

Protective Effects of Diallyl Disulfide Against Experimentally Induced Hepatoma in Mice

Dr. Divya.D¹, Vickram² and Kashinath.R.T³

1

Received: 4 March 2012 Accepted: 2 April 2012 Published: 13 April 2012

Abstract

Many herbal extracts have been reported to modify significantly, the transformation of normal cells into neoplastic cells. Garlic and its extracts are known for their hypolipidemic, hypoglycemic, antiplatelet aggregating effect as well as for its anticancer effects. Many of these health beneficial effects of garlic are attributed to its principle organosulfur compound diallyl disulfide(DADS). It was thought that DADS may be involved in anticarcinogenic antitumorogenic effect of garlic, hence the present work was undertaken to assess the protective effects of DADS in ehrlich ascites carcinoma (EAC) cells induced hepatoma in mice. The study has three groupsnormal group (group1), the EAC cells implanted mice (group 2) DADS-treated EAC cells implanted mice (group 3). The results indicate a significant decrease in ascitic fluid volume, ascitic fluid cell count, liver tissue amino acid nitrogen levels, liver tissue glutaminase activity liver tissue lactate levels as well as a increase in life span observed in group 3 mice as compared to group 2 mice, suggesting that DADS gives a significant protection in group3 mice probably by decreasing the anaerobic glucose utilization as well as by interfering with protein deoxy ribonucleotide synthesis.

Index terms— Herbal extracts, garlic, diallyl disulfide, anti- -tumorogenic effects ., EAC cells, liver, hepatoma

Protective Effects of Diallyl Disulfide Against Experimentally Induced Hepatoma in Mice Divya.D¹, Vickram² & Kashinath.R.T³ Abstract -Many herbal extracts have been reported to modify significantly, the transformation of normal cells into neoplastic cells. Garlic and its extracts are known for their hypolipidemic, hypoglycemic, antiplatelet aggregating effect as well as for its anticancer effects. Many of these health beneficial effects of garlic are attributed to its principle organosulfur compound diallyl disulfide(DADS). It was thought that DADS may be involved in anticarcinogenic & antitumorogenic effect of garlic, hence the present work was undertaken to assess the protective effects of DADS in ehrlich ascites carcinoma (EAC) cells induced hepatoma in mice. The study has three groupsnormal group (group1), the EAC cells implanted mice (group 2) & DADS-treated EAC cells implanted mice (group 3). The results indicate a significant decrease in ascitic fluid volume, ascitic fluid cell count, liver tissue amino acid nitrogen levels, liver tissue glutaminase activity & liver tissue lactate levels as well as a increase in life span observed in group 3 mice as compared to group 2 mice, suggesting that DADS gives a significant protection in group3 mice probably by decreasing the anaerobic glucose utilization as well as by interfering with protein & deoxy ribonucleotide synthesis. compounds derived from garlic (1,18). The chemoprotective activity has been attributed to the presence of organosulphur compounds in garlic (6,25,31). The principle organosulphur compound present in garlic is diallyl disulphide [DADS] (3,22). Hence it was thought DADS may be responsible for garlic's cancer protective activity. The present work was undertaken to assess the chemoprotective effects of DADS in Ehrlich ascites cells induced hepatoma in mice.

1 II.

2 Materials and Methods

3 a) Tumor cell line & their Maintenance :

The inoculum of EAC cells was kindly provided by Amala Cancer Research Institute, Thrissur Kerala (India). EAC cells were thereafter propagated by weekly intraperitoneal injection of freshly drawn ascitic fluid (0.5 ml) from a donor mice bearing ascites tumor of 8-10 days old into healthy swiss albino male mice. Transplantation was carried out using sterile disposable syringes under aseptic conditions

b) Chemicals :
All the chemicals employed in the present study were of Analar grade (A.R). Diallyl disulphide (DADS) was procured from Sigma-Aldrich chemicals Pvt. Ltd. USA.

4 c) Animals :

In the present study, 18 Swiss male albino mice weighing 25-30g were randomly selected from animal house of Basaveshwara Medical College & Hospital, Chitradurga. The experiments were conducted according to the norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi and Ethical clearance was obtained from IAEC (Institutional Animal Ethical Committee) of Basaveshwara Medical College.

