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Antiperoxide effect of S-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats

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Abstract. Treatment of alloxan diabetic rats with the antioxidant S-allyl cysteine sulfoxide (SACS) isolated from garlic (*Allium sativum* Linn), ameliorated the diabetic condition almost to the same extent as did glibenclamide and insulin. In addition, SACS controlled lipid peroxidation better than the other two drugs. Furthermore, SACS significantly stimulated in vitro insulin secretion from B cells isolated from normal rats. Hence it can be surmised that the beneficial effects of SACS could be due to both its antioxidant and its secretagogue actions. The former effect is more predominant and the latter is only secondary. These effects highlight the therapeutic value of garlic, which is a component of many diets.
Key words. Antioxidant; S-allyl cysteine sulfoxide; insulin secretagogue; albino rats; glibenclamide; malondialdehyde; hydroperoxide; superoxide dismutase.

Very little research has been conducted on the biological activity of S-allyl cysteine sulfoxide (SACS), an aminoacid isolated from garlic (*Allium sativum* Linn). SACS, also called allin, is a precursor of allicin with the related formulae $C_3H_5S(O)-CH_2CH-(NH_2)COOH$ and $C_3H_5-S(O)-S-C_3H_5$ respectively. SACS was found to be a strongly hypolipidemic agent by others[2,3]. In the various strains of garlic analysed, SACS was the most predominant S-containing compound, followed by cycloallin and S-methyl cysteine sulfoxide (SMCS)[4]. Polar compounds from garlic, including SACS, do not have any antimicrobial action[5]. Gabhardt[6] found that in a culture of rat hepatocytes incubation with water soluble garlic extracts diminished cholesterol synthesis, but SACS did not exert such inhibition. However, according to his work[6] SACS inhibited fatty acid synthesis when used at a concentration higher than its breakdown products in the whole garlic extract. Hikino et al.[7] found that the volatile oil, and two sulfur-containing aminoacids, S-allyl and S-methyl mercaptocysteines ($R-S-S-CH_2-CH(NH_2)COOH$, where $R = CH_3$ or C_3H_5), extracted from garlic, effectively prevented the damage to liver cells induced by carbon tetrachloride and D-galactosamine, two potent liver toxins. However, the protection offered by SACS (which lacks the disulfide bond) was much less than that provided by the alkyl mercaptocysteines. They further showed in the same work that garlic components exerted their protection by inhibiting the generation of free radicals and by preventing the oxidation of lipid peroxides, and thus served as potent antioxidants.

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The present work is related to the antidiabetic effects of SACS in experimental diabetes. Many reports show that in diabetes, in addition to hyperglycemia, there is an increase in glycosylated haemoglobin[8], serum and tissue lipids[9], serum transaminases[10,11], serum alkaline and acid phosphatases[12] and glucose-6-phosphatase[13], and a decrease in haemoglobin, serum insulin[14], hepatic glycogen[15] and in the activities of hexokinase[16] and lipogenic enzymes[17,18]. In earlier studies reported from this laboratory[2,3] on the effects of SACS on alloxan diabetic rats, we found that it ameliorated the changes in most of the above parameters to an extent comparable to that obtained with glibenclamide and insulin. In this paper we report on the insulin secretagogue effect of SACS in vitro, as well as its in vivo action as an antiperoxide and related effects in diabetic rats.

Materials and methods

SACS was prepared from fresh garlic according to the method of Itokawa et al.[1], with some modifications, using ion exchange resins washed with 1N HCl, 1N NaOH or deionised 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 1R-120 (strong cation exchanger) so as to absorb the garlic aminoacids. The column was washed with deionised water to remove the impurities and the aminoacids were eluted with NH_4OH (2N). Ammonia was removed by concentration of the eluate in a rotary evaporator at 40–43 °C and the concentrate loaded onto a column of amberlite CG-120 (strong cation exchanger). The column was washed with deionised water and the aminoacids eluted with 0.1N NH_4OH. 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 as before and passed through a column of amberlite IRA-45 (weakly basic anion) so that unwanted aminoacids and ammonia were absorbed onto the resin and removed. The effluent was collected and concentrated. Pure SACS was obtained from it after three recrystallisation steps from 80% ethanol. It had a m.p. 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 Rf value of 0.24, similar to that of an authentic sample obtained earlier from the Biochemical Institute in Helsinki.

In a pilot study[3] we had already found that SACS had a dose-related action in normal rats in the dosage from 100 to 400 mg/kg. As 200 mg/kg gave an appreciable (14%) blood glucose reduction this dose was used in the present study.

