Effect of diallyl disulfide on liver nucleotide metabolism in experimentally induced hepatoma in mice

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Abstract

Background: The antitumorigenic effects of active ingredient of garlic, diallyl disulfide (DADS), has been extensively studied & found to retard the growth of neoplastic cells than any other allyl sulfur compounds of garlic. Earlier we have reported antitomorogenic properties of DADS, showing tumor regression by interfering with the liver glucose utilization, protein synthesis as well as lipid synthesis in tumor cells.

Aim: To assess the effect of diallyl disulfide on liver nucleotide metabolism in experimentally induced hepatoma in mice.

Materials & Methods: Swiss albino male mice were divided into four groups - normal, control, preventive and curative groups. Hepatoma was induced by intraperitoneal injection of Ehrlich ascites carcinoma (EAC) cells. DADS (100 mg/kg body weight/mouse/day) was orally fed to protective and curative group mice for a stipulated time period. Mice of all the groups were sacrificed, and liver tissue adenosine deaminase (ADA) activity and uric acid (UA) levels were measured.

Results: The present study shows a significant decrease in ADA activity and UA levels in protective ($p > 0.001$) and curative groups ($p > 0.01$) as compared to control group.

Conclusion: DADS has inhibitory effects on nucleotide metabolism by inhibiting the activities of ADA and xanthine oxidase enzymes, and by reducing the production of deoxy ribonucleotides, probably by involving in thiol-disulfide exchange reactions.

Key words: Diallyl disulfide, nucleotide metabolism, anticancer, hepatoma, Adenosine deaminase

Introduction

Nucleotide metabolism plays a major role in DNA replication, RNA synthesis and in cellular bioenergetics. Cancer cells are highly dependent on De Novo biosynthesis of nucleotides[1]. The biological effects of garlic, more specifically the chief organosulfur compound of garlic, daily disulfide (DADS), are well studied[2]. The antitumorogenic effects of DADS has been studied and found to be far more effective in retarding the growth of neoplastic cells than any other allyl sulfur compounds of garlic[3]. Our earlier studies with DADS[4,5] has reported its antitumorogenic properties showing its interference with the liver glucose utilization, protein synthesis as well as lipid synthesis in tumor cells. The present study was conducted to analyze its effects on liver nucleotide metabolism in Ehrlich ascites carcinoma (EAC) induced hepatoma in mice.

Materials and methods:

Experimental animals and Ethics:

Swiss albino male mice weighing 25-30 g were randomly selected from the Institutional animal house and employed in the present study. The mice were kept in plastic well aerated cages at normal room temperature (27 ± 5°C) and at normal 12-hour light/

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dark cycle. The feed (Amruth Rat Feed, manufactured and supplied by Pranav Agro Industries, Pune, India) and the tap water were provided ad libitum throughout the study. DADS was procured from Sigma-Aldrich Pvt. Ltd. USA. All other chemicals used in the present study were of A.R. grade. Ethical clearance was obtained from Institutional Animal Ethical Committee. The experiments were conducted as per the guidelines of CPCSEA, New Delhi.

Invivo- cell line maintenance and induction of hepatoma:

The EAC tumor-bearing swiss albino mice were kindly provided by the Amala Cancer Research Institute, Thrissur, Kerala. EAC cells were thereafter propagated by weekly intraperitoneal injection of freshly drawn ascitic fluid (0.5 ml) from a donor mouse bearing ascites tumor of 8-10 days old into healthy swiss albino male mice at our Institutional animal house. Transplantation was carried out using sterile disposable syringes under aseptic conditions**.** Liver histology showed areas of fibrotic and necrotic changes with hyperchromatism in EAC bearing mice.

Animal study groups and experimental design:

The animal study groups and the experimental design is same as explained in our earlier study[5], and as follows: The mice were divided into 4 groups - normal group (Group-1), control group [EAC induced hepatoma bearing mice] (Group-2), protective group [DADS treated-EAC induced hepatoma bearing mice] (Group-3) and curative group [EAC induced hepatoma bearing- DADS treated mice] (Group-4).

Group-1: Normal group - consists of 6 healthy Swiss albino male mice fed with 5.0 ml of normal saline/ kg body weight/day orally, using stainless steel round ball tipped mice feeding needles, for 10 days.

Group-2: Control group - consists of 6 healthy Swiss albino male mice to which aliquots of 3 x 10^6 EAC cells/mouse were injected intraperitoneally for inducing hepatoma. These mice were fed 5.0 ml of normal saline/kg body weight/day for 10 days. In a week time, a fully-grown ascites tumor was observed in all mice.

