

The Influence of Garlic and *Spondias mombin* on Sodium Arsenite induced Clastogenicity and Hepatotoxicity in Rats.

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ABSTRACT

The effect of aqueous extract of garlic (GA) and *Spondias mombin* (SM) on the clastogenicity and hepatotoxicity of sodium arsenite (SA) was evaluated in Wistar rat using bone marrow micronucleus assay and monitoring the activities of γ -gamma transferase (γ -GT), alkaline phosphate (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the plasma. 100 mg/kg b.w. of aqueous extract of garlic (GA) and 25mg/kg b.w of SM leaves were fed to rats for 30 consecutive days either alone or simultaneously. 2.5mg/kg b.w. of SA was injected interperitoneally on the 7th, 14th, 21st and 30th day of the experiment, alone and together with GA or SM or a combination of both.

SA significantly ($P < 0.05$) induced the formation of micronucleated polychromatic erythrocytes (mPCEs) and γ -GT, ALP, AST and ALT activities when compared to the control. GA significantly ($P < 0.05$) reduced the degree of mPCEs formation and activities γ -GT, ALT AST and ALP induced by SA. Although, pretreatment with SM significantly reduced plasma activity of γ -GT, ALT and ALP, it however, led to an additive increase in mPCEs formation. Pretreatment with both GA and SM also significantly ($P < 0.05$) reduced the activities of all the four liver function enzymes. GA alone appear to be more effective in modulating the clastogenicity and hepatotoxicity of SA than SM alone or in combination with GA.

(Keywords: sodium arsenite, *Spondias mombin*, garlic, micronucleated polychromatic erythrocytes, γ -gamma transferase, alkaline phosphate, aspartate aminotransferase, alanine aminotransferase)

INTRODUCTION

Arsenic is a common environmental pollutant that is threatening life of many people especially in developing countries of the world [1]. It is widely distributed in nature in many forms and its compounds are used extensively as components of herbicides, insecticides, rodenticides, food preservatives, and drugs [2,3]. Ingestion of the metalloids in drinking water presents the greatest hazard and this has been associated with many cancer and non cancer effects in affected populations [4]. Such effects include tumors at multiple sites including the skin, liver, lungs, urinary bladder and prostate [5-6]. Similarly, inorganic arsenic exposure has been linked to endemic arsenic dermatosis along with hyperkeratosis, maningioma, diabetes, embryotoxicities, spontaneous abortion, adverse pregnancy outcomes, gangrene, and blackfoot disease [7-9].

An early event in arsenic carcinogenesis is molecular alterations both in humans and animals [10-11], which manifests dose dependent chromosomal breaks and alterations [12]. Several reports have implicated oxidative stress in arsenic-induced cytotoxicity and genotoxicity [13]. The liver is not also spare from inorganic arsenite toxicity. In fact, the liver has been reported to be a critical target of arsenic in humans and arsenic exposure is associated with development of hepatocellular carcinomas as well as other toxic lesions [4].

Efforts to prevent and treat arsenic toxicity by therapeutic measures had only limited success [14]. Some studies suggest the use of antioxidants and antioxidant rich foods and herbal

medicinal plant for the management of arsenicosis [15-16] and also for the beneficial effects of antioxidants as antidotes for arsenic toxicities [17]. We therefore, assess the potentials of two medicinal plants widely used in the treatment of some common ailments due to their oxidant property.

Garlic (*Allium sativum* Linn.), has been found to have an important dietary and medicinal role for centuries [18]. The spice has come under extensive use due to its effect on the cardiovascular system as well as antibiotic, anti-diabetic, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and hormone-like effect [19]. Recently, it has been found that the garlic and its compound have anti-mutagenesis and anti-carcinogenesis effects. *In vivo* studies show that garlic and its associated sulfur components suppress the incidence of tumors in rodent models [20-21]. In addition, garlic has been shown to modulate the toxicity of a number environmental carcinogens including arsenic [22].

Spondias mombin L. (Anacardiaceae) also known as hog plum is a plant that grows in almost every part of the world. It is fruitiferous deciduous plant of about 20m high that grows in the rain forest and the coastal area of Africa. It is known locally as "iyeye" and "iyawe" by the Yoruba and Hausa people of Nigeria [23]. The trees are used for fencing and in the construction of yam storage barns. Ripened fruits are eaten by the old and young and processed into ice-cream, cool beverages, wine, jam, and other preservatives [24].