5 d) Experimental design :

The mice were divided into 3 groups (6 animals per group)-normal group (group 1), control group [EAC cells implanted mice] (group 2) and protective group [DADS-treated EAC cells implanted mice] (group 3).

6 i. Normal group

This group consists of 6 swiss albino male mice that received 5.0 ml of normal saline /kg body weight orally by gastric intubation daily for a period of 10 days.

7 Introduction

The transformation of normal cells into neoplastic cells involves at least three distinctive phases, namely-initiation, promotion and progression. Many dietary components have been reported to significantly modify each of these phases (30). Garlic (*Allium sativum*) a common dietary component, is known to modify the cancer process. Epidemiologic and clinical studies have shown that consumption of garlic reduced the risks of cancer incidence (5,17,29). A number of studies have demonstrated the chemoprotective activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic, garlic oil and a couple of organosulfur compounds. Control group This group consists of 6 swiss albino male mice with experimentally induced hepatoma. About 3x 10⁶ EAC cells were injected intraperitoneally into healthy mice. These mice also received 5.0 ml of normal saline / kg body weight orally by gastric intubation daily for a period of 10 days. A well grown tumor was observed within 7-10 days.

iii. Protective group This group consists of 6 swiss albino male mice, received 5.0 ml of warm aqueous solution of DADS (100mg/kg body weight) orally by gastric intubation daily for a period of 4 days. On the 4th day 3x 10⁶ EAC cells were injected intraperitoneally. Later 5.0 ml warm aqueous solution of DADS (100 mg)/kg body weight was given orally further for a period of 6 days.

The mice of all the three groups were maintained on standard lab feed (Amruth Rat Feed, supplied by Pranav Agro Industries, Pune, India) and tap water ad libitum throughout the study. On the 11th day, body weights of mice of all the groups were noted & abdominal circumferences were recorded. Then the mice were anaesthetized & sacrificed. The ascitic fluid was immediately collected in a clean dry graduated tube by puncturing the abdomen. The fluid volume was noted. The ascitic fluid was assayed for total proteins (11) & total cell count was assessed microscopically using Neubauer chamber. The mice were dissected & livers were procured. Blood stains of liver tissues were removed by smooth blotting & were immediately transferred into a clean pre weighed beaker. The weights of liver of individual groups were noted. Later, the liver tissues were refrigerated at 0-2 °C in cold phosphate buffer pH 7.4 till further use. Each individual liver tissue procured was processed to analyze various biochemical parameters as follows: a) To 0. The data entry was carried out using MS Office Excel worksheet and statistically evaluated. The P value was calculated using 'student t' test.

8 III.

9 Results

The results of the present study are given in IV.

10 Discussion

The eukaryotic cell cycle normally consists of series of events involving -growth stimulus, replication & division (3,23,24). It is known that many allyl sulphur compounds of herbal origin reduce the growth rate of neoplastic cells in culture as well as invivo (26), probably by blocking certain events of cell cycle. The results of the present study with 100 mg DADS/ kg bodyweight given to EAC implanted mice (refer table-1) suggests that at this dosage DADS significantly retards the development of ascites. DADS might have interfered with the cell cycle at G2/M phase as it is known that DADS arrest the cell growth at G2/M phase of cell cycle which means of deoxyribonucleotides. This process requires the participation of nucleotide reductase enzyme, which requires thioredoxin, a sulphhydryl compound for its activity. A possible sulphhydryl exchange reaction of DADS with thioredoxin as proposed above may reduce its availability hence decreases the production of deoxy ribonucleotides thus reducing the available DNA levels in cancer cell development which is evident from results depicted in table 1.

