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Hepatoprotective and anticlastogenic effects of ethanol extract of *Irvingia gabonensis (IG)* leaves in sodium arsenite-induced toxicity in male Wistar rats

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Summary: Consumption of arsenic contaminated water has been associated with diverse health defects such as cancer and skin lesions. Some plants of medicinal value have been reported to show protective effects against toxins. In this study, the effects of ethanol extract of the leaves of *Irvingia gabonensis* (IG) against sodium arsenite (SA) induced hepatotoxicity and clastogenicity in male Wistar rats was investigated. Eight groups of five rats each were used for the study. They were administered with 250 or 500 mg/kg body weight of IG with or without SA at 2.5 mg/kg body weight. IG extract has a significant ($p<0.05$) reducing effect on serum liver function enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (γGT) activities. This was corroborated with the histopathological analysis findings. Also the groups treated with both the extract and SA recorded significantly ($p<0.05$) reduced number of micronuclei when compared with the group treated with SA only. IG extract also reduced the oxidative stress induced by SA as measured by the reduced generation of hydrogen peroxide (H_2O_2) and significant ($p<0.05$) difference in the CAT and SOD activities between the groups treated with both SA and extract, and the positive control group administered SA alone. This study therefore shows that the ethanol leaf extract of *Irvingia gabonensis* have hepatoprotective and anticlastogenic effects against sodium arsenite-induced toxicity possibly by enhancing the antioxidant status in the Wistar rats.

Keywords: *Irvingia gabonensis* , Sodium arsenite, Hepatotoxicity, Clastogenicity, Oxidative stress, transaminases.

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INTRODUCTION

Cancer causing substances damage DNA and induce transmittable mutations in both humans and animals (Perera *et al*., 2002). Their interaction with tissue macromolecules such as DNA alters the replication and repair mechanisms (Ames, 1979). For instance, sodium arsenite, a common contaminant of underground water in some regions of the world, is known to be hepatotoxic and genotoxic (Guillamet *et al.*, 2004; Odunola *et al.*, 2008; Gbadegesin *et al.*, 2009).

Attentions have recently shifted to the use of medicinal plants in prophylactic, therapeutic and curative applications. *Irvingia gabonensis* (IG) (AubryLecomte ex O'RorkeBaill) is a plant with promise. It is an indigenous forest tree belonging to the group of plants classified as non-timber forest products. *Irvingia gabonensis* is commonly called bush mango or African mango since the trees bear fruits that look like small mango (Matos *et al*., 2009). Traditionally, the stem bark is used to relieve pain in Sierra Leone (Okolo *et al*., 1995). The aqueous maceration of the leaves is used as antidote for some poisonous substances. In combination with palm oil, the leaves are also used to stop haemorrhage in pregnant women. In Senegal, the decoction of the stem bark is used in the treatment of gonorrhoea, hepatic and gastrointestinal disorders (Hubert *et al*, 2010). The root bark is prescribed in poultrice form to treat wounds. The decoction of the root bark is also used to treat diarrhoea (Osadebe *et al*., 2012). The nut is used in treating type 2 diabetes (Adamson *et al.*,1990). The kernel serves as condiments used in thickening and flavouring soups. The fruit is consumed in the south-western part of Nigeria with less interest in the nuts while the reverse is the case in the eastern part. The stem bark of the tree is added to palm wine as a preservative (NAERLS, 1999). The leaf extracts of the plant has been reported to have

diuretic effect in rats and hypotensive effect in cats (Nosiri *et al.,* 2009a; 2009b)

However, there is dearth of information on the hepatoprotective, antioxidant and anticlastogenic effects of the leaves of this plant. This study was therefore conducted to evaluate the hepatoprotective, antioxidant and anticlastogenic effects of the ethanol extract of *Irvingia gabonensis* (AubryLecomte ex O'RorkeBaill) leaves on sodium arsenite-induced toxicity in male Wistar albino rats.

MATERIALS AND METHODS

Reagents and kits

Sodium arsenite ($NaAsO_2$; BDH chemicals Ltd poole England) was dissolved in distilled water and administered at a dose of 2.5 mg/kg body weight corresponding to $1/10^{th}$ of the oral LD_{50} of the salt (Preston *et al.,* 1987). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (γGT) kits were obtained from Randox Laboratories, Crumlin, UK. All other chemicals and reagents were of analytical grade and were products of Sigma Chemical Co. St. Louis, MO., USA or BDH Chemical Ltd, Poole, England.

