



Dimethylnitrosamine (DMN) exposed rats: *Vernonia amygdalina* pre-treatment enhances immunity, hepatic and renal function

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Abstract

Background: The occurrence of dietary and environmental chemicals such as dimethylnitrosamine (DMN) in drinks and foods including fish and meat as well as fresh supermarket products is well established. This study evaluated protective effect of ethanolic leaf extract of *Vernonia amygdalina* (VAE) on liver synthetic molecules, kidney function and hematological parameters in acute dimethylnitrosamine (DMN)-induced hepatic toxicity in wistar male rats.

Methods: Experimental rats divided into four groups of six rats each were used. The first group was untreated and served as control. The second group was orally administered VAE (400 mg/kg) only for seven days. The third group was pre-treated with VAE (400mg/kg) for 7 days and administered 20mg/kg DMN 24hrs after VAE pre-treatment. Rats of the fourth group were given 20mg/kg DMN alone same time with that of group 3. All rats were sacrificed 48hrs after DMN administration.

Results: In rats administered 20mg/kg DMN, VAE pre-treatment at 400 mg/kg significantly increased total protein, albumin, White blood cell (WBC), Red blood cell (RBC), Hemoglobin (Hb), packed cell volume (PCV) and Platelets while it significantly decreased total bilirubin, urea and creatinine compared to DMN-alone administered rats.

Conclusion: This study suggest that VAE pre-treatment exert its ameliorative effect against DMN-induced hematological and biochemical alterations possibly by preventing the decline of antioxidant defense system and could be prescribed as adjunct to dietary therapy.

Keywords: Dimethylnitrosamine; Hematology; Kidney; Liver; *Vernonia amygdalina*.

1. Introduction

Liver disease and toxicity is common especially with many drug treatments. DMN is a potent hepatotoxin, carcinogen and mutagen (George et al. 2001) which exerts carcinogenic effects and induces hepatic necrosis through metabolic activation by CYP2E1 (Guengerich et al. 1991). Activation of DMN by CYP2E1 in mouse liver has been shown to stimulate Kupffer cells leading to generation of superoxide and other reactive oxygen species (ROS) capable of damaging liver cells (Teufelhofer et al. 2005).

Vernonia amygdalina, popularly called bitter leaf and belonging to the Compositae family, is one of the plants with acclaimed folk medicinal usage and is a widely used local plant in Nigeria for both therapeutic and nutritional purposes. *Vernonia amygdalina* is rich in phytochemicals and antioxidants such as flavonoids, vitamin C, saponins, tannins, alkaloids and steroids as well as minerals including sodium, potassium, calcium, iron, magnesium etc (Usunobun & Okolie 2015). Other than the common metabolites and minerals, *Vernonia amygdalina* also contain several active secondary metabolites including vernodalol, vernodalol, vernolide, luteolin, luteolin 7-O- β -glucuronide, luteolin 7-O- β -glucoside, vernonioside D and E and vernolepin (Igile et al. 1994, Jisaka et al. 1992, Erasto et al. 2006). *Vernonia amygdalina* have been proved in human medicine to possess potent anti-malarial and anti-helminthic properties (Abosi & Raseroka 2003) as well as anti-tumorigenic properties (Izevbigie et al. 2004). This study is aimed at determining the effect of ethanolic leaf extract of

Vernonia amygdalina on liver synthetic molecules, kidney function and hematological parameters in DMN exposed wistar rats.

2. Materials and methods

2.1. Collection, identification, preparation and extraction of plant leaves

Fresh leaves of *Vernonia amygdalina* were purchased from a local market in Benin City, Edo state, Nigeria. The leaves were identified by a Botanist 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, washed and air-dried at room temperature (24°C) and then pulverized, crushed into fine powder and weighed.

Ethanolic extracts of the plant leaves was prepared by soaking 400g of the dry powdered plant leaves in one (1) litre of absolute ethanol at room temperature for 48hrs. The extract was then filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extract was thereafter concentrated using a rotary evaporator with the water bath set at 40°C to one-tenth its original volume and then finally freeze dried. The dried residue (crude extract) was then stored at 4°C and used on each day of our experiments.

