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ANTI CARCINOGENIC EFFECTS OF GARLIC AQUEOUS EXTRACTS IN INDUCED HEPATOMA IN MICE

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Abstract: Garlic is well known for its medicinal properties since a long time. The present work was undertaken to study the anti-carcinogenic and anti-tumorigenic effects of garlic aqueous extracts in experimentally induced hepatoma in mice as well as establish a possible anticancer mechanism of garlic. The protective effects of garlic aqueous extracts against biological induction of hepatoma as well as the curative effects of these extracts in biologically induced hepatoma mice were studied. It is evident from the results that garlic aqueous extracts gives protection against biological hepatoma induction. There is a considerable improvement seen in biological induced hepatoma mice given garlic aqueous extracts. These protective as well as curative effects of garlic aqueous extracts may be due to its principle sulfur compound diallyl disulfide.

Keywords: Garlic, Dially Disulfide, Lipid Profile, Hepatoma, Thiol Enzymes

INTRODUCTION

Garlic is a popular spice added to several edible preparations and is a remedy for a variety of ailments. Epidemiological as well as laboratory studies have shown that garlic consumption reduces certain cancer incidences in the stomach, colon, mammary, cervical etc. Garlic has been shown to metabolized into N-acetyl-s-allyl cysteine, allyl-mercaptan, diallyl disulfide, diallyl sulfide, diallyl sulfoxide, diallyl-sulfone and allyl methyl sulfide. Garlic has been thought to bring about its anticarcinogenic effect through a number of mechanisms, such as the scavenging of radicals, increasing glutathione levels, increasing the activities of enzymes such as glutathione s-transferease, catalase, inhibition of cytochrome p4502E1, DNA repair mechanisms, preventing of chromosomal damage[1]. Combination chemoprevention by dietary agents is a promising approach toward cancer control. Many dietary agents are known to prevent experimental Mutagenesis and carcinogenesis by modulating xenobiotic-metabolizing enzymes. The present study evaluated the combinatorial chemo-preventive effects of tomato and garlic on hamster buccal pouch carcinogenesis induced by 7, 12-dimethyl benz (a) anthracene (DMBA)[2]. There was no credible evidence to support a relation between garlic intake and a reduced risk of gastric, breast, lung or endometrial cancer, very limited evidence supported a relation between garlic consumption and reduced risk of colon, prostrate, esophageal, larynx, oral, ovary or renal cell cancer[3]. Garlic and garlic derived compounds reduce the development of mammary cancer in animals and suppress the growth of human- breast cancer cells in culture. Oil soluble compounds derived from garlic, such as diallyl disulphide (DADS), are more effective than water soluble compounds in suppressing breast cancer. Mechanisms of action include the activation of metabolizing enzymes that detoxify carcinogens, the suppression of DNA adduct formation, the inhibition of the production of reactive oxygen species, the regulation of cell-cycle arrest and the induction of apoptosis[4].

The present work was undertaken to study the ant carcinogenic and anti-tumorigenic effects of garlic extracts in experimentally induced hepatoma in mice, as well as to establish a possible anticancer mechanism of garlic.

MATERIALS AND METHODS

This work was conducted at Department of Biochemistry, Animal house of Dr. B.R. Ambedkar Medical College upon approval of the committee of ethics in animal experimentation (132/1999/CPCSEA)

Experimental animals:

Inbred albino mice of 8 weeks age, weighing 15-20 g were used for the study. The mice were obtained from the stock inbred colony which was maintained by mating of brothers and sisters. The mice were maintained on dry pellets of laboratory feed (Amrut rat feed, Navamaharashtra Chakan Oil Mills Ltd., Pune) and tap water.