Test plant material and extraction procedure

Fresh leaves of the plant were harvested, identified and Voucher specimen deposited at the herbarium of the Department of Botany, University of Ibadan. The harvested leaves were air-dried in a well-ventilated room for about four weeks, before milling. Cold extraction was then carried out on the grounded sample by soaking in 50% ethanol for 72 hours at room temperature. The extract was filtered and the filtrate concentrated using a rotary evaporator at temperature of 40 ^{0}C. The concentrated sample was then freeze-dried and the sample kept at room temperature and administered to the experimental animals as detailed below.

Experimental animals and treatments

Forty male Wistar albino rats weighing between 150 – 190 g raised at the preclinical experimental animal house, Department of Physiology, were purchased and kept in the experimental animal facility of the Department of Biochemistry, University of Ibadan. They were fed with commercial rat pellets (Ladokun Feeds, Mokola, Ibadan, Nigeria) and water *ad libitum*.

The albino rats were allowed to acclimatize for one week prior to the commencement of the study. The rats were randomly distributed into eight groups of five animals each based on the treatment received.

Group 1: These served as control and were administered distilled water only.

Group 2: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite for 2 weeks.

Group 3: The rats in this group were orally intubated daily with 250mg/kg body weight of *Irvingia gabonensis* (IG) extract for 2 weeks.

Group 4: The rats in this group were orally intubated daily with 500mg/kg body weight IG extract for 2 weeks.

Group 5: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite and 250mg/kg body weight IG extract simultaneously for 2 weeks

Group 6: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite and 500mg/kg body weight plant extract simultaneously for 2 weeks.

Group 7: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite for 2 weeks followed by 250mg/kg body weight IG extract for subsequent 2 weeks.

Group 8: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite for 2 weeks followed by 500mg/kg body weight IG extract for another 2 weeks.

Termination of the experiment and extraction of tissues

The rats were sacrificed by cervical dislocation twenty four hours after the last treatment dose. 0.04% colchicine was administered at a dose of 10 ml/kg body weight to the animals 2 hours prior to the sacrifice. Blood samples were collected through retro-orbital bleeding, and put into a plain bottle and allowed to clot. The samples were then centrifuged at 3,000 g for 30 minutes to separate the serum.

The animals were dissected and opened to harvest the liver and femur. A portion of the liver from each sacrificed animal was excised, blotted and then perfused with potassium chloride (1.15%) in order to remove all traces of blood haemoglobin which might contaminate the tissues. The liver samples were preserved and fixed in 10 % buffered formal-saline and processed for paraffin sectioning. Sections of about 5 µm thickness were stained using Haematoxylin-Eosin staining dye and subsequently evaluated in the Veterinary Pathology Department of the University of Ibadan. The remaining portion of each liver sample was homogenized in phosphate buffer and centrifuged at 4 ^{o}C. The supernatant was used immediately or stored at – 20 ^{o}C. The femurs were also excised and bone marrow extruded for micronucleus induction assay.

Assays of liver function enzymes

γ-glutamyltransferase activity.

γGT was assayed in the serum by using the reconstituted γGT diagnostic reagent following the method of (Szasz, 1969). The principles involve 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 (pnitroaniline).

This product absorbs UV at 405 nm thus making a direct kinetic determination of γGT activity possible.

Alanine aminotransferase and aspartate aminotransferase activities

Serum ALT and AST were assayed according to (Reitman and Frankel, 1957) 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).

Histological Analysis

Liver sections were fixed in 4% p-formaldehyde and washed in phosphate buffer pH 7.4 at 4^0C for 12 hours. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye and finally observed under a microscope.

Micronucleus (MN) assay

The femurs from each of the animals were removed and bone marrow was aspirated. Microscopic slides of the bone marrows were prepared according to (Matter and Schmid, 1971). 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).

Antioxidant enzymes and hydrogen peroxide generation assays

H_2O_2 generation assay

50 µl of supernatant fraction of the liver homogenate was vortexed and incubated at room temperature for 30 minutes. This was then shaken to ensure proper mixing and incubated for 30minutes before reading at 560 nm. The concentration of the hydrogen peroxide generated was extrapolated from the standard curve.

