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GENOPROTECTIVE POTENTIAL OF *ALLIUM SATIVUM* AGAINST CHEMICAL CLASTOGENS

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ABSTRACT

Clastogenicity is an induction of changes in structure of chromosomes, which is dangerous to any organisms. In concern to mutation, the clastogenicity is structural chromosome aberrations produced by some clastogens, which cause breaks in chromosomes and resulted in either loss or re-shifts of chromosomal segments. In treating mutagenicity, compared to modern medicine, the traditional herbal medicine based on medicinal plants has some ability to cure without worsening the condition. Chromosomal aberrations based human syndromes are very critical and sometimes leads to lethality. Such syndrome or disorders are often irreversible. In this present study, we evaluated the anticlastogenic effect of *Allium sativum* (garlic) against chromosomal aberrations induced by $K_2Cr_2O_7$ and EMS in bone marrow cells of mice. The results showed that the clastogenic effect was minimized by *Allium* extract in mice model.

KEYWORDS: Clastogenicity, Chromosomal aberrations, Genoprotective, *Allium.*

INTRODUCTION

Herbal drugs contribute about 80% material in the Indian medicines. Endogenous antioxidant defenses are inadequate to prevent damage completely. So, diet derived antioxidants are important. The evidence for a key role of Vitamin E and Vitamin C in free radical scavenging are strong. In India there are over 2,000 plants credited with medicinal properties and only a few of these are cultivated. while others grow in wild state (Kirtikar and Basu 1975a,b,c; Chopra et al 1958). Cytotoxicity and tissue damage in human diseases is caused by free radicals which are generated *in vivo* and cause damage to DNA, lipids, proteins and other biomolecules. Interest is also growing in the role of plant phenolics especially flavonoids. Experimental approaches to the optimization of antioxidant nutrients intake are being proposed. The role of antioxidants in preventing cytotoxicity and tissue damage in various human disease is becoming increasingly recognized. Plant extracts contains substances which have the capacity to protect against mutagens and carcinogens.

Garlic (*Allium sativum)* has been used since ancient times, as a spice and also for its medicinal properties. Garlic (*Allium sativum*) has been claimed to have both hypochlolesterolenic (Bordia, 1981; Augusti, 1977) and hypolipidemic effects. Antioxidant properties have been attributed to garlic (Augusti and Sheela, 1996). The active compound in garlic are sulphur rich S-allyl cysteine sulphoxide and the compound derived from it viz. allicin, ajoene and polysulphides (Block, 1992). Garlic is reported to cure coronary heart disease (CHD) cancer and peptic ulcers. *Allium sativum* has been reported in preventing a wide range of human disease in Indian indigenous system of medicines (Augusti 1990; Chopra et al 1956 a,b,c; Kirtikar and Basu 1975 a,b,c) including high lipid cholesterol level (Mirhadi et al 1991; Sarkar and De, 1983) and cancer induced by Benjopyrene and methyl cholanthrene in animals models(Hussain et al 1990; sadhna et al 1988). Fresh garlic given daily as 7-8 cloves, have been reported to have beneficial effects on blood pressure, cardiovascular, diseases, coagulation and platelet aggregation (Mansell and Reckless, 1991). Aqueous extract of garlic bulb has been found to inhibit the mutagenic effects of ionizing radiations and various clastogens in Chinese hamster cells and mouse *in vivo* (Roy Choudhary et al 1993; Das et al 1996). In general, the effects of crude extract are greater than that of an equivalent amount of any single component indicating that the different component, major or minor are involved in the process. Das et al (1993) observed modification in cytotoxic effects of inorganic arsenic by crude extract of *A. sativum* in mice. The frequency of chromosomal aberrations was significantly lower in animals maintained on crude plant extract as a dietary supplement. Pretreatment with the garlic extract reduced gamma radiation induced chromosomal damage in mice bone marrow cells (Singh et al 1996).

Chromium, a well-recognized environmental Pollutant that may bring about adverse effects on both humans and animals (IARC, 1990; Dayan and Paine, 2001). It is well established human carcinogen based upon epidemiologic studies (Langard, 1990). It induced chromosomal aberrations, mutations and transformation in cultured mammalian cells (De Flora et al1990). The results obtained in the study indicated that K_2CrO_4 has clastogenic and mutagenic potential *in vivo*.

A significant positive correlation was found between the carcinogenic potency, mutagenic and recombinogenic potencies for EMS and some other monofunctional alkylating agents (Quinto et al 1990). EMS has been shown causing a number of gene mutations in *E.coli* (Aaron et al 1980; Quinto et al 1990), and Drosophila melanogaster (Mitchell and Simmons et al 1978).

