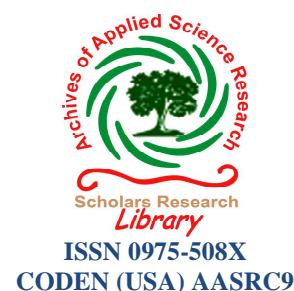




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Protection by garlic extract against lead induced tissue atrophy in albino rats

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ABSTRACT

The anti-mutagenic activity of allium sativum L. extract was studied in bone marrow cells of albino rats using micronucleus assay. The experiment was conducted for a period of 15-days using 100mg/kg body weight of the freshly prepared garlic extract as a dietary supplement via oral gavage. The rats were divided into four groups; group A (distilled water), group B (lead acetate), group C (garlic extract + [12hr] lead acetate), and group D (garlic extract + lead acetate [1:1]). Control groups were given lead acetate and distilled water only. After the short-term exposure, rats were sacrificed by cervical dislocation and chromosome preparations were made from bone marrow according to colchicines hypotonic-fixation air drying Giemsa schedule. The cytogenic end-points studied were chromosomal aberrations and damaged tissues were observed via microscope where the gross appearances of the tissues indicate atrophic changes. The chromosomal aberration induced by lead acetate was reduced significantly in animals fed with the extract in group C (2%) with (0.025 ± 0.053) mean frequency of polychromatic erythrocyte while lead acetate administered to animals in group B was highly mutagenic having total chromosomal aberration of (25%) with mean frequency of (0.013 ± 0.988) . This experiment indicates that crude garlic extract administered orally before or simultaneously with lead acetate protects against tissue atrophy in rats in vivo to a statistically significant level, this could be extrapolated to man.

Keywords: atrophy, lead, garlic extract, micronucleus assay, bone marrow.

INTRODUCTION

Garlic bulb had been used locally for more than 3,000 years in medicine for its antibiotic, anti-arteriosclerotic and anti-thrombic properties (Barone and Tonsey, 1997). Its roles in diet have been reported to reduce cholesterol levels (Sarkar, 1983). In the last ten years, studies by environmental protection agency as well as other international regulatory agencies have shown

that chronic low-level exposure to lead is associated with societal problems such as brain dysfunction in children exposed to lead, neurobehavioral changes in adult, hypertension and chronic liver and kidney diseases (Brautbar, 1985). Increased consumption of garlic has been related to the prevention of gastrointestinal cancer (Horowitz, 1981). Laboratory experiments have shown that its consumption inhibits the growth of *moris hepatomas* and *ehrlisch ascites* cells (Criss et al, 1982) and production of tissue plasminogen activator (TPA) induced tumors (Belman, 1983). However, comparison of the available data suggests that fresh garlic extract in relatively large amount may prevent certain degenerative diseases (Marsell and Reckless, 1991). Aqueous extract of garlic showed anti-mutagenic activity towards ionizing radiation, peroxides and hydroxyl radical generation (Knasmuller et al, 1989). It had been demonstrated in a series of studies that lead-induced hypertension is associated with increase reactive oxygen species (ROS) (Nachman, 1980). This supposition has been based upon the finding of increased lipid peroxidation and tissue nitrotyrosine abundance, which are foot print of excess reactive oxygen specie activity. In this regard, exposure to lead promotes hydroxyl radical generation and lipid peroxidation and as well enhances inactivation of endothelium derived nitric oxide (NO) by locally produced oxygen free radicals and this contributes to hypertension and arteriosclerosis in lead exposed animals. Lipid peroxidation occurs when reactive oxygen species generated close to the membrane, attack the fatty acid side chains of membrane phospholipids (Halliwell, 1989). Transition elements have been implicated in generating reactive oxygen species with subsequent oxidative deterioration of biological macromolecules (Henriksen, 1985). The present investigation was designed to study the degree of protection afforded by *allium sativum* extract against lead induced mutagenic effects in mice as it had been reported that lead is a mutagen capable of inducing chromosomal aberration in man (Sharma and Talukder, 2000). Also it has been shown that most, if not all, cancers are characterized by chromosomal changes that are frequently specific to a particular tumor. Besides, induction of aneuploidy in germ cells is a cause of birth defects, fetal deaths and infertility in animals. The human organism is exposed to various types of environmental contaminants at different stages of life, majority of them are harmful especially lead. Tolerated normal intake of lead is 0.3mg but daily intake of 3.5mg taken for a few month results in toxicity. Lead levels in 93% cases of either sex in Pakistan gave a firm evidence of contamination of water supply, fish and other food sources by industrial effluent in big cities (Manser et al, 1990). Also occupational exposure of lead to men decreases their fertilities as it had been established that prolonged lead exposure initially produces a direct testicular toxicity (Griffin and Fahim, 2002) followed by hypothalamic or pituitary disturbances on long exposures.

