

Original Research Article

Toxicity of Sodium arsenite and Cyclophosphamide in Rats: Protective Effects of Aqueous Extract of *Adansonia digitata* L Fruit Pulp.

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:** Sixty six male Wistar rats were distributed into eleven groups (A-K) of six animals each. Group A received distilled water and normal diet, Groups B received SA at 2.5mg/kg body weight, Group C received CP at 10mg/kg body weight, Groups D –I received the extract alone and with SA or CP. Groups J and K received SA and CP for 14 days and without treatment for another 14 days. **Results:** A statistically significant ($P < 0.05$) higher levels of: mean γ GT, ALT and AST activities, number of micronucleated polychromatic erythrocytes (nMPCEs) scored in the bone marrow cells, 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 confirmed the hepatotoxicity 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 Bombacaceae 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 *Adansonia digitata* 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 has been reported [16]. Fruit pulp extract have been found to have hepatoprotective effects [17]. As the present authors are aware that there is no data on the protective effects of the fruit pulp of *Adansonia digitata* on sodium arsenite- or cyclophosphamide- 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* in sodium arsenite and cyclophosphamide induced toxicities in male Wistar rats.]

2. MATERIAL 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 ($\text{C}_7\text{H}_{15}\text{C}_2\text{N}_2\text{O}_2\text{P}\cdot\text{H}_2\text{O}$) (CycloceITM 500) CELON Laboratories limited, India, was administered at 10mg/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 40°C . The concentrated sample was then freeze-dried and the sample kept for further use.

2.3 Experimental animals and treatments

Sixty-six Male Wistar albino rats (100 and 150g) were purchased and housed in the experimental animal house, Department of Biochemistry, University of Ibadan, Nigeria at $29 \pm 2^\circ\text{C}$, maintained on water and standard diet *ad libitum* with 12 hrs light/dark cycle. All animal 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 eleven 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. Group J and K received SA and CP respectively for 14 days and normal diet for another 14 days. All administration were done daily for 14 days, CP was administered intraperitoneally while other treatments were done orally.

2.5 Liver function enzymes assays

γ -glutamyl transferase activity.

γ GT was assayed in the serum by using the reconstituted γ GT 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 γ GT, thereby yielding a cleavage product (p-nitroaniline). This product absorbs UV at 405 nm thus making a direct kinetic determination of γ GT activity possible.

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 4 °C 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. Livers were washed in ice cold 1.15% potassium chloride (KCl) and weighed. They were then homogenized in 4 volumes of homogenizing buffer containing 50mM 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 -20°C until when needed for analysis.

An aliquot of 0.4ml of the sample was mixed with 1.6ml of Tris-KCl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. 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 532nm. The MDA level was calculated according to the method of Adam-Vizi and Seregi [22].

2.9 Cells analysis

Cells per mm² 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 10,500rpm in a micro-haematocrit centrifuge and volume PCV was measured using microhaetocrit 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 4°C and the blood cells (erythrocytes and leucocytes) were separated into eppendorf tubes. All blood parameters were performed within 12h. 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. Results