MATERIAL AND METHODS

Experimental Animals

10-12 weeks old Swiss albino mice (*Mus musculus*) weighing 25-30g were used as experimental animals. They were kept in cages under controlled temperature(25±5°C) and aseptic conditions. Pallet diet and water was provided *ad libitum*.

a) *Allium sativum* extract

Fresh bulk of Allium sativum were purchased from the local market. They are peeled and 50gms of them are weighed. They were chopped finely. Placed the herb in a saucepan and 750 ml of cold tap water was added. Bring it to boil. Simmer for about 20-30 min. until the liquid is reduced to 1/3 approx. Strained the liquid through a sieve into 500ml beaker. Allowed to cool and final volume is made to 500ml by adding distilled water. Extract is stored in the refrigerator.

b) Potassium dichromate($K_2Cr_2O_7$) Solution

$K_2Cr_2O_7$ was obtained from MERCK India Ltd. Mumbai. It was dissolved in 0.9% NaCl at dose level of 250 mg/kg.b.wt. and administered intraperitoneally(i.p.).

c) Ethyl Methanesulphonate (EMS) solution

EMS was also collected from MERCK India Ltd. Mumbai. It was dissolved in hot 0.9%NaCl at dose level of 250mg/Kg.b.wt. and administered intraperitoneally.

Experimental Design

For the present study *Allium sativum* extract was divided into 12 groups of 3 animals each. One group was taken as control and was given point 0.3 ml of solvent (0.9 % NaCl) intraperitoneally to each animal. Two group were injected with 0.3 ml $K_2Cr_2O_7$ solution at dose of 20 mg/ Kg.b.wt and 0.3% ml of EMS at dose of 250 mg/ Kg.b.wt. respectively. Remaining 9 groups were orally primed with 3 different doses of extract of *A.sativum* for 15 days each. Out of these 9 groups 3 groups with different doses of *A.sativum* extract only, were dissected for further processing. Out of remaining 6 groups, 3 groups were further given intraperitoneally(i.p.) $K_2Cr_2O_7$ (20mg/kg.b.wt.) and 3 groups were further given EMS (250mg/kg.b.wt.) for two days each. After 48hrs all animals were given i.p. 0.3 ml colchicine solution (0.04%). Then, after 3hrs the animals were dissected for bone marrow. The bone marrow cells were processed for a standard procedure (Preston et al 1987) and slides were stained in Giemsa 92%. 50 well spread metaphase plates were scored per animal. Chromosomal aberrations were evaluated in accordance with the method of Ties et al (1987). The percentage of aberrant cell (%AC) and chromosomal aberrations per cell (CA /Cell) were calculated for each animal. Statistical analysis was carried out using student's t-test to detect significant differences in clastogenicity amongst different treatment.

RESULTS

The results of chromosome analysis in bone marrow cells of mice following 15 days of priming with Allium extract and single i.p. injections of $K_2Cr_2O_7$ and EMS and combination of Allium extract with $K_2Cr_2O_7$ and EMS are shown in **Table-1, figure-1a.** For Garlic anticlastogenic agent against $K_2Cr_2O_7$, all the three different doses (10, 50 and 100 mg/kg b.wt.) has shown significant reduction in CA/cell as %AC (**Table-1 Figure-1b**). In case of anticlastogenic effects of Garlic against EMS, dose of 10mg/Kg b.wt of garlic showed no significant reduction in CA/cell and %AC. But higher doses (50 and 100mg/kg b.wt) showed significant reduction in CA/cell and % AC against EMS.

Table 1: Modulation of clastogenicity of $K_2Cr_2O_7$ and EMS in the bone marrow cells of mice orally primed with *Allium sativum* (Garlic).

Chemicals	Treatment Days	Dose mg/kg.b.wt	Total Chromosomal aberrations					Mean±S.E.	
			G	G"	B'	B"	CR	CA/Cell	%AC
GAR	15	10	1	0	1	0	0	0.0133±0.0000511a	0.666±0.513a
GAR	15	50	1	1	0	0	1	0.02±0.000154a	0.333±0.513a
GAR	15	100	1	0	0	0	1	0.0133±0.0000188a	1.666±0.513a
K2Cr2O7	2	20	7	3	19	7	2	0.253±0.00052b	10.00±1.53b
GAR +$K_2Cr_2O_7$	15+2	10+20	5	3	15	6	2	0.20±0.513	5.3330±0.513
GAR +$K_2Cr_2O_7$	15+2	50+20	4	2	9	4	1	0.10±0.000693bc	4.666±0.513bc
GAR +$K_2Cr_2O_7$	15+2	100+20	2	1	5	4	1	0.086±0.0000511bc	3.333±0.513bc
EMS	2	250	16	5	63	15	6	0.70±0.00061d	22.00±6.16d

