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## ORIGINAL ARTICLE

# The chemopreventive effect of diallyl disulphide on N-nitrosodiethylamine induced heptocarcinogenesis

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**Summary**
In the present study we have investigated the anticarcinogenic property of diallyl disulphide (DADS) on hepatic thiobarbituric acid reactive substances (TBARS) and antioxidants such as reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase in control, N-nitrosodiethylamine and diallyl disulphide treated rats. The levels of TBARS and activities of SOD and catalase were decreased in NDEA treated rats whereas GSH and GPx tended to increase in carcinogenic rats. Oral administration of DADS (60mg/kg body wt) tends to normalize these variables in liver. This clearly indicates that DADS could possess chemopreventive effects by modulating the oxidant-antioxidant status of the living system. However, the exact mechanism remains to be elucidated.

*Key Words:* DADS – hepatocarcinogenesis – lipid peroxidation – antioxidants

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world and one of the most lethal (Schaff et al. 1998). N-Nitrosodiethylamine, a potent hepatocarcinogenic dialkyl nitrosamine is present in tobacco smoke, water, cheese, cured and fried meats and in a number of beverages (Rajesh kumar and Kuttan 2000). A review on NDEA reported that a number of animal species including mice, rats, guinea pigs, hamsters, rabbits, dogs and monkeys (Verna, Whysner and Williams 1996) developed liver cancers on exposure. It is metabolized to its active ethyl radical ($CH_3$ $CH_2^+$) by cytochromes and the reactive product interacts with DNA producing mutation and further oncogenesis.

Lipid peroxidation and associated membrane damage are key features of NDEA-induced carcinogenesis (Anis, Rajesh kumar and Kuttan 2001). The antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase, protect membrane and cytosolic components against damage caused by free radicals during carcinogenesis (Banakar et al. 2004). An estimation of lipid peroxidation products and antioxidants has accepted them as significant biomarkers of cancer chemoprevention (Hayes and Pulford 1995).

The potency of garlic (*Allium sativum*) has been acknowledged for more than 5000 years. In

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ancient times the Babylonians, Egyptians, Phoenicians, Vikings, Chinese, Greeks, Romans and Hindus used garlic frequently (Block 1985) as a remedy for intestinal disorders, skin diseases, wounds *etc*.

To date, around 10000 publications from all over the world have confirmed the beneficial effects of garlic and its compounds including the reduction of risk factors for cardiovascular diseases and cancer, a stimulation of the immune function, enhanced foreign compound detoxification and radio protection. Diallyl disulphide, an oil soluble compound, is not present in garlic cloves but it is present in garlic oil (Block 1996, Lawson 1996). It is a product of allicin transformation (Lawson 1996), which is produced when the enzyme allicinase acts on its substrate allin during garlic cutting or crushing (Block 1985, Lawson 1996). DADS have been found in human breath after garlic consumption (Rosen et al. 2001), which shows that this compound is a garlic metabolite *in vivo*.

The anticarcinogenic effects of garlic and its organosulfur compounds have been attributed to modulation of the antioxidant and/or the drug metabolizing enzyme system (Yang et al. 1994). DADS has been demonstrated to exert a chemopreventive activity against human cancers (colon, lung and skin) is an effective inhibitor in the growth of neoplastic CMT-13 cells (Sundaram and Milner 1996) and acts as an effective inhibitor for the promotion phase of DMBA induced skin tumors in mice (Lu et al. 2004).

Hence the present study was carried out to throw more light on the preventive effect of DADS in NDEA induced hepatocarcinogenesis using lipid peroxidation products and antioxidants.

