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## Effect of S-allyl cysteine sulphoxide on lipid metabolism and free radical scavengers in alcohol-fed rats

Alcohol consumption is increasing steadily in humans the world over, and it has become one of the serious health hazards. Several harmful effects of alcohol are known, but liver is the major organ that is most susceptible to the toxic effects of ethanol<sup>1</sup>. Ethanol can also alter the normal function and composition of membranes, which may ultimately lead to serious cellular impairment. Ethanol administration causes alcoholic fatty liver and hyperlipidemia<sup>2</sup>. Hepatic cirrhosis is a major cause of death in young and middle aged individuals who are chronic alcoholics. Partial protection against alcoholic fatty liver has been observed on administration of antioxidants<sup>3</sup>.

Garlic (*Allium sativum* Linn) and compounds derived from it are found to have many therapeutic effects<sup>4</sup>. There are several reports on the effect of garlic extract on lipid metabolism, lipid peroxidation and antioxidant enzymes<sup>5–7</sup>. Members of *Allium* family are reported to have antimutagenic properties and therapeutic values and possess diuretic properties, aid in digestion, act as a heart stimulant and alleviate rheumatic problems<sup>8</sup>. These properties of garlic can be attributed to the presence of sulphur compounds present in it. S-allyl cysteine sulphoxide (SACS), commonly called alliin, is an amino acid isolated from garlic<sup>9</sup> and is found to have a major effect on lipid metabolism.

Garlic was purchased from the local market. SACS was extracted from fresh garlic according to the method of Ito-kawa *et al.*<sup>10</sup> with some modifications, using ion exchange resins washed with 1N HCl, 1N NaOH or deionized water. Fresh garlic was boiled in water to inactivate the enzyme allinase. It was then ground and extracted with 80% methanol, filtered and passed through a column of amberlite IR-120 (strong cation exchanger) so as to absorb the amino acids. The column was washed with deionized water to remove the impurities and the amino acids were eluted with NH<sub>4</sub>OH (2N). Ammonia was removed by concentration of the eluates in a rotary evaporator at 40–43°C and the concentrate was loaded onto a column of amberlite CG-120 (strong cation exchanger). The column was washed with deionized water and the amino acids eluted with 0.1N NH<sub>4</sub>OH. Fractions of the effluent were tested for the presence of SACS by thin layer chromatography (TLC) as described below. The SACS containing fractions were pooled, concentrated and passed through a column of amberlite IRA-45 (weakly basic anion) so that unwanted amino acids and ammonia were absorbed onto the resin and removed. The effluent was collected and concentrated. Pure SACS was obtained from it after three recrystallization steps from 80% ethanol. It had a melting point of 164°C. The yield of SACS was

1.06 g/kg garlic. In TLC on silica gel G, using butanol:acetic acid:water (12:3:5) pure SACS gave an *R<sub>f</sub>* value of 0.24, similar to that of an authentic sample obtained from the Biochemical Institute, Helsinki.

Male albino rats of Sprague Dawley strain weighing 100–120 g were used for the study. They were maintained under environmentally controlled conditions with free access to standard food (Lipton, India) and water. They were divided into three groups of six rats each. A dose of 18% alcohol was administered orally for a period of 50 days. The animals were grouped as follows for the experiment: (1) Group I: Normal diet, ad lib; (2) Group II: Normal diet ad lib and 18% alcohol (4 g alcohol/kg body weight/day) orally; (3) Group III: Normal diet ad lib, 18% alcohol (4 g alcohol/kg body weight/day) along with SACS (500 mg/kg body weight/day) orally.

The rats were maintained on respective diet for a period of 50 days. The normal control group showed a gradual increase in body weight during the experimental period. The alcohol-treated group showed an increase in body weight during the initial period of 2 weeks and later on showed a gradual decrease in body weight up to the end of the experimental period. On the other hand, the SACS-treated group showed a regular increase in body weight during the entire experimental period. At the

end of the experimental period the animals were decapitated and blood and tissues were quickly collected for various biochemical studies.

Ethanol was obtained from E. Merck, and thiobarbituric acid was obtained from BDH laboratories. BSA and tris were obtained from Sigma Chemical Co, USA. All other chemicals and reagents used were of analytical grade.

Cholesterol in the serum was determined by CHOD-PAP method<sup>11</sup> (kit supplied by E. Merck (India) Ltd). The reaction mixture contains 10 µl of serum and standard (200 mg/dl). One ml reaction solution which contains PIPES buffer (pH 7.5, 99 mmol/l), salicylic alcohol (3.96 mmol/l), 4-aminoantipyrine (0.5 mmol/l), peroxidase (≥ 100 U/l) was mixed well and incubated for 5 min at 37°C. Then the absorbance was measured at 546 nm.