Tumour cell lines & their maintenance:

Ehrlich sacites tumor cell line Dalton's lyruphoma Ascites, (DLA) kindly provided by Amla Cancer Research Institute, Trissur was used for the study. The tumor cell line was maintained by serial intra peritoneal

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(IP) transplantation in mice. Full grown tumor cells were aspirated from mice peritoneum, washed thrice with PBS (pH 7.4) and suspended in PBS injected intra peritoneally into a new healthy mouse. All operations were done aseptically.

Preparation of garlic aqueous extracts (GAE):

One part of fresh garlic bulbs were crushed with one part of water (w/w) in waring blender. It was filtered through a gauge cloth. One ml of this filtrate was considered equivalent to an aqueous extract of 500 mg garlic. This was prepared fresh each time.

An optimum dose of 3g/kg body weight was employed in this study. This 3g optimum dosage was arrived at as a result of previous experiments conducted in our lab.

Experimental groups:

For the present study mice were divided into 4 groups, consisting of 6 mice in each group.

Group 1: Normal group- consisting of normal mice maintained on laboratory diet adlibtum.

Group 2: Control group- consisting 6 experimentally induced hepatoma mice.

About 3 x 10^6 Dalton's lyruphoma Ascites tumor cells in PBS (pH 7.4) were injected into the peritoneal cavity. Later the mice were maintained on lab feed adlibtum. A well grown tumor was observed within 10 days. On the 11^{th} day the mice were sacrificed.

Group 3: GAE protective group- consisting 6 mice given aqueous extracts of 3 gm garlic/kg body weight for 10 days prior to tumor implantation. On the 10^{th} day 3 x 10^6 Dalton's lyruphoma Ascites tumor cells in PBS (pH 7.4) were injected into the peritoneal cavity. Later aqueous extracts of 3 gm garlic/kg body weight was given orally to these mice for further 10 days. Later mice were maintained on lab feed adlibtum. On the 21^{st} day mice were sacrificed.

Groups 4: GAE curative group- consisting 6 experimentally induced hepatoma mice. About 3 x 10^6 Dalton's lyruphoma Ascites tumor cells in PBS (pH 7.4) were injected into the peritoneal cavity. These mice were maintained on lab feed adlibtum. After 5 days aqueous extracts of 3 g garlic/kg body weight was given orally to these mice daily upto 10^{th} day. On the 11^{th} day the mice were sacrificed.

Blood from mice was collected with heparin as anticoagulant after the stipulated time by cutting the jugular vein with a sharp blade. Blood samples were centrifuged at 1500 rpm for 10 minutes.

The plasma samples were used for the estimations of glucose[5], uric acid[6], total proteins(TP)[7], albumin[8], total lipids(TL)[9], total cholesterol(TC)[10], triacyl-glycerols (TAG)[11], phospholipids (PL)[12], aspartate transaminase (AST)[13], alanine transaminase (ALT)[14], free fatty acids (FFA)[15], total aminoacid nitrogen (total AAN)[16] and vitamin C (Vit C)[17].

The liver was removed to ice cold containers and processed immediately.

A part of the liver tissue was homogenized with chloroform-methanol (1:1v/v) and the extracts were used for the estimations of lipid parameters TL[9], TC[10], TAG[11] and PL[12].

Another part of the liver tissue was homogenized with phosphate buffer (pH 7.4) and the extracts were used for the estimations of AST[13], ALT[14] and total –SH groups[18].

A third part of the liver tissue was homogenized with 5% TCA and the extracts were used for the estimation of thiobarbituric acid reactive substance (TBARS)[19].

A fourth part of the liver tissue was homogenized with 10% TCA. The extracts were used for the estimations of total DNA[20] and DNA damage[19]. The DNA damage was estimated by estimating TBARS levels of isolated DNA.

A fifth part of the liver tissue was immediately put into buffered formalin for histopathological studies.

Ascetic fluid samples were collected and used for the estimations of TL[9], TP[7], albumin[8], AST[13], ALT[14] as well as for cytological studies.