Determination of catalase activity

1 ml of supernatant fraction of the liver homogenate was mixed with 19 ml distilled water to give a 1: 20 dilution. The assay mixture contained 4 ml of H_2O_2 solution (800 µmoles) and 5 ml of phosphate buffer, pH 7.0 in a 10 ml flat bottom flask. 1 ml of properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1 ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide contents of the withdrawn sample were determined by the method described above.

Determination of Superoxide dismutase (SOD) activity.

1 ml of supernatant fraction of the liver homogenate was diluted in 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

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.

RESULTS

Effect of *Irvingia gabonensis* on the body and liver weights of rats

The initial body weights of the animals were taken before the commencement of treatments. Just before sacrifice, the body weights of the animals were taken and designated as final body weights. These were used to calculate the percentage body weight change. The harvested liver tissues were also weighed and the relative liver weights were determined (Table 1).

Administration of sodium arsenite caused a significant ($p<0.05$) reduction in the percentage weight change as compared with the negative control. There were no significant ($p>0.05$) change in the body weight in other groups, after the four weeks of treatment, as compared with the negative control (Table 1). Also, changes in the relative liver weight of rats were not significant ($p> 0.05$) when compared across all groups.

Hepatoprotective activities of ethanol extract of the leaf of *Irvingia gabonensis* in Wistar rats treated with sodium arsenite.

The protective effect of *Irvingia gabonensis* leaf extract was assessed by evaluating the activities of serum enzymes; γ-glutamyltransferase (γGT), aspartate amino transferase (AST) and alanine amino transferase (ALT) in the groups of rats administered the extract and/or sodium arsenite and the control untreated rats. Administration of sodium arsenite at 2.5 mg/kg body weight resulted in almost double folds increase in the mean serum AST value, more than double ALT value and more than fourfold

increase in γGT when compared with negative control group.

The mean ALT, AST and γGT activities in groups of rats administered graded doses of *Irvingia gabonensis* extract with sodium arsenite decreased significantly ($p<0.05$) when compared with the group treated with sodium arsenite alone (Table 2). The extract alone at the high dose of 500 mg/kg body weight produced significant ($p<0.05$) higher level of serum AST and ALT activities. The results of the histological assessment of the liver cells integrity support the serum enzyme activities patterns of hepatotoxicity in the treated animals (Figure 1). There was marked widespread thinning of hepatic cords in the liver of rats administered sodium arsenite (positive control). There were no visible lesion in the negative control group and the groups administered 250mg/kg and 500mg/kg body weights of the extract (groups 1, 3 and 4 respectively). There were no observable lesions in the groups 5 and 6, administered 250 and 500 mg/kg of extracts respectively with sodium arsenite at 2.5mg/kg. However, there is multifocal centrilobular thinning of hepatic cords in group 7, and moderate Kupffer cell hyperplasia, portal fibrosis and random single-cell necrosis in group 8. Groups 7 and 8 were post-treated with extracts at 250 and 500 mg/kg respectively, after two weeks of sodium arsenite administration.

Effect of *Irvingia gabonensis* on the frequency of micronucleated polychromatic erythrocytes (mPCEs) scored in the bone marrow of rats treated with sodium arsenite.

