Volume-4, Issue-9, Sept-2015 • ISSN No 2277 - 8160

Research Paper Zoology

Protective Effects of Garlic Extract on Cyclophosphamide Induced Genotoxicity Using Micronucleus Test in Mice

K. Rudrama Devi	Department of Zoology, Osmania University, Hyderabad, Telangana, India.
Sri Vani	Department of Zoology, Osmania University, Hyderabad, Telangana, India.
K.Dilip reddy	Department of Zoology, Osmania University, Hyderabad, Telangana, India.
Minny Jael.P	Department of Zoology, Osmania University, Hyderabad, Telangana, India.

ABSTRACT

In the present investigation the antimutagenic effects of Garlic extract has been evaluated against cyclophosphamide induced genotoxicity. Single IP administration of garlic extract at various doses i.e. 125, 250 and 500 mg /kg . When treated individually did not induce micronuclei in polychromatic erythrocytes of mice. A single Intra peritoneal of 50mg/kg of cyclophosphamide induced significant increase in the percentage of micronuclei in bone marrow cells of mice. However after co administration of three doses of garlic extract there was a dose dependent decrease in the % of micronuclei was observed. Thus the results clearly indicate the protective effects of garlic extract against cyclophosphamide induced genotoxicity in bone marrow cells of mice. Therefore the data indicate that Garlic extract is a safer dietary component in cancer chemo preventive strategy.

KEYWORDS : *Garlic extract*, Cyclophosphamide, Micronuclei

INTRODUCTION

A number of antineoplastic drugs are in common use to combat various types of cancers. These are shown to be mutagenic in different test systems and these antineoplastic drugs such as Cyclophosphamide, Cisplatin, Tamoxifen, Gemcitabine and Paclitxel etc., have shown clastogenic effects in various test systems. Potential genetic damage due to drugs and other chemicals is well recognized. Extensive studies have been carried out on mutagenicity of various drugs in microorganisms, insects, mammals and in exposed population (Smorenburg et al.,2001,Padmalatha et al.,2001,Akram et al.,2010&2012, Anuradha et al.,2010, Anil kumar et al., 2011, Deshpande et al., 2013).

Cyclophosphamide (CPM) is a well-known bifunctional alkylating agent widely used in cancer chemotherapy and expresses its genotoxicity when metabolically activated (Fleming RE ,1997). It is extensively used for the treatment of various cancers as well as an immunosuppressant in organ transplantation, rheumatoid arthritis, systemic lupus erythromatosis, multiple sclerosis, and other benign diseases (Perini et al, 2007, Uber et al, 2007). According to the International Agency for Research on Cancer (IARC), CPM is widely used as reference mutagen and has been classified as carcinogenic for animals and humans (IARC, 1987).

Garlic is readily available medicinal herb known for its health benefits. It has a wide range of medicinal properties like antiviral, antifungal, antihelmintic, anti-inflammatory, antidote, anticancer, antimutagenic, hepatoprotective and immunomodualtion etc. (Banerjee et al., 2003; Khanum et al., 2004). Recent studies have shown the antigenotoxic and antimutagenic effects of garlic for various drugs and chemicals (Shukla and Taneja, 2002; Bhuvaneswari et al., 2004, Siddique and Afzal, 2005; Belloir et al., 2006). Studies of the anticarcinogenic effects of garlic on several carcinogens were found to be effective in different ways such as direct inhibition of tumor cell metabolism, inhibition of initiation and promotion phases of carcinogenesis and modulating the post immune response and besides all these garlic acts as a strong antioxidant by its ability to scavenge free radicals, (Wei and Lau, 1998). Sulfur rich

constituents of garlic such as Diallyl Sulfide (DAS) and Diallyl Disulfide (DADS) are known to induce activities of phase II enzymes, which in turn reduce the genotoxicity of several carcinogens. Hence in the present investigation studies were carried out on the protective effects of garlic extract on cyclophosphamide induced micronuclei in bone marrow erythrocytes of mice.

METERIALS AND METHODS

***Animal treatment*:** The study was conducted after taking the approval of Institutional Ethical Committee on twenty adult male swiss albino mice 30 to 50 days old and weighing around to 30 to 40 g were maintained in plastic cages under controlled lighting conditions (12:12 light and dark cycle) relative humidity (50±5%) and temperature (37±2ºC) fed with mice feed and were given ad libitium access to water.

