



International Journal of  
**Cancer Research**

ISSN 1811-9727



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**Inhibitory Effect of Unprocessed/Processed Alliums under  
*in vitro/in vivo* Conditions on Carcinogen Induced  
Mutagenesis Using Different Assays**

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**Abstract:** Three experiments were carried out to assess the antimutagenic potential of garlic or onion. This was tested in, *in vitro/in vivo* models where mutagenicity was induced either with benzo (a) pyrene (BP)/3-methyl-cholanthrene (3MC), using Ames test or the comet assay. Also the effects of cooking processes like boiling/frying on the antimutagenic potential of these alliums were tested by SOS inhibition assay using 4-nitroquinoline-N-oxide (4NQO) as the mutagen. In the first experiment, onion feeding at 1 and 5% in the diet inhibited the formation and excretion of urinary mutagens in dose dependent manner as indicated by decreased frequency of revertants in strains TA 98 and TA100 in the presence of urine belonging to rats fed onion and exposed to BP or 3MC. In the second experiment using SOS induction assay, both processed or unprocessed garlic exhibited dose dependent inhibition of SOS induction. However with boiled/unboiled onion this was not statistically significant. Studies with DAS also showed a dose dependent inhibition of SOS induction. No significant differences were observed in inhibition of SOS responses with processing of onion/garlic. These results indicate that the antimutagenic principle in garlic is not destroyed on heat treatment during cooking processes. In the third experiment, BP treatment resulted in comet induction (as judged by the comet ratio-diameter/length) significantly as compared to control, both in liver and kidney tissues ( $p < 0.001$ ). Allium vegetables fed groups showed values similar to control group. As was observed in the peripheral blood lymphocytes, a trend towards recovery from damage was noted both in liver and kidney tissue due to prior allium feeding. All these results show that alliums whether in the processed or unprocessed form can be used in diets to avert toxic effects of environmental carcinogens/genotoxicants and can be potent chemopreventive agents and cancer prevention is far more desirable alternative than treatment by surgery and drugs. The protection could be achieved even by very low intake level of allium in natural form through diet.

**Key words:** Alliums, antimutagenicity, ames test, SOS response, comet assay

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### Introduction

There is a growing incidence of cancer of various sites which is mainly attributed to the oxidative damage to the host genome by environmental contaminants. Plant constituents particularly vegetables and fruits are rich sources of antioxidant vitamins (Weisburger, 1999). Their role in the prevention of

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free radical induced DNA damage implicated in cancer causation is well proven. Spices and condiments which are part of the Indian diet have chemical constituents which have antioxidant, antimutagenic and anticarcinogenic properties. Of all the spices the most commonly used spices in India are turmeric, garlic and onion. Alliums are mostly popular for their flavour which is characteristic of sulphur containing compounds formed directly either by crushing or following a chemical reaction. Onions and garlic are members of allium family and are rich sources of compounds like diallyl sulfide (DAS), Allyl methyl trisulphide (AMT), Allyl methyl disulphide (AMD) and diallyl tri sulphide (DAT) (Block, 1985) which have all been shown to inhibit benzo(a)pyrene induced forestomach tumors (Sparmins, *et al.*, 1988). However the mechanisms involved in conferring this protective effect is not yet clearly understood. Hitherto there is a recent report demonstrating the antioxidant and antimutagenic effects of onion extracts against mutagens (Shon *et al.*, 2004). Reports from other laboratories have shown that the antimutagenic activity of organosulphur compounds from allium is associated with phase II enzyme induction (Guyonnet *et al.*, 2001). Experiments carried out earlier in our laboratory suggested that regular intake of alliums through dietary route could induce protective enzymes like GST, QR, UDP glucuronyl transferase accompanied by a decrease in BP-DNA binding. (Krishnaswamy and Polasa, 2001; Rajpurohit and Krishnaswamy, 1994).

