# AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 32, NO 1 MARCH 2003

EDITOR
B. O. OSOTIMEHIN

ASSISTANT EDITOR
A. O. UWAIFO

ISSN 1116—4077

*Afr. J. Med. med Sci* (2003) 32, 75-80

# Comparative effects of some local food condiments on sodium arsenite-induced clastogenicity

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**Summary**

The modulatory effects of the aqueous extracts of some locally consumed food condiments namely garlic (*Allium sativum*), ginger (*Zingiber officinale*), sconio (*Pimpinella anisumm* LINNE) and cloves (*Syzygium aromaticum*) on the clastogenic effects of sodium arsenite, a known inorganic clastogen were assessed in mouse bone marrow cells using the micronucleus assay method. Results of preliminary investigation of the clastogenicity of the condiments show that aqueous extracts of these condiments have very mild clastogenic activity in mice in the order garlic > ginger and sconio > cloves and that extracts of ginger and sconio seem to have the same degree of clastogenicity. Pre-treatment of mice for seven days with extracts of the condiments orally before exposure to the oral dose (2.5 mg/kg body wt.) of sodium arsenite resulted in a remarkable reduction of the magnitude of formation of micronuclei in polychromatic erythrocytes of the bone marrow. The degree of reduction of the clastogenic effect of arsenite was of the order ginger > garlic > cloves > sconio. This reduction of arsenite induced clastogenicity by aqueous extracts of the condiments may be due in part to the antioxidant properties of their chemical constituents, thus suggesting that the condiments may be useful in the prevention of arsenite-induced toxicity in areas where arsenic is an environmental contaminant.

**Keywords:** *Sodium arsenite, ginger, cloves; sconio; garlic; clastogenicity*

**Résumé**

Les effects modulatoires des extraits acqueux de certains condiments en consommation locale: l'aile (*Allum Sativum*), Djinger (*Zingiber Officinale*), Percil(*Pimpinella anisum line*) et Cèlerie (*syzguim oromadicum*) sur les effects collastogèniques de l arsènie de sodium , un clastogèn inorganique connu ètaient evaluès sur les cellules de la moèlle osseuse des rats en utilisant l'analayse des micronuclèiques. Les rèsultats des investigations prèliminares sur la clastegènicitè de ces condiments montrent que ces extraits acqueux avaient d'activitè clastogènique très legère sur les rats dans l'ordre l'aile > djinger> percil> cèlerie.Les extraits du djinger et du percil semblent avoir le meme degrè de clastogènecitè, le traitement orale de ces rats pour 7 jours avec ces extraits avant l'administration d'une dose orale d'arsènite de sodium, rèsultait a une rèduction remarquable de la magnitude de formation des micronuclèiques chez les erythrocytes plychromèes dans les moèlles osseuses. Le degrè de rèduction des effects clastogèniques de l'arsènite ètait dans l'ordre Djinger > aile > cèlerie > persil. Le rèduction d'arsènite indusiat la clastogènecitè par les extraits acqueux des condiments,ainsi suggerant que les condiments peuvent etre utilisès dans la prevention de la toxicitè induite par l'arsènite en lieux ou l'arsènite est un contaminant environemental.

## Introduction

Arsenic, a well known human carcinogen, is widely distributed in the environment as a toxicant in coal, soil, drinking water and air [1,2]. The metal is therefore a great threat to public health due to exposure via inhalation in industrial areas and ingestion from food, agricultural products and portable water[3]. For instance, epidemiological evidence has associated intake of arsenic contaminated water in areas such as Inner Mongolia, China, [4 ,5] Taiwan [6], Bangladesh and West Bengal, India [7] with cardiovascular diseases such as hypertension [8] and arteriosclerosis [9], diabetes mellitus [10] and cancer of the skin, lung, bladder and kidney [11-13].

This lethal environmental contaminant which is a widely distributed member of the arsenicals has been used extensively as a component of animal feeds, pesticides, herbicides and as a representative for study [14]. Although, arsenic compounds are detoxified in the body via methylation [15] and are excreted mostly as arsenite salts through feaces and as arsenate in urine, the rate of excretion is generally slow and most of the arsenites remain unmethylated. In this regard, 20-25% of inorganic arsenite usually remains unmethylated [16]. Toxicity is therefore believed to result from the unmethylated and tissue bound arsenicals. For instance, the highest level of arsenite was reported in the liver and kidney of cancer patients twenty hours after intravenous injection of sodium arsenite ($^{76}As$) [17].