RESULTS

Result obtained in the present study are given in tables 1 to 3 & Figs. 1-4

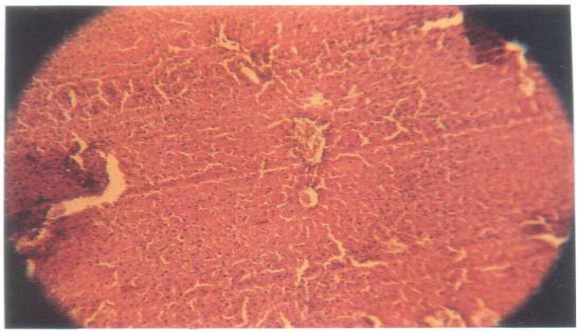

Fig 1 : Normal Group shows normal liver tissue with central vein in mice. (H & E,x 320)

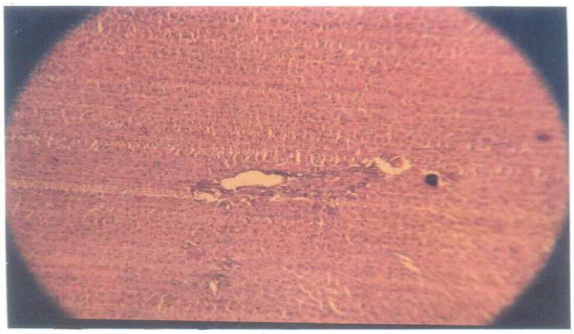

Fig 2 : Control Group (Induced hepatoma) Section from the liver tissue shows hyperplastic hepatocytes and cords of normal hepatocytes in mice. (H & E,x 320)

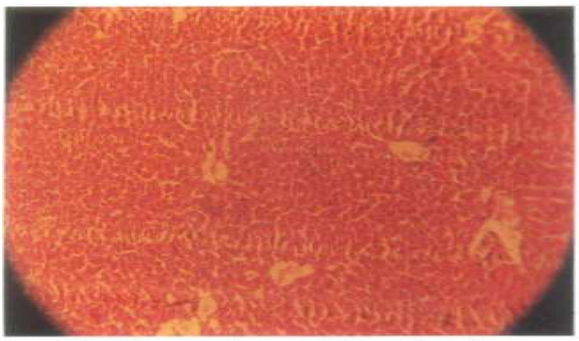

Fig 3 : GAE Protective group section from liver tissue shows normal cords of hepatocytes in lobular pattern in mice. (H & E,x 320)

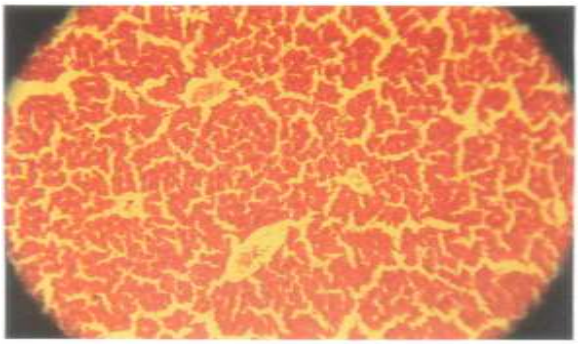

Fig 4 : GAE Curative group section shows cords of liver cells showing mild hyperplasia mice. (H & E, x 320)

As seen from the table ascitic fluid levels of TL, TP, globulins, AST & ALT are significantly raised in protective (group 3) and curative (group 4) as compared to control (group 2). A significant decrease in total cell count in ascetic fluid is seen in both these groups as compared to control group. The reduction in cell count is more pronounced in curative group (group 4).

Figures 1 to 4 shows the cross section of liver in normal mice, garlic aqueous extract treated cancer cells injected mice and experimentally hypatoma induced mice given GAE respectively.

Figures 1 and 2 show normal liver tissue and malignant liver tissue respectively. As seen from the figures, a significant protection against experimental induced hepatoma is seen in mice treated with GAE prior to cancer induction (Fig.3), whereas a significant Control of cancer is seen in hepatoma mice given GAE orally (Fig 4).

