BRIEF REPORT

Dietary protection by garlic extract against lead induced oxidative stress and genetic birth defects

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Abstract
The antioxidant potentials of *Allium sativum* extract was studied in bone marrow cells of albino rats using micronucleus assay with the use of 100 mg/ml crude garlic extract as dietary supplement via oral gavage. The rats were divided into three groups: **A**, distilled water; **B**, lead acetate; **C**, garlic extract + lead acetate. After the short-term exposure, rats were sacrificed by cervical dislocation and chromosomal preparations were made from bone marrow according to colchicines-hypotonic-fixation-air-drying-Giemsa schedule. The cytogenic end points observed were chromosomal aberrations and tissue damage. The chromosomal aberration induced by lead was reduced significantly in animals fed with the extract in group C while lead acetate administered to animals in group B was highly mutagenic. Besides, the antioxidant properties of garlic was further demonstrated in the *in vitro* experiment where it provided significant protection to thiobarbituric reactive substances inhibition, and reduced and oxidized glutathione contents in liver tissues. This suggests their ability to act as free-radical scavengers and in protecting the cell against oxidative stress for normal cellular functions. The results further harp on the involvement of reactive oxygen species in lead toxicity and also revealed the beneficial role of garlic therapeutic efficacy which indicates the antimutagenic and antioxidant potentials of garlic against oxidative stress and mutation.

INTRODUCTION

Chronic exposure to lead leads to its accumulation in vital organs with maximum concentrations reported in kidneys [1]. Developing brain is particularly vulnerable to its toxic effects ranging from behavioral abnormalities, learning impairment, decreased hearing and impaired cognitive functions in human and experimental animals [2]. Oxidative stress has been reported as one of the important mechanisms of lead toxicity [3]. This suggests that changes in glutathione levels as well as antioxidant enzyme activities implicate oxidative stress in lead toxicity. Some earlier studies also indicate that the disruption of reducing status of tissue leads to formation of reactive oxygen species (ROS) which may damage essential biomolecules such as protein, lipids and DNA [4]. This further emphasizes that at high levels; these ROS could be toxic to cells and may possibly contribute to cellular dysfunction and poisoning. On the other hand, the roles of garlic as dietary supplements cannot be over-emphasized as regards restoration and maintenance of the body physiological well-being. Its roles in the diets have been reported to reduce cholesterol levels. Furthermore, the antimicrobial, antithrombotic, and antibiotic properties of garlic have been proven over different diseases such as stroke, atherosclerosis, infertility and prostate cancer [5].

The present investigation was designed to study the degree of protection offered by garlic extract against lead-induced oxidative stress and mutagenic effects as it had been reported that lead is a mutagen capable of inducing chromosomal aberrations in man and animals [6]. Besides, it is also aimed to justify the hypothesis that most, if not all, cancer cells are characterized by chromosomal changes or alterations that are frequently specific to a particular tumor.

MATERIALS AND METHODS

Plant extract

Crude aqueous extract of garlic (*Allium sativum* L) of single clove variety was prepared from bulbs purchased from the market in Ado Ekiti, Nigeria. The clove was sliced, ground into paste and then dissolved in distilled water; 10 mg/ml corresponding to 100 mg/kg of animals was used for the *in vivo* experiment. The garlic concentration was however, varied for the *in vitro* experiment relative to the liver tissue used.

Experimental animals

The *in vivo* experiment was performed using twelve male Wistar rats weighing 120 ± 10 g housed in stainless cages with temperature maintained at 25 ± 2ºC and 12 h alternating day/night cycle, and the rats were

fed standard pellets and water *ad libitum*. The handling and use of the animals were in strict compliance with NIH guide for the care and use of laboratory animals.

Experimental protocols

The animals were divided into three groups with four animals in each group: rats in group **A** serve as control and were treated with distilled water only; those in group **B** received 2.5 mg/kg lead acetate; group **C** rats were fed simultaneously with 2.5 mg/kg lead acetate and 100 mg/kg garlic extract (1:1). The concentration of the lead salt was made equivalent to $^{1}/_{10}$ of the LD_{50} [7]. The dose of garlic extract was equivalent to the exact concentration used for beneficial effects against specific disease conditions [8]. Each dose was administered via oral gavage to the animals on daily basis consecutively for four weeks.

