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Hepatotoxicity and Genotoxicity of Sodium Arsenite and Cyclophosphamide in Rats: Protective Effects of Aqueous Extract of *Adansonia digitata* L. Fruit Pulp

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAO designed the study. Authors MAG, AMA and APO wrote the first draft of the manuscript. Authors AMA and APO performed the statistical analysis. Author AMA managed the literature search. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the protective effects of the aqueous extract of the fruit pulp of *Adansonia digitata* (AEFAD) in sodium arsenite (SA) and cyclophosphamide (CP) induced hepatotoxicity and clastogenicity in rats.
Study Design/Methodology: Fifty four male Wistar rats were distributed into nine groups (A-I) of six animals each. Group A received distilled water and normal diet, Groups B received SA at 2.5 mg/kg body weight, Group C received CP at 10 mg/kg body weight, Groups D –I received the extract alone and with SA or CP.
Results: A statistically significant (P <0.05) higher levels of: mean γGT, ALT and AST activities, number of micronucleated polychromatic erythrocytes (nMPCEs) scored in the bone marrow cells,

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proliferation of hepatic cells and lipid peroxidation were observed in rats exposed to (SA) or (CP) as compared with the control. Treatment with AEFAD along with SA or CP significantly (P <0.05) reduced the effects of the toxins on the above indices. Observations made with histological analysis of the liver sections revealed lesions ranging from general congestion, mild periportal cellular infiltration and hepatic necrosis to severe congestion in the treated groups.
Conclusion: Findings from this study therefore reaffirmed the hepatoxicity and clastogenicity of SA and CP and revealed that AEFAD can ameliorate these toxicities in rats.

Keywords: Adansonia digitata; clastogenicity; cyclophosphamide; sodium arsenite.

1. INTRODUCTION

Arsenite is a proven human and animal carcinogen. Exposure to arsenic, especially through the intake of polluted well water, has been associated with lesions of the skin and certain forms of cancers [1]. In addition, other non-cancer health effects that include neurological disorders and damaged cognitive development in children have been reported [2,3]. On the other hand, cyclophosphamide (CP) is an alkylating agent that is widely prescribed and used in cancer chemotherapy [4]. It has been reported to be cytotoxic [5]. The injury to normal tissues and the numerous side effects is the major limitation to the use of CP in cancer therapy [6,7]. Scientists are working to discover potent compounds with little or no side effects to combat factors that influence carcinogenesis. However, diverse medicinal plants have been used safely without known side effects. Higher plants are now regarded as living chemical factories, providing a vast number of chemical substances that show various biological activities [8,9]. African continent specifically is blessed with a wide array of medicinal plants. *Adansonia digitata* L (baobab) is an example; forming an important part of herbal preparations in Nigeria. Locally it is called Ose or Igi-ose by the Yorubas, kukaa by the Hausas, both are Nigerian tribes. It belongs to the family Bombacacea and the genus Adansonia [10,11]. *Adansonia digitata* L (Baobab) is known in many African countries as the "tree of life" due to its many traditional, medicinal and nutritional uses [12]. The leaves, bark and fruits of the tree are employed traditionally in several African countries as food stuffs and medicinal uses [13] Ramadan et al. [14] reported the anti-inflammatory and antipyretic activities of the fruit pulp of *Adansonia digitata,* also it is used to stimulate or counteract immune responses [14,15]. The profound antioxidant capacity of *Adansonia digitata* fruit pulp and leaves have been reported [16]. Fruit pulp extract has been found to have hepatoprotective effect [17]. At present authors are aware that there is no data on the protective effects of the fruit pulp of *Adansonia digitata* on sodium arsenite- or cychlophosphamide- induced toxicities in rats. The aim of the present study was to evaluate the effects of the aqueous extract of the fruit pulp of *Adansonia digitata* on sodium arsenite and cyclophosphamide induced toxicities in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Reagents and Kits

SA ($NaAsO_2$; BDH chemicals Ltd poole England) was administered at 2.5 mg/kg body weight (corresponding to 1/10th of the oral LD_{50} of the salt) [18]. Cyclophosphamide ($C_7H_{15}C_{l2}N_2O_2P.H_2O$) (CyclocelTM 500) CELON Laboratories limited, India, was administered at 10 mg/kg body weights. All other chemicals and reagents were of analytical grade.

2.2 Test Plant Material and Extraction Procedure

Fresh leaves and the fruits of the plant were used for its identification/confirmation at the Department of Botany, University of Ibadan and Forestry Research Institute, Jericho, Ibadan Nigeria, with FHI NO. 109859. The fruits were broken open and pulp separated from the seeds with the use of a sieve. Cold extraction was then carried out on the pulp by soaking in distilled water for 72 hrs at room temperature. The extract was filtered and the filtrate concentrated using a rotary evaporator at temperature of 40ºC. The concentrated sample was then freeze-dried and the sample kept for further use.

