AMELIORATING EFFECT OF METHANOLIC LEAF EXTRACT OF AZADIRACHTA INDICA (NEEM) ON ARSENIC-INDUCED OXIDATIVE DAMAGE IN RAT LIVER

Oluwole J. OYEWOLE
Department of Biochemistry, Osun State University, Osogbo, Nigeria.
E-mail: ioluoye@yahoo.com. Tel.: +2348066808560.

Received 21 July 2011; accepted 14 August 2011

Abstract
This study investigated the ameliorating effect of methanolic leaf extract of Azadirachta indica on arsenic-induced oxidative damage in rat liver. Twenty four rats were divided into four groups: group 1 (control) received distilled water while 250mg/kg bw of arsenic chloride (AsCl₃) was administered intraperitoneally to test groups (2, 3 and 4) to induce hepatic damage. Group 3 and 4 were treated with oral administration of 100mg/kg bw and 200mg/kg bw of methanolic leaf extract of Azadirachta indica respectively for 14 days. Antioxidant capacity was markedly depleted in rats liver administered with arsenic (group 2) as the drug caused significant decrease in hepatic Vitamin C, superoxide dismutase (SOD), catalase and glutathione-s-transferase (GST) activities. The drug also caused significant increase in serum aspartate amino transferase (AST) and alanine aminotransferase (ALT) which is indicative of liver dysfunction. Co-administration of Azadirachta indica leaf extract with arsenic to rats (group 3 and 4) effectively reduced the extent of liver damage as levels of serum enzymes and hepatic antioxidants were modulated close to normal (group 1). It can be concluded from this study that Azadirachta indica leaf has hepatoprotective properties and might be useful for treating liver diseases.

Key words: Azadirachta indica, arsenic, antioxidants, oxidative damage, liver dysfunction.

INTRODUCTION
Arsenic and many of its compounds are potent poison to the living system as they disrupt ATP production through several mechanisms [1]. Arsenic which is found in air, water, fuels and marine life inhibits pyruvate dehydrogenase and succinate dehydrogenase in the citric acid cycle [2]. It also competes with phosphate and uncouples oxidative phosphorylation, thus inhibiting mitochondrial respiration and ATP synthesis. This impairment of cell respiration can lead to cell swelling, glycogen depletion and fatty change in the liver, kidney and heart [3].

Hydrogen peroxide production is also increased when arsenic in injected, which might form reactive oxygen species and oxidative stress. Daily human intake of arsenic contained in food ranges from 0.5-1mg, with the greatest concentrations coming from fish and crustaceans [4]. Epidemiological studies have suggested a correlation between chronic consumption of drinking water contaminated with arsenic and the incidence hepatic fibrosis and cirrhosis [5].

Azadirachta indica (Neem) is a tree in the mahogany family well known in Africa as one of the most versatile medicinal plants having a wide spectrum of biological activity [6]. Every part of the tree has been used as traditional medicine for household remedy against various human ailments. Neem is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products [7].

Extract of neem leaves and seed is thought to be helpful as malaria prophylaxis as they have been found to be effective against both chloroquin-resistant and sensitive strains of malarial parasite [8]. Neem oil and bark extracts
have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter [6]. This study intends to investigate the ameliorating effect of *Azadirachta indica* on arsenic chloride induced oxidative damage in rat liver.

**MATERIALS AND METHODS**

**Collection and treatment of Plant materials**

*Azadirachta indica* leaves were collected in Iwo town, Osun State, Nigeria and air-dried. It was then ground into powder with an electric blender (model MS-223). The powdered leaf (100g) was soaked in 600ml of 80% methanol with constant stirring by a magnetic stirrer for 48 hr. The mixture was filtered followed by removal of the solvent on the rotatory evaporator to give a dark-brown crude extract.

**Experimental animals**

Twenty four (24) Wistar strain albino rats (average weight 150g) were used for this study. They were obtained and raised in the Biochemistry Animal House of the Department of Chemical Sciences, Osun State University, Osogbo following approval by the Institution’s Ethical Committee. The rats were divided into four groups: group 1 (control) received distilled water while 250mg/kg bw of arsenic chloride (AsCl$_3$) was administered intraperitoneally to test groups (2, 3 and 4) to induce hepatic damage. Group 3 and 4 were treated with oral administration of 100mg/kg bw and 200mg/kg bw of methanolic leaf extract of *Azadirachta indica* respectively for 14 days.

Rats were kept under laboratory conditions (25±2°C and relative humidity of 50±15%) in cages cleaned of metabolic waste twice daily and were allowed to acclimatize for two weeks. They were exposed to 12 hr daylight and darkness, fed rat chow and water *ad libitum*.

**Preparation of Serum**

The rats were sacrificed by cervical dislocation and blood sample collected into clean, dry centrifuge tube. The blood was left for 10 min at room temperature to clot after which it was centrifuged at 3,000g in an MSC (Essex, UK) bench centrifuge. The clear supernatant (serum) was aspirated using a Pasteur pipette into clean, dry sample bottles and then frozen overnight for enzyme analyses.