Preparation of *Garlic* Extract:

Fresh garlic cloves were purchased from the local market and made into coarse powder with mortar and pestle. The powder (about 250gm) was soaked in 500ml of ethanol for 72hrs. The solvent was runned through rotavapour to separate solvent and concentrated through Soxhlet apparatus. Garlic extract were selected in the present study.

Dosage schedule:

In the present study two experiments were conducted. The animals were feed orally with cyclophosphamide and garlic extract and categorized in to following groups

Group I : controls
Group II: garlic extract150mg/kg
Group III: garlic extract 200mg/kg
Group IV: garlic extract 250mg/kg

In the second experiment for modulation studies all the three groups as follows:

Group I : controls
Group II: Cyclophosphamide 50 mg/kg
Group III: garlic extract150mg/kg + Cyclophosphamide 50 mg/kg
Group IV: garlic extract 200mg/kg + Cyclophosphamide 50 mg/kg
Group V: garlic extract 250mg/kg + Cyclophosphamide 50 mg/kg

Micronucleus Test:

The femur bones were removed, made muscle free and cleaned. The bone marrow was extracted in a petridish containing 3ml of human

AB serum by aspiration using a 26G needle. The bone marrow was aspirated by repeated number of flushings until a fine suspension of cells was formed. The same procedure was followed through the distal end of the bone to ensure the full extraction of the bone marrow. The cell suspension was centrifuged at 1000 rpm for 10 mts. The supernatant was removed and to the sediment half a drop of serum was added. The cells in the sediment were carefully mixed by aspiration into capillary part of the fresh Pasteur pipette. A small drop of viscuous suspension was placed on one end of well cleaned slide and smears were prepared. Four slides were prepared per animals and all the slides were coded.

The staining was done within 24hrs of the preparation. The slides were stained for 3 mts. In undiluted may-Gruenwald solution (250 mg May-Gruenwald stain in 100 ml of methanol) Stained for 2mts. In May-Gruenwald solution diluted with distilled water (1:1 ratio) Rinsed in distilled water and again stained for 10 mts in Giemsa stain diluted with distilled water (1:6 ratio). The slides were rinsed in tap water. Dried with filter paper, cleaned with methanol. Cleaned in xylene for 7 mts. And mounted in DPX. Two to three drops of cell suspension were dropped on clean grease free, pre-chilled slides. The slide was blown once across and allowed to dry on aslide warmer. Two slides from each animal were prepared by air drying technique from control and treated animals. The slides were coded and stored in dust free chambers. The staining was done within 24hrs after the slide preparation. The slides were stained with 2% Gieitisa (2ml of Giemsa in 46ml of double distilled water plus 2ml of phosphate buffer* pH 6.8) for 7-8 minutes and later they were rinsed in double distilled water and allowed to dry.

The slides with perfect morphology of the nucleated cells were selected for screening. The slides were screened for the presence of micronuclei in polychromatic erythrocytes and normochromatic erythrocytes and micro photographed (Fig. 1a & b). A total of 2000 polychromatic cells and normochromatic cells in control and treated animals were screened.

Finally the slides were soaked in Xylene for overnight and mounted in DPX mutant. The data obtained in various experiments conducted were statistically analyzed by using t-test

RESULTS

Experiments were designed to assess the mutagenic effects of Cyclophosphamide for the incidence of micronuclei in bone marrow erythrocytes of swiss albino mice. The results obtained are presented in tables 1 and illustrated graphically in 1.The results on the frequency of micronuclei in young bone marrow cells of garlic extract treated animals are presented in Tables 2 and illustrated in graph 2.

The frequency of micronuclei in polychromatic erythrocytes was 0.34%, 0.35% and 0.40% in animals administered with 125, 250 and 500 mg/kg garlic extract treated animals as against 0.31% in control animals. The frequency of micronuclei in normochromatic erythrocytes was 0.19%, 0.24% and 0.27% in animals administered with 125, 250 and 500 mg/kg garlic extract treated animals as against 0.09% in control animals. The P/N ratio was 0.93, 0.93, 0.92 in 125, 250 and 500 mg/kg garlic extract treated groups as against 0.98 in control group. The differences in the frequencies of micronuclei in polychromatic erythrocytes between the control and treated group were found to be statistically insignificant (P>0.05, Table 2).

The results on the frequency of micronuclei in polychromatic erythrocytes of Cyclophosphamide + garlic extract treated animals are presented in Table 2 and illustrated in graph 2.