Chromosomal aberrations

Chromosomes were studied from bone marrow cells following the usual colchicines-hypotonic-fixation-air-drying technique [9]. Animals were sacrificed by cervical dislocation 24 h after the last treatment; 90 min prior to sacrifice, each animal was injected with 1% colchicine. Femurs were removed and the bone marrow was flushed out into centrifuge tubes with freshly prepared and pre warmed (36ºC) solution of 0.56% KCl into 8 ml centrifuge tubes. The fat lumps were removed with fine tipped pipette and was allowed to stand for 1 h to allow cells swell in the hypotonic solution. The cells were later pelleted by centrifugation (1000 rpm) for 5 min and supernatant discarded by the use of a Pasteur pipette [10]. Freshly prepared Clerk's fixative (glacial acetic acid:methanol, 1:3) was added to the cells and was suspended by vigorously agitating the centrifuge tubes [11]. The slides were coded and scored blind and were stained with Giemsa for 15 min, rinsed, dried at room temperature. Each slide was placed on the microscope and scanned carefully under objective (10x) for metaphase spreads while better views were observed under 40x for identifying clearly mitotic spreads of the chromosomes. The identified spreads were viewed using 100x oil immersion objective. The end points scored were chromosomal aberrations and damage cells.

Thiobarbituric acid reactive substances (TBARS)

Production of TBARS was determined by the method of Ohkawa *et al* [12]. The rats were anesthetized with ether, sacrificed by decapitation, and the liver tisssue were quickly removed and placed on ice-blocks. 1 g of the tissue was homogenized in cold Tris-HCl buffer at pH 7.4 (1:10 w/v). The homogenate was centrifuged for 10 min at 1400g and the supernatant was used for the assay. The supernatant was incubated with or without 50 mμ of freshly prepared lead acetate (2.5 mg) and at different concentrations (10-160 mg/ml) of the plant extract, together with an appropriate volume of deionized water amounted to total volume of 300 mμ at 37ºC for 1 h. The color reaction was carried out by adding 200, 250 and 500 mμ each of 8.1% sodium dodecyl sulphate (SDS), acetic acid (pH 3.4), and 0.6% TBA, respectively, and were further incubated at 97ºC for 1 h. The absorbance was read after cooling at a wavelength of 532 nm in a spectrophotometer. The experimental design involves the basal containing no extract and pro-oxidant (normal), control containing pro-oxidant without the extract, while the other test-tubes contain both the extract and pro-oxidant.

Reduced glutathione (GSH)

0.2 ml of sample homogenate (liver tissue) was added to 1.8 ml of distilled water and 3 ml of the precipitating agent sulphosalicylic acid was mixed with the 2.5 ml garlic extract. This was centrifuged at 3000g for 4 min and 0.5 ml of the supernatant was added to 4.5 ml of Ellman's reagent. A blank was prepared with 0.5 ml of the diluted precipitating agent and 4 ml of phosphate buffer and 0.5 ml of Ellman's reagent. The absorbance of the reaction mixture was taken within 30 min of color development at 412 nm against a reagent blank. The concentration of GSH was extrapolated from the GSH standard curve [13].

Oxidized glutathione (GSSG)

The reaction mixture containing 500 mμ phosphate buffer, 100 mμ sodium azide, 200 mμ GSH and 100 mμ H_2O_2 were added to 500 mμ of the sample, after which 600 mμ of distilled water was added and mixed thoroughly. The mixture was incubated at 37ºC for 3 min after which 0.5 ml of TCA was added and centrifuged at 3000 rpm for 5 min. Two milliliters of K_2HPO_4 and 1 ml of dinitrothiocyanobenzene (DNTB) was added each to 1 ml of the supernatants and the absorbance was read at 412 nm against blank.

Statistical analysis

The data from the groups were pooled and analyzed statistically using ANOVA [14] and Duncan's multiple range tests in order to compare significance of differences where P values less than 0.05 were considered significant.

RESULTS

Lead acetate-induced chromosomal aberrations such as chromosomes and chromatic breaks, gap and chromosomal rearrangement were analyzed. Fig.1 shows the frequencies of total chromosomal aberrations and the mean frequencies per cell as well as percentage of damaged cells in rats exposed to lead acetate *in vivo*. The frequency of damaged cells significantly reduced (2%) in group C animals fed simultaneously with both the toxicant and garlic extract. Group B animals showed highly mutagenicity with a total chromosomal aberration rate of 25% while no significant difference was observed in animals of group **A**.