3.1 Hepatoprotective effect of AEFAD in wistar rats treated with SA or CP

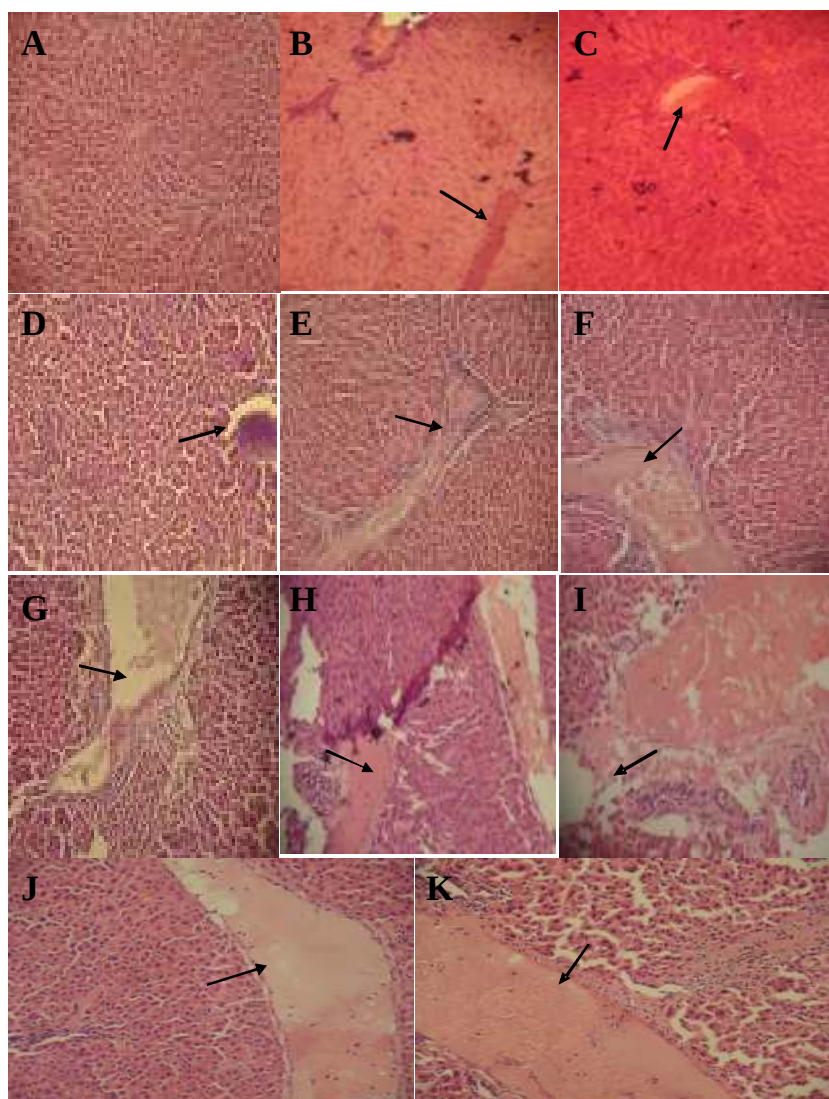
The protective effect of AEFAD on the rat liver integrity was assessed by evaluating the activities of serum enzymes; γ-glutamyl transferase (γGT), aspartate amino transferase (AST) and alanine amino transferase (ALT) (Table 1). In addition, liver histopathological analysis was carried out on the treated and control groups of experimental rats (Figure 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. The results of histopathology examinations also support the trends of the liver function enzyme results. There was no visible lesion in the 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. However, there was a very severe portal congestion, with periportal fibroplasia and cellular infiltration by mononuclear cells in groups J and K.

Table 1.0 Levels of gamma glutamyltransferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in sera of rats treated with AEFAD, CP and SA. Data are expressed as mean $\pm$ sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200mg/kg BW AEFAD, E. 400mg/kg BW AEFAD, F. SA + 200mg/kg BW AEFAD, G. SA + 400mg/kg BW AEFAD, H. CP + 200mg/kg BW AEFAD, I. CP + 400mg/kg BW AEFAD, J. SA for 14 days + normal diet for 14 days, K. CP for 14 days + normal diet for 14 days. BW = Body weight, SA = Sodium arsenite, CP = Cyclophosphamide, AEFAD = Aqueous extract of the fruit pulp of *Adansonia digitata*.

GROUP	GGT	AST	ALT
A	4.63 $\pm$ 0.40	225.00 $\pm$ 13.22	25.65 $\pm$ 1.58
B	24.32 $\pm$ 5.79*	272.00 $\pm$ 2.00*	34.05 $\pm$ 6.19*
C	26.29 $\pm$ 9.60*	252.00 $\pm$ 16.00*	34.05 $\pm$ 7.75*
D	5.79 $\pm$ 0.74	225.33 $\pm$ 13.45	28.75 $\pm$ 3.77
E	12.73 $\pm$ 2.31	234.00 $\pm$ 17.08	28.00 $\pm$ 0.99
F	7.33 $\pm$ 0.66	234.66 $\pm$ 2.30	19.02 $\pm$ 1.87
G	5.79 $\pm$ 1.16	265.00 $\pm$ 1.73	28.25 $\pm$ 0.52
H	9.26 $\pm$ 1.15	236.66 $\pm$ 3.05	39.45 $\pm$ 3.33*
I	13.89 $\pm$ 3.47	228.66 $\pm$ 17.67	43.80 $\pm$ 5.45*
J	5.79 $\pm$ 0.56	72.83 $\pm$ 9.67*	42.10 $\pm$ 8.98*
K	26.05 $\pm$ 0.57*	75.16 $\pm$ 11.02*	42.04 $\pm$ 7.86*

* mean difference is significant ($P < 0.05$) when compared with group A.