Trado-medicine practitioners across Africa use all parts of the plant for medicinal purposes [25]. The fruits decoction is consumed as a diuretic and febrifuge, while the decoctions of the bark and leaves is used as an emetic and in the treatment of diabetic, diarrhea, dysentery gonorrhoea, and leucorrhoea (25-26). Infusion of its leaves has been used for a long time, without any report of collateral effect due to its anti-vitrotic activity against the herpes virus [27]. Recently, Idu *et al*, [29] reported the inhibitory activity of *Spondis mombin* against *Cycas revoluta* induced carcinogenesis. However, there is dearth of information about the health effects of *Spondias mombin* extract against arsenic intoxicification.

Therefore, the present study aims to investigate the influence of garlic and *Spondias mombin* alone or in combination against sodium arsenite

induced clastogenicity and hepatotoxicity in male rat.

MATERIALS AND METHODS

Chemical Reagents

Sodium arsenite (Na_2AsO_2) [Mol wt. 129.9, As 57.6% (As No. 778 4-46-5)] from Sigma Chemical Co., St. Louis, MO was dissolved in distilled water and administered at a dose of 2.5mg/kg body weight (corresponding to 1/10th of the oral LD₅₀ of the salt). All other chemicals used were of analytical grade and were also obtained from Sigma chemical Co. Louis Mo. USA. Gamma-Glutamyl Transferase (γ -GT), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) kits were obtained from Randox Laboratories Ltd., United Kingdom.

Plant Extract

Fresh leaves of *Spondias mombin* collected from the botanical garden of the University of Ibadan, Ibadan and Garlic, *Allium sativum* bulbs obtained from Bodija Market at Ibadan, Oyo State Nigeria were authenticated at the herbarium in the Department of Botany and Microbiology, University of Ibadan, Ibadan. To prepare the extract, fresh leaves of *Spondias mombin* weighing 2g were washed under running water, soaked in double distilled water over night and later blended in an electric blender with the same water. The suspension obtained was filtered and concentrated with a rotary evaporator under reduced pressure 60^oC. A dosage of 25mg/kg body weight was orally administered to the experimental animals. Crude extract of Garlic was prepared from freely sliced cloves, grounded into paste dissolved in double distilled water and filtered. A dose of 100mg/kg body weight was fed to the experimental animals.

Experimental Animals

Thirty five male Wistar albino rats approximately 10-12 weeks old bred in the Central Animal House, College of Medicine, University of Ibadan, Ibadan, Nigeria and having an average weight of 130g were housed five per cage with wood shaven bedding in polypropylene cages under standard environmental conditions of 50 ± 10%

relative humidity, $29 \pm 2^{\circ}\text{C}$ temperature and 12 hour light and 12 hour dark cycle at the Experimental Animal House, Department of Biochemistry, University of Ibadan. They were fed with rat pellet containing at least 20% protein, 3.5% fat, 9.0% fiber, 1.2% calcium, 0.7% phosphorus, vitamin, mineral per mix, antioxidant, antibiotics, carbohydrates, etc., from God First Feed Mill, Bodija, Ibadan, Nigeria and water *ad libitum*.

Experimental Protocol

The experimental rats were randomly divided into seven treatment groups of five animals each. Group I rats served as negative control and were treated with distilled water only for thirty consecutive days. Rats in group II were placed on normal diet for thirty consecutive days and injected interperitoneally with 2.5mg/kg body weight of sodium arsenite on the 7th, 14th, 21st and 30th day respectively. Rats in groups III, IV and VII were treated with 100mg/kg body weight aqueous garlic extract for thirty consecutive days; in addition those in IV and VII were injected interperitoneally with 2.5mg/kg body weight of sodium arsenite on the 7th, 14th, 21st and 30th day. Whereas rats in groups V, VI and VII were pretreated with the aqueous extract of *Spondias mombin* leaves (100mg/kg body weight of rats) for thirty consecutive days and on the 7th, 14th, 21st and 30th day, those in VI and VII were injected interperitoneally with 2.5mg/kg bodyweight of sodium arsenite.

The rats were sacrificed by cervical dislocation twenty-four hours after the last treatments were administered. Blood was collected via cardiac puncture and plasma prepared for enzyme analysis. Femoral bone marrow from each animal was also harvested and used for micronucleus assay.