2.3 Experimental Animals and Treatments

Fifty four Male Wistar albino rats (100 and 150 g) were purchased and housed in the experimental animal house, Department of Biochemistry, University of Ibadan, Nigeria at 29±2ºC,

maintained on water and standard diet *ad libitum* with 12 hrs light/dark cycle. All animals used for the present piece of work were handled in accordance with the University of Ibadan guidelines for the use of experimental animals in research. The albino rats were allowed to acclimatize for one week prior to the commencement of the study.

2.4 Experimental Protocol

The rats were randomly distributed into nine groups of six animals each. Group A served as control and were administered distilled water and normal diet only. Group B was treated with SA at 2.5 mg/kg body weight corresponding to 10% of the oral LD_{50} of the salt [18]. Group C was treated with 10 mg/kg body weight (6.25% of the LD_{50}) CP. Group D and E were treated with 200 and 400 mg/kg body weight AEFAD respectively. Group F was treated with SA and 200 mg/kg body weight AEFAD. Group G received SA and 400 mg/kg body weight AEFAD. Group H received CP and 200 mg/kg body weight AEFAD. Group I was treated with CP and 400 mg/kg body weight AEFAD simultaneously. All administration were done daily for 14 days, CP was administered intraperitoneally while other treatments were done orally.

2.5 Liver Function Enzymes Assays

2.5.1 γ-glutamyl transferase activity

γGT was assayed in the serum by using the reconstituted γGT diagnostic reagent following the method of Szasz. [19] This involves the transfer of glutamyl group from a glutamyl peptide (L-γ-glutamyl-p-nitroanilide) to another peptide (glycylglycine), in a reaction catalysed by γGT, thereby yielding a cleavage product (p-nitroaniline). This product absorbs UV at 405 nm thus making a direct kinetic determination of γGT activity possible.

2.5.2 Alanine aminotransferase and aspartate aminotransferase activities

Serum ALT and AST were assayed according to Reitman and Frankel [20] 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).

2.6 Liver Histological Analysis

Liver sections were fixed in 4% p-formaldehyde and washed in phosphate buffer pH 7.4 at 4ºC for 12 hours. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye and finally viewed under a microscope.

2.7 Micronucleus (MN) 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, [21]. The slides were then fixed in methanol, air-dried, pre-treated with May-Grunwald solution and air-dried again. The dried slides were stained in 5% Giemsa solution and induced in phosphate buffer for 30 seconds. Thereafter, it was rinsed in distilled water and air-dried. The slides were mounted and scored under a microscope for micronucleated polychromatic erythrocytes (MPCEs) following standard procedure at X40 magnification.

2.8 Determination of Lipid Peroxidation

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. This method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde: an end product of lipid peroxide during peroxidation.

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 minutes. The supernatant which is the post-mitochondria fraction was stored at -20ºC 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 0.5ml of 30% TCA was added. Then 0.5 ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80ºC. This was then cooled in ice and centrifuged at 3000 rpm for 15 minutes. 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 Adam-Vizi and Seregi [22].

2.9 Cells Analysis

Cells per mm^2 analysis was carried out by counting the numbers of cells on stained slides prepared from the liver under a Nikon light microscope at x40 with the aid of a grid and tally counter.

2.10 Determination of Hematological Parameters

Whole blood examination includes total leukocytes (WBC), hemoglobin (Hb) content, hematocrits (PCV), lymphocytes and Neutrophils. Haematocrits (PCV) were determined immediately after sampling, using a microhaematocrit centrifugation (10,500 ×g for 5 min). Capillary tube was centrifuged for 5 minutes at 10,500 rpm in a micro-haematocrit centrifuge and volume PCV was measured using microhaematocrit reader. Hemoglobin concentrations (mg/dl) were determined by the cyanhaemoglobin method using a wavelength of 540 nm. The blood plasma was obtained by centrifuging the heparinized blood at 4100 ×g for 10 min at 4ºC and the blood cells (erythrocytes and leucocytes) were separated into eppendorf tubes. All blood parameters were performed within 12 h. The parameters were determined as described by Jane [23].

2.11 Statistical Analysis

The results were expressed as mean ± Standard deviation. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 10.0.1. P-values less than 0.05 were considered statistically significant for differences in mean.