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170 Figure 1.0: Photomicrograph of Histopathology Examination of rat Livers x400.

171 A. Negative control, B. SA only, C. CP only, D. 200mg/kg BW AEFAD, E. 400mg/kg BW AEFAD, F. SA +
 172 200mg/kg BW AEFAD, G. SA + 400mg/kg BW AEFAD, H. CP + 200mg/kg BW AEFAD, I. CP +
 173 400mg/kg BW AEFAD, J. SA for 14 days + normal diet for 14 days, K. CP for 14 days + normal diet for 14
 174 days. BW = Body weight, SA = Sodium arsenite, CP = Cyclophosphamide, AEFAD = Aqueous extract of
 175 the fruit pulp of *Adansonia digitata*.

176 A No visible lesion, B There is diffuse portal and sinusoidal congestion, C There is diffuse portal and
 177 sinusoidal congestion, D There is very mild portal congestion, E There is cellular infiltration by
 178 mononuclear cells, F There is a moderate portal and central venous congestion. There is also a mild
 179 periportal cellular infiltration by mononuclear cells G There is severe portal congestion, with moderate
 180 periportal hepatic necrosis, mild fibroplasia and cellular infiltration by mononuclear cells, H There is
 181 moderate portal congestion, and mild periportal hepatic necrosis and cellular infiltration by mononuclear
 182 cells, I There is moderate portal congestion, and mild periportal hepatic necrosis and cellular infiltration
 183 by mononuclear cells, There is a very severe portal congestion, with periportal fibroplasia and cellular
 184 infiltration by mononuclear cells in J and K.

3.2 AEFAD 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 (Figure 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 200mg 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.

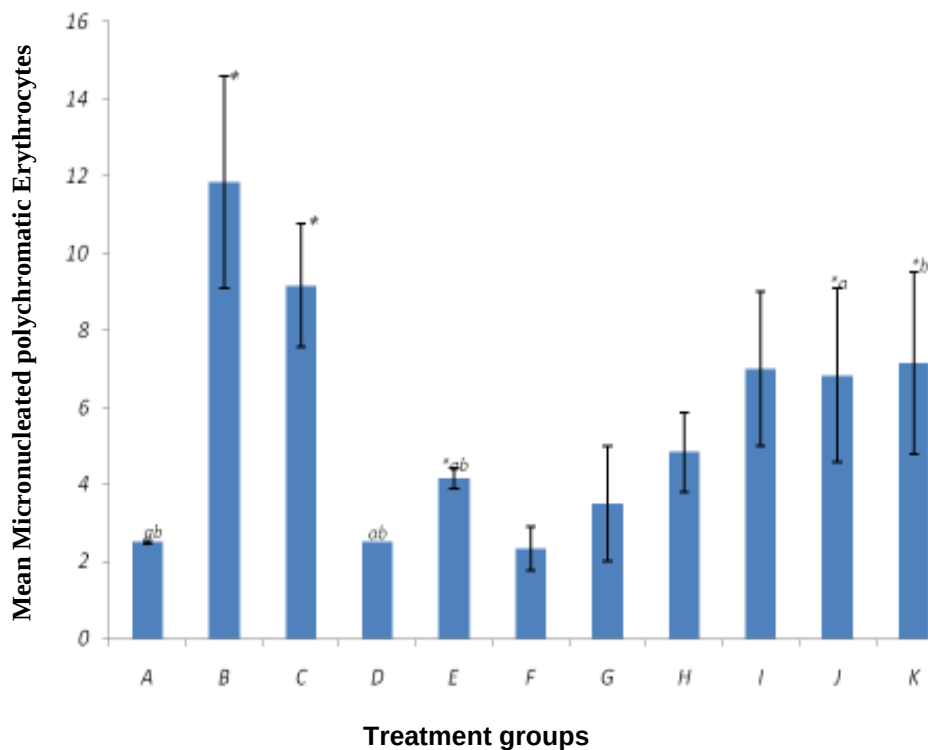


Figure 2.0 Number of induction of micronucleated polychromatic erythrocytes (nMPCEs) in rat bone marrow cells after treatment with SA, CP and AEFAD. Data are expressed as mean $\pm$ sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200mg/kg BW AEFAD, E. 400mg/kg BW AEFAD, F. SA + 200mg/kg BW AEFAD, G. SA+400mg/kg BW AEFAD, H. CP + 200mg/kg BW AEFAD, I. CP + 400mg/kg BW AEFAD, J. SA for 14 days + normal diet for 14 days, K. CP for 14 days + normal diet for 14 days. 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.