Table 1. **Effect of DADS in the levels of TBARS and GSH and the activities of GPx, SOD and catalase in control and experimental animals.**

| Groups | Control | NDEA+$CCl_4$ | NDEA+ $CCl_4$ +DADS | DADS |
|---|---|---|---|---|
| TBARS (nmol/100g tissue) | $0.832\pm0.06^a$ | $0.359\pm0.02^b$ | $0.765\pm0.05^c$ | $0.859\pm0.05^a$ |
| GSH (mg/100g tissue) | $148.1\pm13.2^a$ | $198.5\pm14.2^b$ | $168.8\pm14.21^c$ | $162.0\pm15.1^c$ |
| GPx ($U^A$) | $7.55\pm0.61^a$ | $13.3\pm.72^b$ | $11.6\pm0.49^c$ | $8.16\pm0.31^d$ |
| SOD ($U^B$) | $11.1\pm0.96^a$ | $6.13\pm0.64^b$ | $8.37\pm0.64^c$ | $12.01\pm0.59^d$ |
| CAT ($U^C$) | $118.5\pm0.96^a$ | $63.7\pm6.47^b$ | $96.1\pm6.89^c$ | $128.2\pm9.31^d$ |

Values are mean ± SD; n=6, values not sharing a common superscript differ significantly ($2\alpha$= 0.05; Duncan's multiple range test)
$U^A$- μ moles of glutathione utilized/min/g protein
$U^B$ - enzyme required for 50% inhibition of NBT reduction /mg protein
$U^C$ - μ moles of $H_20_2$ utilized/min/g protein

## MATERIALS AND METHODS

*Animals*

Adult Male Wistar rats (160–180g) obtained from The Central Animal House, Faculty of Medicine, Annamalai University were used in the study. A commercial standard pellet diet (Hindustan Lever Ltd., Bangalore, India) and water were available to animals *ad libitum*. Animals were maintained in a controlled environment (30±2°C) with (12:12h) light- dark cycles in an experimental room simulating natural conditions (Mirunalini and Subramanian 2004). In Annamalainagar (11°24'N, 79°42'E), the light-dark (LD) is almost 12:12h throughout the year. All animal experiments were

approved by the ethical committee, Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition (NIN), Indian Council of Medial Research (ICMR), Hyderabad, India.

*Treatment schedule*

N-nitrosodiethylamine and diallyl disulphide were purchased from Sigma Chemical Co., USA and Lancaster, U.K. respectively. All other chemicals and biochemicals used in the study were of analytical grade. The animals were randomized and grouped into experimental and control rats (n=6 in each group). Group I rats served as controls. Group II (NDEA+$CCl_4$) rats received a single intra peritoneal injection of NDEA (200mg/kg body weight) followed by weekly injections of $CCl_4$ (3ml/kg body weight) for 6 weeks (Sunderasen and Subramanian 2002, 2003a, b). Group III animals received NDEA+$CCl_4$ as in group II and in addition, they were orally administered with 1ml of DADS (60mg/kg body weight thrice a week for 6 weeks (Wu, Kassie and Mersch-sundermann 2001). Group IV rats received DADS alone as in group III.

After 20 weeks of experimental study, the animals were killed by decapitation. The livers from all animals were dissected out, washed in ice cold saline, patted dry and weighed and 10% tissue homogenate was prepared to measure thiobarbituric acid reactive substances (Yagi 1987), reduced glutathione (Ellman 1959), glutathione peroxidase (Rotruck, Pope and Ganther 1973), superoxide dismutase (Kakkar, Das and Viswanathan 1984) and catalase (Sinha 1972) by spectrophotometric methods.

## RESULTS

The levels of TBARS and GSH and the activities of GPx, SOD and catalase are shown in Table 1. Group II (NDEA+$CCl_4$) showed significantly decreased levels of TBARS when compared to group I (Control) rats. Significantly increased levels of TBARS were observed in group III (NDEA+$CCl_4$+DADS) when compared to group II animals. No significant difference was found in the levels of TBARS in groups I and IV.

The levels of GSH and GPx were significantly increased in group II as compared with group I. Groups III and IV showed higher levels and activity of GSH and GPx when compared to group I rats. Reduced activities of SOD and catalase were observed in group II rats when compared with group I; increased activities of SOD and catalase were found in groups II and III, when compared to group II rats.

