International Journal of Pharmacology and Toxicology, 4 (1) (2016) 74-77

International Journal of Pharmacology and Toxicology

Website: www.sciencepubco.com/index.php/IJPT
doi: 10.14419/ijpt.v4i1.6065
Research paper

Dimethylnitrosamine (DMN) pre-treated rats and protective effect of *Vernonia amygdalina* post-treatment on liver function

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Abstract

Background: Liver disease and toxicity is common, especially with many drug treatments. The effect of *Vernonia amygdalina* ethanolic leaf extract post-treatment on rats pre-treated with dimethylnitrosamine (DMN) was evaluated.
Methods: Male wistar rats were orally administered DMN (single dose of 25 mg/kg) on first day and thereafter post-treated with 100 and 200mg/kg ethanolic leaf extract of *Vernonia amygdalina* for seven days. Analysis of serum concentrations of albumin, total protein, total bilirubin and glucose were carried out.
Results: Administration of DMN alone to rats significantly increased glucose and total bilirubin concentration ($P < 0.05$) in the serum while it significantly reduced ($P<0.05$) serum total protein and albumin concentrations when compared with controls. However, post-treatment of DMN administered rats with 100 and 200 mg/kg ethanolic extract leaf of *Vernonia amygdalina* significantly ($P<0.05$) reversed these changes in a concentration dependent manner.
Conclusion: In conclusion ethanolic leaf extract of *Vernonia amygdalina* have a potent hypoglycaemic and hepatoprotective action against dimethylnitrosamine-induced liver damage in rats may be due to its antioxidant properties..

Keywords: *Dimethylnitrosamine; Pre-treatment; Post-treatment; Vernonia amygdalina.*

1. Introduction

Dimethylnitrosamine (DMN) is a potent hepatotoxin, mutagen and carcinogen (Usunobun et al. 2015), whose exposure may happen in large number of places including industries such as tanneries, pesticide manufacturing plant, rubber and tire manufacturing plants, alkylamine manufacture/use industries, fish processing industries, foundries, and dye manufacturing plants (Mitch et al. 2003).
Vernonia amygdalina, commonly known as bitter leaf, is reputed to have several health benefit against amoebic dysentery (Moundipa et al. 2005), gastrointestinal disorders (Akah & Ekekwe 1995), antimicrobial and antiparasitic activities (Akinpelu 1999; Hladik et al. 2005). *Vernonia amygdalina* have been reported to contain saponins, alkaloids, tannins, steroids, terpenes, coumanns, flavonoids, phenolic acid, lignans, and xanthones (Muraina et al.2010, Usunobun & Okolie 2015), edotides (Izevbigie 2003) and sequiterpenes (Kupchan et al. 1969). This study was undertaken to determine the effect of ethanolic leaf of *Vernonia amygdalina* post-treatment on liver synthetic molecules and glucose in rats pre-treated with an acute single dose of DMN.

2. Materials and methods

2.1. Collection, identification, preparation and extraction of *Vernonia amygdalina* leaves

Fresh mature leaves of *Vernonia amygdalina* were obtained from a garden in Ikpoba Hill, Benin City, Edo state. The leaves were identified, sorted, washed and air dried at room temperature and milled into powder and weighed (1500g). Three (3) litres of absolute ethanol was added to the powdered sample and allowed to stand for 48hours. It was filtered using filter paper and re-filtered using cotton wool to ensure purity. The ethanol extract was reduced to one-tenth by rotary evaporator at 40ºC. The extract was further evaporated to dryness at 37ºC in water bath. The extract was stored in a well-sealed container and kept in a refrigerator at 4^{o}c to protect it from light and moisture till it was used.

