

Modulatory of effect of fresh *Amaranthus caudatus* and *Amaranthus hybridus* aqueous leaf extracts on detoxify enzymes and micronuclei formation after exposure to sodium arsenite

Adetutu Adewale¹, Awe Emmanuel Olorunju²

Departments of ¹Biochemistry, and ²Pharmacology and Therapeutic, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

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ABSTRACT

Vegetables are the cheapest and most available sources of important proteins, minerals, vitamins, and essential amino protein. These vegetables are commonly used in Africa for the treatment of illness. This study evaluated the protective effects of *Amaranthus caudatus* and *A. hybridus* against sodium arsenite-induced toxicity in rats. The effects of sodium arsenite and/or the plant extracts were assessed using bone marrow micronucleus assay and by measuring the activities of tumour maker enzymes such as gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) in white albino Wister rats. The study showed that sodium arsenite significantly ($P < 0.05$) induced the formation of micronucleated polychromatic erythrocytes and the activities of ALP and GGT when compared with control. The levels of white blood cell, hemoglobin, and lymphocyte count were altered in sodium arsenite fed rats and were reverted back to near normal levels in rats pretreated with the plant extracts. *A. caudatus* and *A. hybridus* showed significant role in protecting the detoxifying enzymes; also, *A. caudatus* has a more protective effect on reducing the micronuclei formation when compared with *A. hybridus*. This study suggests that *A. caudatus* and *A. hybridus* possess anticarcinogenic effect.

Key words: *Amaranthus caudatus*, *Amaranthus hybridus*, chemoprotective, detoxify enzymes, micronuclei, sodium arsenite

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INTRODUCTION

Arsenic is widely distributed and used extensively as components of herbicides, insecticide, rodenticide, food preservatives, and drugs.^[1] However, unusual ingestion of arsenic in drinking water has been associated with many cancers and noncancer effects in the affected populations.^[2,3] An early event in arsenic carcinogenesis is molecular alterations both in humans and animals, which manifests dose-dependent chromosomal breaks and alterations.^[4] The liver has been reported to be a dire target of arsenic in humans. Arsenic exposure is associated with the growth of hepatocellular carcinomas as well as other

toxic lesion.^[3] Efforts to avert and treat arsenic toxicity by therapeutic measures had only limited success.^[5] Several reports have associated oxidative stress in arsenic-induced cytotoxicity and genotoxicity.^[6] Consequently, some studies propose the use of antioxidant rich foods and phenolic herbal products for the management of arsenic toxicity.^[7]

In rural areas of most developing countries of the world where potable water is barely available, the poor populace is inadvertently exposed to arsenic poisoning especially in the mining areas. Green leafy vegetables are known to contain useful minerals and vitamins that are commonly available and are considered as the corner stones of health care system in alleviating some serious diseases.^[8] In most West African countries, *A. hybridus* and *A. caudatus* leaves are frequently eaten as cooked vegetables. *A. caudatus* and *A. hybridus* are popular vegetables in West Africa and have been reported to possess many antioxidant components

Address for correspondence:

Dr. Adetutu Adewale, Department of Biochemistry,
Faculty of Basic Medical Sciences College of Health Sciences,
Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
E-mail: aadetutu@lautech.edu.ng

and other medicinal values.^[8] Despite the use of these vegetables for treatment of many diseases, there is little information on their chemoprotective potentials and chemical composition. This work is, therefore, aimed at determining the effects of *A. caudatus* and *A. hybridus* leaf extracts on certain hematological parameters, detoxify enzymes, and formation micronucleated polychromatic erythrocytes in rats bone marrow after exposure to sodium arsenite. In addition, this study investigated some of the essential vitamins and minerals in the vegetables.

MATERIALS AND METHODS

Collection of vegetable samples

The plants (*A. caudatus* and *A. hybridus*) were collected from Agricultural farm Ladoke Akintola University of Technology, Ogbomoso. The authentication was done by Dr. Ogunkunle JA, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. The voucher number assigned to *A. caudatus* is LHO 233 and *A. hybridus* is LHO 232. The plant materials were washed with water and air dried for 3 weeks in the laboratory at room temperature. The dried vegetables were then pulverized into fine powder for the experiment.