3. RESULTS AND DISCUSSION

3.1 Hepatoprotective Effect of Aqueous Extract of the Fruit Pulp of *Adansonia digitata* in Wistar Rats Treated with SA or CP

The protective effect of AEFAD on the rat liver integrity was assessed by evaluating the activities of serum enzymes; γ-glutamyl transferase (γGT), aspartate amino transferase (AST) and alanine amino transferase (ALT) (Table 1). In addition, liver histopathological analysis was carried out on the treated and control groups of experimental rats (Fig. 1). Administration of SA at 2.5 mg/kg body weight resulted in significant (P <0.05) increase in the mean serum AST and ALT values as compared with the negative control group. Also there was more than fourfold increase in the mean γGT activities in the group of animals treated with SA compared with the observed mean γGT value in the negative control group. Administration of CP at 10 mg/kg body weight also resulted in significant increase in the mean serum AST and ALT values, and more than fivefold increase in the observed mean serum γGT activities compared with the observation made in the negative control groups. This portrays higher level of hepatotoxicity of CP.

Simultaneous treatment with AEFAD during exposure to SA or CP resulted in significant reduction in the mean γGT activities as compared with the respective groups treated with each of the two toxins alone.

Table 1. Levels of gamma glutamyltransferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in sera of rats treated with AEFAD, CP and SA

Group	GGT	AST	ALT
A	4.63±0.40	225.00±13.22	25.65±1.58
B	24.32±5.79*	272.00±2.00*	34.05±6.19*
C	26.29±9.60*	252.00±16.00	34.05±7.75*
D	5.79±0.74	225.33±13.45	28.75±3.77
E	12.73±2.31	234.00±17.08	28.00±0.99
F	7.33±0.66	234.66±2.30	19.02±1.87
G	5.79±1.16	265.00±1.73	28.25±0.52
H	9.26±1.15	236.66±3.05	39.45±3.33*
I	13.89±3.47	228.66±17.67	43.80±5.45*

** mean difference is significant (P < 0.05) when compared with group A. Data are expressed as mean ± sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200 mg/kg BW AEFAD, E. 400 mg/kg BW AEFAD, F. SA + 200 mg/kg BW AEFAD, G. SA + 400 mg/kg BW AEFAD, H. CP + 200 mg/kg BW AEFAD, I. CP + 400 mg/kg BW AEFAD. BW = Body weight, SA = Sodium arsenite, CP = Cyclophosphamide, AEFAD = Aqueous extract of the fruit pulp of Adansonia digitata*

