

# Methanol Extract of *Adansonia digitata* Leaf Protects Against Sodium Arsenite-induced Toxicities in Male Wistar Rats

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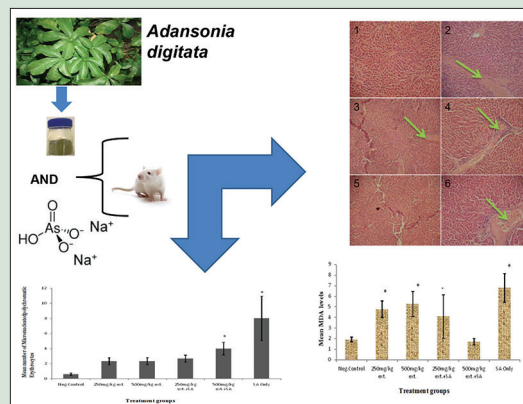
## ABSTRACT

**Background:** Human and animal population exposure to arsenic through the consumption of arsenic contaminated water is rampant in many parts of the world. Protective agents of medicinal plants origin could provide maximum protection against toxicities of various kinds. **Objective:** The protective role of orally administered methanol extract of the leaves of *Adansonia digitata* (MELAD) on sodium arsenite (SA) - induced clastogenicity and hepatotoxicity in male Wistar rats was evaluated. **Materials and Methods:** Thirty male Wistar rats divided into six Groups (1–6) of five animals each were used for the study. Group 1 (negative control) received distilled water and normal diet only, Groups 2–6 received the extract (at 250 or 500 mg/kg body weight) and/or SA at 2.5 mg/kg body weight. **Results:** There was statistically significant ( $P < 0.05$ ) increase in the number of micronucleated polychromatic erythrocytes and lipid peroxidation in the SA group as compared with the negative control and treated groups. Administration of the extract reduced the effects of SA on the above parameters. Activities of serum alanine and aspartate aminotransferases did not show statistically significant effects; however, the histological analyses revealed periportal cellular infiltration by mononuclear cells, whereas the MELAD treated groups show mild cellular infiltration and mild portal congestion. **Conclusions:** MELAD protect against SA-induced toxicities in rats, and it may offer protection in circumstances of co-exposure and cases of arsenicosis.

**Key words:** *Adansonia digitata*, alanine aminotransferase, aspartate aminotransferase, number of micronucleated polychromatic erythrocytes

## SUMMARY

- MELAD extract significantly reduce the lipid peroxidation induced by sodium arsenite in the liver of rats.
- MELAD did not show profound effects on the activities of serum alanine (ALT) and aspartate (AST) aminotransferases.
- MELAD offered significant protection against sodium arsenite-induced genotoxicity in the micronuclei induction assay.
- In the circumstances of co-exposure to arsenic contamination, MELAD may protect against sodium arsenite-induced toxicities.



**Abbreviations Used:** MELAD: Methanol extract of the leaves of *Adansonia digitata*, SA: Sodium arsenite, nMPCEs: Number of micronucleated polychromatic erythrocytes; ALT: Alanine aminotranferase; AST: Aspartate aminotranferase, TBARS: Thiobarbituric acid reactive substances, TBA: Thiobarbituric acid, MDA: malondialdehyde, Sodium arsenite (NaAsO<sub>2</sub>), IARC: International Agency for Research on Cancer

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## INTRODUCTION

Human and animal population exposure to arsenic through consumption of water is rampant in many parts of the world,<sup>[1]</sup> especially in Africa and Asia. Long-term exposure to inorganic arsenic could lead to chronic arsenic poisoning (arsenicosis) with the attendant side effects such as skin lesions, peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular disease, and cancer.<sup>[2-6]</sup> Other health effects reported include neurological disorders and damaged cognitive development in children.<sup>[7,8]</sup>