2.2. Experimental animals and administration

Adult male albino rats weighing 140-200g 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 9.00am-10.00am
A total of thirty male rats were assigned into one of the following groups: Group 1 (control) received distilled water, Group 2 received 100mg/kg *Vernonia amygdalina* only for seven days. Group 3 received a single dose of DMN only (25mg/kg) on the first day. Group 4 received a single dose of DMN (25mg/kg) on

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day 1 and also *Vernonia amygdalina* (100mg/kg) for seven days. Group 5 received a single dose of DMN (25mg/kg) on day 1 and also *Vernonia amygdalina* (200mg/kg) for seven days. 24hrs after last administration, rats from each group were sacrificed by cervical dislocation. Blood samples were obtained through heart puncture via a syringe into a plain sterile bottles (for serum) and were allowed to stand for one hour to clot and thereafter centrifuged at 3000rpm for 45 minutes at room temperature to obtain serum for biochemical analysis.

2.3. Biochemical parameters

Serum albumin, total protein, total bilirubin and glucosewere determined by using the RANDOX Kit according to themanufacturer's instructions

2.4. 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

3. Results

As it is shown from figure 1- 4, the level of total protein and albumin were significantly decreased in rats treated with DMN alone compared to control and extract treated rats. Furthermore, the total bilirubin and glucose of DMN-pre-treated rats were statistically increased in comparison to control and other treated groups.

However in rats post-treated with 100mg/kg and 200mg/kg *Vernonia amygdalina* ethanolic leaf extract after DMN administration, the levels of total protein and albumin were significantly increased in concentration dependent manner compared to DMN alone administered rats. Also in rats post-treated with 100mg/kg and 200mg/kg *Vernonia amygdalina*, after DMN administration, the total bilirubin and glucose were statistically decreased compared to DMN alone rats.

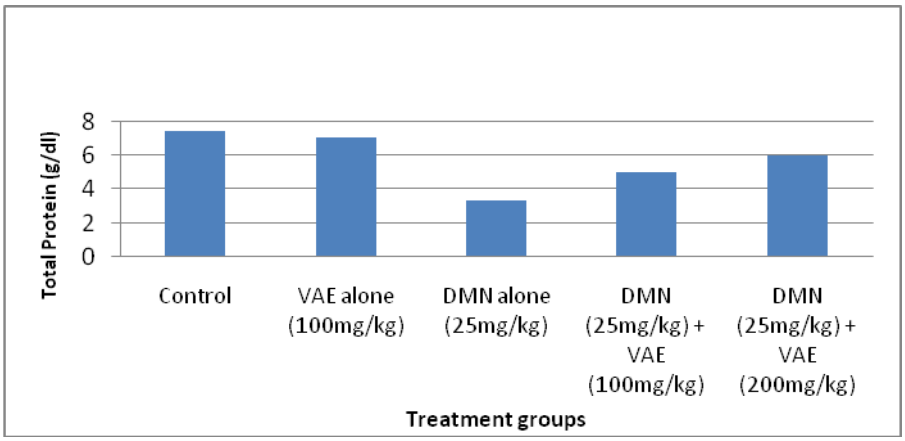


Fig. 1: Effect of *Vernonia amygdalina* Post-treatment on Total Protein in DMN-Pre-treated Rats.

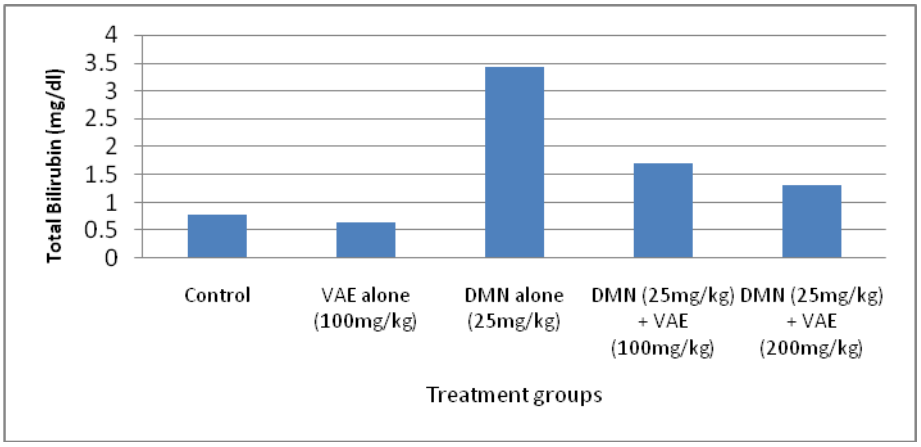


Fig. 2: Effect of *Vernonia amygdalina* Post-treatment on Total Bilirubin in DMN-Pre-treated Rats.