3.3 AEFAD 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) (Figure 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 ($P < 0.05$). 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.

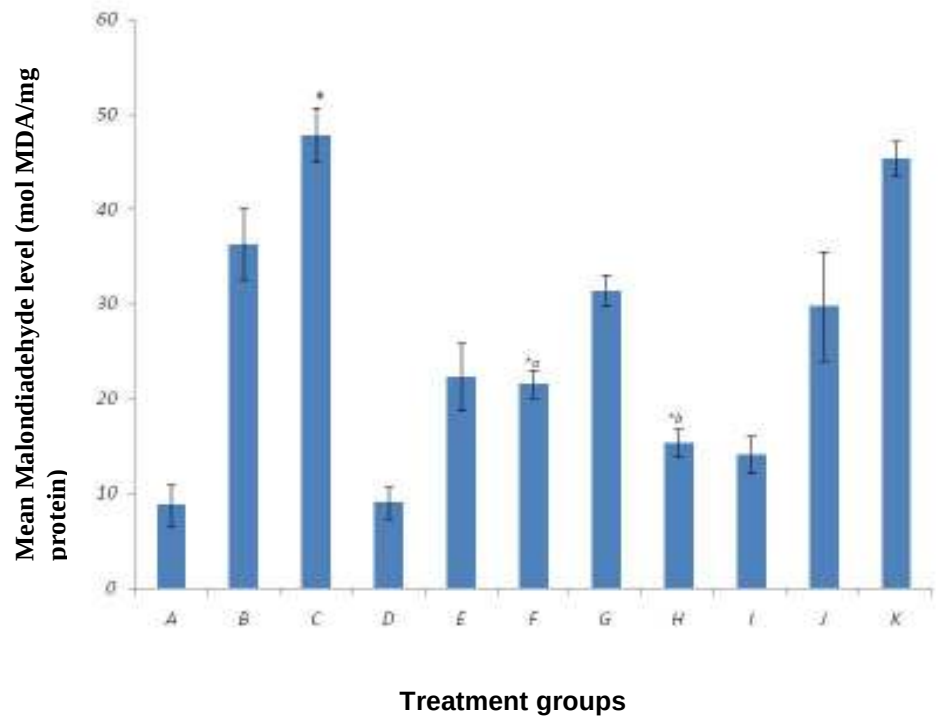


Figure 3.0 Effects of sodium arsenite, cyclophosphamide and *Adansonia digitata* fruit pulp extract on Malondialdehyde level in the liver of treated rats and control. Data are expressed as mean $\pm$ sd ($\times 10^{-8}$) (n=6): A. Negative control, B. SA only, C. CP only, D. 200mg/kg BW AEFAD, E. 400mg/kg BW AEFAD, F. SA + 200mg/kg BW AEFAD, G. SA + 400mg/kg BW AEFAD, H. CP + 200mg/kg BW AEFAD, I. CP + 400mg/kg BW AEFAD, J. SA for 14 days + normal diet for 14 days, K. CP for 14 days + normal diet for 14 days. 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 AEFAD 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 (Figure 4), when compared with the negative control Group (A). The group that received 200mg/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 400mg/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, while there was no reduction in proliferation in groups J and K, expected to have natural recovery. Rather, increase in cell proliferation was observed. 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 400mg/kg body weight with CP.