MATERIALS AND METHODS

Animals and Experimental Design

Sixteen male albino rats littermate were used. The mice were bred in the departmental animal house and were 15-25 weeks old with an average weight of 60g. They were kept four per cage with husk bedding and were fed pellets and water *ad libitum*. The light cycle was 12hr light and 12hr dark. Crude garlic extract was prepared from freshly sliced cloves, dried into powdery form, weighed, and then dissolved in glass distilled water. Rats in group **A** served as control and were treated with distilled water only. Those in group **B** and **C** were respectively treated with 2.5mg/kg lead acetate and 100mg/kg garlic extract + {12hr} lead acetate, while group **D** animals were simultaneously treated with 2.5mg/kg lead acetate + 100mg/kg garlic extract [1:1]. The

concentration of the lead salt was made equivalent to 1/10 of its LD₅₀ (Das et al, 1993). While the dose of garlic extract was equivalent to the high amount used for beneficial effects against specific disease condition (Mansell and Reckless, 1991). Each dose was administered via oral gavage to the animals on daily basis consecutively for 15-days. Animals were sacrificed 24hr after the last treatment.

Chromosome Aberration

Chromosomes were studied from bone marrow cells following the usual Colchicine-hypotonic fixation air drying (Sharma and Sharma, 1994). Animals were killed by cervical dislocation 24hr after the last treatment 90mins prior to sacrifice, each animal was injected with colchicines (0.04%) 2mg/kg (Preston et al, 1987). Femurs were removed and the bone marrow was flushed out and chromosomes were prepared for observation using standard hypotonic (0.075M KCl) – fixative (1:3 glacial acetate ethanol) flame drying technique. The slides were subsequently coded and scored blind. 50 well scattered metaphase plates were scanned from each animal giving a total of 300 metaphases. The end points scored were chromosomal aberrations and damage cells. The chromosomal aberrations included all aberrations such as chromatids, chromosome breaks and rearrangements) which were considered to be equal and counted as one regardless of the number of breaks involved (Das et al, 1993). Damaged cells include all cells with at least one chromosome aberration and stained in Giemsa (1:20) dilution.

Table 1: Group of rats and treatment administered

Group	Treatment administered	Number of mice
A	Distilled water only	4
B	Lead acetate only	4
C	Garlic extract + {12hrs} + Lead acetate	4
D	Lead acetate + Garlic extract {1/1}	4

Statistical Analysis

The data from the groups were pooled and analyzed statistically using one-way analysis of variance (ANOVA), (Sokal and Rohlf, 1987) followed by Duncan's multiple range test in order to compare the significance of different experimental groups which were given as mean \pm standard deviation where P-values $> .05$ were considered significant. The types of chromosomal aberrations screened were chromosome breaks, gaps, isochromatids and polyploidy according to WHO guidelines for evaluation of genetic toxicology (WHO, 1992).

RESULTS AND DISCUSSION

Table 2: Chromosomal aberration recorded following treatment with crude garlic extract and lead acetate.

GROUP	%	G	G'	B	B'	RR	% CA/Cell	MEAN ± STD. DEVIATION
A	4	0	3	0	0		0	0.010 ± 0.004
B	32	3	37	2	25		25	0.013 ± 0.988
C	7	1	12	0	16		2	0.025 ± 0.053
D	11	2	13	0	6		6	0.053 ± 0.010

Total of 300 cells per treatment. G= chromosomal gap; G'=isochromatid break; B= chromatid break; B'= chromosome break; RR= chromosomal rearrangement; CA/Cell= chromosomal aberrations per cell.

Table 2 above, shows the frequencies of total chromosomal aberrations and the mean frequencies of CA (chromosomal aberrations) per cell and percentage of damaged cells in rats exposed to lead acetate in vivo. The frequencies of aberrations and damaged cells were significantly reduced in the rats simultaneously administered the toxicant and the extract (1:1). Similar effect had been observed in mice fed same extract for 30 days and when sodium arsenite was given simultaneously on 7, 14, 21 and 29th day of treatment by subcutaneous injection (Das et al, 1993, Roychoudhury et al, 1993). The principal constituents of the extract are allicin (thio-2-propene-1-sulfonic acid S-allyl esters), allin (S-allyl-L-cysteine sulfoxide, and cysteine, calcium, iron and zinc in addition to other compounds with sulphur moieties (Asmus, 1983; Willson, 1983; Block, 1985). Allicin is formed by enzymatic degradation from its precursor allin present in the stem when the fresh stems are crushed, thus destroying the cellular structure and releasing the antimutagens. The toxicity of divalent lead ions to animals and human beings is thought to be caused by its binding to thiol ions thus inhibiting some enzymatic reactions (Sharma and Talukder, 1987; Leonard, 1991). The significant reduction of the mutagenic effects of lead by crude garlic extract, whether given simultaneously or at time intervals may be attributed to the activity of allicin. Allicin is also known to be partly responsible for the reduction in radiation-induced mutagenesis in *Salmonella typhimurium* TA 102, (Knasmuller et al, 1989). Other compounds of the extract also protect against cytotoxicity e.g thioether dially sulphide inhibits nuclear damage of colon epithelial cells induced by dimethylhydrazine in vivo. The view that *allium sativum* functions in living system primarily as anti-mutagenic as observed in this experiment, it could be deduced that garlic is a viable protective agent against cancer diseases as it is known that every mutant or mutated cell is susceptible to developing cancer. The problem of protection against exposure to lead through drinking water with reference to lead evaluation in well-water and its contributions to toxicity within Ibadan metropolis (Tugbobo, 2007) has assumed considerable importance, due to the deadly effects of lead poisoning. With this experiment, using cytogenic end points, in rats in vivo indicates that crude garlic extract administered orally before or simultaneously with lead acetate reduces the mutagenic effect of the latter to a statistically significant level in the short-term exposure. From the pathological examination of the gross appearances of the kidney, liver and heart tissues, it was observed that

