Original Research Article

Ameliorative effect of ethanol extract of *Annona muricata* leaves in sodium arsenite induced- toxicity in male wister rats

Abstract

Ingestion of arsenic in drinking water causes cancer at multiple tissues and there is no cure. Research is therefore directed at chemoprevention using medicinal herbs for the management of arsenicosis. In this study hepatoprotective activity of ethanolic extract of *Annona muricata* (AM) leaves was assessed against sodium arsenite (SA) induced hepatic injury in albino rats. The animals were pre-treated with either 250 or 500mg/kg body weight of rat before exposure to SA. SA was dissolved in distilled water and administered at a dose of 5 mg/kg body weight on the 7^{th}, 14^{th} and 21^{st} day of the experiment. SA was observed to induce a significant increase ($p < 0.05$) in serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase activities (ALP). However Pretreatments of rats with various doses of AM significantly ($P<0.005$) reduced serum enzyme levels to near normal against SA treated rats. Furthermore, histopathological observations revealed that treatment with AM extract protected the animals from SA induced liver damage. The results indicated that the leaves of *Annona muricata* possess hepatoprotective activity on SA induced hepatic injury in rats.

Keywords: Hepatoprotective, *Annona muricata*, sodium arsenite, transaminase, Oxidative stress

Introduction

Continuous exposure of humans to arsenic through long term ingestion of contaminated water and its attendant health problem has been reported (Waalker et al, 2004). Epidemiological studies conducted in Taiwa (Chiou et al, 1995), Chile (Smith et al, 1998) and Japan (Tsuda et al, 1995) indicated a connection between arsenic exposures from contaminated drinking water among the inhabitants. Arsenic is a well-known human carcinogen, which potentially affects ~160 million people worldwide via exposure to unsafe levels in drinking water (IARC, 2004). It is an element present in food, soil, water and air, and it is released into the environment from both natural and man-made sources (Chakraborti et al., 2004;). Humans may be exposed to arsenic via ingestion through drinking water (major), inhalation and skin absorption (Gupta *et al*., 2005). Arsenic in drinking water is typically inorganic, and can be present either as As^{+3} (arsenite) or As^{+5} (arsenate). However the ingestion of inorganic arsenic is a significant public health hazard in the world. Arsenic toxicity has been reported to be associated with a variety of cancers, dermatitis, cardiovascular diseases, peripheral neuropathy, diabetes mellitus, renal failure and liver dysfunction. Also arsenic toxicity also induces generation of reactive oxygen species (ROS), which may lead to membrane damage, oxidative stress and carcinogenesis of various organs and subsequent cellular damage and organ disorders (Tanju and Madhuri, 2013). The liver is the primary target organ for the metabolism of arsenicals. The major metabolic pathway of inorganic arsenic in humans is its methylation in the liver. The methylation of arsenic has been demonstrated by the presence of monomethylarsonic acid (MMA) and dimethylarsinic

acid (DMA) in the urine and bile (Li et al., 2008; Cui et al 2008). Liver function test is among the most commonly used and primary clinical investigation for the assessment of liver function. Arsenic intoxication in experimental animals has been linked with micronucleus formation and hepatic tumors (Moore and Smith, 1997; Mazumder, 2005). In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available.

Annona muricata L. (soursop), a member of the Annonaceae family, is a widely distributed plant in Central and South America and tropical countries like Nigeria where it is use in the treatment of asthma, cough, fever, headache, hypertension, and toothache. Previous studies have shown that A. muricata is active against several cancer cell lines (Moghadamtousi et al, 2014). The anti cancer activity of A. muricata has been attributed to antioxidant and apoptosis inducing potential in cells (Moghadamtousi et al, 2014). However, there are no scientific evidences regarding the hepatoprotective activity of this plant against sodium arsenite.

Therefore, the aim of this study was to study the mitigating effect of *Annona muricata* leaves against arsenic-induced liver oxidative damage in rats.

Materials and Methods

Reagents and kits

Sodium arsenite ($NaAsO_2$; BDH chemicals Ltd poole England) was dissolved in distilled water and administered at a dose of 2.5 mg/kg body weight corresponding to 1/10th of the oral LD50 of the salt (Preston *et al.*, 1987). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphate kits were obtained from Randox Laboratories, UK. All other chemicals and reagents were of analytical grade and were products of Sigma Chemical Co. St. Louis, MO., USA or BDH Chemical Ltd, Poole, England.

Plant extraction

Fresh leaves of the plant were harvested, identified and Voucher specimen deposited at the herbarium of the Department of Botany, University of Ibadan. Extraction of air dried leaves of *Annona muricata* was carried out in ethanol for 120 hours at room temperature. The extract was filtered, concentrated, freeze-dried and stored at 4^0C.