As majority of cancers are likely to originate as a consequence of series of mutational events (Morris, 1991), protection against mutations could be beneficial at late as well as early stages of cancer development. Bacterial screening procedures for assaying mutagenicity have been in vogue and can be modified to screen for antimutagens. Short term assay especially the Ames Salmonella test (Deflora *et al.*, 1992) and also SOS response assay (Quillardet and Hofnung, 1985) have been extensively used for evaluating the modulation of the mutagenic response. Mutagenicity test of the urine of carcinogen treated animals would reveal the protective effect following *in vivo* metabolism and can be a good bio monitoring method (Polasa and Krishnaswamy, 1997). Yet another method of studying DNA damage and repair is by using the comet assay, also called the 'Single Cell Gel Assay', at the level of single cells (Singh *et al.*, 1988). Therefore using these three different approaches of testing antimutagenicity, we wanted to see if consumption of alliums would be effective in reducing the risk of mutations and thereby the risk of cancer (Fredrik *et al.*, 2003). Evidences in the literature have always focused on the antimutagenic properties of the phytochemicals in the raw form. Since these are consumed through foods which undergo changes during cooking, it is necessary to know if these effects are retained even after boiling/frying-the usual methods of processing. So the present research were carried out.

To assess the antimutagenic potential of onion fed through diet to rats followed by carcinogen exposure and assessment of urinary mutagenicity by the Ames Salmonella/microsome test; to study the modulation of DNA repair gene induction by the mutagen 4NQO and its modulation by unprocessed/processed, onion/garlic using the sophisticated SOS response assay using *E. coli* PQ37 and was carried out to assess the effects of onion/garlic on comet induction by BP in rat tissues by the Comet assay.

## **Materials and Methods**

All the studies are being carried out at National Institute of Nutrition, Hyderabad, India. The experimental procedures used in these studies met the guidelines of the Institute's Animal Ethical Committee.

### *Experiment 1*

#### *Animals*

Inbred male wistar rats (Wistar/NIN) aged 8-10 weeks were obtained from the National Center for Laboratory Animal Sciences (Hyderabad, India). The animals were housed in polypropylene cages at 22±1.2°C with 50-55% relative humidity and a 12 h light/dark cycle. Water and food were given ad lib. They were randomly divided into nine groups with 10 animals in each group. Group I, Group II and Group VII rats were fed with control diet, Group III, Group IV and Group VIII rats were fed with control diet containing onion (1% in the diet) and Group V, Group VI and Group IX rats were fed with control diet containing onion (5% in the diet) for one month. BP 1 mg /rat was injected (i.p) into Group II, Group IV and Group VI rats at the end of 1 month of feeding the respective diets and 24 h urine was collected. Group VII, Group VIII and Group IX were injected 3-methyl-cholanthrene (3MC) (1 mg/rat) at the end of 1 month of feeding the respective diets and 24h urine was collected.

The food intake of the animals was recorded every week throughout the study. The body weights of the animals were recorded at the beginning and at the end of the experiment.

#### *Chemicals*

All the chemicals used in the mutagenicity were obtained from Sigma Chemical Corp (St.louis, MO, USA).

Onion was purchased from the local market and cleaned, sundried, powdered and incorporated into 20% casein based standard diet. Nutritively the control and the experimental diets were similar.

#### *Bacteria*

*Salmonella typhimurium* TA98 and TA100 were provided by professor B.N. Ames, University of California (Berkeley, CA, USA). The strains were maintained and used in the assay according to the procedure of Maron and Ames (1983).

#### *Carcinogen Exposure*

After 1 month of feeding onion diet, the carcinogen treated groups received a single intraperitoneal (i.p) dose of benzo (a) pyrene (BP) /3 Methylcholanthrene (3MC) in groundnut oil. The controls were treated with equal volume of vehicle. The rats were put in metabolic cages and urine samples collected for 24 h after carcinogen injection. The samples were frozen immediately and kept at -80°C until analysis.

Concentration of urinary mutagens, Preparation of S9 fraction, Mutagenicity assay were carried out as mentioned earlier (Polasa and Krishnaswamy, 1997).

### *Experiment II*

#### *Preparation of Fried Garlic/Onion Extracts*

Garlic or onion paste, each of these was suspended in sunflower oil in two different tubes and vortexed well. One of the garlic and one of the ginger containing tubes was heated and after cooling, an equal volume of DMSO (equal to oil) was added to all the tubes (raw and fried of both garlic and onion tubes). All the tubes were then vortexed for 5 min and then centrifuged. The DMSO layer was used for the SOS test.

#### *Preparation of Boiled Garlic/Onion Extracts*

Each of garlic or onion paste was taken in two different tubes in equal amounts. The tubes were vortexed after adding equal amounts of water. One from each of garlic/onion containing tubes was boiled and then cooled. Then the tubes were spun and the supernatant was collected and used for the SOS test.