The frequency of micronuclei in polychromatic erythrocytes was 01.12;0.96;0.86 in animals primed with 125+50 mg/kg, 250+50mg/kg and 500+50 mg/kg garlic extract + Cyclophosphamide as and 1.31 in 50 mg/kg Cyclophosphamide alone treated animals. The frequency of micronuclei in normochromatic erythrocytes were 0.28, 0.35,and 0.37 in animals primed with garlic extract + Cyclophosphamide 125+50, 250+50 and 500+50 mg/kg garlic extract + Cyclophosphamide against 0.74 in 50 mg/kg cyclophosphamdie alone treated animals. The P/N ratio was 0.94, 0.93 and 0.93 in 125+50, 250+50 and 500+50mg/kg Cyclophosphamide + garlic extract and 0.95 in 50mg/kg Cyclophosphamide alone treated animals (Table 2 Graph 3 & 4).

The percentage of inhibition were observed as 14.28,26.66,34.28 after administration of 125+50mg/kg, 250+50 mg/kg, 500+50 mg/kg garlic extract + Cyclophosphamide administered animals when compared with Cyclophosphamide alone treated groups.(Table 2). The results are illustrated in graph 3.

The differences in the frequencies of micronuclei in polychromatic erythrocytes between control and Cyclophosphamide treated groups were found to be statistically significant. Where as the differences between Cyclophosphamide and primed groups III, IV & V groups were found to be significant (P<0.01, Table 2).

Table 1: Results on the frequencies of micronuclei in bone marrow erythrocytes of mice treated with various doses of *garlic* extract.

	Micronuclei in polychromatic erythrocytes	Micronuclei in normochromatic erythrocytes	Micronuclei in total P+N cells	**P/N ratio**
Control -I	50/16000 (0.31)	16/16208 (0.09)	66/32208 (0.20)	0.98
125 mg/ kg GE	54/16000 (0.34)*	34/17106 (0.19)	88/33100 (0.26)	0.93
250mg/ kg GE	56/16000 (0.35)*	42/17160 (0.24)	98/33160 (0.29)	0.93
500mg/ kg GE	64/16000 (0.40)*	48/17182 (0.27)	112/33294 (0.33)	0.92

The values parenthesis are percentages *P>0.05

Table 2: Protective effects of garlic extract in cyclophasophamide induced micronuclei in bone marrow cells of mice.

Groups	Dose	Micronuclei in polychromatic erythrocytes	Inhibition %
Group – I	Control-II	40/16000 (0.25)	-
Group – II	Cyclophosphamide 50mg/kg	210/16000 (1.31)	-
Group – III	125 mg/kg(GE) +50mg/kg Cyclophosphamide	180/16000 (1.12)	14.28
Group – IV	250mg/ kg(GE)+50mg/kg Cyclophosphamide	154/16000 (0.96)	26.66
Group – V	500mg/kg(GE)+50mg/kg Cyclophosphamide	138//16000 (0.86)	34.28

The values in parenthesis are percentages *P>0.05

DISSCUSSION:

The chromosomal damage caused in somatic cells of mice was evaluated using the method of Schmid (1975). Micronuclei originate from chromosomal material that has lagged in anaphase. In the course of mitosis this material is distributed to only one of the daughter cells. It may be included in the main nucleus or form one or more separate small nuclei ie., micronuclei. The micronuclei mainly consist of either acetric fragments (Heddle and Carrano, 1977) or spindle poisons. Micronuclei can be observed in any cell type of proliferating tissue. They are, however, most easily recognized in cells without the main nucleus namely erythrocytes.

The invivo micronucleus test is one of best methods to screen the clastogenic effects of chemicals and drugs (Chaubey et al, 1978) using this procedure the mutagenicity of various alkylating agents (Maier and Schmid, 1976, Rudrama Devi and Reddy 1986) drugs (Rudrama Devi and Reddy, 1995) was also established.

The present results are comparable with that of Asita el al, (2008) who investigated the intraperitoneal injection of mice with a single dose of 40 mg/kg body weight of Cyclophosphamide induced a significant increase in the frequency of MNPCE, 24 h after injection, when compared with animals that received water treatment. The present results are comparable to Santos Renato et al., (2005); who reported that Cyclophosphamide at 135mg/kg dose induced a significant increase in the frequency of micronuclei in polychromatic erythrocytes of male mice Further, the percentage of chromosomal aberrations was 59.33

in 50mg/kg body wt. Cyclophosphamide treated mice (Raja Wasim et al., al 2013).