Preparation of aqueous vegetable extract and NaAsO₂ solution

A total of 10 g of the powdered was soaked with 50 mL of distilled water and left overnight. It was then filtered into another clean bottle with Whatman No.1 filter paper and prepared on daily basis for the period of administration. NaAsO₂ (Signal-Aldrich, USA) was dissolved in normal saline to a concentration of 2.5 mg/kg body weight. Freshly prepared solution was used for each experiment. A total of 1 mL of this prepared solution was administered interperitoneally to each rat except the rat grouped negative control.

Experimental protocol, animal sacrifices, and collection of blood

The experimental rats were randomly divided into four treatment groups of five animals each. Group A served as the negative control and were treated with distilled water only for 14 consecutive days. Rats in group B were placed on normal diet for 14 consecutive days. Rats in group C were treated with 1 mL of prepared aqueous solution of *A. caudatus* for 14 consecutive days. Rats in group D were also treated with 1 mL prepared aqueous solution of *A. hybridus* for the same 14 consecutive days. Rats in group B, C, and D were injected interperitoneally with 2.5 mg/kg body weight of sodium arsenite on the 14th day. The rats were sacrificed by cervical dislocation 24 h after last treatments were administered, while the animals were

kept fasting for this 24 h. Blood was collected via cardiac puncture into clean plane bottles and use for hematological tests immediately. Serum was also prepared for enzyme assay. Femoral bone marrow from each animal was also harvested and used for micronucleus assay.

Micronucleus, enzyme, and hematological parameter assay

The micronucleus assay was performed according to the method of Heddle and Salmon.^[9] The femur of each rat was freed and stripped clean of muscles. Bone marrow smear was made on the slide. The slides were air-dried and fixed in absolute methanol for 5 min and further air dried for a few minutes to remove the methanol. The slides were then stained in 5% Giemsa stain that was initially dissolved in 0.01 M phosphate buffer pH 6.8, after initial staining with 0.4% of May-Grunwald. They were thereafter rinsed in distilled water, air-dried, mounted in dopexamine hydrochloride (DPX) (BDH) and covered with cover glass smeared with xylene. The stained and mounted slides were coded and scored using an Olympus XSZ 107 BN microscope for the presence of micronucleated polychromatic erythrocytes (mPCEs). The gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities was assayed in the serum by using GGT assay kit (Randox Laboratories Ltd., UK) using the procedure described by the manufacturer. The blood collected into clean plane bottles was taking to laboratory and use for hematological tests immediately. White blood cells (WBCs), platelet, hemoglobin (Hgb), percentage plateletcrit, and platelet distribution width was measured on Sysmex Hematology analyzer (model K4500).

Determination of vitamins and minerals in vegetables

Vitamin C, beta-carotene, and vitamin E (tocopherols) in the samples were extracted according to the method described by Abdulnabi *et al.*,^[10] with slight modifications. Riboflavin was extracted according to the method described in AOAC International.^[11] The extracts were filtered through a 0.45 µm nylon filter disc before HPLC analysis. All samples were carried out in triplicates. The mineral elements comprising sodium, calcium, potassium, magnesium, iron, zinc, copper, manganese, and lead were digested according to the method of Shahidi *et al.*,^[12,13] and Nahapetian and Bassiri (1975) with some modifications. The solutions were analyzed for elemental contents by atomic absorption spectrophotometer.

Statistical analysis

Results were expressed as mean ± standard error of the mean. One-way analysis of variance (ANOVA) was used for data analysis and *post-hoc* analysis after ANOVA employed the Duncan's multiple range test. *P* values less than 0.05 were considered statistically significant.

RESULTS

Activities of liver function enzymes (GGT and ALP) in plasma of test and control rats

Sodium arsenite (NaAsO_2) induced toxicity was measured by GGT and ALP assay in both absence and present of *A. caudatus* and *A. hybridus* [Table 1]. Administration of NaAsO_2 alone significantly increases the activity of GGT and ALP in comparison with the negative control. Pretreatment of rats with aqueous extract of *A. caudatus* and *A. hybridus* before NaAsO_2 exposure significantly lowers GGT activity to about 1.9 and 1.6 folds, respectively. Moreover, priming of rats with aqueous extract of *A. caudatus* and *A. hybridus* before NaAsO_2 injection significantly lowered ALP activity to about 1.4 and 1.04 folds, respectively, in comparison with the sodium arsenite-treated group [Table 1].

