Protective effects of *Vernonia amygdalina* against sodium arsenite-induced genotoxicity in rat

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ABSTRACT

Objectives: Contamination of the environment with arsenic (As) from both human and natural sources is known as a global problem. This study investigated the chemoprotective potential of *Vernonia amygdalina* leave extract against sodium arsenite-induced genotoxicity and hepatotoxicity. **Materials and Methods**: Genotoxic effects were evaluated in the rat bone marrow using micronuclei. The gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) activities were assayed in rat serum. **Results**: Pre-treatment with extract of *V. amygdalina* at doses 5 mg/kg and 10 mg/kg significantly decreased the frequency of micronucleated polychromatic erythrocytes (PCEs). The GGT and ALP activities were elevated more than fourfold, in the liver of rats treated with sodium arsenite, while it was reduced almost to half when the sodium arsenit-treated rats were fed fresh *V. amgdalina* leave extracts The phytochemical constituents of *V. amygdalina* assayed in this study may be responsible for high radical scavenging of the DPPH free radical observed. **Conclusion**: The present results indicate that *V. amygdalina* extract is capable of suppressing the chromosomal aberration induced by sodium arsenite in rat. Thus, *V. amygdalina* may be a potent chemoprotective agent against the toxicity of sodium arsenite in rats.

Key words: Anticlastogenicity, micronuclei test, rat, sodium arsenite, *Vernonia amygdalina*

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INTRODUCTION

In many countries, the levels of As in the environment have turned out to be one of concern and many studies have recognized various adverse health effects on populations.[1] In modern days, exposure to sufficiently high concentrations of inorganic As in natural environments such as in water, sediment and soil has proved to be harmful to the organisms.[2,3] The main pathways of exposure to the human beings include ingestion of drinking water and consumption of foods and to a lesser extent, inhalation of air.[4] Residual damage from the exposures to arsenic has been reported to lead to toxic consequences in the body and these toxic effects in the patient's body may lead to the development of secondary cancers.[5,6] In view of the global health problems associated in drinking water and its impacts on the society, it is important to prevent the bioavailability of As in humans.

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In order to overcome the sodium arsenite contaminations in both rural and urban areas, chemoprotective agents need to be developed as a means of preventing the toxicity associated with arsenic contamination. The present study has been intended on leaves of *V. amygdalina*, which has been reported as a potential anticancer extract.[7] *V. amygdalina* plants popularly known as bitter leaves are green and have a characteristic odour and bitter taste. In many parts of West Africa, the plant has been domesticated and used for treatment of many diseases.[8,9]

Previous investigations reported that the leave extracts of *V. amygdalina* and its components possess free radical scavenging, immunodulatory, anticancer, and antitumour properties.[10,11] Thus, *V. amygdalina* and its components may have the potential to become a good chemopreventive agent against the toxicity of the sodium arsenite. In the present work, the chemoprotective potential of *V. amygdalina* has been investigated against the sodium arsenite-induced genotoxicity using the micronuclei test as an end point in bone marrow of Wistar albino rats. In addition, the phytochemical components and DPPH radical scavenging potentials of *V. amygdalina* were assayed.

MATERIALS AND METHODS

Animals

White male albino Wistar rats (weighing 120 ± 2g) were obtained from the Central Animal Facility of the Physiology Department, LAUTECH Ogbomosho, Nigeria. Experimental protocols were approved by the Faculty of Basic Medical Science LAUTECH, Ogbomoso, Animal Ethics Committee. These animals were bred and maintained in the Physiology Department Central Animal House. Standard pellet diet, obtained from Irorun-Agbe Agro Industries, Ogbomoso and water were provided *ad libitum*. They were housed five per cage under controlled temperature (25 ± 3°C), humidity (50 ± 10%) and light (0600 to 1800 h) conditions.

Chemicals and preparation of test materials

Sodium arsenite was purchased from Sigma UK. All other chemicals were of analytical grade and obtained from the BDH laboratory supplies UK. An aqueous suspension of *V. amygdalina* extract was freshly prepared for each treatment using distilled water (0.8%*w*/*v*) and was administered by the oral route. A solution of sodium arsenite, (2.5mg/kg body weight in normal saline) for each treatment was administered by the intraperitoneal route (i.p.) according to the experimental protocol [Table 1].