Triglycerides in the serum were determined by GPO-PAP method<sup>12</sup> (kit supplied by E. Merck (India) Ltd). Ten µl of serum was mixed with 1 ml reaction solution which contains Good's buffer (pH 7.2, 50 mmol/l), 4-chlorophenol (4 mmol/l), Mg<sup>2+</sup> (15 mmol/l), glycerokinase (≥ 0.4 KU/l), peroxidase (≥ 2 KU/l), lipoprotein lipase (≥ 2 KU/l), 4-aminoantipyrine (0.5 mmol/l), glycerol-3-phosphate oxidase (≥ 1.5 KU/l). Ten µl of triglycerides (200 mg/dl) was used as the standard, mixed and incubated at 37°C for 10 min. The absorbance was measured at 546 nm.

Phospholipids in the sample are estimated by Trinder's method<sup>13</sup>. The reaction mixture contains 10 µl of serum, 1 ml of reaction solution which contains 50 mM tris buffer (pH 7.6), dichlorophenol (2 mM), phospholipase D (400 U/l), choline oxidase (2200 U/l), peroxidase (3600 U/l), 4-aminoantipyrine (0.24 mM). Ten µl of phospholipids (300 mg/dl) was used as the standard, mixed and incubated at 37°C and the absorbance was measured at 505 nm.

For estimation of lipid peroxide content, the tissue homogenate was prepared in 0.1 M tris-HCl buffer (pH 7.5). Malondialdehyde (MDA) was estimated by thiobarbituric acid assay method<sup>14</sup>. To 1 ml of tissue homogenate, 2 ml TCA-TBA-HCl reagent was added and heated for 15 min, flocculant precipitate was removed by centrifugation at 2000 rpm and absorbance was read at

535 nm against a blank containing no tissue homogenate. Molar extinction coefficient was  $1.56 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$ . Hydroperoxide was estimated by iodimetric assay<sup>15</sup>. Molar extinction coefficient was  $1.73 \times 10^4 \text{ m}^{-1} \text{ cm}^{-1}$ . Conjugated dienes were estimated by the method of Recknagel and Ghoshal<sup>16</sup>. Molar extinction coefficient was  $2.52 \times 10^4 \text{ m}^{-1} \text{ cm}^{-1}$ .

Catalase was assayed<sup>17</sup> spectrophotometrically following decrease in absorbance at 230 nm. Specific activity was expressed in terms of unit/mg protein and unit of enzyme has been defined as the velocity constant per second. For estimation of superoxide dismutase activity<sup>18</sup>, tissue homogenate was prepared in 0.25 M sucrose and centrifuged at 10,000 rpm at 4°C to get cytosol fraction. Before estimation, initial purification was done by precipitating the protein from the supernatant with ammonium sulphate (90%) and after dialysis against 0.0025 M tris-HCl buffer (pH 7.4). The supernatant was used as enzyme source. One unit of enzyme has been defined as the enzyme concentration required to inhibit optical density at 560 nm of chromogen production by 50% in one minute under the assay conditions and expressed as specific activity in milli units/mg protein. HMG CoA reductase activity was estimated as described by Rao and Ramakrishnan<sup>19</sup> by determining the ratio of HMG CoA to mevalonate. Ten per cent of tissue homogenate was prepared in

saline arsenate. Equal volumes of the homogenate and dilute perchloric acid were mixed and kept for 5 min and then centrifuged for 10 min at 2000 rpm. To 1 ml of the filtrate, 0.5 ml of freshly prepared alkaline hydroxylamine reagent and 1.5 ml of ferric chloride reagent was added, shaken well and absorbance was taken at 540 nm against similarly treated saline arsenate as blank. The ratio between HMG CoA and mevalonate is taken as an index of activity of the enzyme which catalyses the conversion of β-hydroxy β-methyl glutaryl CoA to mevalonate. Protein in the samples was quantitated by the method of Lowry *et al.*<sup>20</sup> using bovine serum albumin as standard.

Statistical analysis was carried out using the Student's *t* test<sup>21</sup>. Values are expressed as mean ± SD. Values having *P* < 0.001 on comparison were considered as significant.

The effect of long-term administration of alcohol and that of SACS on serum lipid levels is given in Table 1. From the data it is clearly seen that the elevated lipid levels in alcohol-fed rats (*P* < 0.001) were brought back to near normal levels on administration of SACS (*P* < 0.001). The values of lipid peroxides, hydroperoxides, and conjugated dienes in the liver of rats treated with alcohol and SACS are given in Table 2. It is observed that the levels of lipid peroxides, hydroperoxides and conjugated dienes were increased on administration of alcohol (*P* < 0.001)

**Table 1.** Concentration of cholesterol, triglycerides and phospholipids in serum of control and treated rats (values expressed as mg/dl are mean ± SD from six rats in each group)

Group	Cholesterol	Triglycerides	Phospholipids
I – Normal	60.52 ± 0.87	11.28 ± 0.50	109 ± 3.0
II – Alcohol	80.56 ± 0.73*	14.0 ± 0.1*	179 ± 5.6*
III – Alcohol + SACS	62.87 ± 0.3*	12.0 ± 0.09*	120 ± 2.8*

Group II is compared with group I and group III is compared with group I.

\**P* < 0.001.