The number of mPCEs per 1000 PCEs scored in the

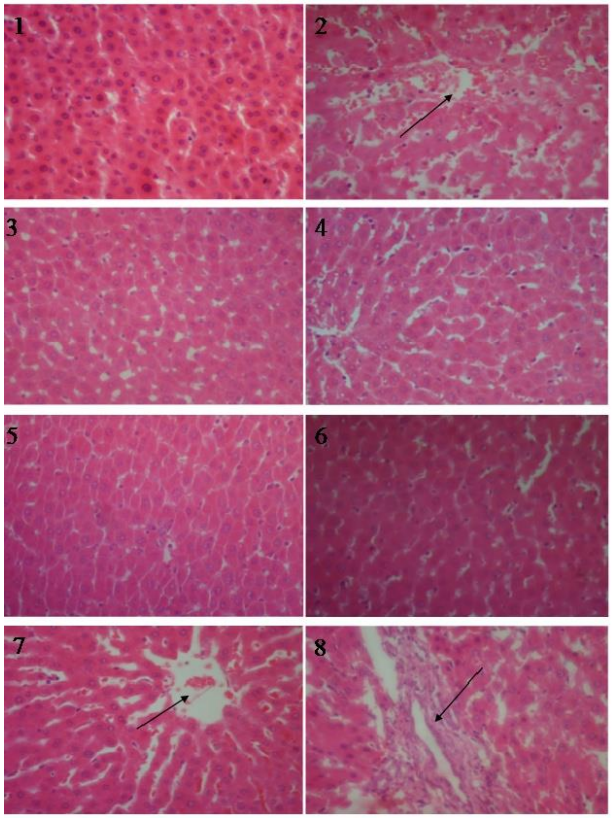

Figure 1. Representative photomicrograph of liver of rats treated with sodium arsenite and/or *Irvingia gabonensis* extract (Magnification= x400). Group numbers are shown. **1** (group given distilled water only). There are no visible lesion. In group **2**, given sodium arsenite, there is marked widespread thinning of hepatic cords. There are no visible lesions in groups 3, 4 (given extract, respectively at 250 and 500mg/kg body weight), and groups 5 and 6 treated with sodium arsenite along with the extract. There is multifocal centrilobular thinning of hepatic cords in group 7. There is moderate Kupffer cell hyperplasia; portal fibrosis and random single-cell necrosis in group 8. Groups 7 and 8 were post treated with the extract at 250 and 500mg/kg body weight respectively after treatment with sodium arsenite for 2 weeks.

Table 1. Body weights, liver weights and percentage body weight changes of experimental rats (values are mean ±SD).

Group/ Treatment	Initial **B.Wt** (g)	Final B.Wt (g)	%Δ**Wt** (g)	Liver W**t**t(g)	**Relative** Liver W**t** (%)
1. Dist. water	156.00±26.07	206.00±26.07	28.66±7.14	5.29±1.00	2.56±0.25
2. SA	188.00±10.95	208.00±4.47	14.82±3.21#	5.76±0.61	2.77±0.25
3.250mg/kg IG extract	156.00±5.48	194.00±13.42	27.39±6.07	5.04±0.71	2.59±0.25
4.500mg/kg IG extract	160.00±0.00	202.00±17.89	29.69±9.37	5.52±0.84	2.72±0.22
5.SA+250mg/kg IG extract	164.00±16.73	198.00±10.95	23.81±5.05	5.15±0.32	2.60±0.13
6.SA+500mg/kg IG extract	160.00±0.00	186.00±19.49	20.31±9.37	4.88±0.84	2.62±0.47
7.SA(2weeks)+ 250mg/kg extract	156.67±11.55	178.00±10.00	22.51±4.75	5.51±0.31	2.89±0.01
8.SA(2weeks)+ 500mg/kg extract	116.00±8.94	170.00±26.46	27.78±9.62	5.09±0.86	2.64±0.31

SA = Sodium Arsenite, IG = *Irvingia gabonensis* # = The mean difference is significant ($p< 0.05$) when compared with control

Table 2. Levels of serum gamma-glutamyltransferase, aspartate aminotransferase, and alanine amino transferase in experimental rats treated with SA and/or *Irvingia gabonensis* extract (values are mean± S.D).

Group/Treatment	AST(U/l)	ALT(U/l)	γGT(U/l)
1. Dist. water	41.84±1.89	8.75±2.13	2.32±1.15
2. SA	78.61±11.84#	22.99±3.36#	10.42±1.15#
3. 250mg/kg IG extract	51.44±18.69	16.78±10.98	3.09±1.76
4. 500mg/kg IG extract	72.00±19.10#	12.79±1.62#	11.19±9.98
5. SA+250mg/kg IG extract	52.61±8.13*	9.00±5.80*	5.21±4.38*
6. SA+500mg/kg IG extract	57.24±12.49*	9.46±6.17*	3.47±3.27*
7. SA (2weeks) + 250mg/kg extract	40.72±9.36*	9.23±1.65*	6.94±3.47
8. SA (2weeks)+ 500mg/kg extract	39.65±6.64*	8.92±2.69*	4.63±1.83*

SA = Sodium Arsenite, IG = *Irvingia gabonensis.* # = The mean difference is significant ($p< 0.05$) when compared with control (group 1). *= The mean difference is significant ($p< 0.05$) when compared with group treated with SA alone (group 2).

Table 3. Intracellular levels of hydrogen peroxide generated, catalase and superoxide dismutase activities in the liver of rats treated with sodium arsenite and/or *Irvingia gabonensis* extract (values are mean ± SD).