Group-3: Protective group - consists of 6 healthy Swiss albino male mice fed 5.0 ml of warm aqueous solution of DADS (100 mg)/kg body weight/day for 4 days. On the 5th day, aliquots of 3 x 10^6 EAC cells/ mouse were injected intraperitoneally for inducing hepatoma. Further, the same dosage of DADS was fed for 6 more days.

Group-4: Curative group - consists of 6 healthy Swiss albino male mice to which aliquots of 3 x 10^6 EAC cells/mouse were injected intraperitoneally for inducing hepatoma. These mice were fed 5.0 ml of normal saline/kg body weight/day for 4 days. From the 5th day, DADS with the same dosage as fed for group-III mice were fed for further 6 days.

On the 11th day, the mice from all the groups were sacrificed and liver tissues were procured. Any blood stains were removed with the help of blotting paper and weights of liver tissues of individual group mice were recorded. Further, each individual liver tissues were processed as follows:

Procedure:

a. To 0.3 g liver tissue, 4.7 ml of cold phosphate buffer, pH 7.4, was added and thoroughly homogenized for 5 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was employed for the estimation of ADA[6].
b. To 0.2 g of liver tissue slice, 1.0 ml of 2/3 N H_2SO_4 + 1.0 ml of 10% sodium tungstate + 7.8 ml of distilled water were added, mixed well and allowed to stand for 10 minutes at room temperature. Later the contents were thoroughly homogenized and centrifuged at 3000 rpm for 5 minutes. The clear supernatant was employed for the estimation of UA[7].

Statistical Evaluation:

Statistical data analysis was done using SPSS software version 24 (IBM, Armonk, NY, USA) and Student 't' test was employed for statistical analysis. The 'p' value < 0.05 was considered significant.

Results:

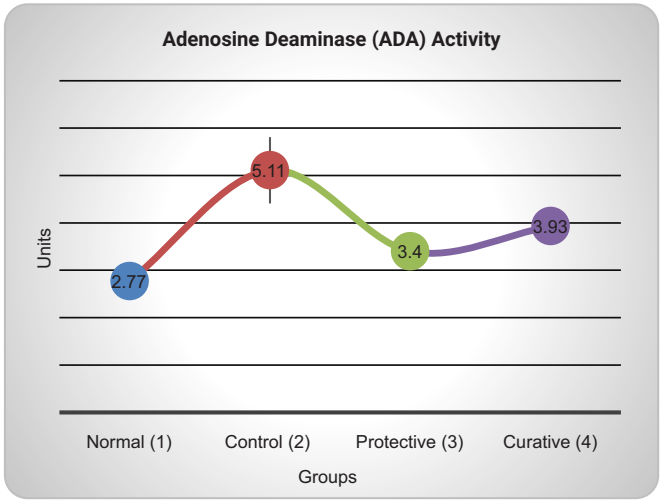
The results of the present study are depicted in table-1, and graphs 1 and 2. It is evident from the table and graph that the ADA activity and UA levels are significantly raised ($p<0.001$) in group-2 mice as compared to group-1 mice, whereas the same parameters are significantly lowered in group-3 ($p<0.001$) and group-4 ($p<0.01$) as compared to group-2 mice.

Table-1 showing liver tissue adenosine deaminase (ADA) activity and uric acid levels in group-1, group-2, group-3 and group-4 mice.

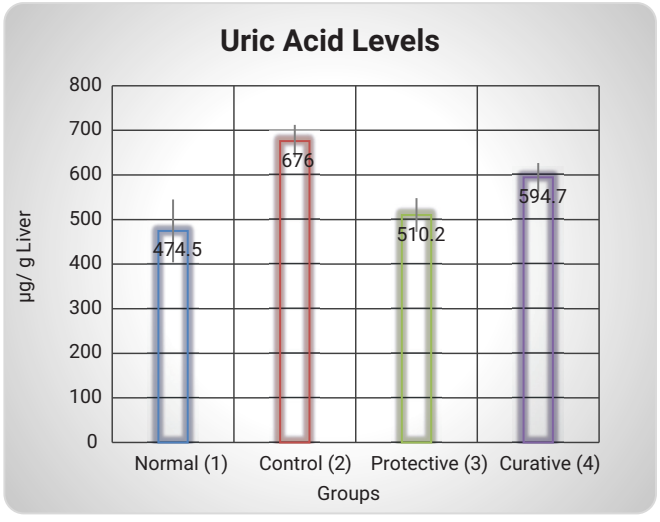
Parameters → Groups ↓	Adenosine deaminase (ADA) (units)	Uric acid (µg/g)
Group-1 Normal group (6)	2.77 ± 0.09	474.50 ± 70.48
Group-2 Control group (6)	5.11*** ± 0.70	676.00*** ± 36.30
Group-3 Protective group (6)	3.40*** ± 0.10	510.20*** ± 37.84
Group-4 Curative group (6)	3.93** ± 0.13	594.70** ± 32.04