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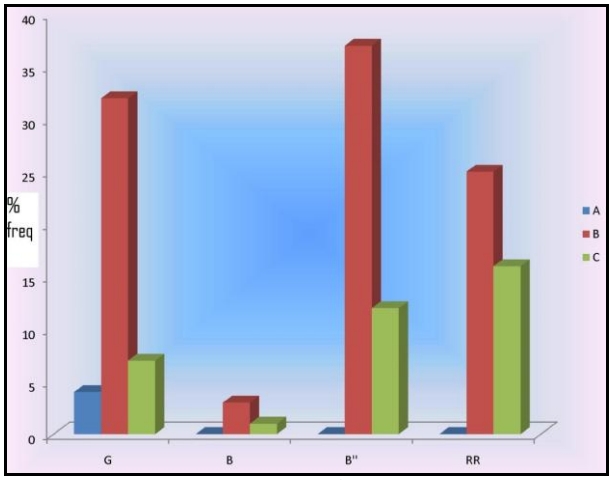

Figure 1. Chromosomal aberrations following treatment with lead acetate and garlic extract [G, chromosomal gap; B, chromosome break; B", chromatid break; RR, chromosomal rearrangement; $P < 0.05$ for the total chromosomal aberrations].

Table 1 shows the interaction (inhibition) of garlic extract with lead acetate induced lipid peroxidation in the liver. There was a statistically significant ($P < 0.05$) increase in the formation of TBARS in lead acetate in control group (B) that contained no extract when compared to normal group (A). Garlic extract significantly inhibited lipid peroxidation in a dose dependent manner from 10 to 160 mg/ml; it was observed that with increased concentration of the extract, there was marked progressive increase in the inhibitory effect ranging from 34% to 74%.

Table 2 and 3 shows the effect of garlic extract on the levels of GSH and GSSG, respectively. It was observed that the aqueous extract caused marked increase in their levels.

Table 1. The inhibitory effect of garlic extract on lead acetate induced lipid peroxidation in rat liver

Extract (mg/ml)	Absorbance	Inhibition (%)
Basal (normal)	0.082 ± 0.00041	-
Control	0.498 ± 0.00041	-
10	0.324 ± 0.00048	34.9
20	0.31 ± 0.00054	37.8
40	0.208 ± 0.00149	58.2
80	0.201 ± 0.00325	59.6
160	0.128 ± 0.012	74.3

Results are Mean ± SD of two determinations

Table 2. Effect of garlic extract on reduced glutathione (GSH) in the liver tissue

Extract concentration	Absorbance	GSH concentration
1 mg/ml	0.824	175.32 µg/ml
2 mg/ml	0.824	175.32 µg/ml
3 mg/ml	0.823	175.11 µg/ml

Table 3. Effect of garlic extract on oxidized glutathione (GSSG) in liver tissue

Extract concentration	Absorbance	GSSG concentration
1 mg/ml	0.58	123.4 µg/ml
2 mg/ml	0.584	124.26 µg/ml
3 mg/ml	0.583	124.04 µg/ml

DISCUSSION

The results in Fig.1 show that allicin, which is the principal constituent of the extract, formed from its precursor allin via enzymatic degradation when crushed and macerated, destroyed the cellular structure releasing the antimutagens. The toxicity of divalent lead ions to the animals is caused by its binding to thiol or suflhydryl group; thus, inhibiting some enzymatic reactions in the body tissue [15]. Hence, the significant reduction of the mutagenic effects of lead by crude garlic extract could be attributed to the activity of allicin.

The anti-mutagenic effect observed in this experiment, indicates that garlic is a viable protective dietary supplement against DNA damage and associated cancer diseases which could later manifest as birth defects, and as it has since been known that every mutant cell is highly susceptible to developing cancer. Besides, generation of highly ROS like hydroxyl radical, hydrogen peroxide, superoxide anions and lipid peroxide aftermath of lead exposure may result in systematic mobilization and depletion of cell intrinsic antioxidant defenses.

The results of the *in vitro* experiment also show a significant increase in the levels of TBARS on lead administration serving as a pro-oxidant. This increase in TBARS levels, especially in the control group, may be a key factor in oxidative deterioration of membrane polyunsaturated fatty acid as well as assault on membrane integrity of the tissue [16]. However, there was a marked recovery in the altered TBARS and GSH levels due to the effects of the garlic extract on the liver tissue. The results obtained show that the effect of the extract is in synergy with the increase in the antioxidant defense system characterized by GSH and GSSG levels, respectively, and as well supporting the hypothesis that administrations of thiol-containing dietary supplements counteract both *in vivo* and *in vitro* oxidative stress posed by lead exposures [17]. Besides, heavy metals such as lead have been reported to cause oxidative stress due to production of ROS and to resist

the oxidative damage, the antioxidant enzymes and certain metabolites present in animal tissues playing an important role leading to adaptation and ultimate survival of the animals during period of stress. This action of the antioxidant enzymes has been complemented by garlic extract fed to the experimental animals in this study where it caused marked increase in the enzymes activities.

In conclusion, this study re-emphasizes the needful consumption of garlic as dietary supplement in protecting the body against oxidative stress which could mutate and render the cancerous cells [18].

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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