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In addition to various epidemiological research activities going on in the arsenic endemic areas of the world, safe drinking water and consumption of foods rich in substances that can protect against arsenic toxicity may play a pivotal role in solving the health problems resulting from arsenite intoxication. Incontrovertible pieces of evidence have shown that consumption of certain foods and adherence to certain dietary customs could protect against diverse human ailments including cancer [18]. In this regard, the consumption of garlic (*Allium sativum*), ginger (*Zingiber officinale*), sconio (*Pimpinella anisumm* LINNE) and cloves (*Syzygium aromaticum*) have been linked to diverse antitoxic properties in areas where they are widely ingested [19-22]. Moreover, aqueous extracts of garlic bulbs have been shown to significantly reduce the clastogenic effects of sodium arsenite [23] and to have antimutagenic activity against ionizing radiation, peroxides, adriamycin and N-methyl-N'-nitrosoguanidine in *Salmonella* tester strain [24].

The present study was therefore, designed to investigate the possible protective effects of aqueous extracts of ginger, sconio and cloves on sodium arsenite-induced clastogenicity in mice.

## Materials and methods

### *Experimental animals*

Male albino mice (*Mus musculus*) litter mates of about 10 – 12 weeks old having an average weight of approximately 22 g were obtained from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. The animals were kept five [5] per cage and were fed pellets (from Ladokun Livestock Feeds Limited, Ibadan, Nigeria) and water ad libitum. The mice were allowed to acclimatize for five [5] days before commencement of the experiment. Room temperature was 29 ± 2 °C with 12 hrs light/dark cycle.

### *Clastogen*

Sodium arsenite ($NaAsO_2$; Loba, Chemie, Co. Bombay, India; mol. wt. 129. 92; As 57.6%) was dissolved in glass-distilled water. The concentration used was 2.5 mg/kg body weight of mice and this corresponds to 1/10$^{th}$ of the oral $LD_{50}$ of the salt in mice [25].

### *Extracts of food condiments*

Garlic bulbs (*Allium sativum* L; single clove variety); ginger (*Zingiber officinale*; the yellow variety); sconio (*Pimpinella anisumm* L.) and cloves (*Syzygium aromaticum)* were purchased from Bodija Market at Ibadan, Oyo State, Nigeria. Garlic extract (Ga) was prepared from freshly sliced cloves grounded into paste which was made up to 2.2% w/v stock suspension. About 0.1ml of this suspension was administered to the mice orally to give a dosage of 100 mg/kg body wt. of mice. This corresponds to a daily human intake of 6.0 g garlic by a 60 kg individual. The dose was also equivalent to the highest concentration of garlic extract that had been used beneficially against certain disease conditions [26]. Ginger extract (Gi) was prepared from freshly peeled and sliced rhizome grounded into paste. The preparation and administration are as described for garlic extract. In preparing extracts of sconio (Sc) and cloves (Cl), these food condiments or additives were separately ground into dry powder. Stock suspension 2.2% (w/v) of each of the extracts were also prepared and 0.1 ml each of the suspension was administered to experimental animals accordingly.

### *Experimental protocol*

Throughout the duration of the experiment, the mice were given pellet and water ad libitum. The mice were divided into ten different groups of five mice each. The mice in group I were given distilled water for seven consecutive days. Those in group II were given distilled water for seven days and on the seventh day, they also were given $NaAsO_2$ (2.5 mg/kg bdwt). The mice in groups III, IV; V and VI were separately fed with aqueous extracts of Ga, Gi, Sc and Cl, respectively, for seven days. The animals in the remaining four [4] groups VII; VIII; IX and X were separately fed with aqueous extract of Ga, Gi; Sc and Cl, respectively, for seven days and on the seventh day they were fed 2.5 mg $NaAsO_2$ / kg body wt.. Twenty-four hours after the last feeding of the extracts and/or $NaAsO_2$, the mice were killed by cervical dislocation.