## DISCUSSION

Our results revealed that lipid peroxidation was found to be decreased in group II rats compared to group I. The level of lipid peroxidation products was found to be decreased in rat tumor cells and tissues when compared with the corresponding normal tissues (Dianzani et al. 1984, Cheeseman et al. 1986). Such a difference (Dianzani et al. 1984, Cheeseman et al. 1986) could be defined by (i) a decreased content of highly unsaturated fatty acids in tumors, (ii) a decreased concentration of cytochrome P-450 that can participate in the initiation of lipid peroxidation, (iii) a decreased content of NADPH content and (iv) changes in the antioxidant status and protective enzymes such as SOD and catalase. Experimental studies suggesting that lipid peroxidation has an inverse relationship with cell proliferation, corroborate our results (Nakagami, Uchida and Ohwada 1999). Moreover, the lipid peroxides leak from the organs or tissues into the blood stream and lead to increased circulatory lipid peroxides (Sunderasen and Subramanian 2003a, Singh et al. 2003). Increased concentration of TBARS in group III was due to the protective role of DADS.

The antioxidant systems are thus a major cell defense against oxygen toxicity and protect membrane and cytosolic components against damage caused by free radicals during diseased conditions. Decreased lipid peroxidation associated with an enhanced GSH and GSH dependent enzymes is a well known phenomenon in carcinogenesis (Anis, Rajesh kumar and Kuttan 2001). GSH occurred primarily in conjunction with foreign compounds or their metabolites for detoxification and transport from body in the cancerous stage, so that GSH concentration and its dependent enzymes were increased in group II rats. Puscas et al. (1999) have suggested that rapidly growing tumor cells exhibit lower SOD and catalase activities, corroborating our present observations. The lower activities of antioxidant enzymes in group II rats may due to increased scavenging of free radicals and superoxides (Sun 1990).

Chemopreventive agents can be divided into two groups: antimutagenic and antiproliferative: Antimutagens reduce the formation of carcinogens or mutagens there by preventing DNA damage through suppression of phase I enzymes or enhancement of Phase II detoxifying enzymes and alternatively chemopreventive agents may exert antiproliferative effects via induction of cell cycle arrest or apoptosis, inhibition of terminal differentiation and inhibition of oncogene activity

or DNA synthesis (Wu, Kassie and Mersch–Sundermann 2004, Wu et al. 2001).

Diallyl disulphide treatment in group III rats increased lipid peroxides and normalized the levels of antioxidants; this might be due to its antiproliferative and anticarcinogenic effects. *Allium* compounds including DADS inhibit cytochromes (Phase I enzymes) and reduce the formation of reactive metabolite, ethyldiazonium ion in liver (Mignard et al. 1996). DADS could trap NDEA and its metabolite by its S-S bond or allyl mercaptan as suggested by Wattenberg et al. (1982). It also enhanced the detoxification process by inducing phase II enzymes including GST, quinone reductase and epoxide hydrolase (Antosiewicz and Singh 2004). DADS is a more effective inducer of total GST and mu GST activities than DAS, DPS and DPDS. It also increases the protein levels of all the major hepatic GST subunits and especially GST of the $\alpha$ class, $\gamma$ GST $\mu_1$ and $\gamma$ GST $P_1$ (Guyonnet et al. 2001). DADS induce apoptosis by increasing intracellular calcium ion concentration (Sundaram and Milner, 1996) or by stimulating the generation of reactive oxygen species (Guyonnet et al. 2001).

In addition the metabolism of DADS leads to different compounds such as allyl mercaptan, allyl methyl sulfide, allyl methyl sulfoxide and allyl methyl sulfate (Germain et al., 2002). The effects of these metabolites on the mechanisms underlying the initiation and promotion of tumors remains to be assessed.

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