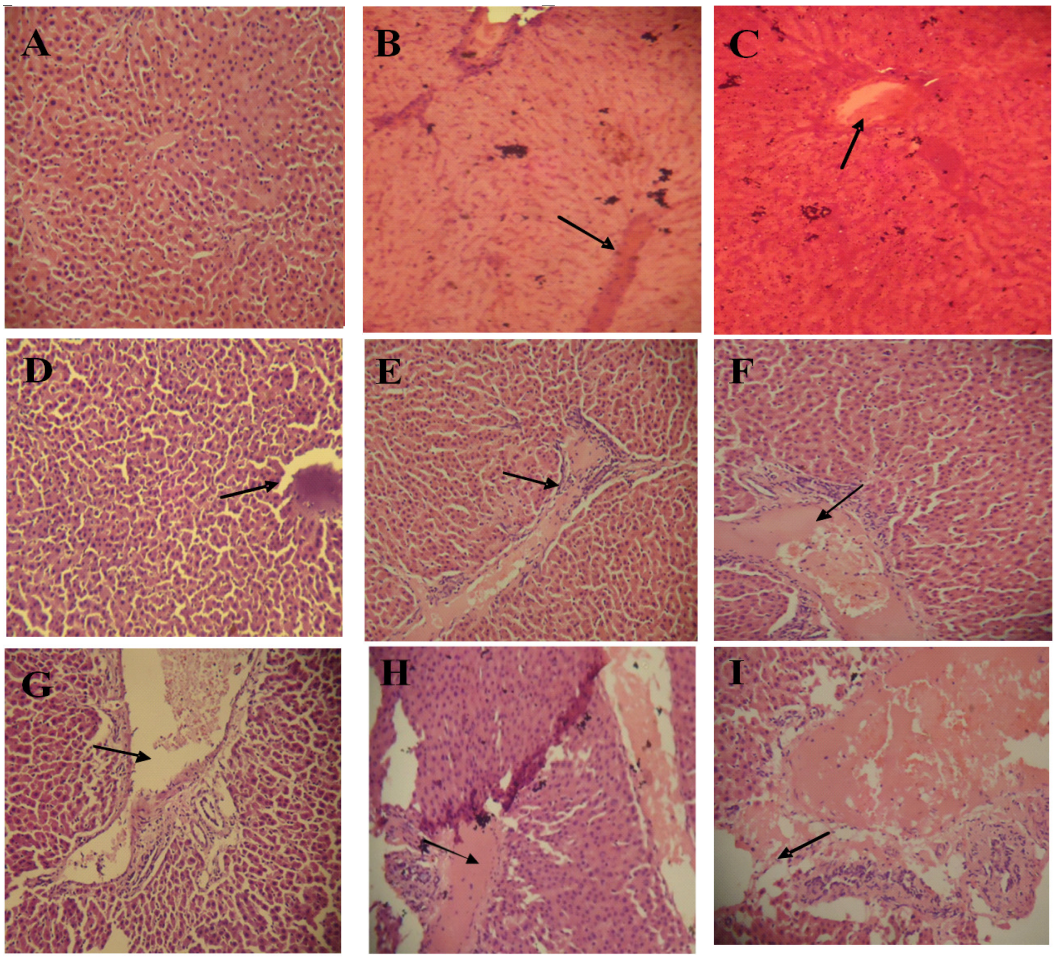

Fig. 1. Photomicrograph of histopathology examination of rat livers x400

A. Negative control, B. SA only, C. CP only, D. 200 mg/kg BW AEFAD, E. 400 mg/kg BW AEFAD, F. SA + 200 mg/kg BW AEFAD, G. SA + 400 mg/kg BW AEFAD, H. CP + 200 mg/kg BW AEFAD, I. CP + 400 mg/kg BW AEFAD. BW = Body weight, SA = Sodium arsenite, CP = Cyclophosphamide, AEFAD = Aqueous extract of the fruit pulp of Adansonia digitata

A No visible lesion, B There is diffuse portal and sinusoidal congestion, C There is diffuse portal and sinusoidal congestion, D There is very mild portal congestion, E There is cellular infiltration by mononuclear cells, F There is a moderate portal and central venous congestion. There is also a mild periportal cellular infiltration by mononuclear cells G There is severe portal congestion, with moderate periportal hepatic necrosis, mild fibroplasia and cellular infiltration by mononuclear cells, H There is moderate portal congestion, and mild periportal hepatic necrosis and cellular infiltration by mononuclear cells, I There is moderate portal congestion, and mild periportal hepatic necrosis and cellular infiltration by mononuclear cells

The results of histopathology examinations also support the trends of the liver function enzyme results. There was no visible lesion in the negative control. Diffuse portal and sinusoidal congestion were observed in SA and CP treated Groups (B and C); very mild periportal cellular infiltration by mononuclear cells was observed in Group D treated with 200 mg/kg body weight AEFAD. At a higher dose of 400 mg/kg body weight of AEFAD, there was central venous congestion (Group E). There is a moderate portal and central venous congestion and a mild periportal cellular infiltration by mononuclear cells in Group F, there is severe portal congestion, with moderate periportal hepatic necrosis, mild fibroplasia and cellular infiltration by mononuclear cells in Group G. There is moderate portal congestion and mild periportal hepatic necrosis and cellular infiltration by mononuclear cells in groups H and I.

3.2 Aqueous Extract of the Fruit Pulp of *Adansonia digitata* Protect against Clastogenicity Induced by SA or CP

We observed that the number of micronucleated polychromatic erythrocytes (nMPCEs) per 1000 PCEs scored in the bone marrow (Fig. 2) is significantly higher in the groups of rats administered SA or CP (Groups B and C) compared with the nMPCEs scored in the control (Group A). The increase in the nMPCEs was reduced close to 80% in the group administered both SA and the 200 mg/kg body weight AEFAD (Group F) and reduced close to 50% in the group administered both CP and 200 mg body weight AEFAD (Group H). In Groups D and E treated with AEFAD alone, there was no significant ($P < 0.05$) increase, in the nMPCEs when compared with the negative control. SA at the dose administered portrays higher clastogenic property than CP, while 200mg/kg body weight AEFAD reduced the clastogenic effect of SA better than that of CP.

3.3 Aqueous Extract of the Fruit Pulp of *Adansonia digitata* Reduces Lipid Peroxidation in Rats Treated with SA and CP

Lipid peroxidation level increased significantly ($P < 0.05$) in the group or rats treated with SA alone (Group B) or CP alone (Group C) when compared with the negative control (Group A) (Fig. 3). There was also increase in malondialdehyde production after SA treatment. The malondialdehyde level observed in the groups treated simultaneously with extract and CP (Groups I and H) reduced significantly ($P < 0.05$). The malondialdehyde level observed in the groups treated simultaneously with SA and AEFAD (Groups F and G) also reduced significantly ($P < 0.05$) when compared with the toxicants groups. It is observed that CP exhibit higher level of lipid peroxidation when compared with SA while the extract reduced the level of lipid peroxidation generated by CP better than that of SA.

