfunction, malnutrition and malabsorption, diarrhea, nephrosis, alpha-1-antitripsin deficiency, acute hemolytic anemia, hypogammaglobulinemia/agammaglobulinemia; severe and loss through the urine in severe kidney disease and pregnancy. Prolonged destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes decrease in the serum levels of total protein and albumin.

Lipids are the most important cellular entities which are not only the constituents of cell membrane but also involved in many cellular functions, metabolic processes and are vital for energy production. Liver is the organ involved in the synthesis of lipoproteins and metabolism of cholesterol. The results of this study have established that, the 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 was resulted from damage of hepatic parenchymal cells that lead to disturbance of lipid metabolism in liver⁽³⁶⁾. 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⁽³⁷⁾. However, rats pretreated with *Vernonia amygdalina* ethanolic 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 acyltransferase *in vitro*. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver⁽³⁸⁾.

The observed protective effect of the plant extract against dimethylnitrosamine may be attributed to the phytochemical, antioxidant and free-radicalical properties of *Vernonia amygdalina*^(39 - 41). The efficacy of any hepatoprotective drug is essentially dependent on its ability in reducing the harmful effects or maintaining the normal hepatic physiology that has been distributed by a hepatotoxin. In this study, we observed that pretreatment of 400mg/kg b.w of ethanolic leaf extract of *Vernonia amygdalina*

to rats decreased the DMN induced elevated ALT and cholesterol as well as increased DMN-induced depressed total protein, globulin and albumin level in the treated groups. This suggests the maintenance of structural integrity of the hepatocytic cell membrane or regeneration of damage liver cells by the extract.

Conclusion

Pretreatment with 400mg/kg *Vernonia amygdalina* before induction of hepatic damage with dimethylnitrosamine (DMN) restored total protein, albumin and globulin levels, minimizes derangement in alanine aminotransferase (ALT), a liver function enzyme as well as total Cholesterol, maintained the hematological parameters studied, thus conferring hepatoprotection and anti-inflammatory effect of *Vernonia amygdalina*. In the future, examination of the protective effect of *Vernonia amygdalina* ethanolic leaf extract against DMN in dose dependant manner could be investigated.

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Conflicts of interest

The author declares no conflict of interests.

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