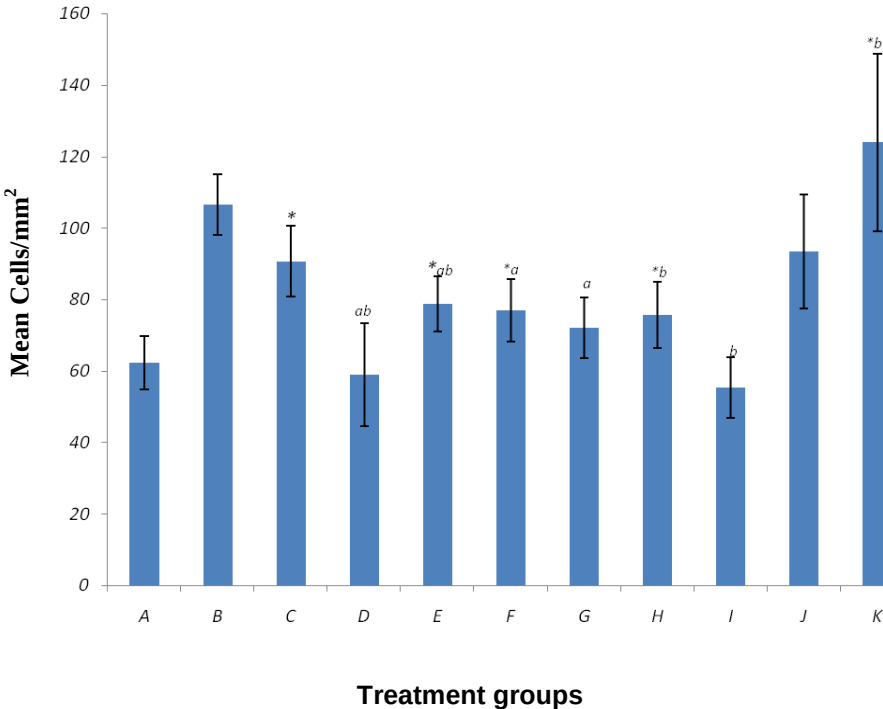


Figure 4.0 Effects of sodium arsenite, cyclophosphamide and *Adansonia digitata* fruit pulp extract on cells/mm² analysis in the liver of treated rats and control. Data are expressed as mean $\pm$ sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200mg/kg BW AEFAD, E. 400mg/kg BW AEFAD, F. SA + 200mg/kg BW AEFAD, G. SA + 400mg/kg BW AEFAD, H. CP + 200mg/kg BW AEFAD, I. CP + 400mg/kg BW AEFAD, J. SA for 14 days + normal diet for 14 days, K. CP for 14 days + normal diet for 14 days. 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.

3.5 Effect of AEFAD 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).

Table 2.0 Effects of sodium arsenite, cyclophosphamide and *Adansonia digitata* pulp extract on hematological parameters of treated rats and control. Data are expressed as mean $\pm$ sd. (n=6): A. Negative control, B. SA only, C. CP only, D. 200mg/kg BW AEFAD, E. 400mg/kg BW AEFAD, F. SA + 200mg/kg BW AEFAD, G. SA + 400mg/kg BW AEFAD, H. CP + 200mg/kg BW AEFAD, I. CP + 400mg/kg BW AEFAD, J. SA for 14 days + normal diet for 14 days, K. CP for 14 days + normal diet for 14 days. BW = Body weight, SA=Sodium arsenite, CP = Cyclophosphamide, AEFAD=Aqueous extract of the fruit pulp of *Adansonia digitata*.

GROUPS	PCV	LYMPHS	WBC	NEUTR	Hb
A	50.33 $\pm$ 2.88	81.00 $\pm$ 5.00	6275.00 $\pm$ 325.00	24.00 $\pm$ 1.73	15.52 $\pm$ 1.50
B	46.66 $\pm$ 3.21	76.33 $\pm$ 1.00	6650.00 $\pm$ 576.62	21.33 $\pm$ 1.15	15.46 $\pm$ 1.53
C	42.00 $\pm$ 10.39	68.33 $\pm$ 7.37	5850.00 $\pm$ 832.16	23.66 $\pm$ 2.51	15.75 $\pm$ 0.07
D	52.33 $\pm$ 0.57	66.00 $\pm$ 7.21	11050.00 $\pm$ 458.25	21.00 $\pm$ 3.60	17.68 $\pm$ 0.43
E	50.66 $\pm$ 2.51	70.00 $\pm$ 13.07	6866.67 $\pm$ 808.29	21.66 $\pm$ 2.51	17.53 $\pm$ 0.15
F	48.00 $\pm$ 1.00	69.00 $\pm$ 6.55	6800.00 $\pm$ 304.13	17.33 $\pm$ 2.51	16.30 $\pm$ 0.65
G	49.00 $\pm$ 3.60	80.00 $\pm$ 4.35	7033.33 $\pm$ 1609.61	14.66 $\pm$ 1.15	16.26 $\pm$ 1.10
H	47.66 $\pm$ 1.52	69.00 $\pm$ 3.60	5875.00 $\pm$ 725.00	28.33 $\pm$ 4.72	16.24 $\pm$ 0.92
I	49.66 $\pm$ 1.52	50.66 $\pm$ 3.05	6733.33 $\pm$ 472.58	20.33 $\pm$ 2.51	16.57 $\pm$ 0.76
J	48.00 $\pm$ 2.00	64.00 $\pm$ 6.92	5375.00 $\pm$ 75.00	27.66 $\pm$ 3.21	15.43 $\pm$ 0.75
K	50.33 $\pm$ 0.57	70.00 $\pm$ 4.58	4750.00 $\pm$ 350.00	18.00 $\pm$ 4.00	16.70 $\pm$ 0.14