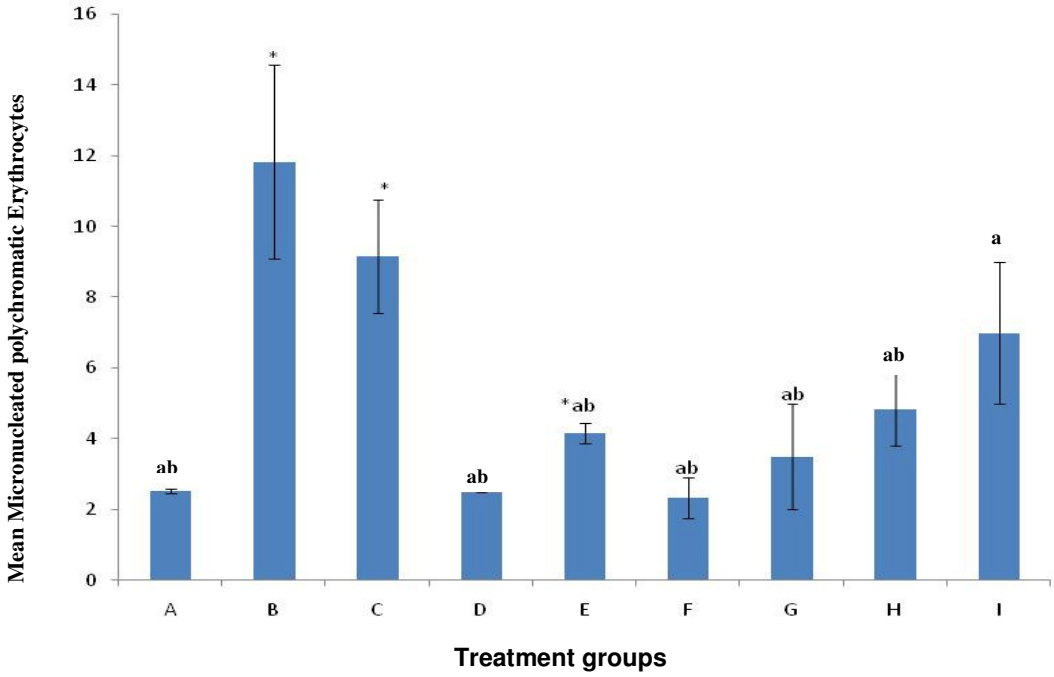

Fig. 2. Number of induction of micronucleated polychromatic erythrocytes (nMPCEs) in rat bone marrow cells after treatment with SA, CP and AEFAD. Data are expressed as mean ± sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200 mg/kg BW AEFAD, E. 400 mg/kg BW AEFAD, F. SA + 200 mg/kg BW AEFAD, G. SA+400 mg/kg BW AEFAD, H. CP + 200 mg/kg BW AEFAD, I. CP + 400 mg/kg BW AEFAD. BW=Body weight, SA = Sodium arsenite, CP = Cyclophosphamide, AEFAD=Aqueous extract of the fruit pulp of *Adansonia digitata*

**mean difference is significant ($P < 0.05$) when compared with group A. [a] mean difference is significant ($P < 0.05$) when compared with group B. [b] mean difference is significant ($P < 0.05$) when compared with group C*

