



EFFECTS OF *TELFARIA OCCIDENTALIS* ON THE FORMATION OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES IN MICE BONE MARROW

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ABSTRACT

Chromosomal mutation and oxidative DNA damage have been associated with some arsenic linked human diseases. This study investigated the effects of *T. occidentalis* on the formation of micronucleated polychromatic erythrocytes in laboratory mice. The mice were treated with or without sodium arsenite and/or *T. occidentalis* and the effects were assessed in the bone marrow of mice using micronucleus assay. In addition, the effects on the activity of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) and certain hematological parameters were also evaluated. Certain vitamins were extracted by chemical methods and their presence was detected using high performance liquid chromatography (HPLC) in the leaf extract of *T. occidentalis*. The mice treated with sodium arsenite alone and those administered with sodium arsenite and *T. occidentalis* had micronucleated polychromatic erythrocyte (mPCEs) counts of 21.00 ± 2.00 and 12.67 ± 2.67 respectively. The ALP and GGT concentrations of the mice treated with *T. occidentalis* and sodium arsenite shows about two times decrease than those treated with sodium arsenite alone. Treatment of mice with sodium arsenite and/or *T. occidentalis* revealed varying degree of deviations in the mice hematological granulocytes (GRAN), lymphocytes (LYMPH), haemoglobin (HGB), platelet (PLT), white blood count (WBC) and mean platelet volume (MPV). Ascorbic acid, α -tocopherol, β carotene and riboflavin were shown to be present in appreciable quantity in the leaf extract as compared to the standard compounds. The result shows that *T. occidentalis* was able to suppress the chromosomal damage caused by sodium arsenite (SA)-induced toxicity in mice. Hence, *T. occidentalis* may be used as food supplement for protection against SA induced toxicity.

KEYWORDS: *T. occidentalis* Arsenic; Hepatotoxicity; Oxidative stress; Oxidative damage markers, Vitamins



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1.0 INTRODUCTION

The natural source of human exposure to arsenic occurs through consumption of drinking water sourced from groundwater that contains dissolved inorganic arsenic (Nandi *et al.*, 2006). Human exposure to arsenic through ingestion and inhalation from herbicides, insecticides, rodenticides and preservatives and byproducts of fossil fuels are also potential sources of toxicity (Flora *et al.*, 2004; Bauer and Blodau, 2006; Jana *et al.*, 2006). One of the mechanisms of arsenic toxicity is the increase of cells reactive oxygen species that may cause oxidative DNA damage (Hei, 2001). Chronic exposure to arsenic causes a wide range of toxic effects and an elevated risk of cancer (Meliker *et al.*, 2007). Many studies have shown that vegetables possess valuable effects against oxidative stress related diseases through scavenging free radicals and by enhancing the activities of antioxidant enzymes (Orozco *et al.*, 2003; Gupta and Flora, 2006; Hord *et al.*, 2009). Many of these vegetables have been shown to have strong antioxidant and anticlastogenic potentials. These biological potentials could be explored in the prevention of arsenic toxicity and inhibition of arsenic oxidative chromosomal in human exposure (Gupta and Flora, 2006). *T. occidentalis* is a popular vegetable consumed in West Africa for its nutritive content and medicinal value (Mohammed and Sharif, 2011). *T. occidentalis* leaves are selected in this study because of the previously reported strong antioxidant and therapeutic properties (Ugochukwu and Babady, 2007; Kayode *et al.*, 2011). Previous studies on chromosomal damage have established that an increase in the formation of mPCE is a clear indication of chromosomal damage in experimental animals (Mersch-Sudermann, 2004). Consequently, the chemopreventive effect of *T. occidentalis* leaf extracts on the frequency of mPCE in mouse bone marrow was investigated in this study. In addition, the effects of the administration of *T. occidentalis* leaf extract and sodium arsenite on some haematological variables, alkaline phosphatase and gamma glutamyl transferase

were assessed. Certain vitamins in *T. occidentalis* leaf extract were extracted and identified.

2.0 MATERIALS AND METHODS

2.1 Plant material and preparation of aqueous extract

Leaves of *T. occidentalis* were collected from Ladoke Akintola University of Technology (LAUTECH) farm Ogbomoso, Nigeria. The collected materials were authenticated and the voucher specimens (LHO 230) were stored in the Department of Pure and Applied Biology herbarium at LAUTECH. The plant materials were washed with water to remove dirt and were air-dried in the laboratory for three weeks. The dried plant was then ground into powder form, using a blender. The aqueous extracts were prepared by maceration; 100g of the dry powdered plant materials was placed in 500mL of distilled water at room temperature for 72 hours. The extracts were filtered through Whatman filter paper and concentrated using a freeze dryer.

