Anticarcinogenic Effects of Garlic Aqueous Extracts in Nitroso R Salt (1-Nitroso-2-Naphthol-3,6-Disulfonic Acid Disodium Salt Hydrate) Induced Hepatoma in Rats

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Research Article

Abstract: Garlic has long been used as a popular remedy for many ailments since a long time .The present work was undertaken to study the anticarcinogenic and antitumorigenic effects of garlic aqueous extracts in experimentally induced hepatoma in rats as well as establish a possible anticancer mechanism of garlic. The protective effects of garlic aqueous extracts against chemical induction of hepatoma as well as the curative effects of these extracts in chemically induced hepatoma rats were studied. It is evident from the results that garlic aqueous extracts gives protection against chemical hepatoma induction. There is a considerable improvement seen in chemical induced hepatoma rats given garlic aqueous extracts. These protective as well as curative effects of garlic aqueous extracts may be due to its principal sulfur compound diallyl disulfide.The principal organosulphur compound of garlic is known to possess many clinical beneficial effects, but its overuse or abuse has been reported to cause certain harmful side effects due to its possible metabolite acrolein. It was thought that the disulphide nature of dially disulphide is responsible for its anticarcinogenic and antitumorigenic effects.

Keywords: Garlic, Hepatoma, Nitroso R salt, diallyl disulfide, lipid profile.

Introduction

Garlic is commonly used as a spicy food item in all parts of the world. It has long been as a popular 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.One of the mechanisms of garlic's cancer prevention effect may be by preventing the formation of nitroso compounds as garlic is known to prevent formation of N-nitroso proline in humans. By this mechanism the sulfhydryl compounds of garlic block the conversion of nitrites into nitrosamines, which are a group of the most potent carcinogens in my opinion diallyl disulfides can also block nitrosamine formation through a similar mechanism. 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 free radicals, increasing glutathione levels, increasing the activities of enzymes such as glutathione s-transferease, catalase, inhibition of cytochromep4502E1, DNA repair mechanisms, preventing of chromosomal damage[1]. The 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 chemopreventive 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].

Materials and methods

The present work was undertaken to study the anticarcinogenic and antitumorigenic effects of garlic

extracts in experimentally induced hepatoma in rats, as well as to establish a possible anticancer mechanism of garlic. 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 adult male albino rats of 3-4 months old, weighing 100-150 g were used for the study. The rats were obtained from the stock inbred colony which was maintained by mating of brothers and sisters. The rats were maintained on dry pellets of laboratory feed (Amrut rat feed, Navamaharashtra Chakan Oil Mills Ltd., Pune) and tap water.

Preparation of garlic aqueous extracts (GAE)

One part of fresh garlic bulbs were crushed with one part of water (w/v) in warring 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 3 g/kg body weight was employed in this study. This 3 g optimum dosage was arrived at as a result of previous experiments conducted in our lab.

Experimental groups

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

Group 1: Normal group- consisting of 6 normal rats maintained on laboratory diet adlibtum.

Group 2: Control group

Hepatoma in rats was induced by Nitroso-R-Salt instead of diethyl nitrosamine (DEN) [5]. Consisting of 6 male albino rats, maintained on lab diet *adiibtum* and given an initial dose of CCl_4 (3mg/kg body weight) was given. On the 7th day a high dose of Nitroso-R-Salt (300 mg/kg body weight) was given. On the l4th day a second dose of CCl_4 (3 mg/kg body weight) was given. Then the rats were maintained on a diet containing phenobarbital (0.05%) for further 16 weeks.

Group 3: AEG Protective group

Consisting 6 albino rats maintained on lab diet *adlibtum* and given 3g aqueous extract of garlic (AEG) as 30 ml warm aqueous solution/kg body weight using gastric tube daily for 3 days. On the 4th day an initial dose of CCl_4 (3 mg/kg body weight) was given. On the 7th day a high dose of Nitroso-R-Salt (300 mg/kg body weight) was given. On the 14th day a second dose of CCl_4 (3mg/kg body weight) was given. Then the rats were maintained on a diet containing phenobarbital (0.05%) for further 16 weeks. All the rats of this group were given daily from 4th day till the end aqueous extract of garlic 3 g/kg body weight.

