Original Research Article

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

Abstract

Ingestion of arsenic, a known contaminant in drinking water causes cancer at multiple tissues and there is no cure. Consumption of arsenic contaminated water has been implicated metalloid-induced carcinogenesis. 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 used 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 anticancer 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 investigate 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 during the month of August, 2018, 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 detected.

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*.

Hepatoprotective study

The rats were divided into six groups of five animals each. Group I, Negative control, was given 1 mL normal saline orally once daily, Group II was treated with 5.0 mg/kg BW of $NaAsO_2$, Group III was administered with 250 mg/kg BW AM only, Group IV was given 500 mg/kg BW 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 orally 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 wax and sections were made of 4-6µm. After staining with hematoxylin and eosin, slides were examined under the microscope (Olympus, Japan) for histopathological changes and photographed.

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: Phytochemical screening of *Annona muricata* leaves.

Constituents	Reaction to Tests/Reagents	Inference
Alkaloids	Positive to Mayer, Dragendoff Wagner and Picric reagent	X
Flavonoids	Acid-alcohol test	XXXX
Glycosides	Reddish-brown colour at the interference	XX
Saponins	Positive to Froth	XXX
Tannins	Turned blue-black precipitate on addition of $FeCl_3$	X

Key: X = present, XX = Moderately present, XXXX = abundance.

Table 2: 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
Negative control	180.00 ± 10.96	204.00 ± 15.00	13.3 3± 2.95	6.89 ± 1.00	3.38 ± 0.25
SA	164.00 ± 16.50	188.00 ± 6.96	14.63 ± 3.01	5.52* ± 0.66	2.94* ± 0.27
SA + 250 mg/kg extract	160.00 ± 18.90	186.00 ± 16.45	16.25 ± 3.22	6.70 ± 0.74	3.60 ± 0.13
SA + 500 mg/kg extract	156.00 ± 9.40	174.00 ± 15.46	11.54 ± 2.86	5.54 ± 0.68	3.18 ± 0.47
250 mg/kg extract	171.00 ± 5.83	195.00 ± 11.20	14.04 ± 3.40	5.80 ± 0.76	2.97 ± 0.01
500 mg/kg extract	186.00 ± 10.86	202.00 ± 12.80	8.60 ± 2.01	6.30 ± 0.54	3.12 ± 0.13

Values are represented as mean ± SD of six animals in each group.

* = The mean difference is significant ($p<0.05$) when compared with the negative control

Table 3: Effects of AM administration on serum level of hepatic alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in rats treated with sodium arsenite

Treatments	ALT/IU	AST/IU	ALP/IU
Negative control	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 ± SD of six animals in each group.
* = Significantly different from the negative control.

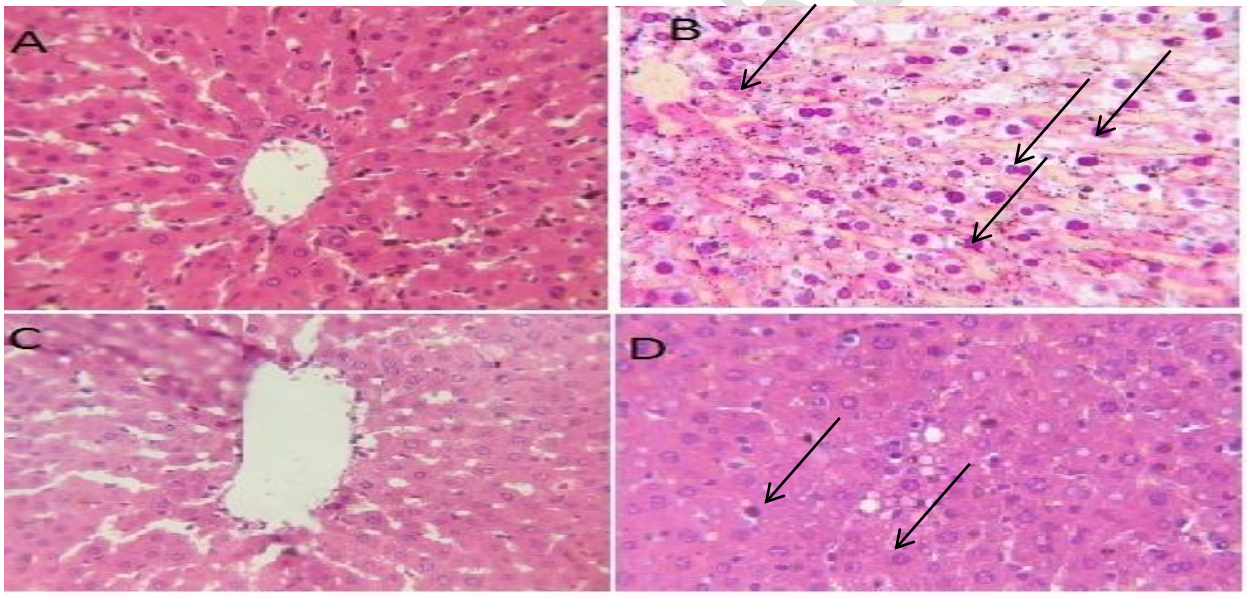

Figure 1: The photomicrograph of liver sections of rats treated with A. Muricata and / or Sodium Arsenite **(**A) Negative control with no visible lesion (B) Animals administered sodium arsenite only showing severe vacuolation (arrow) with moderate congestion of the sinusoid. (C) Animals fed 250 and 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 400**).