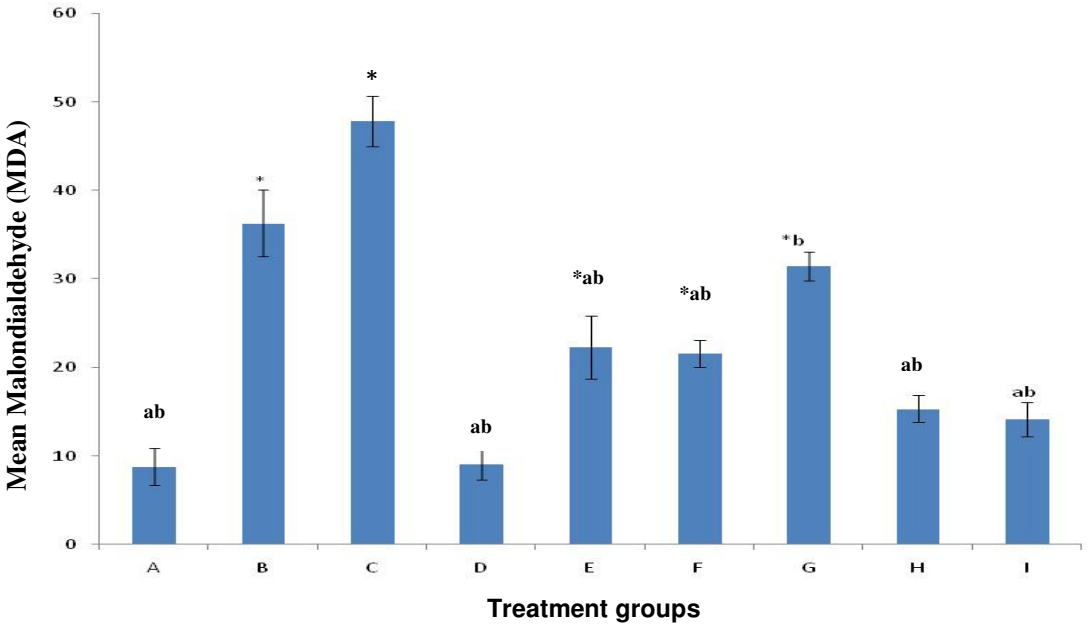

Fig. 3. Effects of sodium arsenite, cyclophosphamide and *Adansonia digitata* fruit pulp extract on Malondialdehyde level in the liver of treated rats and control. Data are expressed as mean ± sd (x 10^{-8}) (n=6): A. Negative control, B. SA only, C. CP only, D. 200 mg/kg BW AEFAD, E. 400 mg/kg BW AEFAD, F. SA + 200 mg/kg BW AEFAD, G. SA + 400 mg/kg BW AEFAD, H. CP + 200 mg/kg BW AEFAD, I. CP + 400 mg/kg BW AEFAD. BW = Body weight, SA= Sodium arsenite, CP = Cyclophosphamide, AEFAD = Aqueous extract of the fruit pulp of *Adansonia digitata*

** mean difference is statistically significant (P <0.05) when compared with group A. [a] mean difference is statistically significant (P <0.05) when compared with group (B). [b] mean difference is statistically significant (P <0.05) when compared with group C*

3.4 Aqueous Extract of the Fruit Pulp of *Adansonia digitata* Modulate Hepatocellular Proliferation Observed in Rats Treated with SA or CP

The result of cell proliferation indicated that SA and CP intoxicated groups (B and C) significantly (*P* <0.05) induced increase in hepatocellular proliferation (Fig. 4), when compared with the negative control Group (A). The group that received 200 mg/kg body weight AEFAD alone has a value almost similar to that of the negative control. There was a slight increase in hepatocellular proliferation in group E which received 400 mg/kg body weight AEFAD but not significant (*P* <0.05) when compared with the intoxicated groups. Groups F, G, H and I which received AEFAD and toxicants all showed reduction in the number of cells per millimeter squared when compared with the intoxicated groups. SA induced higher level of hepatocellular proliferation when compared with CP treated group, in both cases the extract significantly (*P* <0.05) reduced the level of hepatocellular proliferation close to the control, but better at 400 mg/kg body weight with CP.

3.5 Effect of Aqueous Extract of the Fruit Pulp of *Adansonia digitata* on the Haematological Parameters in Rats Treated with SA and CP

The following haematological parameters: Total leukocytes (WBC), hemoglobin (Hb) content, hematocrits (PCV), lymphocytes and Neutrophils, were tested in the treated and control groups of experimental rats. The administration of the extract and exposure to toxicants did not have a statistically significant effect (*P* <0.05) on the values of PCV, lymphocytes, eosinophil and hemoglobin (Table 2); they were neither quantitatively nor qualitatively destroyed. There were no inclusions in the red cells or white cells as observed from the cell morphology. Though the hemoglobin result is not in agreement with the earlier report by Chakraborty et al. [24], that reported a decrease in the hemoglobin level in CP treated rats (Table 2).