Animal treatment protocol

White male albino rats were randomly allocated to groups I to IV as shown in Table 1, each group consisting of five rats. Two doses of *V. amgdalina* extracts (50mg/kg and 100mg/kg body weight) were given by gavaging to the rats in I and II groups for seven consecutive days as a dietary supplement followed by a single dose of sodium arsenite (2.5µg/kg body weight) which were administered intraperitoneally. The rats in the negative control (III), were given distilled water and the positive control group (IV) were given sodium arsenite alone. The animals were sacrificed by cervical dislocation and micronuclei assay was performed to determine the number of micronucleated polychromatic erythrocyte (nPCE) in bone marrow cells of rats.

Table 1: Micronucleated polychromatic erythrocyte (nPCEs) count, GGT and ALP concentration of rats treated with *V. amgdalina* leave extract and sodium arsenite

Groups	n PCE	GGT Conc. (V/L)	ALP Conc, (V/L)
I	14.0 ± 4.97[a]	13.83 ± 1.03[a]	6.72 ± 2.54[a]
II	12.0 ± 4.97[a]	8.70 ± 1.26[a]	6.42 ± 3.3[a]
III	7.5 ± 3.5[b]	23.16 ± 4.02[b]	15.58 ± 3.38[b]
IV	2,0 ± 0.01	6.42 ± 0.03	3.01 ± 0.10

nPCE, nucleated Polychromatic Erythrocyte, conc., concentration
[a]Significantly different from negative control, P < 0.05. [b]Significantly different from sodium arsenite group, P < 0.05. Values represent mean ± S.E.M., *n* = 3.

Micronuclei test

Genotoxic effects were evaluated in the rat bone marrow using micronuclei test described by[12] with some modifications. Bone marrow cells from both femurs were flushed and a fine suspension was prepared in a centrifuge tube containing foetal bovine serum. The cell suspension was centrifuged at 1500 rpm for 10min and the pellet was suspended with the residual fluid. A small drop of the suspension was placed over a clean slide and the smear was prepared and air dried. The slides were stained with May-Gruenwald and Giemsa. Slides were mounted using DPX mountant, dried (20–30°C), cleaned and properly coded. The frequencies of micronuclei (MN) in polychromatic erythrocytes (PCE) were estimated by scoring 1000 PCE per animal.

Serum preparation and gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) assay

Blood was collected by cardiac puncture and samples were kept on ice until analysis. Samples were centrifuged at 1200g for 15 min to obtain the serum. Then, determination of liver damage by measuring the activities of ALP;[13] and GGT[14] were performed. GGT kit of Randox product was used for this study. 1ml of the working reagent was mixed with 0.10ml of serum. The initial absorbance was taken followed by an interval of one minute for 3minutes at 405nm. ALP kit obtained from Agappe diagnostics was used in carrying out the experiment; 1000µl of the working reagent was mixed with 20µl of serum, and allowed to stay for 1 min. The absorbance was read at 405nm at an interval of one minute for 3min.

Histology

Liver samples were taken from all the animals and fixed with 10% formaldehyde in phosphate-buffered saline for 24 h. Tissue pieces were washed with distilled water, dehydrated in alcohol, and embedded in paraffin. 5µm sections were mounted on glass slides previously covered with silane. Masson's trichromic stain was performed in each slide.

Antioxidant assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was done according to the method of[15] with some modifications. The working solution was obtained by mixing 50µl of *V. amgdalina* with 3ml of methanolic DPPH solution (0.1mM). The change in absorbance at 515nm was measured at 0min, 1min, 5min, and every 5 min until it reached 40 min. The antioxidant capacity was calculated as shown below:

$$\% \text{ scavenging activity} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Hence, Abs is the absorbance of blank or control minus absorbance of tested extract solution.

Phytochemical analysis

The following phytochemical analysis was carried out on leaves of *V. amygdalina* using the procedure of[16] as outlined below:

Test for flavonoids

0.5g of plant sample was suspended in 5ml of water and 2.5ml of methanol was added to it and filtered. 1ml of NaOH 10% was added to 1ml of the filtrate. The presence of a yellow precipitate indicated the presence of flavonoids.

Test for tannins

7.5ml of water was added to plant extract (1g) and heated in a water bath. It was then filtered upon cooling. Few drops of iron III chloride ($FeCl_3$) 0.5% were added to 2ml of the filtrate. The appearance of a green or dark-blue precipitate indicated the presence of tannins.

Test for alkaloids

2g of plant sample was heated in a test tube containing 25ml of HCl (1%) for 15 min in a boiling water bath. The suspension was then filtered and 5 drops of Meyer's reagent (potassium tetra iodomecurate) were added into the filtrate (1ml). The formation of a precipitate indicated the presence of alkaloids.