2.2. Experimental animals, DMN and extract administration

Male wistar albino rats divided into four groups of six (6) rats each, weighing between 160-195g were obtained from the Animal Unit facility of the University of Ibadan, Oyo state, Nigeria and housed in wooden cages in the animal house of the Department of Biochemistry, University of Benin. The rats were maintained under controlled environmental conditions (temperature—24°C; relative humidity—50–70%; 12 h light/dark cycle), housed for one week after their arrival to the animal house for acclimatization. The rats had free access to drinking water and normal pellet diet (NPD) ad libitum until they were assigned to individual groups. Institutional Animal Ethical Committee permission was obtained before performing the experiments.

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 (1971).

A total of 24 rats divided into 4 groups were used. Group 1 served as control and was given normal saline, Group 2 received 400mg/kg *Vernonia amygdalina* only for seven (7) days consecutively, Group 3 received 400mg/kg *Vernonia amygdalina* for 7 days consecutively followed by oral administration of a single dose of 20mg/kg DMN (dissolved in 0.15MNaCl), on day 8 (48 hours before sacrifice) while Group 4 received oral administration of single dose of 20mg/kg DMN (dissolved in 0.15MNaCl) on day 8. Before use, the *Vernonia amygdalina* leaf extract was reconstituted in distilled water and administered orally. All rats were sacrificed on the tenth day of the study by cardiac puncture and blood collected via the ocular vein in either EDTA bottles for hematology or in plain tubes. Blood collected in plain bottles were allowed to stand for 45 min before it was centrifuged at 4,000 rpm for 30 min. Serum was stored at -20°C until analyzed.

2.3. Biochemical assays

Serum total bilirubin, total protein, albumin and urea were determined using the RANDOX Kit according to the manufacturer's instructions while serum creatinine was determined using the Jaffe' method. Hematology was carried using full automated blood cell counter PCE -210N (ERMA INC., USA).

2.4. Statistical analysis

Data obtained from the study were expressed as mean value \pm standard deviation. Differences between means of control and tested groups were determined using Statistical Package for social scientist (SPSS). A probability level of less than 5% ($P < 0.05$) was considered significant.

3. Results

The detailed results of the body and tissue weights are presented in Table 1. There was a reduction in body weight gain of DMN administered rats when compared to extract and control rats. However, DMN administered rats pretreated with ethanolic leaf extract of 400mg/kg *Vernonia amygdalina* caused an increase in the body weight gain compared to DMN alone administered rats.

The effect of ethanolic leaf extracts of *Vernonia amygdalina* on acute DMN-induced liver and kidney injury were evaluated by determining the levels of total protein, albumin, total bilirubin, urea and creatinine as shown in Table 2. *Vernonia amygdalina* lowered the levels of urea, creatinine and total bilirubin and significantly increased ($p < 0.05$) total protein and albumin when compared to acute DMN alone group.

Table 1: Effect of *Vernonia amygdalina* on Body and Organ Weight in Acute DMN-Induced Toxicity in Rats.

Treatment	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Relative liver weight (%)	Relative kidney Weight (%)
Control (normal saline)	166.20 \pm 1.47	188.00 \pm 4.55	21.80 \pm 3.08	3.09 \pm 0.07 ^a	0.70 \pm 0.02 ^a
VAE alone (400mg/kg)	165.17 \pm 2.34	194.00 \pm 6.44	28.83 \pm 4.10	3.04 \pm 0.11 ^a	0.69 \pm 0.02 ^a
VAE (400mg/kg) + DMN (20mg/kg)	175.20 \pm 3.41	193.50 \pm 6.01	19.10 \pm 3.00	3.39 \pm 0.08 ^b	0.71 \pm 0.02 ^a
DMN alone (20mg/kg)	192.29 \pm 2.76	199.17 \pm 6.86	6.88 \pm 4.10	3.41 \pm 0.10 ^b	0.73 \pm 0.11 ^a

Values are expressed as Mean \pm SD, (n=6), VAE = *Vernonia amygdalina*, DMN=Dimethylnitrosamine
Mean values in each column having different superscript (a, b, c) are significantly different ($p < 0.05$).