2.2 Experimental protocol

The mice were grouped into three of five mice each. Group A serves as the negative control, group B were administered 2.5mg/kg body weight of sodium arsenite alone (positive control) and group C were administered sodium arsenite and aqueous extract of *T. occidentalis* at 50mg/kg body weight. All the treatment was administered to the mice for fourteen days. The mice had access to pellets and distilled water daily. Twenty four hours after the last treatment with sodium arsenite, the mice were killed by cervical dislocation and the blood was collected directly from the heart. The blood was centrifuged at 3000 rpm for 15 min to obtain serum and used for alkaline phosphatase and gamma glutamyl transferase assays. Gamma glutamyl transferase and alkaline phosphates assay kits were obtained from Randox UK, and the enzyme activity was determined according

to the manufacture's instruction. Clastogenic effects were evaluated in the mouse bone marrow using the micronucleus assay. Bone marrow from the femurs was used for smear preparation on clean slides. The slides were fixed, air-dried, and pre-treated with May-Grunewald solution and subsequently stained with Giemsa solution. The slides were scored for the presence of micronucleated polychromatic erythrocytes (mPCEs) by using a light microscope and a tally counter.

2.3 Extraction and HPLC analyses of vitamins in *T. occidentalis*

The vitamins were extracted according to the method of AOAC International, (1990) and the modified method of Abdulnabi *et al.*, (1997). The extracts obtained were further analyzed using Agilent Technology 1200 series reverse chromatography High Performance Liquid Chromatography system and monitored at 254 nm. The mobile phase composed of 0.015% formic acid buffer and methanol/acetonitrile in the ratio 17:83. The flow rate was 0.2 mL/min, injection volume was 20 μ L.

2.4 Statistical analysis

The values obtained in this study were expressed as mean \pm standard deviation error of the mean (SEM) and analyzed with the one-way analysis of variance (ANOVA). The difference between groups was considered at significant $p < 0.05$ levels.

3.0 RESULTS

3.1 Number of micronucleated polychromatic erythrocytes in mice treated with extract and sodium arsenite

Fig. 1 shows the frequency of mPCEs per 1000 polychromatic erythrocytes in mice treated with extract and sodium arsenite. Mice treated with sodium arsenite alone noticeably induced the formation of mPCEs in the mice bone marrow cells in comparison with the negative control group (Fig. 1). Administration of aqueous extract of *T. occidentalis* significantly reduced ($p < 0.05$) the number of mPCEs in comparison with the sodium arsenite treated mice.

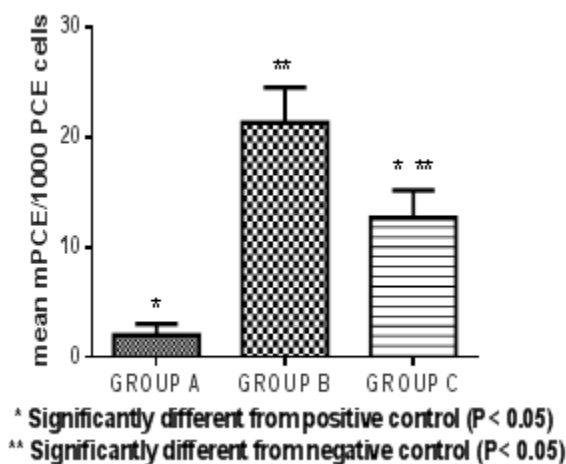


Figure 1
Frequency of micronucleated polychromatic erythrocytes in mice treated with extract and sodium arsenite

Group A (negative control): mice administered with distilled water alone
 Group B (positive control): mice treated with sodium arsenite alone.
 Group C: mice administered leaf extract of *T. occidentalis* and sodium arsenite

3.2. GGT and ALT activities of mice treated with the extract of *T. occidentalis* and sodium arsenite

The Fig. 2 and 3 show the serum activity of the GGT and ALP after administration of aqueous extract of *T. occidentalis* and sodium arsenite. Sodium arsenite alone significantly increased the activity of GGT and ALP in comparison with the negative control group. However, *T. occidentalis* when administered with sodium arsenite decreases the concentration of ALP and GGT in about two times the value of positive control.