It has been suggested that protective agents of medicinal plants origin could provide maximum protection against toxicities of various kinds with practically little or no side effects.<sup>[9]</sup> A number of African plants are traditionally used in different countries during drug- or toxin-induced toxicities.<sup>[10]</sup> *Adansonia digitata* is one of such medicinal plants that have been used for a long time in treating various ailments such as infectious diseases and inflammatory conditions.<sup>[11]</sup>

The leaves, fruit pulp, seeds, and bark fibers have been used traditionally for medicinal and nutritional purposes.<sup>[12,13]</sup> The leaf is eaten as soup condiment and it is said to possess diaphoretic, diuretic, astringent, and antiarrhythmic properties.<sup>[14]</sup>

This study therefore assessed the effects of the methanol extract of the leaf of *A. digitata* (MELAD) on sodium arsenite (SA) - induced toxicities in

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rat. The activities of serum enzymes; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as histological analyses of the liver samples was used to assess hepatotoxicity. Frequency of micronucleated polychromatic erythrocytes (mPCEs) scored in the bone marrow cells of treated and control rats was used for the assessment of genotoxic/antigenotoxic effect of SA/or the extract. Lipid peroxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARS) produced.

## MATERIALS AND METHODS

### Reagents and kits

SA (NaAsO<sub>2</sub>; BDH chemicals Ltd., Poole, England) was dissolved in distilled water and administered at a dose of 2.5 mg/kg body weight corresponding to 1/10<sup>th</sup> of the oral LD<sub>50</sub> of the salt.<sup>[15]</sup> Kits for AST and ALT were obtained from Randox Laboratories, UK. All other chemicals and reagents were of analytical grade and were the products of Sigma Chemical Co., St. Louis, MO, USA.

### Collection of leaves and preparation of extract

Fresh leaves of *A. digitata* were collected from Ajibode extension of the University of Ibadan and used for the identification at the Department of Botany, University of Ibadan, Ibadan, Nigeria. It was also authenticated at Forestry Research Institute, Jericho, Ibadan, with FHI No. 109859. Adequate amount of the leaves was harvested and dried at ambient room temperature of 25°C ± 2°C and the extract was prepared. Essentially, the dried leaves were finely milled and extracted with 95% methanol for 72 h at room temperature. The methanol extract was concentrated in a rotary evaporator at a temperature of 40°C; freeze dried and preserved in the refrigerator until required for use.

### Experimental animals and treatments

Thirty male Wister rats weighing between 100 and 140 g and with no sign of diseases or injury were used. They were housed in the Animal House Department of Biochemistry, University of Ibadan, and they were fed with commercial rat feed and water *ad libitum*. They were allowed to acclimatize for 1 week before the commencement of the experiment. The rats were divided into six groups of five animals each based on the treatments received. Group 1 (negative control) were given distilled water and normal diet, Groups 2 and 3 were administered the extract only at 250 mg/kg and 500 mg/kg body weight respectively, Group 4 was given 250 mg/kg body weight of the extract with SA, Group 5 was given 500 mg/kg body weight and SA, while Group 6 was administered SA only. SA was administered at 2.5 mg/kg body weight; all treatments lasted for 14 days, with 12 h light/dark cycle and temperature of 29°C ± 2°C. Experimental animals were treated and sacrificed following "Guiding Principles in the Use of Animals" as spelt out in the "Standard Rules on the Treatment of Experimental Animals" laid down by the University of Ibadan Ethics Committee on the use of experimental animals.

### Assays

#### Alanine aminotransferase and aspartate aminotransferase activities

Serum ALT and AST were assayed according to the method of Reitman and Frankel.<sup>[16]</sup> This method involves the reaction of pyruvate, the product of transamination reaction catalyzed by ALT or AST, with 2, 4-dinitrophenyl hydrazine to produce intensely colored hydrazone read at 546 nm using a spectrophotometer (Spectronic-20).