Micronucleus Assay

The micronucleus assay was performed according to the method of Heddle and Salmone [30]. The femur of each rat was freed and stripped clean of muscles. The iliac end of the femur was carefully shortened until a small opening to the marrow became visible, a pin of approximate size of the canal was introduced into the marrow canal of the epiphysis end and gently aspirated to extrude the marrow out of the

opening at the iliac end, on to a slide to which a drop of fetal calf serum was added from a Pasteur pipette. The edge of a clean slide was used to thoroughly mix the marrow and serum to homogeneity and the spread as a smear. The slides were air dried and fixed in absolute methanol for five minutes and further air dried for a few minutes to remove the methanol. They were then stained in 5% Giemsa stain that was initially dissolved in 0.01M phosphate buffer pH 6.8, after initial staining with 0.4% of May-Greunwald. They were there after rinsed in distilled water, air dried, mounted in DPX (BDH) and covered with cover glass smeared with xylene. The stained and mounted slides were coded and scored using an Olympus XSZ 107 BN microscope for the presence of micro nucleated polychromatic erythrocytes.

Enzyme Assay

The blood samples collected were transferred into pre-labeled heparinized bottles to prevent coagulation and centrifuged at 3,000g for 30 minutes. The clear supernatants obtained were decanted and used immediately for the determination of plasma enzymes activity or stored at -20°C until required. Gamma-glutamyl transferase (γ -GT) activity was assayed in the plasma by using the reconstituted γ -GT reagent following the method of Szasz, [31]. Essentially, 0.5ml of the plasma was mixed with 0.05 ml of the reconstituted reagent. The absorbance of the mixture was read at 405nm four times with a minute interval between the readings.

The mean changes in absorbance per minute were then obtained. Alkaline phosphatase (ALP) activity was also assayed in the plasma using the reconstituted ALP reagent. 2.5ml of the reagent at 25°C was mixed with 0.05 ml of the sample. The mixture was then incubated at 25°C and the absorbance of the mixture was read twice at a minute interval at 405nm. The change in absorbance per minute was then estimated. ALT and AST activities in the plasma was determined using the ALT and AST kits (Randox Laboratories Ltd., UK) and following the method described by Reitman and Frankel, 1957 [32]. Briefly, 0.1ml of the sample was mixed with 5ml of Solution 1(Buffer; Phosphate buffer, L- Alanine α -Oxoglutarate) and incubated for exactly 30minutes at 37°C . 0.5ml of Solution 2 (2,4-dinitrophenylhydrazine) was added, mixed and allowed to stand for exactly 20mins at 20 - 25°C .

5.0ml of NaOH was added and the absorbance of the sample was read against the reagent blank at 546nm after 5minutes

STATISTICAL ANALYSIS

Results were expressed as mean \pm standard error of the mean. One-way analysis of Variance (ANOVA) was used for data analysis and *post-hoc* analysis after ANOVA employed the Duncan Multiple Range Test. *P* values less than 0.05 were considered statistically significant.

RESULT

The frequency of micronucleated polychromatic erythrocytes (mPCEs) induced in the rat bone marrow cells after administration of aqueous extract of *Spondias mombin* (SM) and Garlic (GA) and / or sodium arsenite (SA) are shown in Table 1.

Table 1: The Frequency of Micronucleated Polychromatic Erythrocytes (mPCEs) per 1000 Polychromatic Erythrocytes (PCES) in Test and Control Rats.

Group	Treatment	MPCES/1000PCES Mean \pm S.EM
I.	Distilled Water Alone	0.60 \pm 0.25*
II.	Sodium Arsenite Alone	16.40 \pm 0.51
III.	Garlic Alone	3.00 \pm 0.31*
IV.	Sodium Arsenite + Garlic	10.20 \pm 0.848***
V.	<i>Spondias mombin</i> Alone	10.40 \pm 0.89***
VI.	Sodium Arsenite + <i>Spondias mombin</i>	26.60 \pm 0.89***
VII.	Sodium Arsenite + Garlic + <i>Spondias mombin</i>	23.20 \pm 1.30***

* Significantly different from rats given distilled water
 ** Significantly different from rats given sodium arsenite

Administration of SA, GA and SM alone markedly induced mPCEs formation in the rat bone marrow cells when compared to negative control. This was observed to be about 27 folds, 5 folds and 17 folds in the rats fed SA, GA, and SM, respectively (Table 1: group II, III, and V). Pretreatment of rats with aqueous extract of GA

significantly decreased the induction on mPCEs by SA to about 17 folds while pretreatment of rats with SM further increased the induction of mPCEs to about 44 folds (Table 1: group IV, and VI). Pretreatment of rats with both SM and GA led to about 39 folds increase in the frequency of induction of mPCEs when compared to the negative control (Table 2: group VII).

Table 2 shows the activities of gamma glutamyl transferase (γ -GT), alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) in the serum after administration of aqueous extract of *Spondias mombin* (SM) and Garlic (GA) and/or sodium arsenite (SA) . SA, GA and SM alone significantly increase the activity of γ -GT by about 2.7, 1.7, and 1.8 folds respectively when compared to the control. Pretreatment of rats with aqueous extract of GA before SA exposure significantly lowered γ -GT activity to about 1.9 folds, while pretreatment of rats with SM lowered it to about 2.0 folds. Simultaneous treatment with SM and GA further decrease γ -GT activity to about 1.6 folds.