#### *SOS Test*

*E. coli PQ 37* was obtained from Hofnung (Pasteur Institute, Paris, France). SOS induction potential of the test compound was carried out by the method as described by Quillardet and Hofnung (Quillardet, P and Hofnung, M, 1985) using 4-nitroquinoline-N-oxide (4NQO) as the mutagen. In brief the *E. coli PQ 37* which carries mutation for SOS responses was used as the test organism. The SOS induction was judged by the  $\beta$  galactosidase (BG) activity, as the lac Z, the structural gene for BG is placed under the control of the *sfIA* gene (SOS responsive gene). Alkaline phosphatase (AP) activity was determined in parallel to take care of the false negative values which may arise if at all there is any modulation of protein synthesis by the test compound. The induction factor of *sfIA:lacZ* expression was taken to be the ratio of BG activity and AP activity at the respective concentration of the test compound, divided by its control value.

4-NQO at 1, 2, 3 nanogram concentration was used as the mutagen. To study the antimutagenic effects of onion, onion was used at 1, 5 and 10 mg concentration (boiled/unboiled) and to study the effects of frying 5, 20 and 50  $\mu$ g of (raw/fried) onion was used. To study the effects of garlic, 0.75, 1.25 and 2.5 mg of boiled /unboiled garlic and to study the effects of frying 10, 40 and 100  $\mu$ g of raw/fried garlic was used.

The active principle of alliums viz., diallylsulphide (DAS) (using at 2.67, 4.45 and 8.9  $\mu$ g concentration) was also tested for its antigenotoxic potential.

All the above concentrations of the carcinogen/onion/garlic/DAS were fixed after initial standardizations done to achieve measurable SOS induction.

#### *Experiment III*

All the chemicals used in the comet assay like the low melting Agarose, Agarose, Tris hydroxy methyl amino methane, Sodium lauryl sarcosinate, Triton X 100, Ethidium Bromide, RPMI 1640 medium with L-glutamine were purchased from Sigma (St.Louis, MO, USA).

#### *Animals*

Inbred male wistar /NIN rats aged 4 weeks were obtained from the laboratory Animal Information Service Center (Hyderabad, India). They were randomly divided into six groups. Group I served as control and received a control diet for one month followed by intraperitoneal (i.p) injection of saline. Group II also received a control diet for one month. At the end of one month this group received 5mg of BP i.p injection. Group III received a control diet containing Garlic 0.1% + Onion 1% for one month followed by i.p injection of saline. Group IV received a control diet containing Garlic 0.1% + Onion 1% followed by 5 mg of BP i.p injection.

Group V received a control diet containing Garlic 0.5% + Onion 5% for one month followed by i.p injection of saline. Group VI received a control diet containing Garlic 0.5% + Onion 5% for one month followed by 5 mg of BP i.p. injection. Groups I and II served as negative and positive controls respectively. Group III served as a control for Group IV and Group V served as a control for Group VI.

Blood and other tissues of interest namely liver and kidney were collected 8 days after saline/carcinogen treatment. DNA damage was studied in Peripheral Blood Lymphocytes (PBL) and the tissue homogenates using single cell gel electrophoresis.

#### *Comet Assay*

The technique as described by Singh (1988) was followed with slight modification. The comet length and diameter were measured. The results are expressed as ratio of cell diameter to tail length.

### Testing for Cell Viability

Cell suspension from all treatment groups were tested for viability by the trypan blue dye exclusion method (Polasa *et al.*, 2004).

### Statistical Analysis

The data was analysed by analysis of variance. Testing of group means was done by Duncan's multiple range test (Maron and Ames, 1983). The homogeneity of variance was found to be not significant for all the parameters by Levene statistic. SPSS 10.0 window version was used for this purpose. Mann-Whitney test was used for comet assay data.

## Results

### Effects of Onion on the Frequency of Revertants Induced by BP/3MC in TA98 and TA100 Bacterial Strains

There was no difference in the weekly food intakes between the groups. No difference was observed in final bodyweight of the animals belonging to different groups. Increased amounts of mutagens were present in the urine of animals fed control diet and exposed to the carcinogen (BP/3MC). This was indicated by elevated reversion frequency following treatment of *S. typhimurium* TA98 and TA100 strains with urine extract. Onion feeding at 1 and 5% inhibited the excretion of urinary mutagens in dose dependent manner as indicated by decreased frequency of reversion in strains TA98 and TA100 in the presence of urine belonging to rats exposed to BP and fed onion. Similar observations were made in the presence and absence of S9. Urine of rats fed onion (1, 5%) and treated with 3MC showed decreased mutant frequency with and without S9 ( $p < 0.05$ ) in both the strains as compared to 3MC treated animals on control diet. However, between the 3MC+ Onion 1% and 3MC + Onion 5% groups, there was no significant difference (Fig. 1-4).