### *Micronucleus assay*

The micronucleus assay was carried out as described by Heddle and Salamone [27] and Heddle *et al.* [28]. Femurs were removed by cutting through the pelvic bones and below the knee. The bones were freed from muscles and the knee and all the surrounding tissues were separated from the shaft in the epiphyseal plate leaving the marrow cavity closed. A needle was inserted into the proximal part of the marrow canal and the marrow was flushed out by gentle aspiration and flushing with fetal calf serum in the syringe. The cell suspension was centrifuged at 1,000 rpm for 5 minutes. The supernatant was removed and the viscous pellet was saved for use. Slides were prepared by smearing the viscous pellet as a thin film on a microscope slide. This was followed by fixation in glacial acetic acid-ethanol (1:3,v\v), air drying and pretreatment in undiluted and diluted May – Gruenwald solution for 3 min and 2 min, respectively. The slides were then stained in Giemsa solution. The stained slides were coded and scored under a compound microscope with the aid of a tally counter for the presence of micronucleated polychromatic erythrocytes.

## Results

Table 1 shows the number of micronucleated polychromatic erythrocytes (PCEs) that were induced in a population of 1000 PCEs in mouse bone marrow after administration of the aqueous extracts of the different food condiments used in this study. As shown in the table, the degree of induction of micronuclei in the PCEs although in the order Ga>Gi and Sc>Cl was only slight when compared with the number of micronucleated PCEs in mice fed distilled water only (negative control group).

**Table 1:** Number of micronucleated polychromatic erythrocytes/1000 polychromatic erytrocytes in mouse bone marrow after administration of food condiments.

| Treatment of experimental animals | Micronucleated PCE/1000PCE slide number | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean ± S.D |
| Distilled water only | 1 | 0 | - | 0 | 0 | 0.25 ± 0.43 |
| Garlic extract (Ga) | 3 | 2 | 2 | 3 | - | 2.50 ± 0.57 |
| Ginger extract (Gi) | 2 | 1 | 1 | 1 | 2 | 1.4 ± 0.48 |
| Sconio extract (Sc) | 2 | 1 | 2 | 1 | 1 | 1.4 ± 0.48 |
| Cloves extract (Cl) | 1 | - | 0 | 2 | 1 | 1.0 ± 0.71 |

*Distilled water or extracts of the food condiments (100 mg/kd bd wt) were administered orally for seven days before the micronucleus assay as described under materials and methods.*

Table 2 shows the effect of the food condiments on sodium arsenite induced formation of micronucleated PCEs in mouse bone marrow. As indicated in the table, sodium arsenite markedly induced micronuclei formation in the PCEs when compared to the negative control group above. However, oral administration of the extracts and sodium arsenite on the seventh day simultaneously led to marked decreases in the population of micronucleated PCEs induced by the toxin.

**Table 2:** Effect of extracts of food condiments on [a]sodium arsenite-induced formation of micronucleated polychromatic erythrocytes in mouse bone marrow.

| Treatment of expenmental animals | Micronucleated PCE/1000 PCE slide number | | | | | | % [b] reduction |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean SD | |
| Distilled water only | 1 | 0- | 0 | 0 | 0 | 0.25 ± 0.43 | - |
| Distilled water + sodiumarsenite | 7 | 7 | 6 | 9 | 7 | 7.2 ± 0.97 | - |
| Ga + sodiumarsenite | 5 | 4 | 4 | 3 | 5 | 4.2 ± 0.75 | 41.7% |
| Gi + sodiumarsenite | 3 | 3 | 5 | 4 | 5 | 4.0 ± 0.89 | 44.4% |
| Sc + sodiumarsenite | 6 | 4 | 5 | 5 | 4 | 4.8 ± 0.75 | 33.3% |
| Cl + sodiumarsenite | 4 | 4 | 5 | 4 | 5 | 4.4 ± 0.49 | 38.9% |

*(a) Sodium arsenite (25 mg/kg bd wt) was administered orally on the seventh day of exposure to distilled water or extracts of food condiments as described under materials and methods.*

*(b) Percentage reduction in the effect of sodium arsenite only by the extracts*

## Discussion

The WHO maximum permissible limit and the U.S. Environmental Protection Agency (USEPA) standard for arsenic in drinking water is 50 µg/L [29, 30]. However, WHO recommended 10 µg arsenic per litre of drinking water as a guideline because at 50 µg/L the risk of dying from cancer of the liver, lung, kidney or bladder is about 13 per 1,000 persons [31, 32]. Up to date here is no remedy for chronic arsenic toxicity. Thus safe drinking water, nutritious food (fruits and vegetables) and physical exercise have been suggested as preventive measures against chronic arsenic toxicity. The present study was therefore designed to assess the modulatory effect of aqueous extracts of ginger, sconio and cloves on sodium arsenite clastogenic effects using mice as a model. *In vivo* chromosomal breakage is easily detectable using the mouse micronucleus assay rather than by the traditional cytogenic techniques [33, 34]. Positive results with the assay demonstrate an *in vivo* genetic activity, specifically chromosome breakage in the bone marrow. Although the result obtained by use of the micronuclei assay does not show conclusively that the agent tested is a mutagen or carcinogen, there is incontrovertible evidence that this is usually the case.