The results indicate non mutagenic nature of garlic extract in bone marrow cells of mice. Similar data was observed when animals treated with *phyllanthus emblica* (Shoba rani 2006. Rudrama devi & Kusum latha., 2012) Carrot juice, Aegelus marmelus fruit extract (Prabhakar reddy and Rudramadevi 2014) showed higher frequency of micronuclei in bonemarrow erythrocytes of mice .

Our results are comparable with that of Abraham and Kesavan (1984) who reported the genotoxic effects of orally administered garlic in bone marrow cells of mice by performing the micronucleus test. Results of the micronucleus test with garlic were not significantly different from control values. Sumiyoshi et al (1984) investigated the influence of garlic extract on the chronic toxicity test orally in Wistar rats for 6months. There were no toxic symptoms due to the garlic extract even at dose level of 2000 mg/kg for 5 times a week during 6months. High dose of garlic extract did not inhibit the body for 5 times a week during 6months. High dose of garlic extract did not inhibit the body weight gain, while the food consumption decreased slightly for the nutritional effects of it in both male and female rats. There were no significant differences in toxic signs were observed on any of the tissues and organs examined.

The above findings clearly indicate that there was no significant increase in the frequency of chromosomal aberrations in somatic cells of garlic treated mice when compared to controls. So it is clear indication that garlic does not exhibit mutagenic effects in somatic cells of mice may be due to the presence of allicin in garlic which is responsible for its antioxidant property. The results showed that there was a gradual decrease in the frequency of various types of chromosomal aberrations with increasing dose and time intervals in somatic cells of cyclophosphamide+garlic treated animals. Thus as a result of various type of chromosomal aberrations the percentage so total chromosomal aberrations at 24h exposure to cyclophosphamide+ garlic wee 2.20 in control and were 9.60 in 60mg/kg of cyclophosphamide treated animals to 8.40,7.00 and 5.60 of cyclophosphamide +garlic treated animals respectively. The anticlastogenic acitivity fo crude extract of garlic (Allium sativum L.) was studied in bone marrow cells of mice.

Male laboroatory-bred Swiss albino mice were given three concentrations from the freshly prepared extract (100mg, 50mg, and 25mg/kg body weight) as a dietary supplement by gavage for 6 consecutive days. On the seventh day the mice were administered a single acute dose of two known clastogens, mitomycin C (1.5mg/kg) and cyclophosphamide (25 mg/kg) or sodium arsenite (2.5 mg/kg), simultaneously with garlic extract. After 24hr, chromosome preparations were made from the bone marrow cells. The mend points studied were chromosomal aberrations and damaged cells. Garlic extract alone induced a low level of chromosomal damage. The clastogenicity of all three mutagens were reduced significantly in the animals which had been give garlic extract as dietary supplement. The extent of reduction was different for the three clastogens and may be attributed to the interaction with the different components of the extract (Das et al., 1995) The endpoints scored were frequencies of chromosomal aberrations and damaged cells induced in bone marrow preparations. These parameters were found to be directly dose dependent and after an initial enhancement at 7 days, were reduced following prolonged exposure for 24hr to the low level observed at 24 hr. Therefore, administration of a low concentration of garlic extract daily is suggested for at least 30 days to obtain the maximum benefit of the extract in protecting against the clastogenic effects of known genototoxicants (Das et al 1995).

Previous studies have shown that the anticlastogenic properties of two dietary supplements, garlic and mustard oil, were screened against the clastogenic activity of sodium arsenite, since diet may contain factors which affect the process of mutagenesis and carcinogenesis. Aqueous extract of garlic (100 mg/kg b.w.) and mustard oil (0.643 mg/kg b.w.) were fed to Mus musculus for 30 consecutive days either singly or simultaneously. Sodium arsenite (0.1 mg/kg b.w.) was injected subcutaneously on days 7, 14, 21 and 30 of experiment, singly and together with the dietary supplements. The animals were sacrificed 24hafter the last exposure to sodium arsenite and clastogeinc effects were observed in the bone marrow cells. The degree of modulation of sodium arsenite-induced chromosomal aberrations was more pronounced in mustard oil than in garlic extract and simultaneous administration of both the dietary supplements reduced the clastogenic effects of sodium arsenite closer to the level of the negative control. The greater efficacy could be due to the interaction of the two dietary supplements and its radical scavenging property. (Choudhry and mitra, 2007).