### Experiment II

#### Effects of Unprocessed/Processed, Onion/Garlic on the DNA Repair Gene Induction by 4NQO

4NQO showed dose response SOS induction at 3, 5 and 10 ng ( $p < 0.001$ ). The active principle of alliums viz DAS (3, 5, 10) when tested for its antigenotoxic potential showed inhibition of 20% to 90.3% depending on the concentration of 4NQO and DAS ( $p < 0.05$ ) (Table 1).

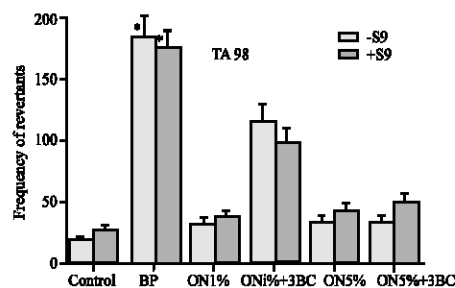


Fig. 1: Effects of feeding onion (ON) on the reversion frequency in BP induced mutagenesis using TA98 strain

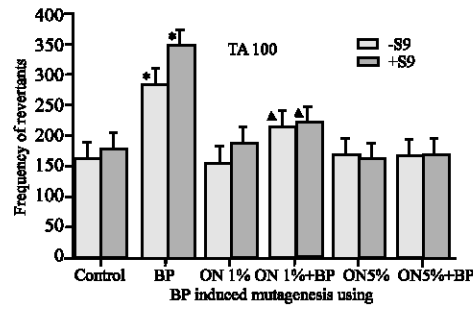


Fig. 2: Effects of feeding onion (ON) on the reversion frequency in BP induced mutagenesis using TA100 strain

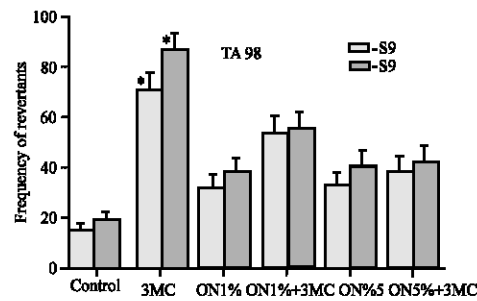


Fig. 3: Effects of feeding onion (ON) on the reversion frequency in 3MC induced mutagenesis using TA98 strain

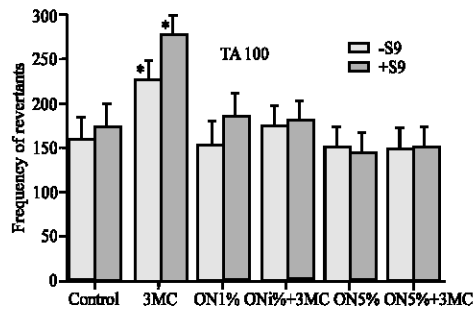


Fig. 4: Effects of feeding onion (ON) on the reversion frequency in 3MC induced mutagenesis using TA100 strain

Both raw and fried onion (5, 20, 50 µg) showed inhibition of SOS induction at the concentrations tested. The extent of inhibition ranged from 7 to 90% at 1-3 ng of 4NQO ( $p < 0.001$ ). No significant differences were observed in percentage inhibition of SOS responses whether raw or fried onions were used for testing (Table 2). The above observations suggest that both raw and cooked form of onion (i.e., even after frying at high temperature) possess antimutagenic property.