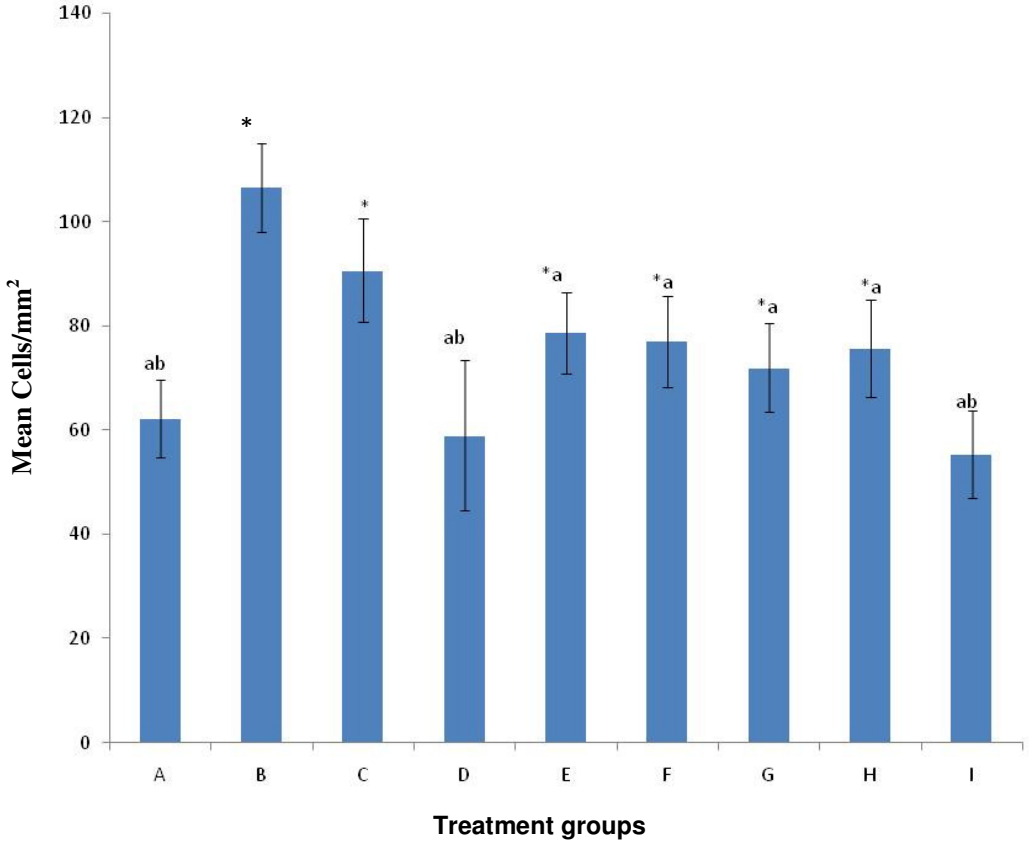

Fig. 4. Effects of sodium arsenite, cyclophosphamide and *Adansonia digitata* fruit pulp extract on cells/mm^2 analysis in the liver of treated rats and control. Data are expressed as mean ± sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200 mg/kg BW AEFAD, E. 400 mg/kg BW AEFAD, F. SA + 200 mg/kg BW AEFAD, G. SA + 400 mg/kg BW AEFAD, H. CP + 200 mg/kg BW AEFAD, I. CP + 400 mg/kg BW AEFAD. BW = Body weight, SA = Sodium arsenite, CP = Cyclophoosphamide, AEFAD = Aqueous extract of the fruit pulp of *Adansonia digitata*

** mean difference is statistically significant (P <0.05) when compared with group A, a mean difference is statistically significant (P <0.05) when compared with group B, b mean difference is statistically significant (P <0.05) when compared with group C*

3.6 Discussion

Exposure to arsenite especially through the intake of polluted well water has been linked with certain forms of cancers [1,3]. On the other hand CP though widely prescribed and used in cancer chemotherapy, has been reported to be cytotoxic [4,5]. The injury to normal tissues is the major limitation in the use of CP because this gives rise to numerous side effects [6,7]. In the present study we evaluate the protective effects of AEFAD in SA and CP models of hepatoxicity and clastogenicity in male Wistar rats.

Serum AST, ALT and GGT are marker enzymes for assessing liver function integrity [25]. Observation made in this study showed that administration of SA or CP resulted in significant (P <0.05) increase in the mean serum AST and ALT, and more than fourfold increase in γGT activities as compared with negative control group. This observation is consistent with earlier observations on the hepatotoxicity of SA [26,27] and of CP [24]. The increase in the activities of serum enzymes is an indication of induction of hepatotoxicity and oxidative stress in the hepatocytes [28,29]. Treatment with AEFAD upon exposure to SA or CP significantly (P <0.05) reduced the induction of γGT activities by about 3 fold and 2 fold, when compared with SA or CP only treated groups, respectively. This further confirmed the hepatoprotective effect of AEFAD reported by Al-Qarawi et al. [17].

Table 2. Effects of sodium arsenite, cyclophosphamide and *Adansonia digitata* pulp extract on hematological parameters of treated rats and control

Groups	PCV	LYMPHS	WBC	NEUTR	Hb
A	50.33±2.88	81.00±5.00	6275.00±325.00	24.00±1.73	15.52±1.50
B	46.66±3.21	76.33±1.00	6650.00±576.62	21.33±1.15	15.46±1.53
C	42.00±10.39	68.33±7.37	5850.00±832.16	23.66±2.51	15.75±0.07
D	52.33±0.57	66.00±7.21	11050.00±458.25	21.00±3.60	17.68±0.43
E	50.66±2.51	70.00±13.07	6866.67±808.29	21.66±2.51	17.53±0.15
F	48.00±1.00	69.00±6.55	6800.00±304.13	17.33±2.51	16.30±0.65
G	49.00±3.60	80.00±4.35	7033.33±1609.61	14.66±1.15	16.26±1.10
H	47.66±1.52	69.00±3.60	5875.00±725.00	28.33±4.72	16.24±0.92
I	49.66±1.52	50.66±3.05	6733.33±472.58	20.33±2.51	16.57±0.76