The result in agreement with the studies on antimutagenic effect of garlic extract (GE) has been evaluated using 'in vivo chromosomal aberration assay' in swiss albino mice. Cyclophosphamide (CP), a well-known mutagen, was given at a single dose of 25

mg/kg b.w. intraperitoneally. Pretreatment with 1, 2.5 and 5% of freshly prepared GE was given through oral intubation for 5 days prior to CP administration animals from all the groups were sacrificed at sampling times of 24 and 48 h and theirbone marrow tissue was analyzed for chromosomal damage. The animals of the positive control group (CP alone) showed a significant increase in chromosomal aberrations both at 24 and 48h sampling time.

GE, alone did not induce aberrations at either sampling time, confirming its non-mutagenicity. However in the GE pre-treated and CP posttreated groups, a dose dependent decrease in cytogentic damage was recorded. A significant suppression in the chromosomal aberrations was recorded following pretreatment with 2.5 and 5% GE administration. The anticytotoxic effects of GE were also evident, as observed by significant increase in mitotic index, when compared to positive control group. Reduction in CP induced clastogenicity by GE was evident at 24 h. Thus results of the present investigations revealed that GE has chemopreventive potential against CP induced chromosomal mutations in swiss albino mice (Shukla et al., 2002).

Consumption of garlic and tomato has been associated with reduced risk of many human cancers. The effects of these two dietary items were studied experimentally on carcinogen [lsqb] DMBA [rsqb] induced clastogenicity in swiss mice. Chromosomal aberrations, which are predictor of cancer risk, were found to be reduced in bone marrow cells of swiss mice exposed to carcinogens. Significant reduction of chromosomal aberrations was noted in bone marrow on day 21 and 30($p<0.02$) although reduction was first evident after 96 hours. This is possibly the first report to suggest that oral administration of garlic and tomato can protect from the damaging effects of carcinogenic insult. It is proposed that one or other of many constituents of garlic and tomato may be responsible for the definite protective effect on chromosomal aberrations (Archana, et al 2002).

In the another study the interactive effects of saffron with two commonly consumed dietary agents, garlic and was evaluated for antigenotoxic effects against cyclophosphamide (CPH) in the mouse bone marrow micronucleus test experimental animals were orally pretreated with saffron (100 mg/kg body weight), garlic (250 mg/kg body weight) in combination for five consecutive days, 2h prior to the administration of CPH. Maximum reduction in the frequencies of micronucleated polychromatic erythrocytes (Mn PCEs) induced by CPH was observed when all the three test compounds were administered together. Furthermore, the protective effects were more pronounced in the garlic-administered groups compared to curcumin and/or saffron administered groups. (Prem Kumar et al., 2004) The anticlastogenic activity of crude extract of garlic (Allium sativum L.) was studied in bone marrow cells of mice. Male laboratory-bred swiss albino mice were given one of three concentrations of the freshly prepared extract (100 mg, 50 mg, and 25 mg/kg body weight) as a dietary supplement by gavage for 6 consecutive days. On the seventh day the mice were administered a single acute dose of two known clastogens, mitomycin C (1.5 mg/kg) and cyclophosphamide (25 mg/kg) or sodium arsenite (2.5 mg/kg), simultaneously with garlic extract. After 24 hr, chromosome preparations were made from the bone marrow cells. The endpoints studied were chromosomal aberrations and damaged cells. Garlic extract alone induced a low level of chromosomal damage. The clatogenicity of all three mutagens were reduced significantly in the animas which had been given garlic extract as dietary supplement. The extent of reduction was different for the three clastogens and may be attributed to the interaction with the differ-

ent components of the extract (Tandras das et al., 2006) The results are comparable with Genotoxic effects of herbal drops of garlic and pasipy were evaluated using the micronucleus test. Maximum Tolerated Dose (MTD) was determined by a dose-response test. For each medicine three treatment groups were considered with doses of MTD, ½ MTD and ¼ MTD according to the CSGMT protocol (1995 Japan). Drugs were administered orally to mice (test groups). Mitomicin C was used as a known genotoxic agent in positive control group. The peripheral blood samples before treatment (zero time samples) were considered as negative control. The appearance of a micronucleus is used as an index for Genotoxic potential. The results obtained indicated that the herbal drops showed genotoxicity effect and it was dose-dependent compared to the negative control group. This genotoxicity was significant ($p<0.05$) but the genotoxic effects of garlic and pasipy were "not significant" compared to the historical negative control group ($p<0.05$). (Kalantari, et al 2007).