Test for saponins

0.5g of plant extract was introduced into a test tube containing 7.5ml of distilled water and the mixture heated for 5 min in a boiling water bath. The solution was then filtered and cooled to room temperature. Five millilitres of the filtrate was introduced into a test tube and agitated for 10s. The formation of persistent foam indicated the presence of saponins.

Test for triterpenes and steroids

0.5g of plant sample was dissolved in chloroform (3ml) and a few drops of acetic anhydride and concentrated H_2SO_4 were added. A purple coloration indicated the presence of triterpenes while bluish-green coloration indicated the presence of steroids. The formation of two layers upon addition of H_2SO_4 is characteristic of the presence of both triterpenes and steroids.

Test for coumarins

One milligram of moistened sample was placed in a test tube and the test tube was covered with a filter paper moistened with 10% NaOH solution. After exposition of the paper to UV light for a few minutes, yellow green fluorescence was indicative of the presence of coumarins

Test for cardiac glycosides

One milligram of plant sample was suspended in 5ml of glacial acetic acid containing one drop of ferric chloride solution. Then 1ml of concentrated sulphuric acid was added gradually along the wall of the test tube. The formation of a brown ring at the interface indicated the presence of a deoxysugar, characteristics of cardenolides.

Statistical analysis

The data were analysed using statistical software (Jandel Sigmastat, for windows version 2.03). The results are expressed as mean ± SEM for each group. The statistical significance was determined by one-way analysis of variance (ANOVA), followed by the Tukey test. The level of statistical significance was set at $p < 0.05$ and < 0.01.

RESULTS

Effects of *V. amygdalina* leave extract on the sodium arsenite induced micronuclei

The protective effects of *V. amygdalina* leave extract on bone marrow micronuclei after seven days pre-treatment are shown in Tables 1. Sodium arsenite at a dose of 2mg/kg induced significant MN over the negative control [Table 1]. Pre-treatment with 50mg/kg *V. amygdalina* leave extract significantly reduced sodium arsenite induced MN in bone marrow by 70%, compared with the frequency of MN induced in the bone marrow of rat treated with sodium arsenite alone. Similarly, pre-treatment with 100mg/kg *V. amygdalina* leave extract significantly reduced sodium arsenite induced MN in bone marrow by 74%, compared with the frequency of MN induced in the bone marrow of sodium arsenite alone

Scavenging effects of the *V. amgdalina* on DPPH radical

The DPPH radical scavenging activity of *V. amygdalina* extract (IC_{50} value of 4.0 ± 0.1μg/ml) is comparable to the IC_{50} value gallic acid (3.8 ± 0.2μg/ml), which is a well-known antioxidant [Table 2]. Scavenging of DPPH radical was found to rise with increasing concentration of the extracts [Table 2].

Histology

The photomicrography of the liver reveals that the treatment of rats with sodium arsenite produced altered normal architecture of the parenchyma [Figure 1a]; group treatment with sodium arsenite plus *V. amygdalina* reduced the necrotic areas [Figures 1c and 1d] in comparison with negative control group [Figure 1b].

Phytochemical constituents of crude extracts of *V. amygdalina*

The test for the secondary metabolite showed the presence of flavonoids and saponin. Tripeternes and cardiac glycosides were also present in appreciable trace while tannins and alkaloids were present in low quantity. Coumarin is absent [Table 3].

Table 2: Scavenging effects of the *V. amgdalina* on DPPH radical

Sample	% Inhibition at concentration (µg/ml)								
	500.0	250.0	125.0	62.5	31.3	15.6	7.8	3.9	IC_{50}
V. amgdalina extract	93.3 ± 42	93.1 ± 311	92.6 ± 30	92.6±25	91.0 ± 20	91.3 ± 30	90.2 ± 32	89.1 ± 16	4.0 ± 01
Gallic acid	95.5 ± 30	95.8 ± 36	94.5 ± 4.0	94.7±46	93.1 ± 4.2	92.0 ± 3.9	91.5 ± 3.0	90.0 ± 1.8	3.8 ± 0.2

Values represent mean ± S.E.M., n = 3

Table 3: Phytochemical constituents of crude extracts of *V. amygdalina*

Plant extract	Flavonoids	Tannins	Alkaloids	Saponins	Triterpenes	Steroids	Coumarins	Cardiac glycosides
Vernonia amygdalina	+++	+	+	+++	++	++	–	++

+ = Presence of constituent; – = Absence of constituent, +++ detected in high quantity, ++detected in medium quatity, +detected in trace amount