Data are expressed as mean ± sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200 mg/kg BW AEFAD, E. 400 mg/kg BW AEFAD, F. SA + 200 mg/kg BW AEFAD, G. SA + 400 mg/kg BW AEFAD, H. CP + 200 mg/kg BW AEFAD, I. CP + 400 mg/kg BW AEFAD. BW = Body weight, SA=Sodium arsenite, CP = Cyclophosphamide, AEFAD=Aqueous extract of the fruit pulp of Adansonia digitata

On the other hands, histopathological analyses of the liver samples corroborate the observations made with the enzyme markers of hepatotoxicity: There were no visible lesions in Group A. Diffuse portal and sinusoidal congestion were observed in SA or CP treated groups (B and C). Very mild periportal cellular infiltration by mononuclear cells was observed in Group D, while 400mg/kg body weight AEFAD produced central venous congestion Group E. There is cellular infiltration by mononuclear cells in Group F. There is a moderate portal and central venous congestion and also a mild periportal cellular infiltration by mononuclear cells in Group G. There is severe portal congestion, with moderate periportal hepatic necrosis, mild fibroplasia and cellular infiltration by mononuclear cells in Group H. There is moderate portal congestion, mild periportal hepatic necrosis and cellular infiltration by mononuclear cells in Group I.

Some studies have implicated SA and CP to be genotoxic [30,31]. The effects of AEFAD on their genotoxicity were assessed using the bone marrow micronucleus assay, a predictive index for evaluating the carcinogenic potential of environmental and occupational chemical exposures [32,33]. Environmental contaminants and clastogens are known to effect the formation of micronuclei by inducing chromosomal breaks and interfering with spindle formation [34,35]. Findings from the present study reaffirmed the clastogenicity of SA and CP. Treatment with SA (group B) or CP (group C) alone, induced significantly (P <0.05) higher nMPCEs in the bone marrow cells of rats when compared with the negative control. In Groups D and E treated with AEFAD alone, there was no significant (P <0.05) increase in nMPCEs scored in the bone marrow cells when compared with the negative control. Co-treatment with AEFAD and SA or CP produced significant (P <0.05) reduction in the nMPCEs scored in the bone marrow cells of rats when compared with groups treated with the respective toxicant alone. This shows that AEFAD is protective against SA and CP induced clastogenicity in rats.

We also assessed the degree of lipid peroxidation (LPO) as a biomarker of oxidative stress [36], in the treated rats. The lipid peroxidation level (measured by malondialdehyde production) significantly (P <0.05) increased in the SA or CP alone treated groups when compared with the negative control suggesting increased generation of reactive oxygen species in the treated animals. Earlier reports have indicated the characteristic induction of lipid peroxidation by SA and CP in the liver, lung and serum of mice and rats with the observation linked with generation of reactive oxygen species [37-45]. The malondialdehyde level observed in the groups treated with AEFAD and SA or CP were significantly (P <0.05) lower when compared with the SA or CP alone treated groups (Group B or C).

In addition, treatment with SA or CP produced significant (P <0.05) increase in hapatocellular proliferation when compared with the control. The induction of hepatocellular proliferation may be an adaptation by the rats to get rid of the toxicants. AEFAD ameliorate the hepatocellular proliferation, producing reduction in the number of cells scored per mm^2 when compared with the intoxicated groups.

The administration of AEFAD and exposure to toxicants did not have significant (P <0.05) effect generally on the values of PCV, lymphocytes,

eosinophil and hemoglobin. They were neither quantitatively nor qualitatively affected. There were no inclusions in the red cells or white cells as observed from the cell morphology. The hemoglobin analysis is not in agreement with the earlier report by Chakraborty et al. [24], who obsereved a decrease in the hemoglobin level in CP treated rats. Our results suggest that AEFAD is able to ameliorate SA- and CP-induced toxicities in male wistar rats.

5. CONCLUSION

Findings from this study therefore reaffirmed the hepatoxicity and clastogenicity of SA and CP and revealed that AEFAD can ameliorate these toxicities in rats. Moderate consumption of AEFAD may play an important role in human hepatoprotection and resistance to liver damage. AEFAD supplementation may possibly influence the response to chemotherapy and ameliorate/ reduce development of adverse side effects that result from the treatment of cancer with CP in patients.

CONSENT

It is not applicable for this study.

ETHICAL APPROVAL

All animal used for this work were handled in accordance with the University of Ibadan guidelines for the use of experimental animals in research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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