Table 1: Inhibitory effect of DAS on , SOS induction\* by 4 NQO

4 NQO (ng)	Diallylsulphide (DAS) ( $\mu\text{g}$ )			
	0	2.67	4.45	8.9 $\mu\text{g}$
1	6.16 $\pm$ 2.5*	4.90 $\pm$ 0.10	3.00 $\pm$ 0.66	0.60 $\pm$ 0.17
2	8.06 $\pm$ 0.72*	6.27 $\pm$ 0.91	4.57 $\pm$ 0.21	0.60 $\pm$ 0.26
3	8.80 $\pm$ 0.70*	6.80 $\pm$ 0.92	5.27 $\pm$ 0.35	0.92 $\pm$ 0.49

\*SOS induction expressed as induction factor., \*Values are mean $\pm$ SD (n = 3). DAS treated groups are significantly different from control (p<0.05)

Table 2: Inhibitory effect of onion extract (not fried/fried) on, SOS induction\*by different concentrations of 4 NQO

4NQO (ng)	Onion (not fried)				Onion (fried)		
	Onion 0 $\mu\text{g}$	5 $\mu\text{g}$	20 $\mu\text{g}$	50 $\mu\text{g}$	5 $\mu\text{g}$	20 $\mu\text{g}$	50 $\mu\text{g}$
1	4.25 $\pm$ 0.21*	3.75 $\pm$ 0.35	1.95 $\pm$ 1.06	0.68 $\pm$ 0.39	3.95 $\pm$ 0.64	1.75 $\pm$ 0.64	0.64 $\pm$ 0.40
2	6.55 $\pm$ 0.64*	5.95 $\pm$ 1.20	2.4 $\pm$ 1.7	0.65 $\pm$ 0.35	6.20 $\pm$ 0.71	2.35 $\pm$ 0.49	0.70 $\pm$ 0.28
3	7.7 $\pm$ 0.42*	7.0 $\pm$ 1.41	2.6 $\pm$ 2.12	0.8 $\pm$ 0.42	6.85 $\pm$ 0.64	2.45 $\pm$ 1.06	0.75 $\pm$ 0.21

\*SOS induction expressed as induction factor., \*Values are mean $\pm$ SD (n = 3). Onion (not fried/fried) treated groups are significantly different from control (p<0.001). Onion (not fried) vs Onion (fried) groups are not significantly different

Table 3: Inhibitory effect of onion extract (unboiled/boiled) on, SOS induction\* by different concentrations of 4 NQO

4 NQO (ng)	Onion (unboiled) <sup>®</sup>				Onion (boiled) <sup>®</sup>		
	Onion 0 mg	1 mg	5 mg	10 mg	1 mg	5 mg	10 mg
1	5.2 $\pm$ 1.13*	2.17 $\pm$ 0.85	1.27 $\pm$ 0.21	2.43 $\pm$ 0.91	3.5 $\pm$ 0.70	3.7 $\pm$ 0.75	3.9 $\pm$ 0.75
2	7.03 $\pm$ 0.90*	3.53 $\pm$ 0.57	3.1 $\pm$ 0.95	3.50 $\pm$ 0.61	5.6 $\pm$ 0.8	5.6 $\pm$ 0.87	5.13 $\pm$ 0.38
3	9.73 $\pm$ 1.53*	5.80 $\pm$ 1.55	4.9 $\pm$ 0.80	5.5 $\pm$ 1.12	8.55 $\pm$ 3.19	7.6 $\pm$ 0.98	6.5 $\pm$ 1.90

\*SOS induction expressed as induction factor., \*Values are mean $\pm$ SD (n = 3). Onion (unboiled/boiled) treated groups are significantly different from control. (p<0.001). <sup>®</sup>Onion unboiled vs boiled values are significantly different (p<0.05 ) at all dose levels

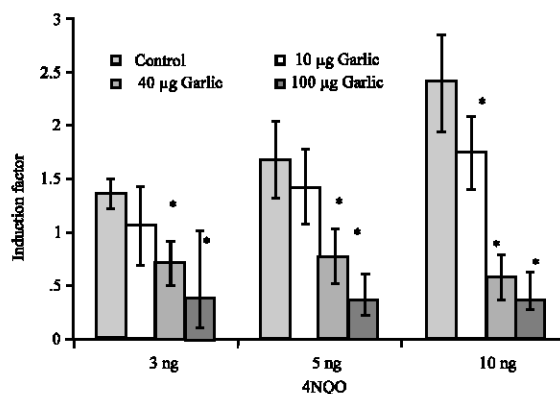


Fig. 5: Dose response effect of garlic on, SOS induction\* by different concentrations of 4 NQO. \*SOS induction expressed as induction factor. Values are mean $\pm$ SD Control (n=3), Garlic treated (n=6). \*Garlic treated groups are significantly different from control (p<0.05)

Both unboiled and boiled onions (1, 5, 10 mg) showed inhibition in SOS induction system. The unboiled was however more effective than boiled (p<0.05) at all dose levels (Table 3).