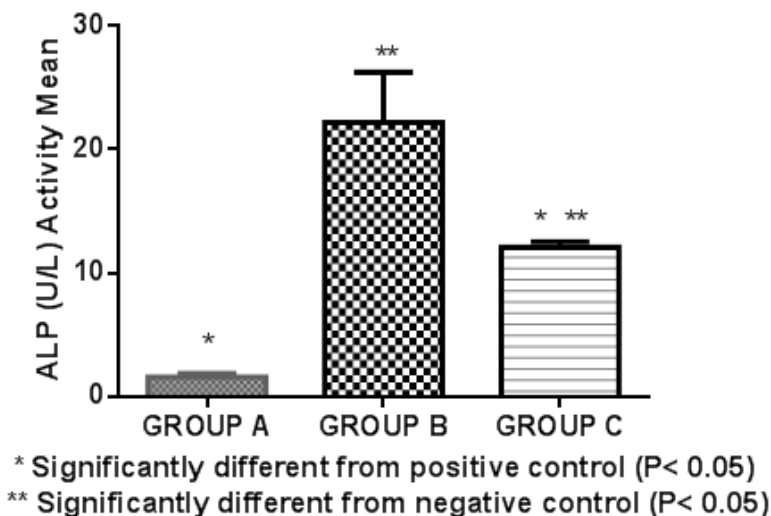


Figure 2

Activity of the ALP in mice treated with extract and sodium arsenite

Group A (negative control): mice administered with distilled water alone
 Group B (positive control): mice administered distilled water for 14 days and sodium arsenite
 Group C: mice administered leaf extract of *T. occidentalis* and dose of sodium arsenite

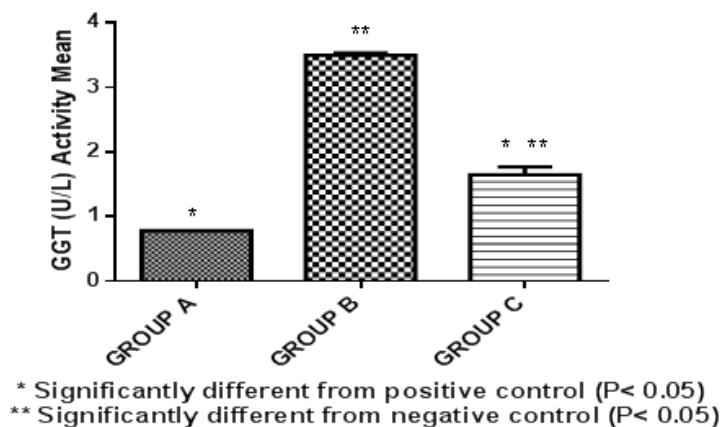


Figure 3

GGT concentration in rat treated with extract and sodium arsenite.

Group A (negative control): mice administered with distilled water alone
 Group B (positive control): mice treated with sodium arsenite alone.
 Group C: mice administered leaf extract of *T. occidentalis* and sodium arsenite

3.3. Effects of *T. occidentalis* on NaAsO₂ induced changes in certain hematological variables in mice

Significant changes were observed in some hematological variables in the mice after exposure to As especially in WBC, PDW, PLT, as well as a rise in HGB concentration. The increase in value of most of the haematological parameters was comparatively reduced in mice treated with the extract of *T. occidentalis* when compared to the positive control mice (Fig. 4).

3.4 Determination of vitamins

Vitamin C (ascorbic acid) had the highest composition in the leaf of *T. occidentalis* followed by vitamin E (alpha tocopherol). Vitamin A (β carotene) and vitamin B₂ (riboflavin) were present in the moderate composition as compared with the standard (Fig. 4).