DISCUSSION/CONCLUSION

Feeding GAE (3 gm/kg body weight) daily for ten days prior to induction of hepatoma gives a fair protection as seen from Fig.3 This hepatoma protective effects as garlic extracts may be due to its sulfur compounds-thiols and disulfides, these sulfur compounds may undergo an exchange reaction[21-22] with thiol enzymes as follows.

$$R_1\text{- S –S – } R_1 + R_2 \text{ – SH ---} R_1 \text{ – S –S - } R_2 + R_1\text{-SH}$$

Such an exchange reaction with fatty acid synthase, HMGCoA reductase, glycerol-3-phosphate dehydrogenate and probably with nucleic acid ligase decreases fatty acid synthesis, cholesterol, synthesis, triacyl glycerol synthesis and nucleic acid production respectively, thereby causing a decrease in these levels which is evident from tables 1 to 3.

The principal disulfide present in garlic is diallyl disulfide (DADA)[23]. This may be metabolized as any disulfide in the body to give rise to allyl thiols which may be involved in maintenance of glutathione levels which is evident from tables 1 to 3. Such a metabolic degradation of DADS consumes NADPH resulting in a decrease of cellular NADPH. This may result in decreased fatty acid, cholesterol and triacyl glycerol synthesis. The decrease in these in part may be due to direct inhibition of synthesis by allicin, alliin and by DADS of garlic extracts[24].

Table.1 : Gives the plasma levels of glucose, uric acid, TP, albumin, globulins TL, TC, TAG, PL, FFA, AAN, Vit C, AST & ALT in normal (group 1), control (group 2), protective (group 3) & curative (group 4) mice, As per the table, glucose, albumin, TL, TC & Vit C are lowered in control group as compared to normal group, whereas all these parameters are raised in both protective as well as curative groups as compared to control group.

Analyte	Group 1 (Normal)	Group 2 (Control)	Group 3 (Protective)	Group 4 (Curative)
Plasma				
1. Glucose (mg/dl)	61.5±3.2	32.49±2.1***	55. ±4.3***	58.4±6.93***
2. Uric acid (mg/dl)	9.13±3.8	9.76±3.3	10.31±3.5	9.6±0.21
3. Total Proteins (g/dl)	5.75±2.2	7.09±3.1	3.93±2.42***	10.2±0.32***
4. Albumin (g/dl)	2.0±0.1	0.82±0.18***	1.51±0.8***	2.77±2.15**
5. Globulins (g/dl)	3.75±0.8	6.26±3.92***	2.42±0.72***	7.5±3.12
6. Total Lipids (TL) (mgldl)	134.13±83.6	70.18±59.12**	90.62±15.61	107.68±40.94***
7. Total Cholesterol (TC)(mg/dl)	166.66±36.6	123.4±78.1*	252.0±114.49***	397.33±82.1***
8. Triacylglycerols (TAG) (mg/dl)	24.88±7.5	51.01±17.67***	43.41±6.21	43.9±10.98
9. Phospholipids (PL) (mg/dl)	3.47±0.2	6.81±1.2***	24.24±3.21***	38.31±4.12***
10. Free Fatty acids (FFA) (mg/dl)	1.2±0.2	2.75±0.52***	2.02±0.31***	2.11±03***
11. Total Amino acid Nitrogen (AAN) (mg/dl)	2.21±0.3	2.25±0.61	2.13±0.41	2.86±0.51***
12. Vit C (mg/dl)	1.2±0.1	0.3±0.01***	0.65±0.2***	0.5±0.1***
13. AST (units/ml)	25.9±1.7	31.78±2.3***	37.1±6.5***	65.2±7.07***
14. ALT (units/ml)	8.95±3.8	9.98±3.9	10.6±4.1	25.43±9.19***

Results are expressed as mean ± S.D. No. of animal in each group is 6.
Group 2 compared with Group 1, group 3 & 4 compared with Group 2.
*P<0.02; **P<0.01; *** P<0.001

The levels of TAG, PT, AST, ALT & FFA are elevated in control group as compared to normal group, whereas the levels of PL, AST & ALT are raised in protective & curative groups as compared to control group, but TAG & FFA are decreased in these groups as compared to control group.

