EFFECT OF DIALLYL DISULFIDE ON LIVER LIPID ALTERATIONS IN EXPERIMENTALLY INDUCED HEPATOMA IN MICE

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ABSTRACT

BACKGROUND

Diallyl disulfide (DADS) is an established hypolipidemic and cancer protective organosulfur compound of garlic. Lipids are essential for cell growth and proliferation, apart from providing energy to the cells. Increased rate of lipid synthesis seen in cancer cells is a prerequisite and key for cancer development and metastasis. Present study was undertaken to assess the effects of DADS on liver lipid alterations in Ehrlich Ascites Carcinoma (EAC) cells induced hepatoma in mice.

MATERIALS AND METHODS

Mice were divided into normal, control, curative and protective groups. Hepatoma was induced by intraperitoneal injection of 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 groups were sacrificed, and liver tissue total lipids, total cholesterol and HMG-CoA reductase enzyme activity were estimated.

RESULTS

This work showed a significant decrease ($p<0.001$) in liver tissue total lipids, total cholesterol and HMG-CoA reductase activity in DADS fed mice groups compared to control mice group.

CONCLUSION

Probably by reducing the cellular NADPH levels as well as by involving in disulfide exchange reactions with key enzymes (thiol enzymes) of lipid metabolism, DADS showed lipid lowering as well as tumour regressive effects. The study suggests that utility of DADS targeting lipid metabolism could be a novel strategy for cancer prevention and treatment.

KEYWORDS

Diallyl Disulfide, Lipid Metabolism, Cancer, Hepatoma, EAC Cells.

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BACKGROUND

Lipids, in addition to energy supply, promote cell growth and proliferation. They are also active players in the signalling processes that are involved in cell transformation and tumour development.[1] An increased rate of lipid synthesis in cancerous cells has long been recognised as one of the most important metabolic alterations.[1] Diallyl disulfide (DADS), a chief organosulfur compound of garlic, is known to exhibit hypolipidemic[2,3] and anticancer properties.[4,5,6] The present study was undertaken to assess the effects of DADS on liver lipid alterations in Ehrlich ascites carcinoma (EAC) cells induced hepatoma in mice.

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MATERIALS AND METHODS

Chemicals

A.R. Grade chemicals were used in the present work. Diallyl disulfide was purchased from Sigma-Aldrich Chemicals Pvt. Ltd. USA.

Experimental Animals

In the present study, Swiss male albino mice weighing 25-30 g were randomly selected from animal house of the B M C H, Chitradurga (1284/ac/09/CPCSEA). These mice were maintained on a standard small animal feed supplied by Amruth feeds, Bangalore and kept in well aerated plastic cages at room temperature with food and water available ad libitum.

Cell Line Maintenance and Induction of Hepatoma

The EAC bearing stock Swiss albino mice were obtained from Amala Cancer Research Institute, Thrissur, Kerala (India). For the in vivo EAC cells maintenance and for inducing hepatoma, freshly aspirated ascitic fluid aliquots (0.5 ml), from donor mice of 8-10 days old with well grown ascites tumour, were injected intraperitoneally in to healthy mice

using syringe under aseptic conditions. Liver histology showed areas of fibrotic and necrotic changes with hyperchromatism in EAC bearing mice.

Ethical Consideration

The experiments were conducted according to the guidelines of CPCSEA, New Delhi and Ethical clearance was obtained from Institutional Animal Ethics Committee of B M C H, Chitradurga.

Experimental Design

The mice were divided into 4 groups –normal mice (Group-I), control mice (EAC induced hepatoma bearing mice) (Group-II), protective mice (DADS treated-EAC induced hepatoma bearing mice) (Group-III) and curative mice (EAC induced hepatoma bearing- DADS treated mice) (Group-IV).

1. **Group-I: Normal Mice -** 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.
2. **Group-II: Control Mice -** 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 tumour was observed in all mice.
3. **Group-III: Protective Mice -** 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.
4. **Group-IV: Curative Mice -** 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.