Treatment	H_2O_2 (µmol/min./mg protein)	CAT (Units/mg protein)	SOD (Units/mg protein×10^{-2})
1. Dist. water	13.81±1.14	65.21±3.40	0.14±0.02
2. SA	17.29±2.89#	76.53±4.29#	0.13±0.004
3.250mg/kg IG extract	13.29±0.64	67.66±7.99	0.15±0.05
4.500mg/kg IG extract	14.54±0.27	65.90±11.18	0.15±0.03
5.SA+250mg/kg IG extract	14.09±1.14*	71.31±7.46	0.15±0.02
6.SA+500mg/kg IG extract	15.06±0.66	70.11±5.09	0.15±0.01*
7.SA(2weeks)+ 250mg/kg extract	16.81±2.91	65.92±6.06*	0.15±0.03*
8.SA(2weeks)+ 500mg/kg extract	15.00±1.79	67.51±6.94*	0.15±0.02*

SA = Sodium Arsenite, IG = *Irvingia gabonensis.* # = The mean difference is significant (p< 0.05) when compared with control (group 1). *= The mean difference is significant (p< 0.05) when compared with group treated with SA alone (group 2).

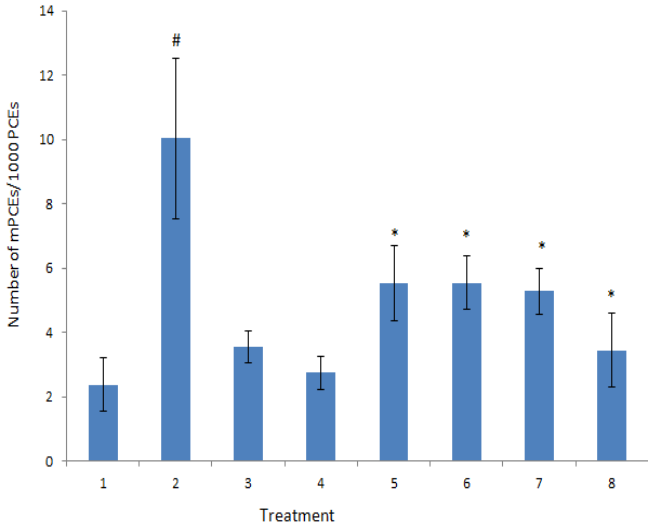

Figure 2. Frequency of micronucleated polychromatic erythrocytes (mPCEs) scored in the bone marrow cells of rats treated with sodium arsenite and/or *Irvingia gabonensis* extract (IG). Group 1 Negative control given distilled water only. Group 2: Given only sodium arsenite (at 2.5mg/kg body weight), Group 3: 250mg/kg body weight (IG). Group 4: 500mg/kg body weight IG, Group 5: Sodium arsenite and 250mg/kg body weight IG simultaneously, Group 6: Sodium arsenite and 500mg/kg body weight IG simultaneously, Group 7: Sodium arsenite (2 weeks) followed by 250mg/kg body weight IG (2 weeks). Group 8: Sodium arsenite (for 2 weeks) followed by 500mg/kg body weight IG (2 weeks). #= the mean difference is significant (p< 0.05) different when compared with control (group 1). *= the mean difference is significant (p< 0.05) when compared with the group given sodium arsenite only (group 2).

bone marrow cells (Figure 2) is significantly higher ($p < 0.05$) in the groups of rats administered sodium arsenite (groups 2) when compared with the negative control given only distilled water (group 1). Treatments with the extract caused a significant ($p<0.05$) reduction in the number of mPCEs scored in the bone marrow cells in the groups administered both the extract and sodium arsenite when compared with the group given sodium arsenite only. When compared with the positive control, given sodium arsenite only, the extract was able to significantly ($p<0.05$) reduce the number of mPCEs both when it was given along with sodium arsenite (groups 5 and 6) or given two weeks after sodium arsenite (groups 7 and 8).

Table 4. Phytochemical constituents of the ethanol extract of *Irvingia gabonensis* leaves.

Phytochemicals	Value
Tannins (mg/100g)	1431.7
Alkaloids (mg/100g)	33.3
Flavonoids(mg/100g)	663.3
Saponins (mg/100g)	23.0
Total phenols (GAE/100g)	85.0
Terpenoids (mg/100g)	13.0

GAE= Gallic Acid Equivalent (conventional units for phenols)

Effect of *Irvingia gabonensis* on intracellular levels of generated hydrogen peroxide (H_2O_2), catalase (CAT) and superoxide dismutase (SOD) activities in liver of the rats treated with sodium arsenite (SA).