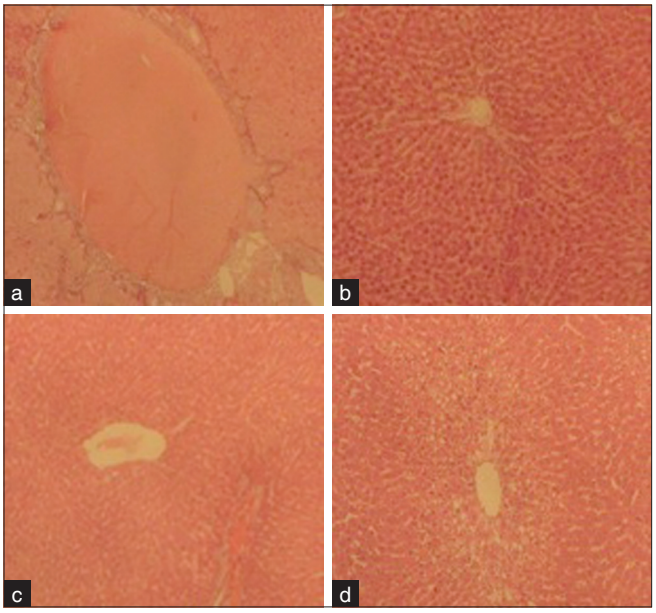

Figure 1: Representative of liver sections obtained from rats treated with sodium arsenite alone (a), distilled water alone (b), *V. amgdalina* (50 µg/ml) plus sodium arsenite-treated rats (c) and *V. amgdalina* (100 µg/ml) plus sodium arsenite-treated rats (d)

DISCUSSION

The genotoxicity of arsenic is well known as it induces chromosomal damage in different test systems.[17,18] The present results confirmed the genotoxic potential of sodium arsenite as evident from the significant induction of MN in rat bone marrow. Pre-and co-treatment with *V. amygdalina* at doses of 50 and 100mg/kg, b.w. for 7 days significantly reduced the sodium arsenite induced MN frequencies in rat bone marrow.

GGT and ALP are found in many tissues, the most notable one being the liver, and has significance in medicine as a diagnostic marker. GGT plays a key role in drug and xenobiotic detoxification. Oxidative injury and lipid peroxidation can be monitored by measuring liver or blood GGT and ALP concentration.[19] Consequently, the effect of pre-treatment of *V. amgdalina* on concentration of ALP and GGT was evaluated in this study. Similarly, with the same treatment *V. amygdalina* significantly reduced the concentration of ALP and GGT medium in serum of the rat. The increase of ALP and GGT concentration with sodium arsenite group (positive control) was marginal over the saline treated groups (negative control). However, with the co-treatment of sodium arsenite with *V. amygdalina* the concentration of the enzymes was decreased. The effects of *V. amgdalina* leave extract discussed until here are important mediators to have beneficial response to prevent liver fibrosis induced by sodium arsenite administration and may have an impact in the prevention of diseases.[20] As the *V. amgdalina* treated group avoided the liver fibrosis the main markers of cholestatic damage, GGT and ALP were normal at the end of the treatment. This study shows the beneficial response to the treatment with *V. amgdalina* leave extract in the experimental model of rat liver fibrosis induced by administration of sodium arsenite.

An antioxidant dietary supplement can reduce the level of DNA oxidative damage and protect normal cells against the adverse side-effects of some carcinogens.[21] In this study *V. amygdalina* leave extract exhibited strong radical scavenging property and previous reports demonstrated that *V. amygdalina* extract scavenges hydroxyl radicals and exhibit chain breaking antioxidant activity against metal ion induced lipid peroxidation.[22] Furthermore, it has been determined that the antioxidant effect of plant products is mainly due to radicals scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes.[23] In this study, some of these phytochemicals were found to be essential components of *V. amygdalina* leave extract. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important

role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[24] Therefore, the reduction in the toxicities and clastogenicity exhibited in this study might be due to counteracting effect of antioxidant components of *V. amygdalina* on reactive species generated by sodium arsenite in rat. This further suggests that *V. amygdalina* has a potent anticlastogenic effect against sodium arsenite induced genotoxicity. The present report suggests the antigenotoxic effect of *V. amygdalina* and the suppression of genotoxicity may be either due to the free radical scavenging mechanisms or different DNA reparative processes against sodium arsenite. Further investigations are needed to reveal the exact mechanistic basis of this type of chemoprotective effect exhibited by *V. amygdalina*. Additional work in terms of different genotoxic end-points with specific fractions and components of *V. amygdalina* is in progress to strengthen the chemoprotective potential of *V. amygdalina* against the toxicity induced by different clastogens.

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