Table.2 : Gives the tissue levels of AST, ALT, TC, TAG, PL, TBARS, Total-SH groups, DNA damage and total DNA, in normal (group 1), control (group 2), protective (group 3) and curative (group 4) mice. As per the table, TC and total –SH groups are lowered in control group as compared to normal group.

Analyte	Group 1 (Normal)	Group 2 (Control)	Group 3 (Protective)	Group 4 (Curative)
Tissue				
1. AST (units/g)	515.2±51.3	526.1±48.3***	556.8±61.2	640.7±32.5***
2. ALT (units/g)	413.7±18.6	415.9±13.4***	501.1±11.3***	829.7±86.9***
3. Total Lipids (TL) (mg/g)	103.6±46.26	155.5±25.72***	125.5±17.13***	133.33±12.3**
4. Total Cholesterol (TC)(mg/g)	8.88±3.2	6.16±2.7***	9.44±0.73***	13.89±0.88***
5. Triacyl Glycerol's (TAG) (mg/g)	84.8±14.15	181.4±32.44***	103.8±18.63***	75.4±18.6***
6. Phospholipids (PL) (mg/g)	11.78±1.2	18.77±2.3***	78.31±5.6***	81.12±4.8***
7. TBARS (m mol/g)	10.02±1.8	14.46±2.2***	4.4±0.53***	4.76±2.08***
8. Total SH groups (m mol/g)	23.42±3.6	11.96±2.8***	13.3±2.43	12.43±2.27***
9. DNA damage (TBARS equi)	22.56±4.2	34.86±6.2***	5.6±1.44***	14.18±6.5***
10. DNA (mg/g)	5.51±1.7	6.89±2.1*	5.11±1.1***	5.45±1.3**

Results are expressed as mean ± S.D. Number of animals in each group is 6.
Group 2 compared with Group 1.
Group 3 & 4 compared with Group 2.
* P < 0.02; ** P<0.01; *** P<0.001

The levels of AST, ALT, TL, PL, TBARS, DNA damage and total DNA are raised in control group as compared to normal group, whereas these parameters except AST, ALT, PL total – SH groups are decreased in both protective as well as curative groups as compared to control group.

Table.3: Gives the value of TL, TP, albumin, glboulins, AST, ALT as well as cell count in ascetic fluid of control (group 2), protective (group 3) and curative (group 4) mice.

Analyte	Group 2 (Control)	Group 3 (Protective)	Group 4 (Curative)
Ascitic Fluid			
1. Total Lipids (TL) (mg/dl)	41.37±8.69	52.4±9.8***	90.86±31.96***
2. Total Proteins (TP) (g/dl)	4.67±0.8	6.831.3***	6.49±1.2***
3. Albumin (g/dl)	1.94±0.88	0.98±0.2***	2.44±0.3**
4. Globulins (g/dl)	2.73±1.1	5.85±2.3***	4.05±1.8**
5. AST (units/ml)	16.34.2	64.41±18.3***	49.2±16.82***
6. ALT (units/ml)	11.37±2.6	12.28±3.8	38.14±4.88***
7. Cell Count	4200 Cells/CM	2200 Cells/CM	800 Cells/CM

Results are expressed as mean ± S.D Number of animals in each group is 6.
Group 3 & 4 compared with Group 2. (*P<0.02; **P<0.01; ***P<0.001)

Further investigations using specific disulfide like diallyl disulfide, may throw more light on these garlic effects.

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