Tumor cells do act as nitrogen trappers (??2) which is a required phenomenon for increased protein synthesis essential for rapid cell proliferation as well as cell multiplication. Liver tissue protein levels in group 2 shows a significant raise ($P<0.001$) as compared to group 1 (refer table 2), indicating a rise in protein synthesis, a normal requirement of increased cell multiplication. The amino acids which are essential for increased protein synthesis might have derived from an increased proteolysis of host tissue. The results given in table 2 shows a significant raise ($p<0.001$) in liver tissue amino acid nitrogen levels. This increase may partly be due to increase in glutamic acid formation through an increased activity of enzyme glutaminase (28). A significant decrease in liver tissue amino acid nitrogen levels, seen in the present studies, in group 3 mice as compared to group 2 mice suggests that DADS might have interfered with host tissue proteolysis hence causing a decrease in liver tissue amino acid nitrogen levels. This decrease in liver tissue amino acid nitrogen levels in group 3 mice, in part, may be due to decreased glutaminase activity (refer table 2) resulting in a lowered glutamic acid levels.

It is known that tumor cells prefer anaerobic glycolytic breakdown of glucose as compared to glucose oxidative pathways. The observed increase in liver tissue Lactate content in group 2 mice is clearly suggestive of the above statement whereas a significant decrease ($P<0.001$) in liver tissue lactate levels in group 3 mice as compared to group 2 mice (refer table 2) indicates probably DADS might have interfered with cellular glycolytic pathways. Many enzymes of glycolytic pathway including hexokinase, phospho fructo kinase (PFK) & pyruvate kinase (PK) are thiol enzymes (09). DADS, a disulfide might have undergone sulphhydryl exchange reaction similar to any other disulfide (27) as proposed above with glycolytic thiol enzymes hence reducing their activities which results in decreased anaerobic glycolysis thus a decrease in output. This decrease in lactate level in group 3 mice as compared to group 2 mice may also be due to lowered cellular NADPH or NADH levels in group 3 mice as DADS is known to undergo reductive cleavage to its thiols using cellular NADPH or NADH, thus reducing the available NADPH or NADH causing a decrease in lactate formation.

A reliable criteria for assessing the potential use of any anticancer agent is the prolongation of life span of animals (16). Andreani et al (2) has suggested that an increase in lifespan of ascites bearing animals by 25% is considered as indicative of significant drug activity. An increase in life span by 50% i.e. 25 (20) to evaluate the effect of DADS on life span of hepatoma induced mice. The percent increase in life span was found to be 50% and inhibitory growth rate percent was found to be 45.43% suggesting that DADS has a significant inhibitory effect on tumor development.

In conclusion, DADS by interfering with protein synthesis as well as with the glucose breakdown in cancer cells, results in reduced cancer cell proliferation & multiplication. Thus shows significant protection against EAC induced hepatoma bearing mice.

11 Global Journal of



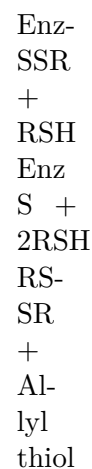
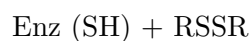
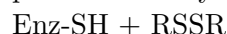
Figure 1: ?

1

glutaminase activity in liver tissue are significantly raised ($p < 0.001$) in group 2 as compared to group 1, whereas the same parameters are significantly lowered ($p < 0.001$) in group 3 as compared to group 2. It is also seen from the table that a significant decrease ($p < 0.001$) in liver glycogen content & total thiol groups observed in group 2 as compared to group 1 & the same parameters are significantly raised ($p < 0.001$) in group 3 as compared to group 2. However there is no significant change seen in transaminases (ALT & AST) activity in all the three groups.

Figure 2: table 1 &

in human colon cancer (18, 4, 19), by decreasing the kinase activity of CDK1/cyclin B complex. Further DADS 6 is a disulphide and like any other disulphides can undergo sulphydryl exchange reactions with cellular proteins & enzymes (33) as follows-



A similar sulphydryl exchange reaction with kinases & other growth factors involved in cell cycle may suppress cell multiplication causing a reduction in cell proliferation. DADS 6 has chemoprotective action against EAC induced hepatoma in mice, which is evident from the results obtained in the present studies (refer table 1 & 2). Cell proliferation as well as cell multiplication requires increased DNA produ-

o

Figure 3:

Figure 4:

1

2
-ction (14)

2
increased synthesis

Figure 5: Table 1 :

Figure 6: ?