4. 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 hepatotoxicity 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 γ GT 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 γ GT 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].

Two weeks after the administration of SA or CP, the observed serum ALT and GGT activities in the group K were significantly higher as compared with the control group. This is an indication of lack of natural recovery from the hepatotoxic effects of SA and CP within the time frame. Though there is reduction in the values of GGT and AST for group J, this is not supported by ALT value and histological analysis.

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. Focusing on possibility of natural recovery; there were very severe portal congestion, with periportal fibroplasia and cellular infiltration by mononuclear cells in groups J and K. This again indicates that there was no observable natural recovery from the hepatotoxic effects of SA and CP.

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 hepatocellular 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 significant reduction ($P < 0.05$) in the number of cells scored per mm² when compared with the intoxicated groups. Fourteen days experimental natural recovery period did not reduce the rate of hepatocellular proliferation in Groups J and K.

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 observed 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. High dose of the extract may be hepatotoxic.]

4. CONCLUSION

[Findings from this study therefore confirmed the hepatotoxicity 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 (WHERE EVER APPLICABLE)

It is not applicable for this study.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

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

REFERENCES

1. Jayanthika BW, Gandolfi AJ. Alan P and Lantz RC. Sodium Arsenite Enhances AP-1 and NFkB DNA Binding and Induces Stress Protein Expression in Precision-Cut Rat Lung Slices. *Toxicol. Sci.* 2001; 61: 283–94.
2. Valentine JL. Review of health assessments for US/Canada populations exposed to arsenic in drinking water. In: *Arsenic Exposure and Health* (a special issue of *Environmental Geochemistry and Health*, (Chappell, W.R., Abernathy, C.O., Cothorn, C.R., eds). Surrey, UK: Laws and Stimson Associates.1994; 16: 139-52.
3. Parvez F, Chen Y, Maria A, Hussain AI, Hassina M, Dhar R, et al. Prevalence of arsenic exposure from drinking water and awareness of its health risks in a Bangladeshi population; results from a large population-based study. *Environ. Health Perspect.* 2006; 114 (3): 355-59.
4. Baumann F, Preiss R. (2001). Cyclophosphamide and related anticancer drugs. *J Chromatogr B: Biomed Sci. Appl.* 2001; 764: 173-92.
5. Hales BE. (1982). Comparison of the mutagenicity and teratogenicity of cyclophosphamide and its active metabolites, 4-hydroxycyclophosphamide, phosphoramidate mustard and acrolein. *Cancer Res.* 1982; 42: 3016-21.
6. Bukowski R. The need for cytoprotection. *Eur J Cancer.* 1999; 32A: S2- 4.
7. Fraiser LH, Kanekel S, Kehrer JP. Cyclophosphamide toxicity; characterizing and avoiding the problem. *Drug.* 1991; 42: 781-95.
8. Taiye RF, Faderera AA, Joseph AA and Iheanyi OO. Antiviral Potentials of *Enantia chlorantha* Extracts on Yellow Fever Virus. *Nature and Science.* 2001; 9(9):99-105.
9. Udobi CE, Onaolapo JA. Phytochemical analysis and antibacterial evaluation of the leaf, stem bark and root of the African locust bean (*Parkia biglobosa*). *J of Med. Plants Res.* 2009; 3(5): 338-44.
10. Wickens GE. The Baobab – Africa's upside-down tree. *Kew Bulletin.* 1982; 37: 173-209.