Table 2: Effect of *Vernonia amygdalina* on Serum Liver Synthetic Ability and Kidney Function in Acute DMN-Induced Toxicity in Rats

Treatment groups	TP (g/dl)	Albumin (g/dl)	TB (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Control (normal saline)	7.50 \pm 0.28 ^a	4.53 \pm 0.05 ^a	0.69 \pm 0.11 ^a	0.70 \pm 0.08 ^a	30.00 \pm 6.56 ^a
VAE alone (400 mg/kg)	7.83 \pm 0.53 ^a	4.27 \pm 0.26 ^a	0.71 \pm 0.17 ^a	0.60 \pm 0.03 ^a	32.33 \pm 7.23 ^a
VAE (400mg/kg) + DMN (20mg/kg)	5.27 \pm 0.91 ^b	2.40 \pm 0.24 ^{cd}	1.51 \pm 0.09 ^b	1.10 \pm 0.08 ^b	72.26 \pm 6.43 ^b
DMN alone (20mg/kg)	3.97 \pm 0.60 ^c	1.73 \pm 0.87 ^c	2.94 \pm 0.03 ^c	1.27 \pm 0.05 ^c	92.00 \pm 4.56 ^c

Values are means \pm SD; n=5, VAE = *Vernonia amygdalina* ethanolic extract, DMN = Dimethylnitrosamine, TP = Total Protein, TB = Total bilirubin
Mean values in each column having different superscript (a, b, c) are significantly different ($p < 0.05$).

Results in Table 3 given below shows that rats administered DMN significantly had decreased WBC, RBC, Hb, PCV and Plt Count whereas *Vernonia amygdalina* leaf extract pre-treatment significantly attenuated the hematological parameters in DMN administered rats towards the control values.

Table 3: Effect of *Vernonia amygdalina* on Haematological Parameters in Acute DMN-Induced Toxicity in Rats.

Treatment groups	WBC (x 10 ³ / μ l)	RBC (x 10 ⁶ / μ l)	Hb (g/dl)	PCV (%)	Plt Count (x 10 ³ / μ l)
Control (normal saline)	5.91 \pm 0.13 ^a	6.10 \pm 0.46 ^a	14.22 \pm 0.53 ^a	52.80 \pm 1.05 ^a	499 \pm 15.62 ^a
VAE alone (400mg/kg)	7.75 \pm 1.55 ^a	6.26 \pm 0.66 ^a	14.13 \pm 0.68 ^a	50.03 \pm 1.60 ^a	536 \pm 12.98 ^a
VAE (400mg/kg) + DMN (20mg/kg)	3.87 \pm 0.87 ^b	4.96 \pm 0.77 ^b	10.03 \pm 0.68 ^b	32.33 \pm 0.25 ^b	91.01 \pm 2.16 ^b
DMN alone (20mg/kg)	2.21 \pm 0.11 ^d	3.17 \pm 0.29 ^d	7.09 \pm 0.94 ^d	21.50 \pm 2.12 ^d	35.99 \pm 5.17 ^d

Values are means \pm SD; n=3, VAE = *Vernonia amygdalina* ethanolic extract, DMN = Dimethylnitrosamine, WBC = White blood cells, RBC = Red blood cells, Hb = Hemoglobin, PCV = Packed cell volume, Plt count = Platelet count.

Mean values in each column having different superscript (a, b, c, d) are significantly different ($P < 0.05$).