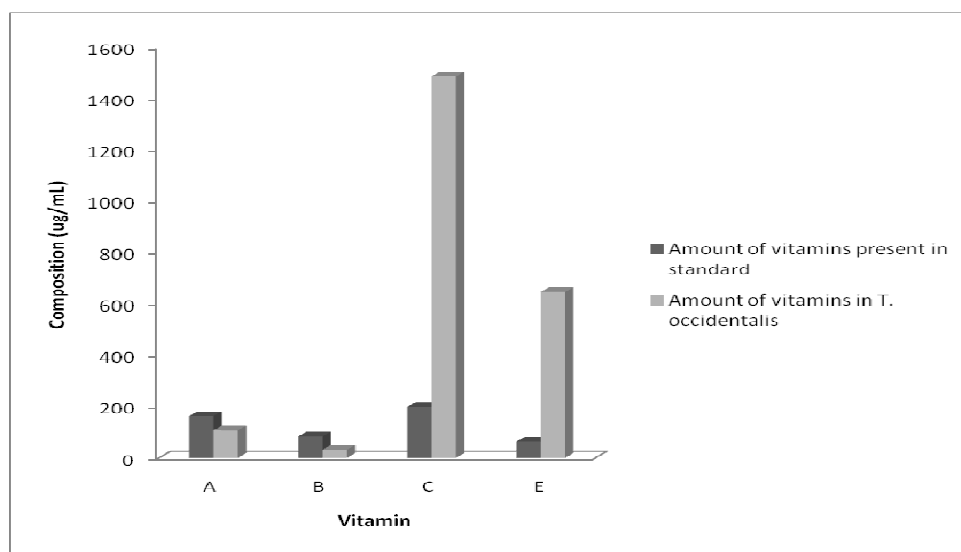


Figure 4
Composition ($\mu\text{g/mL}$) of vitamins in the *T. occidentalis* leaf extract as compared with the standard

4.0 DISCUSSION

Exposure to arsenic has been linked to diverse effects in both experimental animals and humans (Longnecker *et al.*, 2001). Arsenic intoxication in experimental animals has been associated with hepatic tumours (Kitchin, 2001), increased levels of liver enzymes, (Kapaj *et al.*, 2006) as well as with severe metabolic disorders such as diabetes in humans (Longnecker *et al.*, 2001). According to the International Agency for Research on Cancer, there is adequate evidence for the carcinogenicity of arsenic compounds in animals and human (Pershagen *et al.*, 1984). Significantly elevated standard mortality from cancer of the bladder, lung, liver, kidney, skin

and colon were found in the population living in an area of Taiwan, China and some part of Africa where arsenic contamination of the water supply was endemic (Azcue and Nriagu, 1995; Meliker *et al.*, 2007; Asaolu and Asaolu, 2010). Chemoprevention of inadvertent exposure to arsenic poisoning in human especially in some rural areas of the world may be an imperative solution to curb consequential arsenic chromosomal damage in human. Based on the traditional and medicinal uses of *T. occidentalis* as antioxidants and antitumour agent, this study assessed the chemopreventive potentials of the aqueous extract of *T. occidentalis* against SA-induced toxicity in mice. Increased GGT and

ALP activity in serum has been associated with hepatotoxicity (Duthie *et al.*, 1996), chromosomal aberrations, (Edenharder *et al.*, 2006) and oxidative stress in cells (Lee *et al.*, 2004). In this study, the extract of *T. occidentalis* exhibited anticlastogenic properties by reducing SA-induced micronuclei formation in polychromatic erythrocytes in the bone marrow. Consequently, the aqueous extract of *T. occidentalis* has shown the potential of reducing the effect of arsenic toxicity in mice. This study demonstrated that the administration of the leaf extract of *T. occidentalis* to mice may reduce the tendency of chromosomal damage and hepatotoxicity in the treated animals. Dietary antioxidants in the form of nutrients appear to be of great importance in controlling damage by free radicals. In this study, β -carotene, riboflavin, ascorbic acid and α -tocopherol were found to be present in the *T. occidentalis* leaves. These vitamins are known to act as antioxidants, thereby contributing to the hepatoprotective and anticlastogenic effects of the leaf extract of *T. occidentalis*. Vitamins have diverse biochemical functions and the largest number of vitamin functions as precursors for enzyme cofactors. Previous studies have shown that vitamins protect the

DNA from oxidative damage caused and radiation (Uwaegbule, 1989; Berger, 2005). Evidence suggests that vitamin C plays an important role in the human immune system by protecting various types of cells, such as phagocytes, lymphocytes and neutrophils, within the immune system (Collins *et al.*, 2001). While the defined mechanisms underlying the antioxidant effects of the vitamins against sodium arsenite induced toxicities have yet to be identified, previous research reported that consumption of vitamins in the diet can significantly supplement the oxidative defense system. Thus, the antioxidants in dietary vegetable would play a major role in the treatment of toxic effect of arsenic. Hence, the presence of vitamins in the extract of *T. occidentalis* assisted in counteracting the deleterious effect of SA-mediated toxicity. The reduction in the frequency of mPCEs and enzyme concentrations after administration of *T. occidentalis* may be attributed to its antioxidant activities that help in scavenging ROS generated from sodium arsenite metabolism. These results demonstrate that *T. occidentalis* leaf extract can be considered as a useful food which effectively inhibits many of the adverse effects of arsenic intoxication.

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