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Reduction of the Clastogenic Effect of Inorganic Arsenic by Extracts of some Dietary Additives

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Abstract

The clastogenic effects of aqueous extracts of Garlic (*Allium sativum.L*) (Ga), African pepper (*Xylopia aethiopica*) (Ap), Bush pepper (*Piper guineense*) (Bp) and African nutmeg (*Monodora myristica*) (An) at 100mg/kg body weight each administered orally either alone and in combination with a single oral dose of sodium arsenite (2.5mg/kg body weight, $^1/_{10th}$ of LD_{50}) were investigated in mouse bone marrow cells. The results obtained show that Ga induced micronucleus formation in the polychromatic erytrocytes (PCEs) of the bone marrow by about 12 folds followed by Bp (7 folds), Ap (4 folds) and An (1 fold) in comparison with animals exposed to distilled water only. These results indicate that Ga, Bp and Ap have mild clastogenic activity in mice. In contrast, sodium arsenite, a known clastogen, induced micronucleated PCEs formation by about 90 folds. Interestingly treatment of mice with extracts of the dietary additives Ga, Bp, Ap and An, markedly reduced the clastogenic activity of sodium arsenite in the order Ap > Ga > An > Bp. Maximum reduction of arsenite effect was about 60% with Bp. It may be concluded therefore from these findings that garlic, African pepper, bush pepper and African nutmeg may be useful in dietary manipulation of arsenic intoxications.

Introduction

Chronic arsenic (As) toxicity resulting from the consumption of arsenic contaminated ground water all over the World and especially in Bangladesh and West Bengal, India (1) has resulted into the investigations on different methods of intercepting or preventing the harmful effect of such exposures. Epidemiological evidences have associated long term consumption of well water contaminated with As with diverse diseases such as cancers of the bladder, liver, lung and kidney (2, 3). There is no medication for chronic arsenic toxicity. Safe drinking water, nutritious food and physical exercise have been suggested as the only preventive measures to fight chronic arsenic toxicity. (1). Presently, dietary inhibitors of mutagenesis and/or carcinogenesis are being explored for their usefulness in the prevention of human cancers (4). In this regard, dietary intervention programmes have been instituted in As endemic areas of the World. Dietary supplementation with crude extracts of garlic (5, 6) and *Emblica officinalis* fruits (Indian goose berry) (7) iron (8) and dietary oils such as mustard oil (9) has been shown to be highly effective in reducing the cytotoxic effects of chronic exposure to arsenic in the form of sodium arsenite. In our laboratory, dietary administration of the crude extracts of garlic and certain spices namely ginger (*Zingiber officinale*), sconio (*Pimpinella anisumm. L*) and cloves (*Syzygium aromaticum*) has been shown to reduce the clastogenic effects of sodium arsenite in mice (10).

Furthermore, African pepper (*Xylopia aethiopica*), Bush pepper (*Piper guineense*) and African nutmeg (*Monodora myristica*) are some other spices that are highly consumed locally in Nigeria. For instance, African pepper has been used as a principal ingredient of cough mixtures and in the relieve of respiratory ailments (11, 12). Bush pepper has been used as a carminative agent and it has been shown to have antifungal activity against some pathogenic fungi such as *Basidiobulus haptosporus* (13). African nutmeg has been used in the relieve of constipation and as a disinfectant (14). Complete inhibition of tomatoketup spoilage bacteria and yeast by African nutmeg has also been reported (15). There is however a dearth of information on the clastogenic/anticlastogenic properties of these spices.

The present investigation therefore examines the clastogenic potentials of these spices and their effect on sodium arsenite-induced clastogenicity in order to complement the existing dietary intervention attempts in As endemic regions.

Materials and Methods

Experimental Animals

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

Chemicals

Sodium arsenite ($NaAsO_2$; mol. wt. 129. 92; As 57.6%; CAS No 7784–46-5 Loba, Chemie, Co. Bombay, India;) was dissolved in glass-distilled water. The concentration used was 2.5mg/kg body weight of mice and this corresponds to $1/10^{th}$ of the oral LD_{50} of the salt in mice (16). This concentration of sodium arsenite has been shown to be highly clastogenic (5). Giemsa and May-Grunwald stains were obtained from Aldrich Chemical Co., Inc. Mil. Wisconsin, U.S.A. All other reagents were of the highest purity grade and were purchased from the British Drug Houses Ltd. Poole, England or Hopkin and Williams Essex, U.K.

Preparation of Extracts of Food Condiments

Garlic bulbs (*Allium sativum* L; single clove variety); African pepper (*Xylopia aethiopica*); Bush pepper (*Piper guinense*) and African nutmeg (*Monodora myristica*) were purchased from Bodija Market at Ibadan and certified at the herbarium in the Department of Botany and Microbiology, University of Ibadan, Oyo–State, Nigeria. Garlic extract (Ga) was prepared from freshly sliced cloves grounded into paste and made up to 2.2% w/v stock suspension. A dose of 100mg/kg body weight of mice was fed to the mice based on a daily human intake of 6.0g garlic by a 60kg individual. The dose was also equivalent to the highest concentration of garlic extract that had been used beneficially against certain ailments (17). In preparing extracts of African pepper (Ap). Bush pepper (Bp) and African nutmeg (An), these food condiments or additives were separately grounded into dry powder. 2.2% (w/v) stock suspension of each of the extracts were also prepared and 0.1ml each of the suspension were administered to experimental animals accordingly.