4. Discussion

The liver not only synthesizes the proteins for its needs but produces numerous export proteins among which is serum albumin which plays important biological roles including maintenance of plasma oncotic pressure (Peter 1996). In this study, DMN induced liver damage in rats as indicated by the decrease in levels of serum albumin and total protein similar to our previous study (Usunobun 2014). However, *Vernonia amygdalina* pre-treatment significantly protected the rats against DMN-induced hepatotoxicity as evidenced by the significant increase in serum total protein and albumin compared to DMN-alone treated rats. In an earlier study by Muthulingam (2002), oral administration of chloroform and ethyl acetate fractions of *Astercanthalongifolia* and also silymarin to CCl₄ treated rats showed significantly increased serum protein level. Also in a report by Ekam et al (2012), there was a significant increase ($P < 0.05$) in total protein in all group treated with the various fractions of *Vernonia amygdalina* compared to the paracetamol alone group.

Bilirubin is a well-known metabolic breakdown product of blood heme with great biological and diagnostic values (Chowdhury et al. 1989). The increase in total bilirubin in this study corroborate our previous study where we reported increased liver function enzymes in rats administered single dose of 20mg/kg or 12mg/kg DMN (Usunobun et al. 2015, Usunobun, 2014). Thus the increase in total bilirubin level in the serum of DMN-alone induced rats indicates damage to hepatic cells. On the other hand, DMN-administered rats orally pre-treated with ethanolic leaf extract of 400mg/kg *Vernonia amygdalina* caused significant decrease in level of total bilirubin compared to DMN-alone treated rats. The leaf extract decreased ($P < 0.05$) DMN-induced elevated total bilirubin levels, indicating protection of liver structural integrity. It is possible that *Vernonia amygdalina* induced UDP-glucuronosyltransferase that catalyzed the conjugation of bilirubin thereby increasing its hydrophilicity and subsequently excretion. The protective effect of *Vernonia amygdalina* may be due to presence of active compounds such as flavonoids as well as minerals and other antioxidants (Usunobun & Okolie 2015).

In this study, there was also a marked decrease in body weight gain in rats administered DMN alone compared to controls. However, *Vernonia amygdalina* pre-treatment prior to DMN administration caused an increase in body weight gain compared to DMN-alone administered rats. There was also a significant increase in relative liver weight in rats administered DMN alone compared to control rats. The results was similar to that of Ismail et al (2009) who reported that relative liver weight for CCl₄ group showed significant increase as compared to the control groups. Also, the present results were similar with those of Hashimoto et al (1999), who showed that treatment with CCl₄ and phenobarbital causes liver deformity and high percentage of liver weight in the group injected with CCl₄ and fed on basal diet. However, *Vernonia amygdalina* pre-treatment prior to DMN administration led to a decrease of relative liver weight compared to rats administered DMN alone.

WBCs carry out the many tasks required to protect the body against disease-causing microbes and abnormal cells. Platelets play a very important role in the coagulation of blood to help stop bleeding. Hemoglobin, a substance formed with iron, carries oxygen through the blood. In this study, DMN administration decreased WBCs, Platelets, RBCs, PCV, and Hb in DMN treated group compared to control groups similar to our previous study (Usunobun, 2014). However, *Vernonia amygdalina* pre-treatment prior to DMN administration enhanced the hematological indices, thus indicating that *Vernonia amygdalina* improve immunity function and decrease inflammation.

Renal function indices such as serum urea, creatinine and uric acid can be used to evaluate the functional capacity of the nephrons of animals (Yakubu et al. 2003). The increase in serum creatinine and urea contents of rats in DMN administered group observed in this study may be attributed to a compromised renal functional capacity. DMN intoxication might have interfered with creatinine and

urea metabolism leading to increased synthesis or it might have compromised all or part of kidney functional capacity in tubular and glomerular excretion (Zilva et al. 1991). However, pre-treatment with extracts of *Vernonia amygdalina* prior to DMN administration significantly decreased serum urea and creatinine levels, thereby enhancing kidney protection.

In conclusion the protective effect observed by *Vernonia amygdalina* against acute DMN toxicity may be due to bioactive compounds such as flavonoids, saponins and tannins as well as the plant ability to maintain endogenous antioxidants.

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