There was significant ($p<0.05$) high level H_2O_2 CAT activity in the group of rats treated with SA (group 2) compared with the negative control group 1 (Table 3). Treatment with extract resulted in reduced generation of hydrogen peroxide (H_2O_2) and significant ($p<0.05$) difference in the CAT and SOD activities when comparisons were made between the groups treated with both SA and extract and the group administered SA alone.

Phytochemical analysis of ethanol extract of *Irvingia gabonensis* leaves.

Phytochemicals present in the ethanol extract of the leaves of *Irvingia gabonensis* in significant amounts are tannins, alkaloids, favonoids, saponins, polyphenols and terpenoids (Table 4). Tannin has the highest value of 1431.7 mg/100g followed by flavonoids (663.3 mg/100g). Tannin is more than double the value of flavonoids. Terpenoids are present in the least amount.

DISCUSSION

Arsenicals especially the trivalent forms are highly recognized as potent environmental toxicants (Abernathy *et al.,* 1999). Exposure to arsenic via the intake of contaminated water has been linked with diverse heath defects like certain forms of cancer, skin lesions, and non-cancer health effects such as

neurological disorders and impaired cognitive development in children (Abernathy *et al.*, 1999).

The liver is an important target organ for arsenic toxicity (Parvez *et al.*, 2006). Arsenic intoxication in experimental animals has been linked with micronucleus formation and hepatic tumors (Moore and Smith, 1997; Mazumder, 2005). This study examined the effect of the ethanol leaf extract of *Irvingia gabonensis* (Aubry-Lecompte ex O'RorkeBaill) in sodium arsenite model of hepatotoxicity and clastogenicity in Wistar rats.

Administration of sodium arsenite alone for four weeks produced significantly ($p<0.05$) lower change in percentage body weights compared with the negative control group. This suggests that sodium arsenite interfered with systemic activities in the body of the rats. The extract was able to reverse the above effect of sodium arsenite on growth of the experimental rats.

Aspartate aminotransferase (AST) and Alanine aminotransferase (AST) are members of transaminase family of enzymes. They are also known as aminotransferases, they catalyze the transfer of amino groups between L-alanine and glutamate for physiological purpose. ALT and AST are found in large amount in the liver and also small amount are found in the heart, kidney and muscles. When the liver is injured or inflamed as the case may be via its exposure to various forms of toxic substances, the level of ALT and AST in the blood is usually elevated. The level of these enzymes in the blood is directly related to the extent of the tissue damage (Lum and Gambino, 1972).

Gamma-glutamyltransferase (γGT) is the enzyme actively responsible for the extracellular catabolism of glutathione, in the main mammalian cells (Huseby, 1978). γGT is present on the outer surface of plasma membrane of most cell type and in blood, where it has been shown to form complexes with several plasma components particularly with albumin and lipoprotein (Huseby, 1982). The determination of serum γGT activity is well established diagnostic test for hepatobiliary diseases, and is used as a sensitive marker of liver damage. Elevated serum γGT activity is associated with diseases of the liver, biliary system and pancreas (Betro *et al.*, 1973; Huseby, 1982).

The results obtained from the assessment of the serum levels of AST, ALT and γGT indicate that sodium arsenite significantly ($p<0.05$) induced these serum enzyme activities as compared with the control. Increased gamma glutamyltransferase activity has been linked with hepatotoxicity, oxidative stress and chromosomal aberrations in cells (Lum and Gambino, 1972; Dinari *et al.*, 1979; Karmaker *et al.*, 1999). The observations made here are consistent with the findings from previous findings (Mallick *et al.*, 2003; Odunola, 2003; Odunola *et al.*, 2007). The hepatoprotective effect of the extract was independent of whether the extract is administered at the same time with sodium arsenite or two weeks after sodium arsenite. This indicates that the effect could be preventive or curative. Histopathological analysis of liver organs showed that there was no visible lesion in the liver organs of the animals in the control group and in the groups that were treated with sodium arsenite and leaf extract simultaneously (Groups 5 and 6) also in the groups that were treated with extract only (Groups 3 and 4). In the group that was treated with sodium arsenite only (group 2), there was a marked widespread thinning of hepatic cords which indicates the necrotic cell death of the hepatocytes. This is consistent with earlier reports on the hepatotoxicity of sodium arsenite (Odunola *et al.*, 2008; Gbadegesin *et al.*, 2009). There was multifocal centrilobular thining of the hepatic cords in the group that was post-treated with 250 mg/kg body weight (Group 7). There was also moderate kupffer cell hyperplasia in the liver organs of the animals in the group that was post-treated with 500 mg/kg body weight of extract (Group 8).