#### Lipid peroxidation

Lipid peroxidation was determined by measuring the TBARS produced during lipid peroxidation. This method is based on the

reaction between 2-TBA and malondialdehyde (MDA), an end product of lipid peroxide during peroxidation. On heating in acidic pH, the product formed is a pink complex which absorbs maximally at 532 nm. Livers were washed in ice cold 1.15% potassium chloride (KCl) and weighed. They were then homogenized in 4 volumes of homogenizing buffer containing 50 mM Tris-HCl mixed with 1.15% KCl using a Teflon homogenizer. The homogenate was centrifuged at 10,000 g for 20 min. The supernatant which is the postmitochondria fraction was stored at -20°C until when needed for analysis.

An aliquot of 0.4 ml of the sample was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% trichloroacetic acid was added. Then, 0.5 ml of 0.75% TBA was added and placed in a water bath for 45 min at 80°C. This was then cooled in ice and centrifuged at 3000 rpm for 15 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The MDA level was calculated according to the method of Adám-Vizi and Seregi.<sup>[17]</sup>

### Micronucleus assay

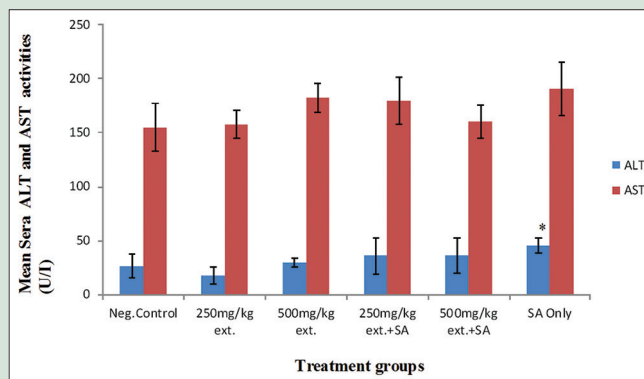
The femurs from each of the animals were removed and bone marrow was aspirated with a syringe. Microscopic slides of the bone marrows were prepared according to Matter and Schmid.<sup>[18]</sup> The slides were then fixed in methanol, air-dried, pretreated with May-Grunwald solution, and air-dried again. The dried slides were stained in 5% Giemsa solution and induced in phosphate buffer for 30 s. Thereafter, it was rinsed in distilled water and air-dried. The slides were mounted and scored under a microscope for mPCEs.

### Histological analysis

Liver sections were fixed in 4% p-formaldehyde and washed in phosphate buffer pH 7.4 at 4°C for 12 h. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with hematoxylin and eosin dye and finally observed under a microscope.

### Statistical analysis

The results were expressed as mean ± standard deviation. Differences between the groups were analyzed by one-way analysis of variance with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, IL, USA, Standard version 18.0.1. *P* < 0.05 was considered statistically significant for differences in mean.



**Figure 1:** Serum alanine and aspartate aminotransferase activities in the sera of rats after treatment with *Adansonia digitata* leaf extract and/or sodium arsenite. Data are expressed as mean ± standard deviation (*n* = 5). The star on bar within group 6 could be defined as \* = significantly different from negative control group (1)



## RESULTS

### Effect of methanol extract of the leaves of *Adansonia digitata* on sodium arsenite induced hepatotoxicity

Serum ALT activity in Group 6 (SA only) was higher when compared with the negative control [Figure 1]. Administration of the extract alone at both 250 and 500 mg/kg body weight did not produce any significant increase in the activities of the enzyme as compared with the negative control. However, co-administration of extract and SA reduced the activities of the enzyme in the sera when compared with the group given SA only.

Moreover, SA increased the activities of AST in the sera of rats when compared with the negative control. Administration of the extract alone at 250 mg/kg body weight produced the same enzyme activity as the control, while administration of the extract at 500 mg/kg body weight gave an enzyme activity close to that of SA only. Co-administration of the extract and SA at 250 mg/kg body weight produced a slightly lower enzyme AST activity when compared with the SA only group, while co-administration of SA and the extract at 500 mg/kg body weight produced enzyme activity similar to that of the control [Figure 1].