Experimental Protocol

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 were also given sodium arsenite (2.5mg/kg body weight). The mice in Groups III, IV; V and VI were separately fed with aqueous extracts of Ga, Ap, Bp and An, respectively, for seven days. The animals in the remaining four Groups VII; VIII; IX and X were separately fed with aqueous extract of Ga, Ap, Bp and An respectively, for seven days and on the seventh day they were fed 2.5mg sodium arsenite / kg body weight. Twenty-four hours after the last feeding of the extracts and/or sodium arsenite, the mice were killed by cervical dislocation. All the mice had access to pellets and water *ad libitum* throughout the duration of the experiment.

Micronucleus assay

The micronucleus assay was carried out as described by Heddle and Salamone (18) and Heddle et al (19). 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 min. 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 -- Grunenwald 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 direct light compound microscope (Leitz Wetzlar model) with the aid of a tally counter for the presence of micronucleated polychromatic erythrocytes.

Results

The data presented in Table 1 show the number of micronucleated polychromatic erythrocytes (mPCEs) per 1000 polychromatic erythrocytes (PCEs) in mouse bone marrow after administration of distilled water or extracts of garlic, African pepper, bush pepper and African nutmeg. From this table, it is clear that Ga, Bp, Ap and An induced micronucleus formation in the PCEs by about 12, 7, 4 and 1 folds respectively, when compared to the mPCEs formed in the negative control mice fed distilled water only.

Table 2 shows the modulating effect of dietary supplementation with Ga, Bp, Ap and An on sodium arsenite-induced micronucleus formation in the PCEs. In this regard, sodium arsenite alone induced mPCEs formation in the mouse bone marrow cells by about 90 folds in comparism to the negative control. However, simultaneous oral administration of sodium arsenite on the seventh day of daily feeding of the extracts led to a significant reduction in the potency of sodium arsenite to induce mPCEs. The degree of reduction of the potency of sodium arsenite by the extracts is in the order Ap > Ga > An > Bp. Specifically Ap, Ga, An and Bp reduced sodium arsenite – induced formation of mPCEs by about 41%, 44%, 52% and 60%, respectively.

Table 1: Number of micronucleated polychromatic erythrocytes (mPCEs) 1000 polychromatic erythrocytes in mouse bone marrow after administration of dietary supplements

Treatment on experimental animals	mPCEs /1000PCEs Mean ± S. D
Distilled water only	0.07 ± 0.01
Garlic extract (Ga)	0.85 ± 0.15
African paepper (Ap)	0.30 ± 0.07
Bush pepper (Bp)	0.50 ± 0.09
African nutmeg (An)	0.09 ± 0.01

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

Table 2: Modulating effect of extracts of the dietary supplements on [a]sodium arsenite-induced formation of micronucleated polychromatic erythrocytes (mPCEs) in mouse bone marrow.

Treatment on experimental animals	mPCEs /1000PCEs Mean ± S. D	% [b]reduction
Distilled water only	0.07 ± 0.01	-
Distilled water + $NaAsO_2$	6.30 ± 0.34	-
Ga + $NaAsO_2$	3.50 ± 0.12	44.4
Ap + $NaAsO_2$	3.70 ± 0.15	41.3
Bp + $NaAsO_2$	2.50 ± 0.10	60.3
An + $NaAsO_2$	3.00 ± 0.11	52.4

(a) Sodium arsenite ($NaAsO_2$) (2.5mg/kg bd. wt. was administered orally on the seventh day of exposure to distilled water or extracts of African pepper (Ap), Bush pepper (Bp) and African nutmeg (An) as described under materials and methods.

Percentage reduction in the effect of sodium arsenite only by the dietary supplements.

Discussion

In the present investigation, sodium arsenite at $^{1}/_{10}^{th}$ of the oral LD50 (i.e. 2.5mg/kg body wt.) induced the formation of micronucleus in the polychromatic erythrocytes (PCEs) of the mouse bone marrow as previously observed in our laboratory (10) and by other investigators. The degree of this induction was about 90 folds as compared with the micronucleated polychromatic erythrocytes (mPCEs) observed in the group of mice fed with distilled water only. Crude extracts of garlic (Ga), bush pepper (Bp), African pepper (Ap) and African nutmeg (An) also induced certain degree of micronucleus formation in the PCEs when compared with the negative control. This induction is about 12, 7, 4 and 1 folds for Ga, Bp, Ap and An respectively. Of these extracts, garlic extract seem to be the most active and African nutmeg was the least active in the induction of chromosomal aberration. In vivo chromosomal breakage is easily detected by the micronucleus assay (18). The ability of crude garlic extract to induce chromosomal damage is well documented (5, 6, 9).

Oral administration of the crude extracts for seven days followed by simultaneous oral administration of sodium arsenite on the seventh day greatly reduced the degree of clastogenic effect of sodium arsenite in the order Ap > Ga > An > Bp. (Table 2). The degree of reduction being about 41%, 44%, 52% and 60% respectively. Bush pepper (Bp) therefore seem to be the most active in reducing sodium arsenite induced chromosomal damage in the experimental animals. The reduction by garlic had been attributed to the antioxidant and free radical scavenging properties of allicin (thio-2-propene-t-sulfonic acid-S-ally ester) an enzymatic degradative product of allin (S-ally-L-cysteine sulfoxide) which is found in garlic (20, 21). The activity of the extracts of African pepper, African nutmeg and especially bush pepper may also be attributed to antioxidant properties of their constituents. This work is being continued to isolate and purify the components of the extracts and to determine their mechanism of action. Effect of long-term supplementation with the extracts is also being studied.

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