- 391 11. Moiselet D. The Baobab Tree. 1998. Accessed 2012. Available: [http://www.senegal-](http://www.senegal-online.com/anglais/pares-fauneflore/baobab.htm)
392 [online.com/anglais/pares-fauneflore/ baobab.htm](http://www.senegal-online.com/anglais/pares-fauneflore/baobab.htm).
- 393 12. Wickens GE and Lowe P. The Baobabs: Pachycauls of Africa, Madagascar and Australia,
394 Springer: 2008. Accessed 2012.
- 395 13. Etkin NL and Ross PJ. "Food as medicines and medicines as food" Soc.sci med.1982; 16, 1559-
396 73.
- 397 14. Ramadan A, Harraz FM, El-Mougy SA. Anti-inflammatory, analgesic and antipyretic effects of the
398 fruit pulp of *Adansonia digitata*. Fitoterapia. 1993; 65(5), 418-22.
- 399 15. Ajose FOA. Some Nigerian plants of dermatologic importance. Int. J. Derm. 2007; 46 (suppl 1):
400 48-55.
- 401 16. Vertuani S, Braccioli E, Buzzoni V, Manfredini S. *Antioxidant capacity of Adansonia digitata fruit*
402 *pulp and leaves*. Acta Phytotherapeutica. 2002. 2 (V), 2-7.
403
- 404 17. Al-Qarawi AA, Al-Damegh MA and El-Mougy SA. J of Herbs, Spices & Med. Plants. 2003;10 (3)
405 by the Haworth Press, Inc. All rights reserved. 10.1300/J044v10n03_01
- 406 18. Preston RJ, Dean BJ, Galloway S, Hoden H, Mcfee AF, Shelby M. Mammalian in vivo
407 cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells. Mutat Res. 1987; 189:
408 157-65.
- 409 19. Szasz G. A kinetic photometric method for serum gamma glutamyl-transferase. Clin.Chem.1969;
410 124, 124-36.
- 411 20. Reitman S and Frankel S. A colorimetric method for the determination of serum level of
412 glutamate-oxaloacetate and pyruvate transaminases. Am J Clin. Pathol. 1957; 28:56–63.
- 413 21. Matter, B. and W. Schmid. (1971). Bone Marrow Toxicity. Mutation Res 12:417-25.
- 414 22. Adam-vizi V and Seregi M. Receptor dependent stimulatory effect of nor-adrenaline on Na⁺, K⁺-
415 ATPase in rat brain homogenate. Role of lipid peroxidant. Biochem. Pharmacol. 1982; 31, 2231-36.
- 416 23. Jain N.C.: Schalm's Veterinary Haematology. 4th edition, Lea Febiger, Philadelphia. Pp. 1221,
417 1986.
- 418 24. Chakraborty P, UgirHossain Sk, Nabendu M, Jayanta K, Smarajit P, and Sudin B. Modulation of
419 Cyclophosphamide-Induced Cellular Toxicity by DiphenylmethylSelenocyanate In Vivo, an Enzymatic
420 Study. J of Cancer Mol. 2009; 4(6): 183-9.
- 421 25. Adaramoye OA, Osaimoje DO, Akinsanya MA, Nneji CM, Fafunso MA, Ademowo OG. Changes
422 in antioxidant status and biochemical indices after acute administration of artemether, artemether-
423 lumefantrine and halofantrine in rats. Authors J. Compilation: Basic Clin. Pharmacol. Toxicol. 2008;
424 102:412-8.
- 425 26. Mallick P, Mallick JC, Guha B, Khuda-Bukhsh AP. Ameliorating effect of micodoses of potentizes
426 homeopathic drug, arsenic album, on arsenic-induced toxicity in mice. BMC Complementary and
427 Alternative Med. 2003; 3: 7.
- 428 27. Odunola OA, Kazeem AK, Ibegbu DM. The Influence of Garlic and Spondias mombin on Sodium
429 Arsenite induced Clastogenicity and Hepatotoxicity in Rats. The Pacific J of Sci. and Tech. 2011; 12(2),
430 401-9.