Histopathology analysis presented a clearer description of the hepatotoxicity of SA and the hepatoprotective role of the extract. There was a reflection of normal hepatocytes in negative control group and extract treated group at 500 mg/kg body weight while in the positive control group (SA only), there was a periportal cellular infiltration by

mononuclear cells. The extract alone treated group at 250 mg/kg and those co-treated with SA had mild periportal cellular infiltration by mononuclear cells [Figure 2].

### Antigenotoxic effect of methanol extract of the leaves of *Adansonia digitata* in sodium arsenite treated rats

Administration of SA to animals in Group 6 significantly ( $P < 0.05$ ) increased the frequency of mPCEs in the bone marrow of rats by about 15 folds when compared with the control while administration of the extract alone at 250 and 500 mg/kg body weight gave an increase in the frequency of mPCEs but not comparable with that of the toxicant [Figure 3].

Co-administration of the extract and SA at 250 mg/kg body weight gave a sharp and statistically significant ( $P < 0.05$ ) decrease in the frequency of mPCEs by about 3 folds, when compared with SA only group, while co-administration of the extract at 500 mg/kg body weight also gave a statistically significant ( $P < 0.05$ ) decrease in the frequency of mPCEs when compared with SA only group by about 2 folds [Figure 3].

### Effect of methanol extract of the leaves of *Adansonia digitata* and/or sodium arsenite on lipid peroxidation

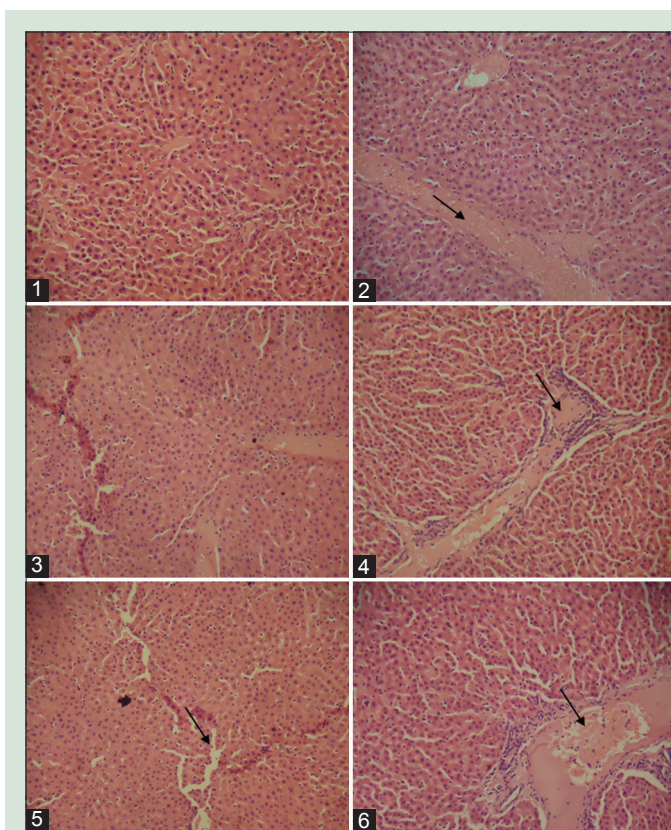
Administration of SA significantly ( $P < 0.05$ ) increased lipid peroxidation in the liver of rats by over 3 folds when compared with the negative control, administration of the extract alone at both doses produced higher levels of lipid peroxidation but not as high as that of the toxicant. On the other hand, co-administration of the extract and SA at 500 mg/kg body weight significantly ( $P < 0.05$ ) reduced lipid peroxidation to normal, also at 250 mg/kg, there was a statistically significant ( $P < 0.05$ ) reduction in lipid peroxidation but not as sharp as that of the 500 mg/kg body weight [Figure 4].