GAR +EMS	15+2	10+250	14	5	58	12	6	0.63±0.000359d	22.66±1.514d
GAR +EMS	15+2	50+250	10	4	50	8	4	0.507±0.000359de	16.666±0.514de
GAR +EMS	15+2	100+250	8	3	44	7	2	0.427±0.000359de	10.666±0.514de

Values in vertical columns followed by same letter(a,a) or (d,d) are not significant at the 5% level, while values followed by different letter (b,bc) are significant at the 5% level. EMS – Ethylmethane sulphonate, G'- Chromatid gap, G''- Isochromatid gap, B' – Chromatid break, B''- Chromosome break, CR – Chromosomal rearrangement, CA – Chromosomal aberrations, AC – Aberrent cell.

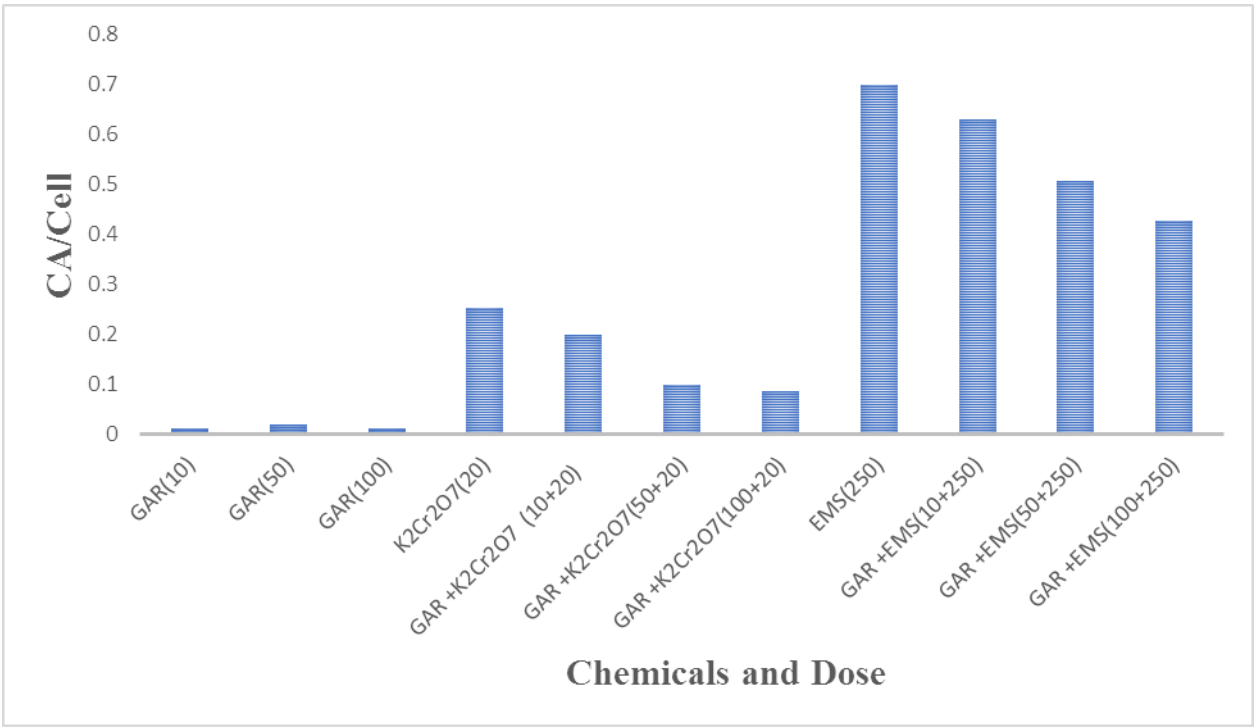

Figure 1a: Modulation of clastogenicity of $K_2Cr_2O_7$ and EMS in the bone marrow cells of mice orally primed with *Allium* extract.