The antioxidant nature of garlic has been attributed to the presence of organosulfur compounds such as s-allylcysteine, daillylsulphide, allylmethylsulphide, smethylcysteine (Ide and Lau, 1999; Wei and Lau, 1998; Chandra Mohan et al, 2004). These volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic (Chandra Mohan et al, 2004). Imai et al 1994 reported the antioxidant properties of garlic preparations and organosulfur compounds in garlic. Among the variety of organosulfur compounds, sallylcysteine and s-allylmercaptocysteine, found in aged garlic extract, showed radical scavenging activity in both chemiluminescence and 1, 1-dipheny-2-picrylhydrazyl assays, indicating that these compounds may play an important role in the antioxidative activity of aged garlic.

ACKNOWLEDGMENT

The author Srivani is thankful to Prof. Sugita Mathur, Head, Department of Zoology, University College of Science, Osmania University for providing for Laboratory facilities.

REFERENCES

1. Smorenburg CH, Sparreboom A, Bontenbal M and Verweij J. 2001. Combination chemotherapy of the taxanes and antimetabolites:Its use and limitations. Eur J Cancer, 37:2310-23. | 2. Padmalatha Rai S, and KK Vijaylakshmi. 2001. Tamoxifen citrate induced sperm shape abnormalities in the in vivo mouse. Mut. Res. 492: 1-6. | | 3. Akram H, Ghaderi Pakdel F, Ahmadi A, Zare S 2012. Beneficial effects of American ginseng on epididymal sperm analyses in cyclophosphamide treated rats. Cell J. Summer;14(2):116-21. | 4. Akram H, Samad Zare, Firouz Ghaderi Pakdel, Abbas Ahmadi 2010. Effects of Vitamin E and Ginseng Extract on Fertility Changes Inducedby Cyclophosphamide in Rats. J Reprod Infertil. 11(4):342. | 5. Anuradha 2010. Modulatory effects of turmeric and garlic against cisplatin induced genotoxicity Ph.D. thesis .Osmania University. | 6. Anil Kumar, Jyotsna D and Anup Singh 2011. A review on spice of life curcuma longa (turmeric)JournalofInternational Applied Biology and Pharmaceutical Technology. Oct – Dec; Volume: 2: Issue-4:371-379. | 7. Deshpande SS, Kewatkar SM, Paithankar VV. 2013. Anticlastogenic activity of flavonoid reach extract of CassiaauriculataLinn. on experimental animal. Indian J Pharmacol.; 45(2):184-6. | 8. Fleming RE., 1997. Pharmacotherapy17:1465–1545. | 9. Perini P, Calabrese M, Rinaldi L, Gallo P. 2007 . Expert Opin Drug Saf 6:183–190. | 10. Uber WE, Self SE, Van Bakel AB, Pereira NL. 2007. Am J Transplant 7:2064–2074 | 11. IARC monographs on the evaluation of carcinogenic risks to humans. Supplement 7, 1987. | 12. Banerjee, S.K., P. K. Mukherjee and S.K. Maulik 2003. Phytother Res. 17: 97-106. | 13. Khanum F, Anilakumar KR, Viswanathan KR 2004. Anticarcinogenic properties of garlic: A review. Crit. Rev. Food. Sci. Nutr., 44: 479-488. | 14. Shukla, Y and P. Taneja 2002. Cancer Lett. 176(1): 31-36. | 15. Bhuvaneswari, V, Velmurugn B, Abraham SK and Nagini S 2004. Tomato and garlic by gavage modulate 7, 12-dimethylbenze(a)anthracene induced genotoxicity and oxidative stress in mice. Brazillian journal of Medical and biological research.37: 1029-1034. | 16. Siddique YH, Afzal M 2005. Antigenotoxic effect of allicin against methyl methanosulphonate induced genotoxic damage. J. Environ.Biol., 26(3): 547-550. | 17. Boller, K. and W. Schmid Hum. 1970. . Chemische mutagenese beim Säuger. Das knochenmark des Chinesischen hamsters als in vivo-Testsystem. Hämatologische befunde nach behandlung mit trenimon. Humangenetik ;11:35-54. | 18. Wei Z, Lau BHS. 1998. Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. Nutr Res; 18:61. | 19. Schmid, W. 1976. "The micronucleus test for cytogenetic analysis pp. 31–53. (7). | 20. Heddle, J.A. 1973. A rapid in vivo test for chromosomal damage. Mut. Res., 18: 187-190. | 21. Chaubey, R. C., Kavi, B. R., Chauhan, P. S. and Sundaram, K. 1978. Micronucleus Test. Evaluation of chemicals and other environmentalagents for in vivo chromosomal damage. In: Proceedings of International Symposium on Environmental agents and their biological effects . 3:415-418. | 22. Maier, P. and Schmid, W. 1976. Ten model mutagens evaluated by micronucleus test.Mut. Res. 40(4): 325-327. | 23. Rudrama Devi K and Reddy PP. 1986. Dose response relationship for sperm abnormalities induced by thiotepa in mice.Cell and chromosome research. 9 (3):77-78. | 24. Asita Okorie A, Mann E. Dingann and Sibusisiwe Magama 2008. Lack of modulatory effect of asparagus, tomato, and grape juice on cyclophosphamide-induced genotoxicity in mice, AfricanJournalofBiotechnology Vol. 7 (18), pp. 3383-3388. | 25. Santos-Mello, Renato; Deimling, Luiz Irineu; Lauer Junior, Claudio AndCarvalho, Thaís Rieger de. 2005. Chemoprotective effect of cysteamine against the induction of micronuclei by methyl methanesulfonate and cyclophosphamide.Genet. Mol. Biol. vol.28, n.1: pp. 156-160. | 26. Raja Wasim, R.C. Agrawal and M. Ovais. 2013.Prevention of Cyclophosphamide-Induced Micronucleus Formation in Mouse Bone Marrow by Solanum lycopersicum Extract. American-Eurasian Journal of Scientific Research 8 (6): 244-247. | 27. Shoba Rani M and Rudrama Devi K. 2006. Induction of chromosomal aberrations in bone marrow cells of mice.Trends in life science, vol. 21 (1 &2). | 28. Prabhakar Reddy, Sushma Ch., and K. Rudrama Devi. 2014. "Evaluation of Antimutagenic Potential of Aeges Marmelos Fruit Extract in Cyclophosphamide Induced Genetic Damage in Somatic Cells of Mice",. Innovative Journal of Medical and Health Science. (In press). | 29. Abraham SK and Kesavan PC 1984. Genotoxicity of garlic, turmeric and asafoetida in mice Mutat. Res. 136(1):85-88 | 30. Sumiyoshi H, Kanezawa A, Masamoto K, Harada H, Nakagami S, Yokota A, Nishikawa M, Nakagawa S. 1984; [Chronic toxicity test of garlic extract in rats] J Toxicol Sci. 9:61–75.Das T, Khan NS, Sooranna SR 1995. Current medical research opinion. 13:257-263. | 31. Choudhry AR and Mitra C 2007. Cancer letters. 121(1), 16:45-50. | 32. Archana Sengupta, Sharmistha Ghosh, Sukta Das 2002. Administration of garlic and tomato can protect from carcinogen induced clastogenicity. 22(7): 859-866. | 33. Prem Kumar K, Kavitha S, Santhiya ST, ramesh AR, Suwanteerangkul 2004.interative effects of saffron with garlic against cyclophosphamide induced genotoxicity in mice. J Asia Pac J Clin Nutr. 13, 3,: 292-294. | 34. Tandras das, Arati Roychoudhury, Archana Sharma, Geeta Talukder 200.6 Environmental and Molecular Mutagenesis. 21 (4):383-388. | 35. Kalantari, H, Larkii A Latifi SM Hung, 2007 Ata physiol. 94(3):261-6. | 36. Ide N1, Lau BH. 1999. Aged garlic extract attenuates intracellular oxidative stress.Phytomedicine. May;6(2):125-31. | 37. Chandramohan, KVP, Abraham SK, and Nagini S, 2004: Dose-dependent protection by tomato against 7,12-dimethylbenz(a)anthracene- induced genotoxicity and oxidative stress in miceJournal of Medicinal Food. 7(1): 55-60. | 38. Rudrama devi .K * and Kusum lata chamyal 2012. protective effects of phyllanthus fruit extract in adriamycin induced genotoxicity in bone marrow cells of mice research article international journal of pharma and bio sciences, vol 3/issue 1/jan – mar