the positive control tissues were normal, and well vascularised with intact size and weight. While these differ for the treated animals where there was reduced size, pale appearance, and tough texture of the tissues which showed resistance on cutting. These are indicative atrophic changes induced by lead. From this experiment, animals treated with lead only (group B) lost 16% of body weight and this significant loss in weight was due to loss of appetite and gastrointestinal disruption induced by lead (Harvey, 2002). Also the tissues appeared pale as a result of reduced vascular of the animal system due to inhibition of Delta aminolevulinic acid dehydratase and thus hemoglobin is not formed. This work is being further continued with long-term supplementation of isolated garlic extract against lead induced toxicity.

This study gives preliminary information about the vital needful consumption of *allium sativum* in diet against chronic lead poisoning as advocated by WHO that garlic is a supportive dietetic measure for cancer prevention and as well as complementary therapy for cancer patients as it is known that every mutant cell is susceptible to developing cancer. However, levels of lead in the body must be properly monitored to guide against its toxicity.

Diagrams of the atrophic changes on liver tissues

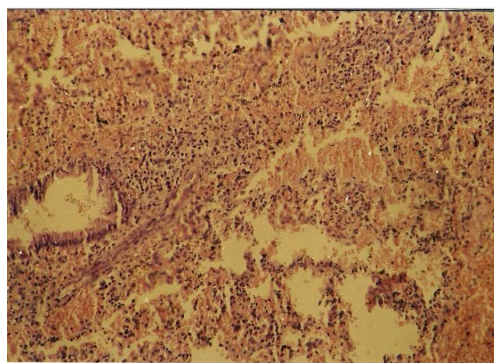


Figure 1: Diagram of aberrated Liver Tissue.
Group D animals fed with garlic extract + lead acetate [1:1].

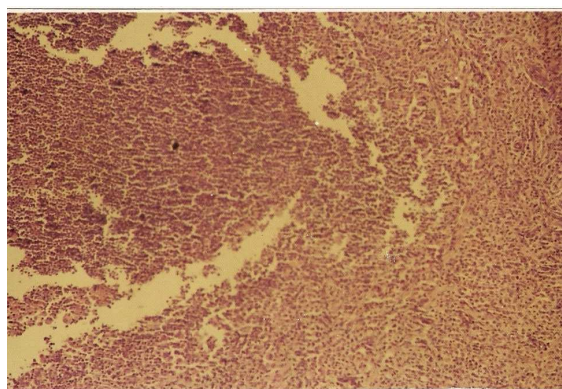


Figure 2: Diagram of Averagely Normal Liver tissue.
Group C animals treated with garlic extract after [12hrs] + lead acetate.

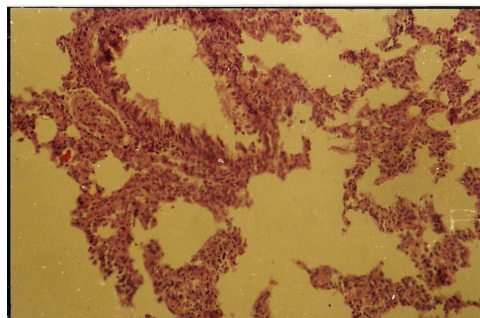


Figure 3: Diagram of aberrated Liver Tissue.
Group B animals treated with lead acetate only.

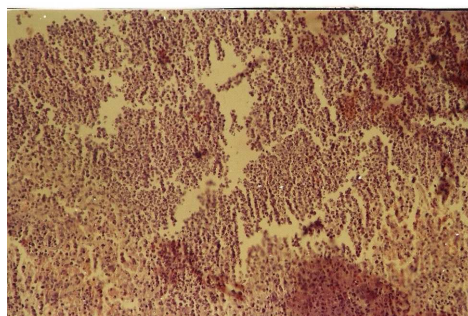


Figure 4: Diagram of Normal Liver.
Group A animals treated with distilled water only.

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