## DISCUSSION

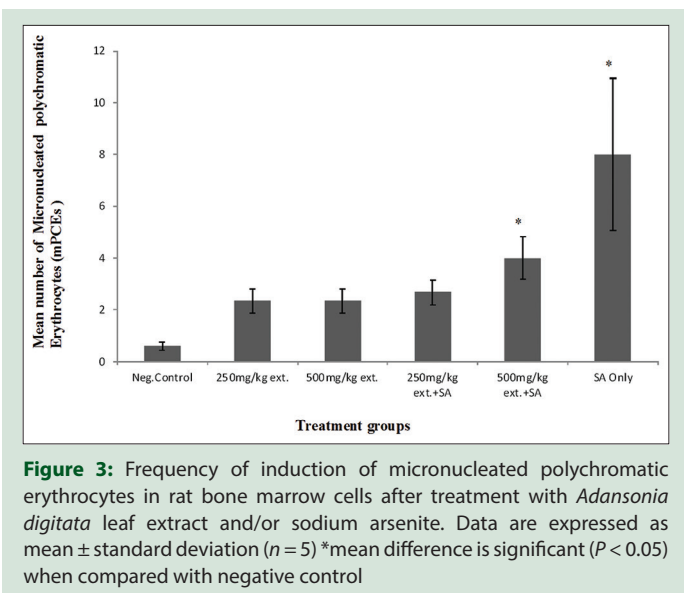
The carcinogenic status of SA ( $\text{NaAsO}_2$ ) is well established and this has also been evaluated by the International Agency for Research on Cancer.<sup>[19-21]</sup> Epidemiological investigations demonstrate that long-term exposure to  $\text{NaAsO}_2$  via the consumption of contaminated water leads to different types of cancer in skin, lung, liver, kidney, and bladder.<sup>[22]</sup> Oxidative stress is a relatively new and widely recognized theory of  $\text{NaAsO}_2$  carcinogenesis.<sup>[23]</sup> Supplementation with antioxidants such as retinol, Vitamin C and E etc., may help people from the developing countries to mop up free radicals and thereby combat oxidative stress and cancers that emanate due to oxidative stress. Overtime, diverse medicinal plants have shown high content of antioxidants.

In this study, we assessed the hepatoprotective, lipid peroxidation and antigenotoxic activities of the MELAD by comparing the levels of serum aminotransaminases, AST, and ALT in the treated groups and control as an index for hepatotoxicity. AST and ALT are found primarily in the liver, also in the heart, kidney, and muscles. When the liver is injured or inflamed after exposure to various forms of toxic substances, the level of ALT and AST in the blood is usually increased. The serum activities of these enzymes are directly related to the extent of the tissue damage.

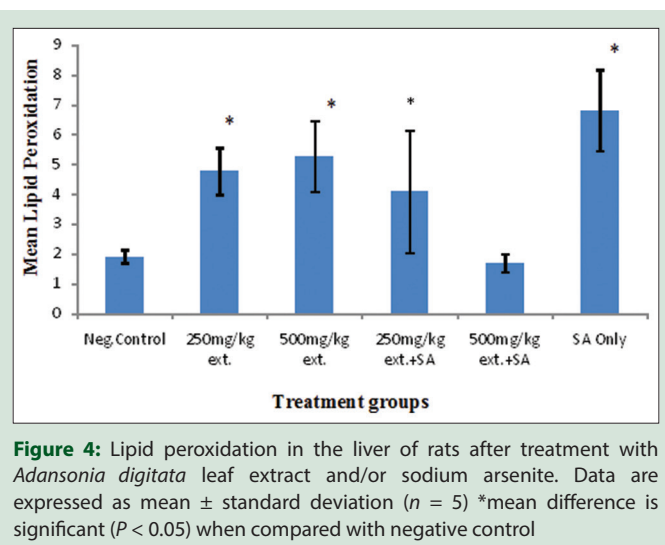
Serum ALT activity in the group administered SA was higher when compared with the negative control. Administration of the extract alone at both 250 and 500 mg/kg body weight did not produce any statistically significant increase in the activities of the enzyme as compared with the negative control, suggesting the safety of the extract. However, co-administration of extract and SA reduced the activities of the enzyme