431 28. Dinari G, Cohen MI, Mc Namara H, Kochen JA. Effect of lead exposure on the activity of some
432 hepatic enzymes in rat. *Pediatr. Res.*1979 (5Pt1): 644-46.

433 29. Jens JJ, Hanne H. A Review on Liver Function Test. The Danish Hepatitis C 2002; Available:
434 [http://home3.inet.tele.dk/omni/hemochromatosis iron.htm](http://home3.inet.tele.dk/omni/hemochromatosis%20iron.htm)

435 30. Odunola OA. Comparative effects of some local food condiments on sodium arsenite induced
436 clastogenicity. *Afr. J. Med. Med. Sci.*2003; 32: 75-80.

437 31. IARC. Cyclophosphamide. In Overall Evaluations of Carcinogenicity. IARC Monographs on the
438 Evaluation of Carcinogenic Risk of Chemicals to Humans, suppl. 7. Lyon, France: International Agency
439 for Research on Cancer.1987; 182-4.

440 32. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus
441 frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis* 2006;
442 28 (3):625-31.

443 33. Celik A, Ogenler O, Comelekoglu U. The evaluation of micronucleus frequency by acridine
444 orange fluorescent staining in peripheral blood of rats treated with lead acetate. *Mutagenesis*. 2005;
445 20(6):411-5.

446 34. Eastmond DA, Tucker JD. Identification of aneuploidy inducing agents using Cytokinesis blocked
447 human lymphocytes and antikinetochore antibody. *Environ. Mol. Mutagen*. 1989;13, 34-43.

448 35. Gudi RD, Sandhu SS, Athwal SR. Kinetochore identification in Micronuclei in mouse bone
449 marrow erythrocytes. An assay for detection of aneuploidy-inducing agent. *Mutat. Res*. 1990; 234, 263-8.

450 36. Halliwell B, Gutteridge JM. Free radicals in biology and medicine, 2nd ed. Oxford: Clarendon
451 Press.1989.

452 37. Yamanaka K, Hoshino M, Okanato M, Sawamura R, Hasegawa A, Okada S. Induction of DNA
453 damage by dimethylarsine, a metabolite of inorganic arsenic is for the major part likely due to its peroxyl
454 radical. *Biochem. Biophys. Res. Commun*. 1990; 168, 58-64.

455 38. El- Demerdash FM, Yousef MI, Radwan FME. Ameliorating effect of curcumin on sodium arsenite
456 induced oxidative damage and lipid peroxidation in different rat organs. *Food and Chem. Toxicol*. 2009;
457 47, 249-54.

458 39. Morakinyo AO, Achema PU, Adegoke OA. Effect of Zingiber Officinale (Ginger) on Sodium
459 Arsenite Induced Reproductive Toxicity in Male Rats. *Afr. J. Biomed. Res*. 2010; 13, 39 – 45.

460 40. Patel JM, Block ER. Cyclophosphamide-induced depression of the antioxidant defense
461 mechanisms of the lungs. *Exp Lung Res*. 1985; 8: 153-165.

462 41. Venkatesan N, Chandrakasan G. Modulation of cyclophosphamide induced early lung injury by
463 curcumin, an anti-inflammatory antioxidant. *Mol. Cell Biochem*. 1995; 142: 79-87.

464 42. Kaya H, Oral B, Ozguner F, Tahan V, Babar Y, Delibas N. The effect of melatonin application on
465 lipid peroxidation during cyclophosphamide therapy in female rats. *ZentralblGynakol*. 1999; 121: 499-502.

466 43. Lear L, Nation RL, Stupans I. Effects of cyclophosphamide and adriamycin on rat hepatic
467 microsomal glucuronidation and lipid peroxidation. *Biochem. Pharmacol*. 1992; 44: 747-53.

468 44. Mathew S, Kuttan G. Antioxidant activity of *Tinosporacordifolia* and its usefulness in the
469 amelioration of cyclophosphamide induced toxicity. *J Exp Clin Cancer Res*. 1997; 16: 407-11.

470 45. Premkumar K, Pachiappan A, Abraham SK, Santhiya ST, Gopinath PM, Ramesh A. Effect of
471 Spirulina fusiformis on cyclophosphamide and mitomycin-C induced genotoxicity and oxidative stress in
472 mice. Fitoterapia. 2001; 72: 906-11.
473
474
475
476