Table 2: Activities of Liver Function Enzyme in Plasma of in Test and Control Rats.

Group	S γ -GT (U/L) MEAN \pm S.EM	SALP (U/L) MEAN \pm S.EM	SALT (U/L) MEAN \pm S.EM	SAST (U/L) MEAN \pm S.EM
I.	11.58 \pm 0.38	14.12 \pm 0.71	32.31 \pm 0.57	40.37 \pm 0.39
II.	31.27 \pm 0.30*	70.52 \pm 0.69	71.42 \pm 0.89	70.99 \pm 1.84
III.	19.39 \pm 0.38	21.17 \pm 0.54	40.44 \pm 0.63	48.45 \pm 0.64
IV.	22.00 \pm 0.42	14.80 \pm 0.35	47.76 \pm 0.55	45.61 \pm 1.16
V.	20.35 \pm 0.41	49.32 \pm 0.43	43.39 \pm 0.68	51.18 \pm 0.68
VI.	23.05 \pm 0.48	37.17 \pm 0.43**	54.59 \pm 0.74	40.81 \pm 0.60
VII.	18.23 \pm 0.15	23.19 \pm 0.40	59.10 \pm 0.89	56.53 \pm 0.52

* Significantly different from rats given distilled water
 ** Significantly different from rats given sodium arsenite

SA, GA, and SM alone significantly increase the activity of ALP by about 5.0, 1.5, and 3.5 folds, respectively, when compared to the control. Pretreatment of rats with aqueous extract of GA before SA exposure significantly lowered ALP activity to about 1.1 folds, while pretreatment of

rats with SM lowered it to about 2.6 folds. Simultaneous treatment with SM and GA lowered ALP activity to about 1.6 folds.

SA, GA, and SM alone significantly increase ALT activity by about 2.2, 1.3, and 1.3 folds, respectively, when compared to the control. Pretreatment of rats with aqueous extract of GA before SA exposure significantly lowered γ -GT activity to about 1.5 folds, while pretreatment of rats with SM lowered it to about 1.7 folds. Simultaneous treatment with SM and GA decrease ALT activity to about 1.8 folds.

SA, GA, and SM alone significantly increase the activity of AST by about 1.8, 1.2, and 1.3 folds, respectively, when compared to the control. Pretreatment of rats with aqueous extract of GA before SA exposure significantly lowered AST activity to about 1.1 folds, while pretreatment of rats with SM lowered it almost to the level of the control. Simultaneous treatment with SM and GA decrease AST activity to about 1.3 folds.

DISCUSSION

In consonant with earlier our studies and that of others, SA significantly increased ($P < 0.05$) the frequency of micronucleated polychromatic erythrocytes (mPCEs) when compared with the negative control. This observation in SA treated group may reveal the clastogenic potency of SA in polychromatic erythrocytes of bone marrow cells. In the same vein, at the tested concentration, aqueous extract of GA significantly increased ($P < 0.05$) the frequency of formation of micronuclei when compared with the control. The mild clastogenicity of GA extract is well documented [33]. Similarly, feeding of rats with aqueous extract of SM induced the frequency of micronuclei formation in PCEs when compared with the negative control. This marked induction suggests that SM extract at the tested concentration is clastogenic.

The presence of anti-nutritional factors like alkaloids, saponins and anthraquinones in SM may account for this observation [25, 27, 34]. Mori et al [35] showed that a variety of pyrrolizidine alkaloids are genotoxic in rat, mouse and hamster. Similarly, anthraquinones have been observed to bind to DNA and thereby inducing oxidative stress [36].

The result of the combination experiments confirm earlier observation that pretreatment with the extract of GA significantly reduced ($P < 0.05$) the frequency of mPCEs formation in bone marrow cells induced by the administration of sodium arsenite. For instance, Das et al. [37] reported similar decrease in the degree of mPCEs formation in mice pretreated with garlic before sodium arsenite challenge.

Most of the inhibitory properties of GA have been attributed to the allyl sulfides and diallyl sulfides present in it. These compounds have been shown to exhibit anti-genotoxic effects against a variety of carcinogens [22, 38]. In contrast, the degree of mPCEs formation was further increased ($P < 0.05$) in rats pretreated with SM before SA administration. This increase was found to be additive under our experimental conditions. This may demonstrates the inherent health hazards that are associated with co-exposure to both SA and SM.