Protection of *A. caudatus* and *A. hybridus* leaf extracts against sodium arsenite-induced change in some hematological variable in rats

Change in hematological variables, following NaAsO_2 exposure, induced a significant increase in WBC, lymphocyte (LYMPH), together with a rise in Hb concentration of group B rats [Table 2]. However, no significant changes were observed in groups C and D in comparison with the negative control group.

mPECs in test and control rat bone marrow cells

Administration of sodium arsenite alone markedly induced micronucleated polychromatic erythrocytes formation in the rats bone marrow cells compared to negative control (treated with distilled water alone). Pretreatment of rats

with aqueous extract of *A. caudatus* significantly decreased the induction on mPCEs by sodium arsenite to about 1.54 fold as compared to positive control. In addition, the administration of rats with *A. hybridus* also reduced the induction of mPCEs to about 1.1 folds in comparison with the positive control [Figure 1].

Mineral compositions of *A. hybridus* and *A. caudatus*

Mineral composition of *A. hybridus* and *A. caudatus* are as shown in Table 3. The leaf extract of *A. caudatus* is richer in sodium, potassium, calcium, and iron in comparison with *A. hybridus*, while trace minerals like copper, manganese, and zinc are more prominent in *A. hybridus* than *A. caudatus*. Major minerals like sodium, calcium, and

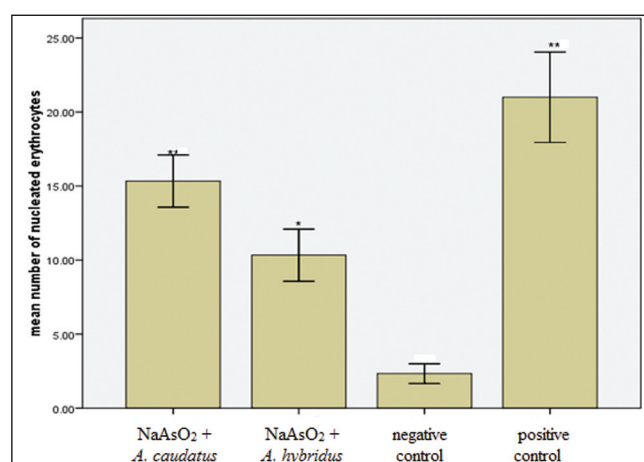


Figure 1: Frequency of mPCEs per 100 in test and control rats. Values are expressed as mean $\pm$ standard error of the mean, *Significantly different from negative control ($P < 0.05$), **Significantly different from negative control ($P < 0.01$)

Table 1: Activities of liver function enzymes (GGT and ALP) in plasma of test and control rats

Group	Treatment	GGT (U/L)	ALP (U/L)
A	Distilled water alone	0.78 $\pm$ 0.005	1.66 $\pm$ 0.0300
B	Sodium arsenite alone	3.50 $\pm$ 0.025*	23.19 $\pm$ 0.0100*
C	Sodium arsenite+A. <i>caudatus</i>	1.82 $\pm$ 0.002**	17.14 $\pm$ 0.0100**
D	Sodium arsenite+A. <i>hybridus</i>	1.17 $\pm$ 0.222**	22.62 $\pm$ 1.520**

Values are expressed as mean $\pm$ standard error of the mean; *Significantly different from rats given distilled water; **Significantly different from rats given sodium arsenite; ALP=Alkali phosphatase; GGT=Gamma glutamyl transferase

Table 2: Hematological parameters in rat serum after treatment with aqueous extracts of *A. caudatus* and *A. hybridus* and/or NaAsO_2

Groups	Total WBC/ μL	LYMPH (%)	GRAN (number)	HGB (g/dL)	PLT (μL)	MPV (fL)	PCT (%)
A (treated with distilled water)	5.3 $\times 10^9 \pm 312$	1.9 $\times 10^9 \pm 2141$	1.7 $\times 10^9 \pm 2001$	11.4 $\pm$ 1.9	553 $\times 10^3 \pm 655$	2.8 $\pm$ 0.1	0.154 $\pm$ 0.01
B (treated with NaAsO_2)	7.1 $\times 10^9 \pm 200^*$	4.9 $\times 10^9 \pm 4531$	1.7 $\times 10^9 \pm 2366$	13.8 $\pm$ 2.1*	572 $\times 10^3 \pm 454$	3.0 $\pm$ 0.3	0.171 $\pm$ 0.01
C (treated with NaAsO_2 +A. <i>caudatus</i>)	4.9 $\times 10^9 \pm 132^*$	2.9 $\times 10^9 \pm 2432$	1.6 $\times 10^9 \pm 3100$	9.3 $\pm$ 2.3**	522 $\times 10^3 \pm 345$	2.8 $\pm$ 0.2	0.146 $\pm$ 0.02
D (treated with NaAsO_2 +A. <i>hybridus</i>)	7.7 $\times 10^9 \pm 231$	5.7 $\times 10^9 \pm 342^{***}$	1.5 $\times 10^9 \pm 2211$	14.3 $\pm$ 2.3**	526 $\times 10^3 \pm 243$	2.8 $\pm$ 0.1	0.147 $\pm$ 0.02