The results indicated that cooked form of onion also has retained the antigenotoxic potential and can therefore be used in diets to avert toxic effects of environmental carcinogens/genotoxicants.



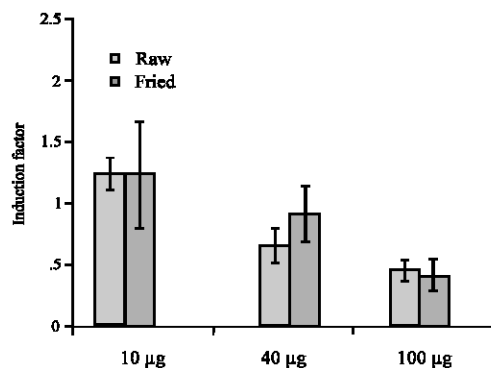


Fig. 6: Effects of raw/fried garlic extracts on, SOS induction\* by 3ng of 4NQO. \*SOS induction expressed as induction factor. Values are mean±SD (n = 6)

Table 4: DNA damage in PBL of rats fed alliums and exposed to carcinogen (5 mg i.p)

Groups	No. of rats	Treatments	Comet ratio Diameter/Length Mean±SD
1	6	Control	0.975±0.014
2	6	BP	0.888±0.048*
3	6	Garlic 0.1% + Onion 1%	0.967±0.017
4	6	Garlic 0.1% + Onion 1% + BP	0.900±0.050*
5	6	Garlic 0.5% + Onion 5%	0.969±0.015
6	6	Garlic 0.5% + Onion 5%+ BP	0.915±0.054*

\* Significantly different from control at P < 0.001 (by Mann-Whitney test)

Table 5: Effect of Allium feeding on tissue DNA damage due to BP administration

Groups	No. of rats	Treatments	Comet Ratio:Diameter/LengthMean±SD	
			Liver	Kidney
1	6	Control	0.969±0.14	0.955±0.034
2	6	BP	0.899±0.65*	0.885±0.080*
3	6	Garlic 0.1%+Onion 1%	0.986±0.03	0.988±0.017
4	6	Garlic 0.1%+Onion 1% + BP	0.910±0.03	0.917±0.026
5	6	Garlic 0.5%+Onion 5%	0.972±0.03	0.974±0.026
6	6	Garlic 0.5%+Onion 5% + BP	0.920±0.05	0.925±0.052

Significantly different from control at P < 0.001 (by Mann-Whitney test)

Both raw and fried garlic exhibited dose dependent inhibition of SOS induction. There was significant inhibition at 10, 40 and 100 µg of garlic (significantly different from control at p<0.05). The inhibition ranged between 10 to 71% with 3 ng of 4NQO. The inhibition of SOS induction at 5 and 10 ng of 4 NQO by garlic was similar to the effect observed with 3 ng of 4 NQO. At 10 ng concentration of 4 NQO, 100 µg garlic could exert 83-85% inhibition (p<0.05) (Fig. 5). There were no significant differences in the inhibition potential between raw and fried garlic extracts (Fig. 6). There was a trend towards dose dependent inhibition of SOS induction with boiled/unboiled garlic extracts at the concentrations used. There were no significant differences in the inhibition potential between boiled and unboiled garlic extracts (Fig. 7). These results indicate that the antimutagenic principle in garlic is not destroyed on heat treatment during cooking processes.

### Experiment III

#### Effects of Onion/Garlic on Comet Induction by BP in PBL of Rats

BP treatment induced significant induction of comets (0.88±0.048) as compared to control (0.97±0.014) (p<0.02). Allium vegetables fed group showed comet values similar to control groups.