Group 4: AEG Curative group

Consisting of 6 albino rats, maintained on lab diet *adlibtum* and given an initial dose of CCl_4 (3mg/kg body weight) was given. On the 7th day a high dose of Nitroso-R-salt (300 mg/kg body weight) was given. On the 14th day a second dose of CCl_4 (3 mg/kg body weight) was given. Then the rats were maintained on a diet containing phenobarbital (0.05%) for further 16 weeks. All the rats of this group (group 4) were given daily from 16 weeks till the 32 weeks 3g aqueous extract of garlic (AEG) as 30 ml warm aqueous solution / kg body weight using gastric tube daily for 32 weeks. Blood from rats 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[6], uric acid[7], total proteins (TP)[8] , albumin[9], total lipids(TL)[10], total cholesterol(TC)[11], triacyl-glycerols (TAG)[12], phospholipids (PL)[13], aspartate transaminase (AST)[14], alanine transaminase (ALT)[15], free fatty acids (FFA)[16], total aminoacid nitrogen (total AAN)[17] and vitamin C (Vit C)[18]. The liver was removed to ice cold containers and processed immediately. A part of the liver tissue was homogenized with chloroform- methanol (1:1 v/v) and the extracts were used for the estimations of lipid parameters TL[10], TC[11], TAG[12]and PL[13] Another part of the liver tissue was homogenized with phosphate buffer (pH 7.4) and the extracts were used for the estimations of AST[14], ALT[15] and total –SH groups[19]. 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)[20]. A fourth part of the liver tissue was homogenized with 10% TCA. The extracts were used for the estimations of total DNA[21]and DNA damage[20]. 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.

Statistical Analysis

Data obtained were analyzed comparing the results of groups using students ‘t’ test Probability values less than 0.02 were considered as significant.

Results

Results obtained in the present study are given in tables 1 to 2 & Figs. 1-4. Figures 1 to 4 shows the cross section of liver in normal rats, garlic aqueous extract treated chemically induced hepatoma rats and experimentally hepatoma induced rats given GAE respectively. Figures 1 & 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 rats treated with GAE prior to cancer induction (Fig 3), whereas a significant.

Control of cancer is seen in hepatoma rats given GAE orally (Fig 4).

Table 1 gives the levels of TAG , PL, 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 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.

Discussion and Conclusion

Feeding GAE (3 gm/kg body weight) daily for seven 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[22-23]with thiol enzymes as follows.

R_1- S –S – R_1 + R_2 – S------→R_1 – S –S - R_2 + R_1-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 2.

The principal disulfide present in garlic is diallyl disulfide (DADS)[24]. 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 2. 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[25].