*Significantly different from rats administered distilled water; **Significantly different from rats administered sodium arsenite, GRAN=Granulocytes; HGB=Hemoglobin; LYMP=Lymphocyte; MPV=Mean platelet volume; PCT=Percentage plateletcrit; PCT=Relative volume of thrombocytes; PLT=Platelet; WBC=White blood cell

potassium are also present in *A. hybridus* but with lesser content in *A. caudatus*.

Vitamin composition of *A. hybridus* and *A. caudatus*

Result from this study show that *A. caudatus* is richer in beta carotene, riboflavin, ascorbic acid, and tocopherol compared with *A. hybridus* [Table 4].

DISCUSSION

There is growing evidence that sodium arsenite toxicity can compromise the integrity of the liver in mouse, rat, fish, and goat.^[14] Additional evidences have shown that exposure to low-level arsenic exposure in drinking water can result in physiological disturbances and hepatocellular carcinoma in man.^[15] On the basis of popular consumption of *A. caudatus* and *A. hybridus*, as sources of essential nutrients and vitamins, their potentials in reducing liver toxicity were evaluated in this study. The plant extracts have no clastogenic and hepatotoxic effects as observed in the micronucleus and enzyme assays. The increase in serum concentration of GGT and ALP in the rats exposed to sodium arsenite in comparison with the negative control is indicative of oxidative stress and cytogenetic damage in animals. The elevation and leakage of the enzymes (GGT and ALP) to the plasma might be due to the increased permeability of the plasma membrane, increased synthesis of the enzymes by the liver, inflammation, and cellular necrosis in the liver.^[16] In addition, increased activity of GGT has been associated with hepatotoxicity, oxidative stress, and chromosomal aberrations in cells.^[14,16]

Table 3: Mineral composition of *A. hybridus* and *A. caudatus*

Mineral element	Composition in <i>A. caudatus</i>	Composition in <i>A. hybridus</i>
Sodium (Na) (ppm)	636.80±10.2	700.21±40.9
Potassium (K) (%)	3.79±0.4	4.33±0.3
Calcium (Ca) (%)	2.41±0.1	3.90±0.2
Magnesium (Mg) (%)	1.21±0.0	0.93±0.1
Iron (Fe) (ppm)	1035.20±123.2	149.80±11.2
Zinc (Zn) (ppm)	38.08±5.2	54.45±56
Lead (Pb) (ppm)	0.07±0.0	0.02±0.0
Manganese (Mn) (ppm)	1.44±0.1	74.91±8.2
Copper (Cu) (ppm)	1.21±0.0	3.93±0.5

Table 4: Vitamins in *A. caudatus*

Vitamins	Amount in standard (µg/mL)	Amount in <i>A. Caudatus</i> (µg/mL)	Amount in <i>A. hybridus</i> (µg/mL)
Vitamin C	198.63±10.4	5298.95±87.0	4487.08±103.8
Riboflavin	84.263±21	102.068±7.9	30.696±2.3
β-carotene	160.87±12.0	1611.59±56.4	106.58±4.5
Tocopherol	60.77±4.2	1375.46±83.2	646.85±9.8

However, pretreatment of rats with aqueous extract of *A. caudatus* or *A. hybridus* prevents hepatic cells against sodium arsenite-induced damage as observed in the significant ($P < 0.05$) reduction in the activities of the ALP and GGT in comparison with the test control rats. The chemoprotective properties of *A. caudatus* and *A. hybridus* might be related to free radical scavenging activities of the crude extract. The vitamins and minerals in the vegetables [Table 3 and 4] might have played a pivotal role in stabilizing lipid oxidation and exhibit some inhibitory action over sodium arsenite-induced hepatic damage.