The relative number of micronucleated polychromatic erythrocytes scored in the rat bone marrow cells show that sodium arsenite significantly ($p<0.05$) induced the formation of micronuclei in the polychromatic erythrocytes of the rat bone marrow cells. This clastogenic effect of sodium arsenite is in line with previous reported findings (Mallick *et al.*, 2003; Odunola, 2003; Odunola *et al.*, 2007). There were also significant decrease ($p<0.05$) in the number of micronuclei scored in the groups treated with the leaf extract (250 mg/kg body weight and 500 mg/kg body weight respectively) after treatment with sodium arsenite, when compared with the group treated with sodium arsenite only. The observed decreased numbers of micronuclei in these groups indicate that the extract is anticlastogenic.

Findings from the present study supported the reports that sodium arsenite generate free radicals like H_2O_2 in living systems (Ramanathan *et al.*, 2002; Usoh *et al.*, 2005). Endogenous enzymes constitute the first line of cellular defense and provide a mutually supportive team of defence against reactive oxygen species (Ramanathan *et al.*, 2002). In sodium arsenite induced hepatotoxicity, the balance between the production of reactive oxygen species and these antioxidant defence systems may be lost, thus leading to oxidative stress due to the overwhelming power of prooxidants generated, which through a series of events deregulates the cellular functions that leads to hepatic necrosis (Amresh *et al.*, 2007). Catalase is responsible for the degradation of hydrogen peroxide which is a reactive oxygen species produced during metabolism. Catalase catalyzes the removal of

hydrogen peroxide formed during the reaction catalyzed by superoxide dismutase (Manna *et al*., 2007). Superoxide dismutase is an enzyme that protects against the superoxide radical which can cause oxidative stress. SOD speeds up the dismutation of superoxide radical to hydrogen peroxide which is then removed by catalase (Usoh *et al.*, 2005). SOD can therefore function as a primary defense and inhibits further production of free radicals. The increased amount of hydrogen peroxide, and therefore total reactive oxygen species, generated when rats were treated with sodium arsenite demonstrates treatment of experimental animals with sodium arsenite would contribute to oxidative stress. It is possible that the extract enhanced the antioxidant system in the treated rats therefore speeding up the mopping up of the reactive oxygen species.

From the results obtained for catalase (CAT) activity and superoxide dismutase (SOD) activity, the catalase activity was significantly ($p<0.05$) induced in the group treated with sodium arsenite only when compared with the control. The explanation to this may be that catalase activity was significantly induced as an adaptive response to the increased amount of hydrogen peroxide generated in the group as catalase is known to mop up hydrogen peroxide which is a reactive oxygen species. There was however no significant differences in catalase activities in the groups treated simultaneously with both sodium arsenite and extract suggesting that an immediate mopping up of the reactive oxygen species by the extract enhanced antioxidant system is inefficient. However, treatment with the extract, two weeks after sodium arsenite administration resulted in CAT activities that are significantly different from sodium arsenite treated group. Similar trends were observed form SOD activities across all the groups.

The result obtained from the phytochemical analysis of ethanol extract of *Irvingia gabonensis* (Aubry-Lecompte ex O'RorkeBaill) leaves indicated the presence significant amount of tannins, saponins, alkaloids, terpenoids, flavonoids and phenols. Tannin is more than double the value of flavonoids. Tannin has been reported to have anti-inflammatory and antiulcer property in rodents, showing a strong antioxidant property (Souza *et al*., 2006). In conclusion, ethanol extract of leaves of *Irvingia gabonensis* (Aubry-Lecompte ex O'RorkeBaill) showed potent hepatoprotective and anticlastogenic activities in sodium arsenite induced toxicity in rats. The extract also enhanced the enzymatic antioxidant status of the treated rats. These biological activities may be due to the phytochemicals present in the extract. Further studies to isolate, purify and characterise the active compounds in the extract are significant.

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