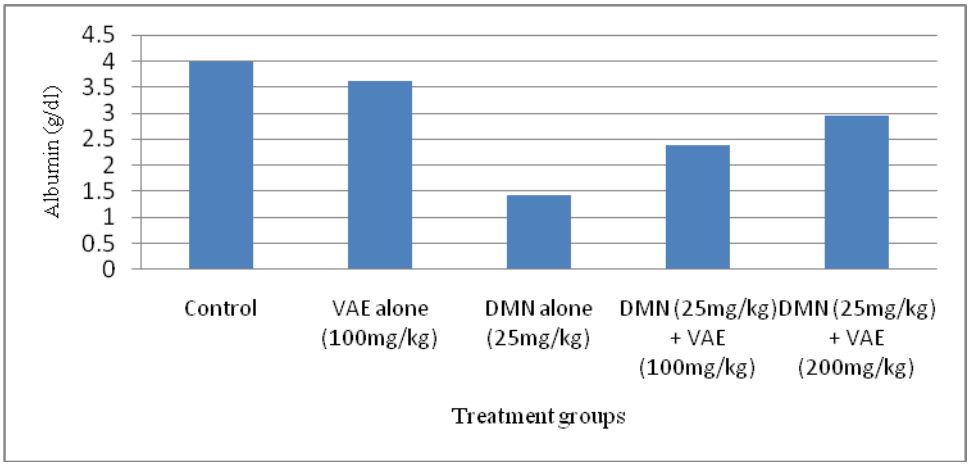


Fig. 3: Effect of *Vernonia amygdalina* Post-treatment on Albumin in DMN-Pre-treated Rats.

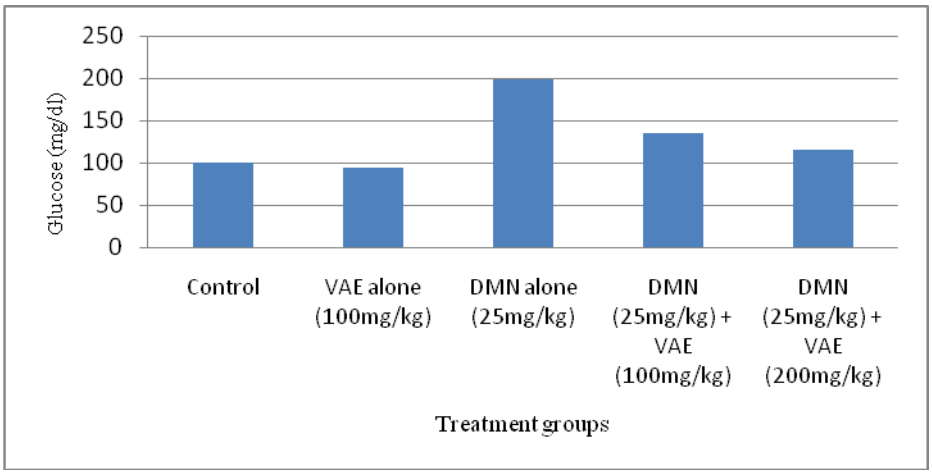


Fig. 4: Effect of *Vernonia amygdalina* Post-treatment on Serum Glucose in DMN-Pre-treated Rats.