It has been suggested that the baseline frequencies of micronucleated cells are usually within the 0.5-2.5 micronuclei/1,000 cells range. Ten cells with multiple micronuclei are rare in healthy subjects but become more common in individuals exposed to radiation or other genotoxic agents.^[16,17] Therefore, the assessment of micronuclei in cells is a promising tool for the study of epithelial carcinogens.^[18] The assay can be used to detect chromosome breakage or mitotic interference, thought to be relevant to carcinogenesis.^[18,19]

In this study, sodium arsenite-treated rats showed an increase in the level of mPCEs in comparison with the negative control rats suggesting an oxidative damage to the DNA. The result of this study confirms the previously reported arsenic induction of micronuclei in animals.^[20] Arsenic toxicity is known to be involved in the reaction leading to an enhanced lipid peroxidation; fluidity and membrane potential which ultimately leads to loss of cellular function and clastogenicity.^[21] Nevertheless, pretreatment of rats with aqueous extract of *A. caudatus* and *A. hybridus* significantly decreased the frequency of mPCEs. The antigenotoxic properties exhibited by these vegetables might be due to the presence of some phytochemicals, vitamins, and minerals as detected in the selected vegetables in this study [Tables 3 and 4]. Furthermore, previous reports have suggested that consumption of polyphenolic compounds from vegetables can inhibit the processes of mutagenesis and carcinogenesis.^[22]

The hematological system is one of the most delicate targets for toxic compounds and a vital index of pathological status in man and animal.^[23] In hematological analysis, there were no differences between the plant extracts treated group and negative control group. This indicates that the administration of the extract might not be able to produce toxic effects on the hematological system. The hematological variables, following NaAsO₂ exposure, induced a significant increase in WBC, LYMPH in this study, and this may be related to arsenic toxicity. However, the plant extract presented some levels of changed on the hematological parameters which indicated various level

protections against sodium arsenite-induced toxicity. In this study, the aqueous crude extracts of *A. caudatus* caused a statistically significant decrease in the levels of WBC and hemoglobin concentration, while significant increase was observed in the percentage of LYMPH and hemoglobin concentration of *A. hybridus*. Hence, this shows that the leaves of this *A. caudatus* may not provide protection against anemia induced by sodium arsenite. For *A. hybridus*, the extract may provide required protection against toxicity induced by sodium arsenite. All other hematological and biochemical analysis values in rat blood samples are considered normal in comparison to the control group. Thus, confirming the administration of *A. hybridus* and *A. caudatus* to the rats prior to sodium arsenite-induced toxicity may reduce the risk of certain hematological diseases.

In this study, *A. hybridus* and *A. caudatus* were found to be rich in certain vitamins and minerals which are known to modulate the toxicity of arsenic and other environmental toxicant. Most of the vital vitamins and minerals known for antioxidant and chemopreventive properties were detected in *A. caudatus* and *A. hybridus* leaf extracts, and their previous medicinal values have been highlighted in previous studies. For instance, beta carotene and tocopherol found in large amount in vegetables, fruits, and medicinal herbs have been reported to prevent sodium arsenite-induced fibrosis in the liver by inhibiting lipid peroxidation, improving antioxidant status, and decreasing the activity of ALP in the serum.^[24] Similarly, vitamin C has been shown to reduce arsenic-mediated ovarian and uterine toxicity.^[25] Vitamin C acts as a direct scavenger of free radicals and suppresses the formation of carcinogen.^[26] β -carotene is an antioxidant and has in this way cancer preventing properties.^[25] Tocopherol is also necessary for the formation of red blood cells and maintenance of muscle and other tissue. It also protects polyunsaturated fatty acids that are essential for the body, vitamin E, the lipid soluble chain-breaking antioxidant scavenges free radicals, and prevents lipid peroxidation, thus stabilizing the cell membrane.^[27]

Consequently, the detection of some known antioxidants (vitamins) and essential micronutrients in these vegetables suggested that they possess many components that helped in their chemopreventive properties as exhibited in the micronucleus, enzymes, and hematological assays as observed in this study. Generally, green leafy vegetables are consumed either in the cooked form or served as salads. It can be seen that these vegetables possess antioxidant components at varying levels. The estimation of vitamins and minerals components with biological antioxidant role is of great importance and may be the components responsible for chemopreventive purpose. These preliminary results suggest that vitamins, elemental components, and aqueous extractable constituents of *A. hybridus* and *A. caudatus*

protect the mutagenic effects of sodium arsenite in the test animals. Previous studies and the results of this study suggest that the chemopreventive activities may be due to the presence of a single mineral and/or vitamin or to their synergistic effect. Hence, future work will be channeled into the isolation of active principle(s) responsible for their chemopreventive potentials.

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