The result of the present investigation confirms that twenty-four hours after administration, 2.5 mg arsenite/kg body wt. induced micronuclei formation in polychromatic erythrocytes (PCEs) in the bone marrow of mice (Table 2, Fig.1). The increase in the population of micronucleated PCEs was highly significant when compared to the control. These results suggest an *in vivo* chromosomal breakage in the bone marrow induced by arsenite because micronuclei

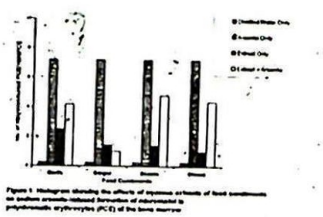

**Fig.1:** Histogram showing the effects of aqueous extracts of food condiments on sodium arsenite-induced formation of micronuclei, in polychromatic erythrocytes (PCE) of the bone marrow.

arise from chromosomal fragments that are not incorporated into daughter nuclei at the time of cell division. Before assessing the modulatory effects of the four food condiments used in this study, preliminary investigation was carried out to determine if the condiments on their own have any clastogenic effect. Although crude extracts of garlic had earlier on been shown to induce some degree of chromosomal damage in mouse bone marrow cells [35], there is a dearth of information on the clastogenic or carcinogenic potentials of Gi, Sc and Cl. The data generated in this study on the level of micronucleated PCEs observed in the bone marrow smear of mice fed with crude extracts of the food condiments (i.e., Ga, Gi, Sc and Cl) show evidence of a somewhat mild clastogenic activity of the order Ga > Gi and Sc > Cl when compared with the negative control group (Table 1 and Fig. 1). Extracts of Gi and Sc seem to have the same potency while Cl extract is the least active.

An assessment of the modulatory effect of these extracts on the toxicity of sodium arsenite shows clearly that pretreatment of the mice by feeding the crude extracts of the condiments for seven days before exposure to sodium arsenite markedly reduced the population of micronuclei in the PCEs and this is an indication of a decrease in chromosomal damage. From the data presented in Table 2 and the histogram (Fig. 1) the degree of reduction of formation of micronuclei by sodium arsenite in the presence of the crude extracts of the condiments is in the order Gi > Ga > Cl > Sc. However, there is no significant difference in the degree of reduction from one condiment to the other. The reduction of clastogenic effect of sodium arsenite by crude extracts of garlic has been attributed to the inactivation of active oxygen species by allin (thio-2-propene-1-sulfonic acid S-allyl esters) an enzymatic degradation product of allin (S-ally-L-cysteine sulfoxide) which is present in garlic [24, 36]. Similarly, the ability of crude extracts of ginger to reduce the clastogenic effect of sodium arsenite may be attributed to the gingerol and sesquiterpene components of ginger. These two components have been shown to possess antiviral, antifungal and antioxidant properties [37, 38]. The gingerols have also been shown to inhibit pentahydrate-induced vomiting in leopards and frogs [39]. In addition, clove extracts contain sesquiterpenes eugenol, eugenol acetate and caryophyllene which have been suggested to have antioxidant and anticarcinogenic properties [40, 41]. The effect of clove extract in the present investigation may also be due to the effects of these sesquiterpenes. Although little is known about the anticarcinogenic and anticlastogenic properties of sconio, its antifungal, antipesticidal and antioxidant properties are well documented [42]. Thus, its antioxidant properties may account for its ability to reduce micronuclei formation and thus offer some protection against sodium arsenite-induced chromosomal damage.

In view of the observation that arsenic toxicity may be prevented by the binding of arsenic to thiol groups through the use of compounds such as British anti-Lewisite-2,3-dimercaptopropanol (BAL) [43], the active ingredients in the extracts of Ga, Gi, Sc and Cl could be purified and used to protect against arsenite toxicity. In this connection, further investigation will be carried out to establish the usefulness of purified components of the extracts in arresting arsenite toxicity.

**Acknowledgements**

This work was supported in part by the University of Ibadan Senate Research Grant (SRG/COM/1996/16A). The technical assistance rendered by Mr. J.M. Omorogbe is appreciated.

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