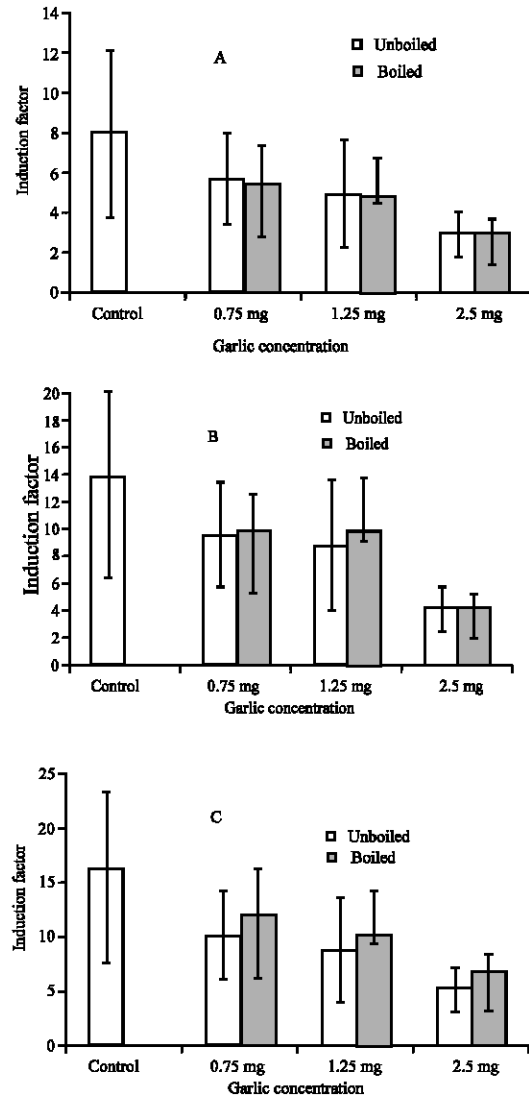


Fig. 7: Effects of unboiled/boiled garlic on, SOS induction\* by (A) 1 ng (B) 2 ng (C) 3 ng concentrations of 4 NQO. \*SOS induction expressed as induction factor. Values are mean $\pm$ SD (n = 3)

Allium feeding at both the levels showed some protective effect although this is not statistically significant (Table 4).

#### *Effects of Onion/Garlic on Comet Induction by BP in Liver and Kidney of Rats*

BP treatment induced significant induction of comets as compared to control both in the liver and kidney tissues ( $p < 0.001$ ). Allium vegetables fed groups showed values similar to control group. As was observed in the peripheral blood lymphocytes, a trend towards recovery from damage was noted both in liver and kidney tissues due to prior allium feeding (Table 5).

## **Discussion**

Diet being a major environmental risk factor, the contribution of diet and nutritional status to cancer risk and thereby its role in prevention and treatment of cancer has attracted the attention of researchers and public health policy makers. Many naturally occurring compounds particularly of plant origin possess excellent antimutagenic and thereby anticarcinogenic and chemopreventive potential (Middlebrooks, 1977; Tripathi *et al.*, 2005). Hence it is apparent that in principle, cancer and other mutation based diseases can be prevented not only by reducing human exposure to risk factors but also by enhancing host defenses through consumption of foods rich in protective agents. Although epidemiological evidences can address the relation of diet to cancer, laboratory studies gain importance as they provide empirical evidences to workout the exact quantity of micro/macro nutrient needed to counteract the toxic effect of xenobiotics.

It must be noted that many substances that cause mutations among micro organisms also cause cancer in animals and humans. This observation underlines the influence of microbial mutagenicity tests which are widely used to study the mutagenic/antimutagenic effects of the components of human diets (Surh, 1999).

Allium vegetables particularly onion/garlic are supposed to possess hypoglycemic, hypolipidemic, antiatherosclerotic and antibacterial properties. More recently their possible anticarcinogenic potency has received attention. Although the organosulfur compounds present in their oils have been reported to inhibit tumorigenesis, the mechanism of their chemopreventive action remains obscure (US Food and Drug Administration, 2000).

The *Salmonella* test is a highly validated assay to detect mutagens and has been recommended to test the mutagenicity of body fluids of treated animals and thus an indirect *in vivo* screening procedure for antimutagens/anticarcinogens (Sengupta *et al.*, 2004). Thus our experimental protocol would help in identifying chemopreventive agents as increased or decreased excretion of mutagens would be indicative of enhancement or inhibition of neoplastic process.

In the present study, the reduced urinary excretion of mutagens in the allium fed, carcinogen treated animals is a clear evidence to show that this could be one of the mechanisms by which the allium compounds exert their anticarcinogenic effects in animal models. This fact is further strengthened by the inhibitory effects of unprocessed/processed, onion/garlic on the DNA repair gene induction by 4NQO. Also a trend towards recovery from damage noted both in liver and kidney tissues due to prior allium feeding of the carcinogen treated animals is indicative of the role of allium compounds at the level of DNA repair.