Phytochemical screening

A preliminary phytochemical screening of *annona muricata leaveas* was carried out. The phytochemical profile was performed as described by Kokate, 1994 and Harborne, 1998. The presence of alkaloids, flavonoids, glycosides, tannins and saponins were analyzed.

Experimental animals

Thirty male Wistar albino rats (180–200 g) were obtained from the animal house of the Physiology Department, University of Ibadan, Ibadan. The animals were grouped and housed in cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with light and dark cycles of 12 and 12 h, respectively. They

were allowed free access to the standard dry pellet diet and water *ad libitum*. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Hepatoprotective study

The rats were divided into five groups of six animals each. Group I, normal control, was given 1 mL normal saline orally once daily, Group II was treated with 5.0 mg/kg bwt of $NaAsO_2$, Group III was administered with 250 mg/kg bwt AM only, Group IV was given 500 mg/kg bwt AM only, Group V was given 250 mg/kg of AM and $NaAsO_2$, Group VI was administered with 500 mg/kg AM and $NaAsO_2$. The $NaAsO_2$ was given once on days 7, 14 and 21, while AM was administered orally daily for 21 days. The dose of SA used corresponds to $1/5^{th}$ of the oral LD_{50} of the salt (Preston *et al.*, 1987). The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957) using commercial diagnostic kits. This method involves the reaction of pyruvate, the product of transamination reaction catalysed by ALT or AST, with 2, 4 –dinitrophenyl hydrazine to produce intensely coloured hydrazone read at 546 nm using a spectrophotometer (Spectronic-20). and serum alkaline phosphatase activity (ALP) was estimated according to Kind and King (1954).

Histopathological analysis of liver

For histological studies, liver tissues were fixed with 10 % phosphate-buffered neutral formalin, dehydrated in graded (50–100 %) alcohol and embedded in paraffin. Thin sections were cut and stained with hematoxylin and eosin stain for microscopic assessment.

Statistical analysis

Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. The statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS) (SPSS, 17.0). Differences between means were considered significant at $P < 0.05$.

Results

Table 1: Influence of AM treatment on body weight and relative change in organ weight of rats treated with sodium arsenite.

Treatments	Initial body weight (g)	Final body weight (g)	% Weight change	Liver weight (g)	% Liver weight
Distilled water only	180.00 ± 10.96	204.00 ± 15.00	13.33	6.89	3.38
SA	164.00 ± 16.50	188.00 ± 6.96	14.63	5.52	2.94
SA + 250 mg/kg extract	160.00 ± 18.90	186.00 ± 16.45	16.25	6.70	3.60
SA + 500 mg/kg extract	156.00 ± 9.40	174.00 ± 15.46	11.54	5.54	3.18
250 mg/kg extract	171.00 ± 5.83	195.00 ± 11.20	14.04	5.80	2.97
500 mg/kg extract	186.00 ± 10.86	202.00 ± 12.80	8.60	6.30	3.12

Values represent mean ± SEM of six animals in each group.

Table 2: Effects of AM administration on serum level of hepatic transaminases (ALT, AST and ALP) in rats treated with sodium arsenite

Treatments	ALT/IU	AST/IU	ALP/IU
Distilled water	11.00 ± 2.0	13.00 ± 1.8	29.5 ± 2.5
SA	39.5 ± 2.6*	38.5 ± 2.0*	47.0 ± 2.3*
SA + 250 mg/kg extract	30.00 ± 1.8	29.00 ± 2.5	41.00 ± 1.8
SA + 500 mg/kg extract	26.8 ± 1.6	28.7 ± 3.1	39.00 ± 1.5
250 mg/kg extract	26.34 ± 1.2	25.00 ± 1.3	33.80 ± 1.8
500 mg/kg extract	24.50 ± 2.2	23.80 ± 1.4	31.00 ± 1.2

Values represent mean ± SEM of six animals in each group.
* Significantly different from the negative control.