**Figure 2:** Photomicrographs of liver histopathology (×400) 1: Normal hepatocytes, no visible lesion, 2: Mild periportal cellular infiltration by mononuclear cells. 3: Normal hepatocytes 4: Mild portal congestion and moderate portal cellular infiltration by mononuclear cells. 5: Mild portal congestion and extensive portal cellular infiltration by mononuclear cells. 6: Periportal cellular infiltration by mononuclear cells



**Figure 3:** Frequency of induction of micronucleated polychromatic erythrocytes in rat bone marrow cells after treatment with *Adansonia digitata* leaf extract and/or sodium arsenite. Data are expressed as mean  $\pm$  standard deviation ( $n = 5$ ) \*mean difference is significant ( $P < 0.05$ ) when compared with negative control



**Figure 4:** Lipid peroxidation in the liver of rats after treatment with *Adansonia digitata* leaf extract and/or sodium arsenite. Data are expressed as mean  $\pm$  standard deviation ( $n = 5$ ) \*mean difference is significant ( $P < 0.05$ ) when compared with negative control

in the sera when compared with the group given SA only, this show that the extract has ameliorative properties on SA-induced hepatotoxicity.

Moreover, SA increased the activities of AST in the sera of rats when compared with the negative control. Administration of the extract alone at 250 mg/kg body weight produced the same enzyme activity as the control, while administration of the extract at 500 mg/kg body weight gave an enzyme activity close to that of SA only, this is pointing toward possible toxicity of the extract at a higher dose. Co-administration of the extract and SA at 250 mg/kg body weight produced a slightly lowered AST activity when compared with the SA only group, while co-administration of SA and the extract at 500 mg/kg body weight produced enzyme activity similar to that of the control.

Histopathology analysis presented a clearer description of the hepatotoxicity of SA and the hepatoprotective role of the extract. There was a reflection of normal hepatocytes in negative control group and extract treated group at 500 mg/kg body weight, whereas in the positive control group, there was a periportal cellular infiltration by mononuclear cells. The extract alone treated group at 250 mg/kg and those co-treated with SA had mild periportal cellular infiltration by mononuclear cells.

Findings from the liver histological analyses support the observations made with the enzyme markers. There was a reflection of normal hepatocytes in negative control group and extract treated group at 500 mg/kg body weight, whereas in the positive control group, there was a periportal cellular infiltration by mononuclear cells. The group given the extract alone at 250 mg/kg and those co-treated with SA had mild periportal cellular infiltration by mononuclear cells.

SA has been shown to induce the formation of mPCEs in experimental animals and consequently therefore a biomarker for clastogenicity.<sup>[19]</sup> The result obtained in this study is in line with the earlier observations and those from our laboratory.<sup>[20,21,24,25]</sup> SA significantly ( $P < 0.05$ ) induced micronuclei formation by about 15 folds when compared with the control while administration of the extract at 250 mg/kg body weight with SA brought about significant ( $P < 0.05$ ) reduction in the frequency by about three folds, when compared with the positive control (Group 6). The reductions in the frequency of micronuclei suggest the anticlastogenic effect of the extract.

SA significantly ( $P < 0.05$ ) increased lipid peroxidation in the liver of the treated rats by over 3 folds as compared with the negative control, on the

other hand, the extract at the two doses significantly ( $P < 0.05$ ) reduced the lipid peroxidation to about the level observed for the negative control.

## CONCLUSIONS

Taken together, the MELAD has the potential to mitigate or remediate toxicities resulting from SA intoxication in rats, and it may offer protection in circumstances of co-exposure and cases of arsenicosis. However, further work is required to identify the active principle.

## Financial support and sponsorship

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## Conflicts of interest

There are no conflicts of interest.

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