BP is one of the ubiquitously present environmental contaminant. It is metabolized to reactive derivatives capable of binding to cellular macromolecules. This step is one of the key events in tumorigenesis. The polycyclic aromatic hydrocarbons including BP are converted to reactive metabolites following activation by microsome mixed function oxidase and epoxide hydrolases (Schreiner, 1983). Most of the mutagens/carcinogens undergo this type of transformations in the body and act in a similar fashion like BP. Stimulation of detoxification enzymes, inhibition of microsomal mixed function oxygenases have been suggested as possible mechanisms of cancer chemoprevention (Yang and Gelboin, 1976). Host susceptibility to react to the toxic effect of genotoxicants or chemical carcinogen depends on the balance between detoxification and metabolic activation of the xenobiotic (Manson *et al.*, 1997). So compounds which are capable of modulating the activity of enzymes responsible for the metabolism of carcinogens are of interest as they reduce the level of activation enzymes and enhance the activities of detoxification enzymes. DAS and other organosulfides

administered orally could induce glutathione-S-transferase in mouse tissues (Sparmins *et al.*, 1988). Our experiments suggested that regular intake of alliums through dietary route could induce protective enzymes like GST and QR. In another study conducted by Rajpurohit and Krishnaswamy (1994) it was observed that administration of vegetables like spinach, amaranth, gogu, cabbage and onion at 20% of the diet for 4 weeks resulted in increase in the activity of hepatic UDP glucuronyl transferase and glutathione S-transferase accompanied by a decrease in BP-DNA binding (Krishnaswamy and Polasa, 2001).

In this context our observations on the antimutagenic effect of onion and garlic on BP derived mutagens is significant. The active principle present in the allium vegetables namely the diallyl sulfides (DAS) fed to rats through diet at 200 mg kg<sup>-1</sup> body weight for 15 days resulted in increase in hepatic drug metabolizing enzymes (Gross-Steinmeyer *et al.*, 2004). However, this dose is higher than the normally consumed concentration in human diet (500-700 µg of DAS/g garlic). So it was of interest to explore if smaller doses can produce similar effect. Our results with 0.1% ≈ 60 µg DAS and 0.5% garlic indicated that even at ten times lower intake level antimutagenic effect could be observed (Polasa and Krishnaswamy, 1997).

The results presented in this study and earlier studies (Krishnaswamy and Polasa, 2001) demonstrate that short term screening *in vivo* assay coupled with GST assay may be used as a guide for quick screening of natural products. The active leads thus selected can be further taken up for studies demonstrating their inhibitory activities in the long term animal tumorigenesis experiments.

In this study, rats fed allium vegetables and exposed to 3-methyl cholanthrene were protected against formation and excretion of urinary mutagens indicating thereby that allium could not only counteract the mutagenicity of BP but also were active against the genotoxicity of 3-MC which besides being a carcinogen is also a potent inducer of mixed function oxidases (MFO). These enzymes are responsible for the formation of highly reactive electrophilic substances which can damage DNA. From our studies it is evident that alliums can enhance the levels of tissue detoxifying enzymes and it can be envisaged that they might be inhibiting the activation of oxidative pathway of xenobiotics resulting in toxic metabolites (Krishnaswamy and Polasa, 2001). It has been suggested that carcinogens produce nuclear damage and preadministration of DAS prevented nuclear damage induced by some carcinogens. These carcinogens have to be converted to their reactive form before they can bind to DNA. The above observation suggests that DAS inhibits the conversion of procarcinogens to ultimate carcinogens (Haber *et al.*, 1994; Amagase and Milner, 1993; Yang *et al.*, 2001; Green *et al.*, 2003).

Thus present results demonstrate that allium vegetables feeding to rats inhibited BP and 3MC mutagenicity. This could be attributed to stimulation of protective enzymes like GST and QR in target organs (Krishnaswamy and Polasa, 2001). The protection could be achieved even by very low intake level of allium in natural form through diet as compared to treatment with higher amounts of DAS and DADS and other active organosulfur substances.

From epidemiological studies carried out (Fleischauer and Arab, 2001; Dorant *et al.*, 1996), it is evident that alliums provide protection against stomach cancer. Experimental, clinical and epidemiological studies thus provide unequivocal evidences to show alliums can be potent chemopreventive agents and cancer prevention is far more desirable alternative than treatment by surgery and drugs.

### **Acknowledgement**

The technical assistance rendered by Mrs. Amulya Rao and Mr. P. Sathish Babu is gratefully acknowledged.

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