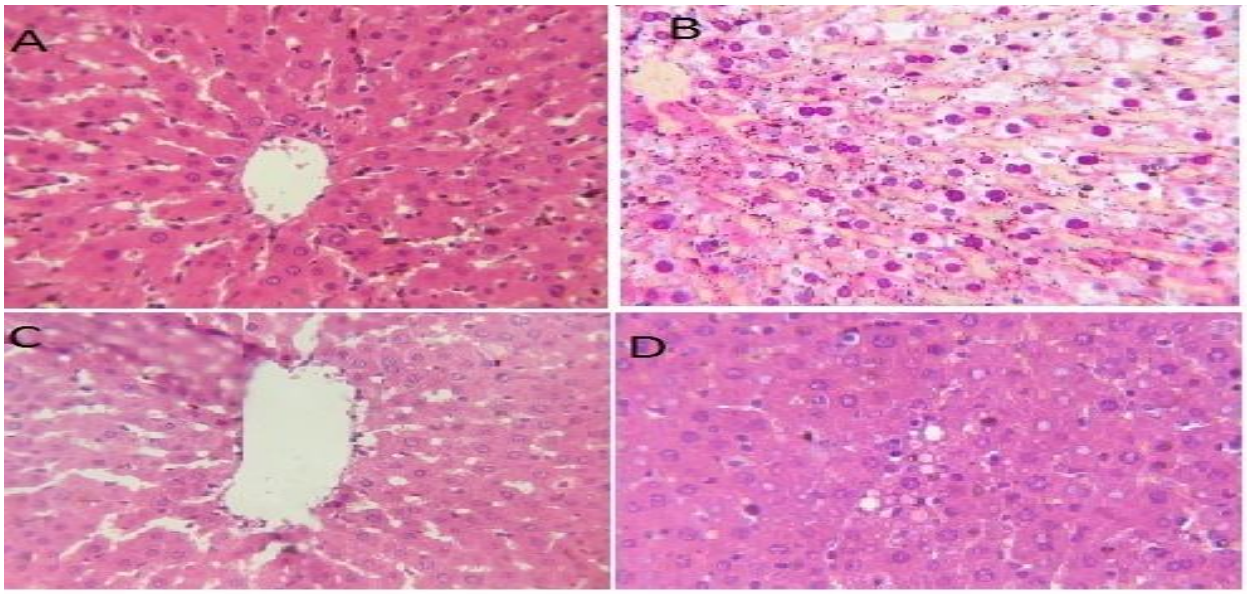

Figure 1: The photomicrograph of liver sections of rats treated with A.Muricata and / or Sodium Arsenite (A) Negative control with no visible lesion (B) Animals administered sodium arsenite only showing severe vacuolation (arrow) with moderate congestion of the sinusoid. (C) Animals fed 250 and 500 mg/kg bwt A.Muricata with no visible lesion *Annona muricata* L. (D) Animals exposed to sodium arsenite and 250 and 500mg/kg bwt A.Muricata with mild vacuolation (arrow). **Mx 100**).

Discussion

The development of new methods for assessing the level of arsenic toxicity due to the deleterious action of arsenic on human body is on the increase. The most sensitive test employed in the detection of acute liver damage is by monitoring the activities of serum enzymes like alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP). Table 1 shows no significant increase in body weight throughout the course of the study however there was a significant decrease in liver weight of SA-only-treated group when compared with control. This resulted from arsenic toxicity in the liver and increased hepatic metabolism to eliminate it. These observations are consistent with reports that SA toxicity can compromise the integrity of the liver in mouse, rat, and goat (Sharma et al., 2009: Roy et al 2009). Liver weights and relative liver weights of groups treated with SA and graded doses of AM extrac*t* were similar to those of the control. This observation indicated that the extract of AM exhibited a potent protective effect on hepatocytes and in response to SA-induced toxicity mitigated SA-induced toxicity on hepatocytes.

In the assessment of liver damage, the determination of enzyme levels such as AST and ALT is used. Necrosis or membrane damage releases these enzymes into circulation which can then be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis and muscle injury. Moreover AST and ALT are more specific to the liver thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhan, 1978). Administration of SA caused a significant ($P<0.05$) elevation of enzyme levels such as AST, ALT and ALP when compared to control. The mean ALT, AST and ALP activities in groups of rats administered graded doses of *annona muricata* extract with sodium arsenite decreased significantly ($p<0.05$) when compared with the group treated with sodium arsenite alone (Table 2). That is the pre-treatment with the extract of *annona muricata* reduced

significantly the elevated serum enzymes when compared with the negative control. The reversal of increased serum enzymes in SA-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987). Also the efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Furthermore histological observations indicated hepatoprotective role of *annona muricata* leaves against sodium arsenite induced hepatoxicity and the results confirmed the findings of hepatoprotective action of AM as presented in figure 1.There were no visible lesion in the negative control group and the groups administered 250mg/kg and 500mg/kg body weights of the extract however there was marked extensive vascular degenerative changes and centrilobular necrosis in hepatocytes of the liver of rats administered sodium arsenite only (positive control). There was mild degenerative changes and absence of centrilobular necrosis in the pre- treatment groups administered with graded dose of AM extract and SA when compared with control .

All these results indicated a hepatoprotective potential of the extract of *annona muricata*. A further detailed study at molecular level is needed to know the exact mechanism of *annona muricata* protective mechanism.

Conclusion

Based on the results obtained pretreatment with the extract was also found to protect the liver from sodium arsenite induced damage which may be attributed to the rich antioxidant nature of the leaf extract. It may be concluded that the extract of *annona muricata* has a significant effect on liver injuries resulting in improved serum biochemical parameters such as ALP, AST and ALT and hence may be use in mitigating arsenic induced toxicity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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