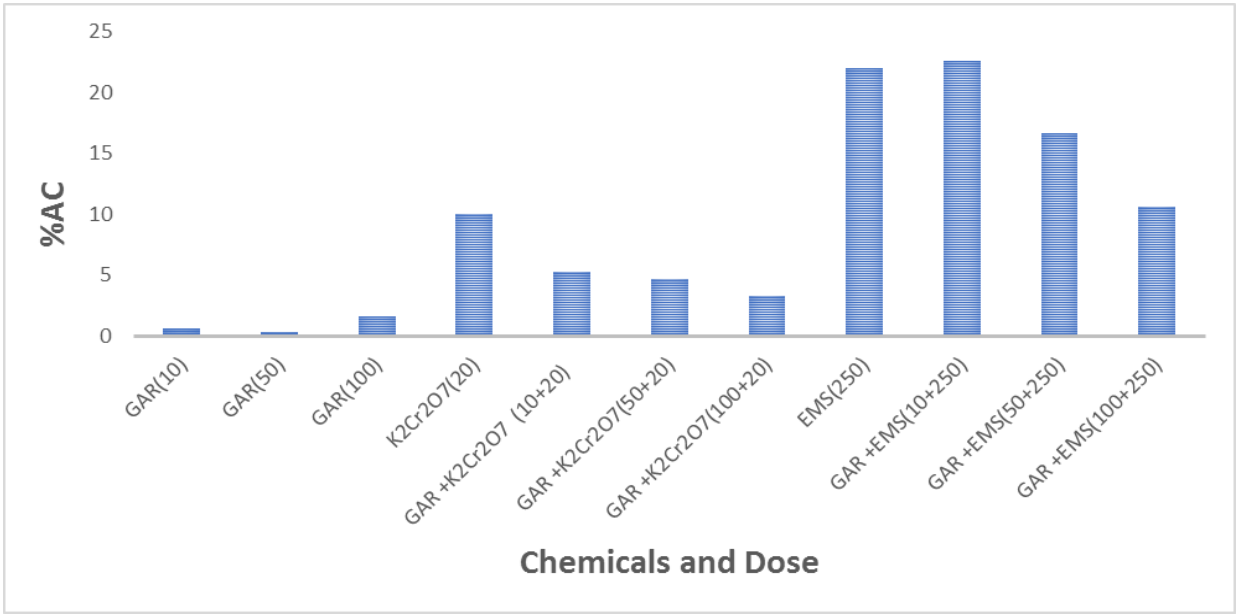

Figure 1b: Modulation of clastogenicity of $K_2Cr_2O_7$ and EMS in the bone marrow cells of mice orally primed with *Allium* extract.

DISCUSSION

Therapeutic properties of garlic(*Allium sativum*) and its chemical composition, is considered a very versatile product in the pharmaceutical, food, and cosmetic market; therefore, different products can be produced, which have been classified into five groups as follows: (a) consumption of fresh garlic, (b) essential garlic oil, (c) macerated garlic oil, (d) garlic powder and (e) aged garlic extract (Rana et al 2011). Ingesting fresh garlic, the gastric juices start the process of transformation to different bioactive compounds, which together with the rest of the partially digested garlic are directed to the small intestine for greater decomposition and to be absorbed into the organism, whence thereafter they are distributed by the blood stream to different places, including the liver, kidneys, plasma and muscles (Colin et al 2012). The benefits of garlic may be attributed to its active principles, viz sulphoxide derivatives of cystenine and their degradation products like allicin and polysulphides including ajoene which acts as antioxidant (Augusti, 1977; Block, 1992). When garlic is used in diet as supplement for prolonged periods and in low concentration the clastogenic effects are minimized (Das et al 1996). The present investigation also indicates towards the genoprotective effect as significant results have been obtained. In the light of literature reviewed and our present results it is suggested that regular intake of 5-8 cloves of garlic daily may give protection against genotoxic effects of various clastogens and thus may protect us from many genetic defects. The mechanism by which plant crude extracts and plant-derived compound suppress chemically induced mutagenesis is not yet known. Some of the mechanisms suggested are (1) alternation of metabolism is of the mutagen/carcinogen resulting in decreased activations and increased detoxification, (2) scavenging of active molecular species as to prevent their reaching critical target sites in the cells, (3) competitive inhibition (Wattenberg 1978). The possible alternation of the pharmacokinetics of the chemicals by the extract cannot be ruled out.

CONCLUSION

The result has shown that garlic extract has reduced the clastogenic effects of both $K_2Cr_2O_7$ and EMS. So, it may be concluded that the garlic extract undertaken for present study are able to minimize the genotoxicity of various genotoxic agents. Thus, in conclusion it may be said that garlic, taken in daily 5-8 cloves in diet may protect from any possible genotoxicity and hence possible genetic defects which otherwise lead to genetic diseases.

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