2

Group	Glycogen Content (mg/g)	Lactate Content (mg/g)	Total Proteins (mg/g)	Aminoacid nitrogen (µgAAN/g)	Total SH groups (mg SH/g)	Glutaminase units	ALT (IU)	AST (IU)
Group 1	12.29	1.81	144.09	550.0	2.24	18.37	21.1	28.8
n=6	± 2.01	± 0.27	± 12.17	± 32.86	± 0.20	± 1.17	± 0.4	± 1.17
Group 2	1.17***	2.91***	201.28***	680.0***	1.28***	31.12***	19.14	30.05
n=6	± 0.24	± 0.10	± 8.50	± 17.88	± 0.15	± 1.52	± 0.45	± 1.04
Group 3	3.14***	2.20***	164.92***	586.6***	1.78***			
n=6	± 0.43	± 0.10	± 7.84	± 27.32	± 0.17			

Figure 7: Table 2 :

¹© 2012 Global Journals Inc. (US) © 2012 Global Journals Inc. (US)
²© 2012 Global Journals Inc. (US)
³© 2012 Global Journals Inc. (US) © 2012 Global Journals Inc. (US) Protective Effects of Diallyl Disulfide
Against Experimentally Induced Hepatoma in Mice

[Hawk and Chemistry ()] , 's Physiological Hawk , Chemistry . *Blood Analysis* 1965. XIV-Edition. 29 p. .

[Hartwell and Kastan ()] , L H Hartwell , M B Kastan . *Cell cycle control& cancer* .*Science* 1994. 266 p. .

[Rivera et al. ()] 'Amino acid metabolism in tumor-bearing mice'. S Rivera , J Azcon-Bieto , F L Lopez-Soriano . *Biochem J* 1988. 249 p. .

[Gupta et al. ()] 'Antitumor activity & Antioxidant Status of Caesalpinia bonducella Against Ehrlich Ascites Carcinoma in Swiss Albino Mice'. Malaya Gupta , Upal Kanti Mazumder , Etal . *Journal of Pharmacological sciences* 2004. 94 p. .

[Molinari ()] 'Cell cycle checkpoints and their inactivation in human cancer'. M Molinari . *Cell Prolif* 2000. 33 p. .

[David] *Chapter-IX, Carbohydrates. The isolation & assay of glycogen from the liver & skeletal muscle of rats*, T David . McGraw-Hill publishing Company Ltd. p. . (Plummer: An introduction to Practical Biochemistry)

[Varley©1969 ()] *Chapter-XI :Non-protein Nitrogen, Pg 190-191*, Harold Varley©1969 . 1988. New Delhi,India: CBS Publishers. (Practical Clinical Biochemistry)

[Varley© ()] 'ChapterXIII : Enzymes, pg 287-297'. Harold Varley© . *Practical Clinical Biochemistry* 1969. 1988. CBS Publishers. (IV-edition)

[Knowles and Milner ()] 'Depressed p34cdc2 kinase activity and G2/M phase arrest induced by diallyl disulfide in HCT-15 cells'. L M Knowles , J A Milner . *Nutr Cancer* 1998. 30 p. .

[Knowles and Milner ()] 'Diallyl disulfide inhibits p34(cdc2) kinase activity through changes in complex formation and phosphorylation'. L M Knowles , J A Milner . *Carcinogenesis* 2000. 21 p. .

[Raul and Ondarza ()] 'Enzyme Regulation by Biological Disulfides'. N Raul , Ondarza . *Bioscience Reports* 1989. 9 (5) .

[Colowick ()] 'Enzymes in Lipid Metabolism in'. Kaplan Colowick . *Methods in Enzymology* 1957. 1 p. .

[Thomson and Ali ()] 'Garlic [Allium sativum]: a review of its potential use as an anti-cancer agent'. M Thomson , M Ali . *Curr Cancer Drug Targets* 2003. 3 (1) p. .