Co-exposure to both substances is not uncommon. It is a known fact that plants accumulate toxic concentrations of arsenic [39]. Also, human population may be co-exposed through arsenic contaminated well or underground water used in preparing leaf tonics and decoctions that is a common drink in certain populations. However, extract of GA appear to have some inhibitory action over the combined clastogenicity of both SA and SM as observed in the slight reduction in the degree of mPCEs formation in rats fed GA alongside SA and SM.

There is growing evidences that SA intoxicification can compromise the integrity of the liver in mouse, rat, fish, and goat [40-43]. Additional evidences have shown that exposure to low level arsenic exposure in drinking water can result in physiological disturbances and hepatocellular carcinoma in man (4). As observed in these earlier studies, the significant increase in plasma γ -GT, AP, ALT, and AST in this study when compared with the control, may be an indication of toxicity of SA in the liver.

Elevation of these enzymes in the plasma might be due to the increased permeability of plasma membrane, increased synthesis of the enzymes by the liver, inflammation, cellular necrosis and cholestasis in the liver [44-45]. In addition, the rise in plasma γ -GT activity of rats exposed to SA when compared to the control in this study is indicative of oxidative stress and cytogenetic

damage in animals exposed to SA. Increased activity of γ -GT has been associated with hepatotoxicity, oxidative stress and chromosomal aberrations in cells [46-47]. Similarly, administration of both aqueous extract of GA alone and SM alone significantly ($P < 0.05$) increased the activities of the four liver function markers although not to the level observed for SA. This may be an indication that both extracts contains substance that may compromise some vital function in the liver at the tested doses. The toxicity of SM may be related to the anti-nutrients present in it. For instance, pyrrolizidine, some alkaloids and anthraquinones found in herbal products have been implicated in hepatic toxicities and one of the mechanism by which they cause the hepatic injury is by elevating the activity of γ -GT, ALP and transaminases [48-49].

Paradoxically, pretreatment of rats with either GA and/or SM prevents hepatic cells against SA-induced damage as observed in the significant ($P < 0.05$) reduction in the activities of the four enzymes in the test control animals. This observation indicates that aqueous extract of GA and SM as well as a combination of the two extracts exhibit some inhibitory action over SA induced hepatic damage. This however, varies in the different liver function markers. Aqueous extract of GA was most effective in preventing SA induced leakage of ALP and ALT into the serum, while aqueous extract of SM was most effective in preventing SA induced serum AST rise.

Simultaneous administration of the two extracts before challenge with SA was the most effective in decreasing serum γ -GT activity. The ability of GA to protect the liver against a number of environmental toxicants is well documented [20-22] and this protective ability has been attributed to the mixture of antioxidant compounds including allicin and other diallyl thiosulfonates as well as volatile compound of sulphur present in the extract of GA [22, 50].

Extract of GA has also been shown to inhibit lipid peroxidation and improve cellular antioxidant defense system in SA intoxicated rats [51]. In addition, the detoxifying effects of GA are related to their ability to inhibit phase I enzymes and induce phase II enzymes or bind to exogenous toxins through sulfhydryl groups [50]. Furthermore, extract of GA contains trace amount of germanium and selenium, which improves host immunity and normalize oxygen utilization in cells [52]. The ability of the SM to reduce serum

enzymes activity may be related to antioxidant compounds present in it.

SM has been found to be rich phenolic compounds and vitamin C, which have been shown to modulate the toxicity of arsenic and other environmental toxicant. For instance, quercetin a polyphenolic flavonoidal compound found in large amount in vegetables, fruits and medicinal herbs have been reported to prevent sodium arsenite induced fibrosis in the liver by inhibiting lipid peroxidation, improving antioxidant status and decreasing the activity of ALT and ALP in the serum [53]. Similarly, vitamin C has been shown to reduce arsenic mediated ovarian and uterine toxicity [54]. Although the interaction between the different compounds found in both extracts of GA and SM have marked effect in reducing SA induced hepatic damage, it was however, not as effective as that produced by the extract of GA alone.

CONCLUSION

In conclusion, our results suggest that pretreatment with aqueous GA extract alone was more effective than either aqueous extract of SM or a combination of both GA and SM in protecting against SA induced clastogenicity and hepatotoxicity under our experimental conditions.

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SUGGESTED CITATION

Odunola, O.A., K.A. Akinwumi, and D.M. Ibegbu. 2011. "The Influence of Garlic and *Spondias mombin* on Sodium Arsenite induced Clastogenicity and Hepatotoxicity in Rats". *Pacific Journal of Science and Technology.* 12(2):401-409.



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