During the study period, all the four groups' mice were provided with food and water ad libitum. 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, individual liver tissues were processed as follows:

Procedure

a) To 0.2 g of liver tissue slice, 4.8 ml saline/arsenate solution was added, mixed well and homogenized thoroughly. To 5.0 ml of homogenate, 5.0 ml of diluted perchloric acid was added, mixed well and allowed to stand for 5 minutes and centrifuged at 3000 rpm for 10 minutes. The clear supernatant was employed for estimation of HMG-CoA reductase activity.[7]
b) To 0.5 g of liver tissue slice, 4.5 ml of chloroform: methanol (1:1) was added, mixed well and thoroughly homogenized for 5 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was employed for the estimation of total lipids[8] and total cholesterol.[9]

Statistical Evaluation

The data entry was carried out using Microsoft Office Excel worksheet and statistically analysed. The 'p' value was calculated from Student 't' test.

RESULTS

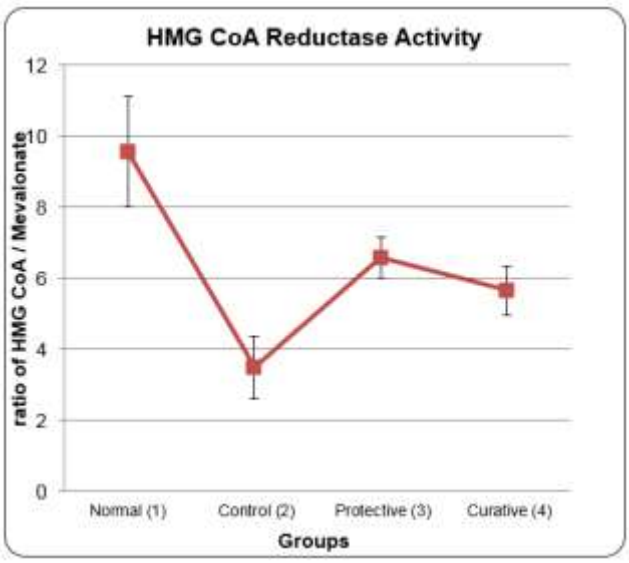
The results of the present study are depicted in table-1 and graphs 1 and 2. It is evident from the table and graphs that the levels of liver tissue total lipids, total cholesterol as well as HMG-CoA reductase activity (the ratio of absorbance of HMG-CoA/mevalonate is taken as index for HMG-CoA reductase activity; ratio is inversely proportional to enzyme activity) are significantly raised ($p<0.001$) in Group-II as compared to Group-I, whereas the same parameters are significantly lowered in Group-III ($p<0.001$) and Group-IV ($p<0.01$) as compared to Group-II mice.

Parameters → / Groups ↓	Total Lipids (mg/g)	Total Cholesterol (mg/g)	HMG-CoA Reductase Activity (Ratio of Liver HMG-CoA/Mevalonate)
Group-I (n=6)	30.63 ± 4.89	6.95 ± 0.68	9.58 ± 1.56
Group-II (n=6)	54.85*** ± 4.98	9.21*** ± 0.47	3.50*** ± 0.89
Group-III (n=6)	42.69*** ± 3.40	7.24*** ± 0.73	6.58*** ± 0.58
Group-IV (n=6)	44.76** ± 3.80	7.93** ± 0.61	5.66*** ± 0.68

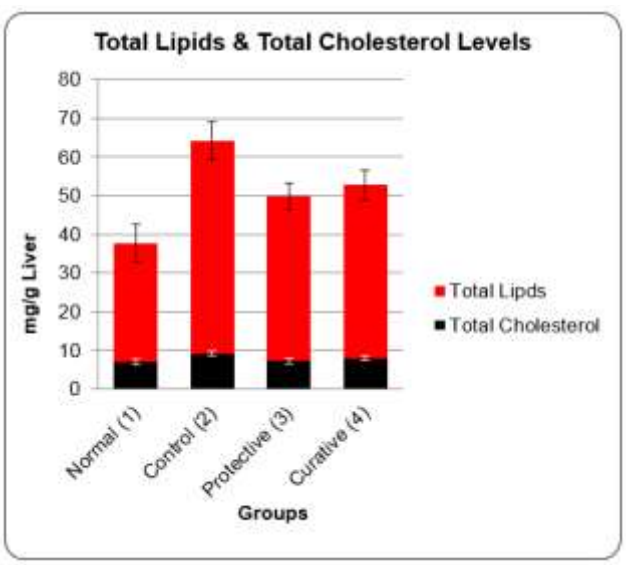
Table 1. Liver Tissue Total Lipids, Total Cholesterol and HMG-CoA Reductase Activity in Group-I, Group-II, Group-III and Group-IV Mice

Note-

- The number in parentheses indicates the number of mice.
- The values are expressed as their mean ± SD.
- Statistical evaluation: probability level - * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
- HMG-CoA reductase activity: ratio of HMG-CoA/mevalonate is inversely proportional to enzyme activity.