[Arunkumar et al. ()] 'Garlic compound, diallyl disulfide induces cell cycle arrest in prostate cancer cell line PC-3'. A Arunkumar , M R Vijayababu , N Srinivasan , M M Aruldas , J Arunakaran . *Mol Cell Biochem* 2006. 288 p. .

[Milner ()] 'Garlic: its anticarcinogenic and antitumorigenic properties'. J A Milner . *Nutr. Rev* 1996. 54 p. .

[Nakagawa et al. ()] 'Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines'. H Nakagawa , K Tsuta , K Kiuchi , H Senzaki , K Tanaka , K Hioki . *Carcinogenesis* 2001. 22 p. .

[Hogland ()] 'Hematological complications of cancer chemotherapy'. H Hogland . *Semi. Oncol* 1982. p. .

[Newall et al. ()] *Herbal medicines: a guide for health-care professionals*, C A Newall , L A Anderson , J D Phillipson . 1996. London; New Delhi,India: CBS Publishers. p. .

[Sadhana et al. ()] 'Inhibitory action of garlic oil on the initiation of benzo[a]pyrene-induced skin carcinogenesis in mice'. A S Sadhana , A R Rao , K Kucheria , V Bijani . *Cancer Lett* 1988. 40 p. .

[Hong et al. ()] 'Inhibitory effects of diallyl sulfide on the metabolism and tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A/J mouse lung'. J Y Hong , Z Y Wang , T J Smith , S Zhou , S Shi , J Pan , C S Yang . *Carcinogenesis* 1992. 13 p. .

[Ziegler ()] 'M: Role of Reversible Oxidation-Reduction of Enzyme Thiols-Disulfides in Metabolic Regulations'. D Ziegler . *Ann. Rev. Biochem* 1985. 54 p. .

[Schorah ()] 'Micronutrients, vitaminutes, and cancer risk'. C J Schorah . *Vitam. Horm* 1999. 57 p. .

[Herman et al. (2007)] 'Molecular targets of Cancer Chemoprevention by Garlic -derived organosulfides'. Anna Herman , Anna A Powolny , V Shivender , Singh . *Acta Pharmacol Sin* 2007 Sep. 28 (9) p. .

[Belman ()] 'Onion and garlic oils inhibit tumor promotion'. S Belman . *Carcinogenesis* 1983. 4 p. .

[Andreani et al. ()] 'Potential Antitumor agents. IX synthesis & antitumor activity of 2 analogues of Ketocaine'. A Andreani , G & S , Galatwar . *J-pharm. Sci* 1983. 72 p. .

[Varley©1969 ()] 'Practical Clinical Biochemistry'. Harold Varley©1969 . *Chapter -XII: The Plasma Proteins*, (New Delhi,India) 1988. CBS Publishers. p. . (IV-edition)

[Murray ()] 'Recycling the cell cycle: cyclins revisited'. A W Murray . *Cell* 2004. 116 p. .

[Midner ()] 'Some aspects of nitrogen & energy metabolism in cancerous subjects'. G Midner . *Cancer Res* 1951. 11 p. .

[Froede ()] 'Studies On Heart Phosphofructokinase: Thiol Groups And Their Relationship To Activity'. H C Froede . *J. Biol. Chem* 1968. 243 p. .

- 190 [Tan et al. ()] 'The initiation of G2/M checkpoint by diallyl disulfide requires the activation of p38 MAP kinase
191 in HL-60 cells'. L M Tan , M X Zhang , H M Luo , Y Zeng , J Li , Z Cui . *Zhonghua Xue Ye Xue Za Zhi*
192 2004. 25 p. .
- 193 [Block ()] 'The organosulfur chemistry of the genus Allium-implications for the organic chemistry of sulfur'. E
194 Block . *Angew. Chem. Int. Ed. Engl* 1992. 31 p. .
- 195 [Agarwal ()] 'Therapeutic actions of garlic constituents'. K C Agarwal . *Med.Res. Rev* 1996. 16 p. .
- 196 [Varley©1969 ()] Harold Varley©1969 . *Chapter IX: Blood and urine urea*, (New Delhi,India) 1988. 1988. CBS
197 Publishers. p. . (Practical Clinical Biochemistry)