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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) rats, As per the table, glucose, albumin, TP,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)	75.5±2.3	45.63±4.2***	64.2±4.2***	69.43±5.23***
2.Urica cid (mg/dl)	5.19±3.57***	8.33±3.31***	6.71±5.54*	3.33±1.75**
3.TotalProteins (g/dl)	7.26±3.12	4.49±2.12***	4.52±0.66**	8.27±0.28*
4. Albumin (g/dl)	5.63±0.26	2.72±0.38***	1.64±0.26*	3.68±1.08***
5. Globulins(g/dl)	1.63±0.38	1.77±0.38***	2.88±0.48**	4.59±0.97*
6.Total Lipids (TL) (mg/dl)	144.41±7.58	98.18±3.12**	135.55±7.18***	116.66±5.04***
7.Total Cholesterol (TC) (mg/dl)	107.33±10.6	72±5.91***	104.00±2.9***	110.2±15.2***
8.Triacylgycerols (TAG) (mg/dl)	95.60±3.5	184.85±9.87**	128.16±4.62***	148.41±5.43***
9.Phospholipids (PL) (mg/dl)	13.09±0.75	17.68±1.89***	28.68±2.95***	16.06±3.25***
10. Free Fatty acids (FFA) (mg/dl)	1.75±0.2	2.91±0.21***	1.56±0.31***	1.91±0.2***
11.Total Amino acid Nitrogen(AAN) (mg/dl)	6.5±0.35	3.17±0.04***	5.65±0.21**	7.17±1.71***
12. Vit C (mg/dl)	0.55±0.1	0.3±0.01***	0.65±0.2***	0.5±0.1***
13. AST (units/ml)	20.69±8.3	31.78±2.3***	28.16±4.2***	65.2±7.07***
14. ALT (units/ml)	18.17±1.6	9.98±3.9	22.6±3.13***	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

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) rats. 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)	484.4±279.4	762±12.13***	1461.3±75.5	752.8±21.5***
2. ALT (units/g)	419.2±48.8	551.3±6.4***	981.3±51.1***	813.7±32.9***
3.TotalLipids(TL) (mg/g)	99.99±5.7	171.66±8.57***	57.77±7.18***	100.0±5.0**
4.TotalCholesterol (TC)(mg/g)	2.4±0.86	4.08±0.36***	3.24±0.41*	3.53±1.66*
5.TraiacylGlycerol's(TAG) (mg/g)	29.26±10.17	106.33±9.38***	28.28±7.6***	63.07±3.34***
6.Phospholipids (PL) (mg/g)	12.2±1.2	19.69±1.64***	69.8±3.98***	8.03±0.52***
7.TBARS (m mol/g)	12.04±1.4	16.64±3.1***	6.62±0.76***	6.93±2.08***
8. Total SH groups (m mol/g)	27.24±2.3	12.96±1.9***	15.3±2.54***	13.24±2.2***
9.DNA damage (TBARS miliequi/g)	6.27±0.3	10.9±2.2***	7.79±2.3***	8.64±0.2**
10. DNA (mg/g)	6.5±1.2	8.3±1.45***	4.5±0.3***	3.0±0.5**

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

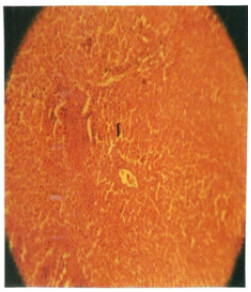

Figure 1: Section studied shows central vein, portal triad and cords of hepatocytes – normal liver tissue in rats. (H & E, x320)

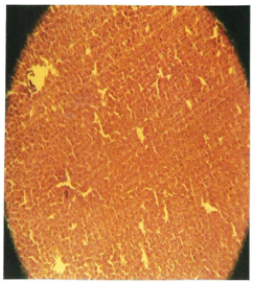

Figure 2: The liver tissue shows over crowding and mallignant hepatocytes. Features of hepatocellular carcinoma of liver in rats. (H & E x320)

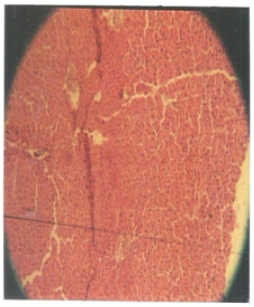

Figure 3: The Liver tissue showing normal and hyperplastic hepatocytes in rats. (H & E, x 320)

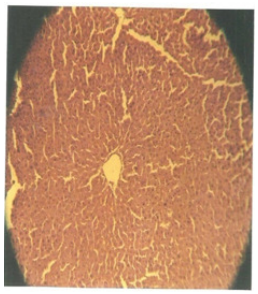

Figure 4: Section from the liver tissue shows cords of liver cells in normal lobular pattern in rats. (H & E, x 320)