**Table 2.** Concentration of malondialdehyde, hydroperoxides and conjugated dienes in liver of control and treated rats (values expressed as mmol/100 g wet tissue is mean ± SD from six rats in each group)

Group	Malondialdehyde	Hydroperoxide	Conjugated dienes
I – Normal	0.63 ± 0.02	9.79 ± 0.38	52.5 ± 1.30
II – Alcohol	1.090 ± 0.39*	14.98 ± 0.20*	54.5 ± 0.58*
III – Alcohol + SACS	0.70 ± 0.28*	10.79 ± 0.08*	51.0 ± 2.73*

Group II is compared with group I and group III is compared with group I.

\**P* < 0.001.

## SCIENTIFIC CORRESPONDENCE

**Table 3.** Activity of superoxide dismutase and catalase in the liver of control and treated rats. (Activities expressed as units/mg protein, values are mean  $\pm$  SD from six rats in each group)

Group	Superoxide dismutase	Catalase
I – Normal	9.16 $\pm$ 0.21	60.2 $\pm$ 0.98
II – Alcohol	5.3 $\pm$ 0.28*	46.8 $\pm$ 1.30*
III – Alcohol + SACS	7.5 $\pm$ 0.13*	56.9 $\pm$ 2.01*

Group II is compared with group I and group III is compared with group II.

\* $P < 0.001$ .

**Table 4.** Activity of HMG CoA reductase expressed as HMG CoA/mevalonate in liver of control and treated rats. (Values are expressed as ratio of mean  $\pm$  SD from six rats in each group.) (The ratio HMG CoA/mevalonate is inversely proportional to HMG CoA reductase activity)

Group	HMG CoA/mevalonate
I – Normal	1.94 $\pm$ 0.31
II – Alcohol	2.59 $\pm$ 0.44*
III – Alcohol + SACS	1.80 $\pm$ 0.12*

Group II is compared with group I and group III is compared with group II.

\* $P < 0.001$ .

and the values decreased to normal levels on treatment with SACS ( $P < 0.001$ ). The activities of antioxidant enzymes, catalase and superoxide dismutase were found to be decreased on treatment with alcohol ( $P < 0.001$ ) and these values increased on administration of SACS ( $P < 0.001$ ; Table 3). The activity of HMG CoA reductase in the liver was lowered on administration of alcohol ( $P < 0.001$ ) which increased to near normal level on administration of SACS ( $P < 0.001$ ; Table 4).

Ethanol feeding has been found to enhance the endogenous synthesis of triglycerides and to reduce the utilization of dietary lipids, resulting in their accumulation in the liver and blood plasma. In the present study we found that SACS present in garlic was able to counteract the above-mentioned effect of ethanol. Garlic protein is rich in sulphur-containing amino acids like methionine, cystine and SACS. These amino acids in turn define the anti-atherogenicity of garlic<sup>10</sup>. Another finding is that these sulphur-containing amino acids and their derivatives could have counteracted the hyperlipidemic and oxidant effects of alcohol. It is also pointed out that sulphur-containing amino acids have a special role as hypolipidemic agents.

In the present study we found that the administration of alcohol has considerably increased the serum lipid levels,

lipid peroxidation products such as MDA, hydroperoxides and conjugated dienes. Whereas antioxidant enzymes such as catalase and superoxide dismutase were found to be decreased on administration of alcohol. On administration of alcohol, the ratio of HMG CoA to mevalonate is found to be increased, i.e. HMG CoA reductase activity is decreased in alcohol-fed rats. This shows that increased serum cholesterol levels in alcohol-fed rats may not be due to increased cholesterologenesis in the liver, but due to decreased esterification and utilization<sup>22</sup>. Administration of SACS to the alcohol-treated rats is found to reverse the above-mentioned effect of ethanol.

The decreased value of lipid levels on the administration of SACS is due to hypolipidemic effect of garlic<sup>23</sup>. Lipid peroxidation products such as MDA, hydroperoxides and diene conjugates were found to be increased in alcohol-fed rats in the present study. The administration of SACS significantly decreased lipid peroxidation. Effect of ethanol administration on lipid peroxidation products has been suggested to enhance the generation of oxygen-free radical during its oxidation in the liver<sup>24</sup>. These free radicals are responsible for the oxidation of the LDL molecule, causing lipid peroxidation<sup>25</sup>.

Antioxidant enzymes such as catalase and superoxide dismutase were found to

be decreased in alcohol-fed rats. These effects were counteracted by the administration of SACS. This is possibly due to the antioxidant action of the compound. The mechanism involved may be that SACS has counteracted the oxidant effect of alcohol, as the sulphhydryl group present in the compound is able to scavenge the free radicals.

HMG CoA reductase, which plays a major role in the cholesterol biosynthesis, functions as the lipogenic enzyme in the tissues. A decrease is observed in the activity of HMG CoA reductase in alcohol-fed rats. This may be due to the alcoholic fatty liver. This effect of alcohol is reversed by the administration of SACS.

The present study indicates that SACS extracted from garlic has antioxidant and hypolipidemic effects.

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