Graph 1. Liver Tissue HMG-CoA Reductase Activity in Group-I, Group-II, Group-III and Group-IV Mice



Graph 2. Levels of Liver Tissue Total Lipids and Total Cholesterol in Group-I, Group-II, Group-III and Group-IV Mice

DISCUSSION

The proliferating cells in general and cancer cell in particular require de novo synthesis of lipids for membrane synthesis and its assembly.[10,11] It is evident from the table-1 and graph-2 that there is a significant raise ($p<0.001$) in total lipids in Group-II mice liver compared to Group-I mice liver suggesting increased lipid synthesis in these tumour cells. This raise in lipid synthesis including fatty acid synthesis is a normal requirement in tumour cells,[12] as they use fatty acids to modify membrane targeted proteins and for bulk membrane synthesis, hence this increased fatty acid synthesis in tumour cells influences cell signalling or cell growth.[1] It is known that tumour cells synthesize fatty acid from glucose[13,14] which involves two important pathways: 1) Anaplerosis, where in acetyl CoA is exported to cytosol in the form of citrate for construction of fatty acids. 2) Increased NADPH production which is contributed by two enzymes, glucose-6-phosphate dehydrogenase and cytosolic malic enzyme.[15] Glutaminolysis is also predicted to produce enough NADPH for fatty acid synthesis.[16] Further it is observed in the present study that there is a significant decrease ($p<0.001$ and $p<0.01$) in total lipids in Group-III and Group-IV mice liver as compared to Group-II mice liver, suggesting that DADS might have interfered in lipid synthesis by reducing the cellular NADPH levels as DADS catabolism requires NADPH.[17] This decrease in NADPH level may also be due to disulfide exchange reaction of DADS with glucose-6-phosphate dehydrogenase (thiol enzyme)[18] thus causing a net reduction in total lipid content.

Cholesterol accumulation is a general feature of cancer, and it has been well correlated with cancer progression.[19,20,21] It is also known that malignant cells have elevated levels of mevalonate synthesis because of high activity of HMG-CoA reductase which fulfils the high requirement of cholesterol by rapidly proliferating cells for new membrane synthesis.[22] This is evidenced by significant raise ($p<0.001$) in cholesterol level as well as HMG-CoA reductase activity observed in Group-II mice liver as compared to Group-I mice liver (table-1, graph-1 and 2).

DADS is an established hypolipidemic and hypocholesterolaemic substance[2,3,23] and at the dosage employed in the present study may induce hypocholesterolaemic effects in Group-III and Group-IV mice (table-1, graph-1 and 2) probably by reducing the cellular NADPH levels as the catabolism of DADS to its allyl thiol do requires NADPH.[17] This effect of DADS may also be due to inactivation of thiol (-SH) group enzymes such as HMG-CoA reductase, fatty acid synthase complex and others.[18,24,25] The thiol – disulfide exchange reaction may be the cause for the inactivation of these enzymes hence, causing a significant decrease ($p<0.001$ and $p<0.01$) in liver tissue total cholesterol and HMG-CoA reductase levels as seen in Group-III and Group-IV mice as compared to Group-II mice.

CONCLUSION

Hence, it may be concluded that DADS at the dosage employed in the present study, showed lipid lowering effects in liver tissues of Group-III and Group-IV mice, probably by reducing the cellular NADPH levels and by inactivating key enzymes of lipid metabolism, thereby may reduce tumour growth. Further, it is also noted that the protective effects of DADS are slightly more significant when compared to the curative effects. This study with DADS implies that targeting lipid metabolism could be a novel strategy for cancer prevention and treatment.

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