4. Discussion

Medicinal plants have been used as traditional treatment for numerous human diseases for thousands of years in many parts of the world. In rural areas of developing countries, herbal materials continue to be used as the primary source of medicines (Chitme et al. 2003).The present study revealed that administration of 25 mg/kg DMN induced hepatotoxicity manifested biochemically by a significant increase of serum glucose and bilirubin concentration in addition to a significant decrease in serum total protein and albumin. The liver is the key site of metabolism of xenobiotics and also plays a role in synthesis of albumin. Liver malfunction will impair its synthetic role and could have led to decreased levels of albumin in serum as observed in DMN administered rats in this study. The administration of DMN alone may have adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins such as albumin in the liver. The decrease in serum protein may be due to changes in protein and free aminoacids and their synthesis in the injured liver cells and/ or increased protein degradation. However, post-treatment with 100mg/kg and 200mg/kg *Vernonia amygdalina* significantly ($p<0.05$) reversed these changes, maybe by increasing protein synthesis. This indicates the hepatoprotective activity of *Vernonia amygdalina* leaves against damage by DMN. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates regeneration of cells (Awang 1993).

Bilirubin is the main bile pigment that is formed from the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile (Nelson & Cox 2000). Malfunctioning of the liver was evidenced by the significant increase ($p<0.05$) in the level of total bilirubin in the serum of the group treated with DMN when compared with control and extract treated groups (Figure 2). Increase in the level of total bilirubin in the blood may result from a defect in the function of the liver to conjugate the bilirubin being produced (Marks & Dennis 1996). The increase in serum total bilirubin may also be due bile ductules blockage as a result of inflammation as well as conjugated bilirubin regurgitation from the necrotic hepatocytes to sinusoids. The concentration dependent significant reduction ($p<0.05$) of total bilirubin level upon post-treatment with 100mg/kg and 200mg/kg *Vernonia amygdalina* indicates that the conjugating function of the liver was improved. The reduction of the total bilirubin level by the extracts suggests that *Vernonia amygdalina* may activate constitutive androstane receptor (CAR) which is a key regulator in bilirubin clearance in the liver. The primary function of CAR in the bilirubin clearance pathway is to direct a coordinate response to elevated levels of bilirubin by increasing the hepatic expression of each component of the pathway (Moore et al. 2004). Thus the ability of *Vernonia amygdalina* post-treatmentto significantly reduce ($p<0.05$) the level of total bilirubin when compared with DMN - treated group suggests the potential of the *Vernonia amygdalina* in clearing bilirubin from the serum when its level is elevated.

Also in this study, administering DMN to rats resulted in a statistically significant increase of serum glucose levels. The progressive accumulation of glucose revealed that rats exposed to DMN may become hyperglycemic. Hyperglycemia is usually associated with insulin resistance during gastrointestinal cancers and in the present study, it is possible that the observed increase in glucose levels could be due to the toxicity caused to the pancreatic islet cells and due to the alteration in carbohydrate metabolism by the administration of DMN. The hyperglycemia observed in the present results might be due to reduced insulin availability. Furthermore, with regards to the fact that DMN has increased serum glucose level, it could be suggested that DMN prevented the influx of

glucose into the cells of peripheral tissues. Increased blood glucose concentration results from an imbalance between the hepatic output of glucose and the peripheral uptake of the sugar (Coles 1980). However, the significant reduction in glucose levels observed in groups receiving 100m/kg and 200mg/kg *Vernonia amygdalina* maybe due to the activity of its bioactive principles such as flavonoids, saponins and alkaloids (Usunobun & Okolie 2015; 2016).

The findings in this study point to the fact that *Vernonia amygdalina* is hypoglycaemic and hepatoprotective against DMN- induced liver damage. Hence it is possible that a probable mechanism of hypoglycaemic and hepatoprotection against DMN- induced damage is the antioxidant activity as our studies have shown the presence of alkaloids, saponins, tannins, flavonoids and polyphenols in *Vernonia amygdalina* leaves.

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