**Preparation of liver homogenate**

The liver were quickly excised from the rat and immediately placed on a blotting paper to remove blood stains. It was then rinsed in 1.15%KCl to remove haemoglobin followed by homogenization in 4 volumes of ice-cold 0.01M potassium phosphate buffer, (pH 7.4) using the Teflon homogenizer. The homogenate was centrifuged at 12,500g for 20 minutes at 4°C to obtain supernatants (post-mitochondrial fractions) which was stored at 8°C till required for assay.

**Assay of hepatic antioxidants**

The level of superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich [9] based on the ability of the enzyme to inhibit auto-oxidation of epinephrine at pH 10.2 and 30°C. The 2, 4-dinitrophenyl -hydrazine method described by [10] was used for the determination of ascorbic acid concentration. Catalase activity was determined based on the method of Sinha [11] which measure the reduction of dichromate in acetic acid to chromic acetate at 570nm. The method of Habig et al. [12] was employed in determining Glutathione–s-transferase (GST) activity using 1,2-dichloro 4-nitrobenzene (CDNB) as substrate.

**Determination of serum Aminotransferases**

Serum AST and ALT activities were determined using Randox diagnostic kits based on the principle described by Reitman and Frankel [13].

**Statistical analysis**

All values were expressed as mean ±SD and subjected to statistical analysis using SPSS window version 9.0. Comparison was done using one-way analysis of variance (ANOVA). P values of <0.05 were considered statistically significant.

**RESULTS**

The concentration of hepatic antioxidants and serum enzymes in rats administered arsenic and methanolic leaf extract of *Azadirachta indica* is indicated in Table 1. Administration of arsenic to rats (Group 2) caused significant reduction in hepatic antioxidants as follows: Ascorbic acid (45.5%), Catalase (44.9%) and GST (37.4%). There was also a significant elevation of serum AST and ALT (51.4%) compared to the control group. The results also showed that the reduced levels of serum hepatic antioxidants and elevated serum aminotransferases (AST and ALT) were significantly normalized on administration of the methanolic leaf extract of *Azadirachta indica* (group 3 and 4). There was no significant difference in hepatic antioxidants and serum enzymes between rats administered with different concentrations of the extract (100mg/kg bw and 200mg/kg bw).

**DISCUSSION**

Data obtained in this study showed that administration of arsenic chloride to rats caused oxidative damage in rats liver as revealed by significant reduction in antioxidants level. Oxidative stress is caused by an imbalance between production of reactive oxygen species and biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage [14]. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all
components of the cell, including proteins, lipids, and DNA [15].

Arsenic may produce cellular damage through a variety of mechanisms. It caused increase in hydrogen peroxide production which forms reactive oxygen species and oxidative stress [16]. These metabolic interferences may lead to multi-system organ failure probably from necrotic cell death. Arsenic also binds to sulfhydryl groups of key metabolic enzymes and forms a stable ring, which deactivates these enzymes. The process of enzyme deactivation may cause widespread endothelial cellular damage [17].

Liver function in rats administered with arsenic was also disrupted as levels of AST and ALT was significantly elevated compared with the control. Determination of enzyme levels such as AST and ALT is largely used in the assessment of liver damage [18]. Necrosis or membrane damage releases the enzymes into circulation and hence it can be measured in the serum. High level of serum AST and ALT indicate liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury [19, 20]. Enzyme elevation in the serum has been attributed to membrane destruction which cause cytosolic enzymes to leak out of the membrane into the intracellular fluid [18]. Free radical generated during arsenic administration can modify membrane lipid through peroxidation which may eventually lead to membrane damage [21].

Methanolic leaf extract of Azadirachta indica was found to protect arsenic induced liver damage in rats in this study. The reduced levels of hepatic antioxidants indicative of oxidative stress and elevated levels of serum AST and ALT indicative of liver damage were found to be significantly normalized on administration of the neem leaf extract. The efficacy of any hepatoprotective drug depend on its capacity to either reduce the harmful effect or restoring the normal hepatic physiology caused by a hepatotoxin. The observed restoration of antioxidant capacity and regeneration of damaged liver cells by Azadirachta indica indicates its protective roles on the structural integrity of hepatocytic cell. The antioxidant activity of neem leaf extract has been demonstrated in vivo during horsegrain germination, which is associated with low levels of lipoxygenase activity and lipid peroxides. Singh et al. [8] reported that aqueous extract of neem leaf offer protection against paracetamol-induced liver necrosis in rats as the extract significantly reduced serum AST, ALT and gamma glutamyl transpeptidase

CONCLUSION

Results obtained in this study showed that Azadirachta indica posses hepatoprotective properties as it prevent loss of antioxidant and liver damage caused by arsenic administration. The usefulness of Azadirachta indica in the treatment of malaria and other diseases caused by free radical is hereby justified in this study.

REFERENCES


Source of support: Nil; Conflict of interest: None declared