Discussion

Cancer disease is increasing annually worldwide, creating some concerns regarding the efficacy of the present treatment study. Arsenic toxicity has been linked to diverse defects in both experimental animals and in humans (Waalkes *et al*., 2003) and exposure to arsenic via the intake of contaminated water has been linked with diverse heath defects like certain forms of cancer, skin lesions, and non-cancer health effects such as neurological disorders and impaired cognitive development in children (Abernathy et al., 1999).

Arsenic intoxication in experimental animals has been linked with micronucleus formation and hepatic tumors (Mazumder, 2005) and the liver is an important target organ for arsenic toxicity (Parvez et al., 2006). Consumption of food products rich in antioxidants have been suggested and shown to hold promise in mitigating disease onset and chemical-induced carcinogenesis. Annona muricata is commonly known as soursop or graviola which produces an edible fruit. This study reports the effect of the ethanolic extract of Annona muricata leaves on SA-induced toxicity on albino Wistar rats. The result obtained from the phytochemical analysis of ethanol extract of *Annona muricata* leaves indicated the presence significant amount of flavonoids, tannins, saponins, alkaloids and glycosides as presented in table 1. These chemicals have been documented with antitumor, antiparasitic and antimicrobial activities. Annona muricata leaves is very rich in flavonoids which have antioxidants effects associated with various diseases such as cancer, Alzheimer's disease, atherosclerosis (Burak and Imen, 1999). Alkaloid is known to possess antioxidant properties that can mitigate cellular macromolecule damage from oxidative radical species (Lambert et al., 2005). Such as DNA strand damage, resulting in genomic instability implicated in carcinogenesis. Saponins have been reported to have hemolytic properties, although they are not toxic when administered orally. Polyphenolic tannins have been reported to exhibit metal ions chelating properties that can help in mopping up trivalent arsenite upon exposure (Okonkwo et al., 2010). However, tannins can be poisonous by depriving the body of essential metal ions needed for proper functioning.

There was no significant increase in body weight throughout the course of the study (Table 2). Compared with the negative control, there was significant decrease in liver weight of SA-only-treated group. 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 AM and AM only were similar to those of the negative control, indicative of AM exhibiting a potent protective effect on hepatocytes on the one hand and in response to SA-induced toxicity thereby ameliorating SA-induced toxicity on hepatocytes.

Exposure to SA had been shown to increase activity of liver transaminases in the blood (Chattopadhyay, 2001), this is an index of hepatotoxicity which might have resulted from oxidative stress-related damages to hepatocytes membrane and leakage of hepatic transaminases into extracellular spaces ultimately finding their way into the blood from the liver [40]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are members of transaminase family of enzymes. They are also known as aminotransferases, they catalyze the transfer of amino groups between L-alanine and glutamate for physiological purpose. ALT and AST are found in large amount in the liver and also small amount are found in the heart, kidney and muscles. When the liver is injured or inflamed as the case may be via its exposure to various forms of toxic substances, the level of ALT and AST in the blood is usually elevated. The level

of these enzymes in the blood is directly related to the extent of the tissue damage (Lum and Gambino, 1972). The induction of ALT and AST activity following sodium arsenite has been well documented (Mallick *et al.,* 2003).

From the results presented in (table 3) there is a significant increase in the activities of AST, ALP, and ALT ($P < 0.05$) in the serum of SA-treated rats when compared with the negative control. The observations made here are consistent with the findings from previous findings (Mallick et al., 2003; Odunola, 2003;). However, there is a significant decrease ($P < 0.05$) in serum activities of AST and ALP in the SA and AM-treated group compared with negative control. This is suggestive of hepatocytes protection from AM co-treatment against SA-induced damages. This can be attributed to the antioxidant present in AM. This is in accordance with some recent studies that recommend the use of antioxidants and antioxidant-rich foods and herbal medicinal plant for the management of arsenicosis (Das and Sengunpta, 2008). The activities of the liver enzymes (AST, ALT, and ALP) were significantly low in the AM-only-treated group confirming protective property of AM. 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).

Our finding from liver histopathology suggests SA-induced toxicity and ameliorative potentials of AM on SA induced hepatocytes damage. Histological examinations of liver sections of treated animals showed that SA was potentially hepatotoxic as reflected by severe vacuolation with moderate congestion of the sinusoid. Liver sections from SA and AM-treated group exhibited very mild vacuolation, while those of the AM-treated group showed no visible lesions confirming a modulatory effect of AM on SA-induced hepatocytes damage

All these results indicated a hepatoprotective potential of the extract of *annona muricata* and these biological activities may be due to the phytochemicals present in the extract. Further studies to isolate, purify and characterize the active compounds in the extract are significant and also a 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.

Ethical Approval

All procedures of this study described were reviewed and approved by the Institutional Animal Ethical Committee.

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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