Note

- The number in parentheses indicates the number of mice.
- The values are expressed as their mean ± SD.
- Statistical evaluation: probability level - * p<0.05, ** p< 0.01, *** p<0.001.
- ADA: 1 unit = mg NH_3/g liver/hr



Graph-1 showing the liver tissue ADA activity in group-1, group-2,



Graph-2 showing the liver tissue uric acid levels in group-1, group-2, group-3 and group-4 mice.

Discussion:

Cell proliferation as well as cell multiplication requires increased DNA production which means increased synthesis of deoxy ribonucleotides[1]. It is known that tumor cells divert glycolytic carbon towards ribose -5- phosphate, a precursor for nucleotide biosynthesis[8-11]. Several studies have confirmed that the tumor cell pyruvate kinase-M2 isoform (key enzyme of glycolysis) expression predicts a substantial contribution of the non-oxidative pathways to ribose-5-phosphate[12,13]. Further it is known that ADA and xanthine oxidase are important markers of cell proliferation and differentiation[14-19]. Earlier studies have shown that feeding 5% garlic juice to rats has greatly inhibited liver xanthine oxidase enzyme[20]. The results of the present study indicate a significant raise (p<0.001) in ADA and UA levels in group-2 as compared to group-1 mice (refer table-1 and graphs 2 and 3), is in agreement with the above statements. It has been also observed in present study that there is a significant decrease (p<0.001 and p<0.01) in liver tissue ADA and UA levels in group-3 and group-4 as compared to group-2 (refer table-1 and graph-1 and 2) suggesting that DADS might have interfered with cell proliferation, thereby interfering with tumorogenesis. Nucleotide biosynthesis requires the participation of nucleotide reductase enzyme, which requires thioredoxin, a sulfhydryl compound for its activity. Thioredoxin has two sulfhydryl groups which are oxidized to a disulfide bond during the process[21]. In order to restore the thioredoxin to its reduced form so that it can be reused, thioredoxin reductase and NADPH are required[22] (refer figure-1).

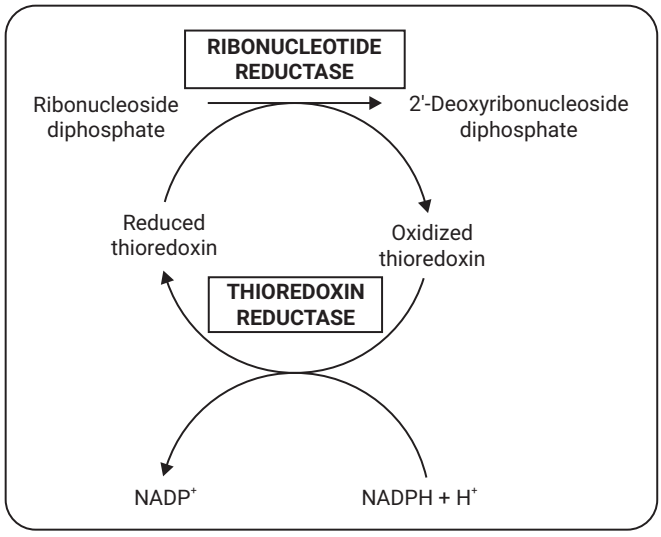


Figure-1: conversion of ribonucleotides to deoxyribonucleotides

A possible sulfhydryl exchange reaction of DADS with thioredoxin may reduce its availability hence decrease the production of deoxy ribonucleotides[23] or DADS may lower the available NADPH or NADH levels, as it is known to undergo reductive cleavage to its thiols using cellular NADPH or NADH, thus reducing the available DNA levels in cancer cell development. DADS may also undergo disulfide exchange reaction with pyruvate kinase, a thiol enzyme[23] thus reduces the availability of ribose-5- phosphate for nucleotide biosynthesis.

Conclusion:

Hence, from the present study it may be concluded that DADS at the dosage employed has interfered with the liver nucleotide synthesis by inhibiting the ADA activity and probably inhibiting the activity of xanthine oxidase enzyme as evident by decreased UA levels. Further, it is also observed that the protective effects of DADS are slightly more significant when compared to the curative effects. Further we believe that, focusing on clinical trials with organosulfur compounds in future cancer research studies with molecular level approach may help researchers and clinicians to understand the cancer preventive and therapeutic actions of garlic constituents in a much deeper way.

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