Male albino rats (Sprague-Dawley strain) weighing 120–150 g were made diabetic by a subcutaneous injection of alloxan monohydrate (180 mg/kg body weight)[19]. After a fortnight, 24 rats with moderate diabetes which exhibited glycosuria and hyperglycemia (blood glucose 200–250 mg/100 ml) were divided into four groups of six. A control group of six normal rats were also taken. The rats were maintained on normal rat feed supplied by Gold Mohur Lipton India Ltd., Bangalore. The diabetic rats and a control group were treated for one month as shown below.

1. Normal rats given normal saline (10 ml/kg/day)
2. Diabetic rats given normal saline (10 ml/kg/day)
3. Diabetic rats given SACS (200 mg/kg/day)
4. Diabetic rats given glibenclamide (500 μg/kg/day)
5. Diabetic rats treated with insulin (5 units/kg/day).

Glibenclamide was supplied by Boehringer Knoll Ltd., Bombay, and insulin by Boots company Ltd., India. The drugs other than insulin were administered daily as a suspension in saline (saline 10 ml/kg/day) through a stomach tube. Daily food consumption was noted. After one month the change in body weight was recorded, and fasting blood glucose[20] and urine sugar (Benedict's qualitative test) were assessed. The rats were then killed by decapitation and blood and tissues were collected for various estimations which were carried out according to standard methods.

Glycosylated haemoglobin[21], serum insulin[22], and haemoglobin[23] were measured in blood. Glutathione[24], and lipid peroxidation levels (malondialdehyde (MDA)[25], hydroperoxide (HP)[26] and conjugated dienes[27]) were assessed in liver, kidney and heart and the activities of superoxide dismutase[28] (SOD), and catalase[29] in liver.

In vitro effects of SACS on B cells

This study was conducted to assess the insulin secretagogue effects of SACS on β cells, isolated from rat pancreas according to the method of Lacy and Kostianovsky[30]. The assay of insulin secreted on incubation of B cells with different concentrations of SACS in KRB Eagle's medium, containing 2 mM glucose and 20 mM glucose at 37 °C, was made separately according to the method of Morgan and Lazarow[31]. All the results were analyzed using ANOVA followed by Tukey's HSD (Honestly Significant Difference). Pairs of groups sig-

Table 1. Fasting blood glucose, urine sugar*, haemoglobin, glycosylated haemoglobin and serum insulin in treated and untreated alloxan diabetic rats. Values are mean ±S.D. for six rats in each group.

Groups	Fasting blood glucose (mg/100 ml)		Total haemoglobin (g/100 ml)	Glycosylated haemoglobin μmoles of fructose/mg/Hb.	Serum insulin (μu/ml)**
	initial	final			
1. Normal	79.2 ± 3.8^{a}	82 ± 3.9^{a}	14.8 ± 2.3	3.0 ± 0.18^{d}	82.5 ± 5.9
2. Diabetic control	22.7 ± 7.3 (+++)	292 ± 8.8 (+++)	10.7 ± 1.7^{c}	7.06 ± 0.33^{e}	21 ± 1.5†
3. Diabetic + SACS	233 ± 7.1 (+++)	162 ± 4.4^{b} (+)	13.7 ± 2.0	$4.0 \pm 0.27^{d,e}$	50 ± 3^{f}
4. Diabetic + glibenclamide	240 ± 8.6 (+++)	123 ± 5.1^{b} (+)	13.8 ± 1.7	$4.3 \pm 0.22^{d,e}$	65 ± 6^{f}
5. Diabetic + insulin	226 ± 6.9 (+++)	120 ± 4.5^{b} (+)	14 ± 1.1	3.8 ± 0.22^{d}	62 ± 5.5^{f}

ANOVA followed by Tukey's HSD is used to assess the significant results.
Level of significance 0.05.
[a]Significantly lower as compared to the diabetic groups.
[b]Significantly lower as compared to the diabetic control.
[c]Significantly lower as compared to all other groups.
[d]Significantly lower as compared to the diabetic control.
[e]Significantly higher as compared to the normal.
[f]Significantly higher as compared to the diabetic control but lower as compared to the normal.
†Significantly lower as compared to the other groups.
*Urine sugar: (+) indicates traces and (+++) indicates more than 2% sugar as depicted under blood glucose.
**μu = microunits.

Table 2. Superoxide dismutase (SOD) and catalase activities in the liver, and glutathione content in tissues of treated and untreated alloxan diabetic rats. (Values are means ±S.D. for six rats in each group).

Groups	Glutathione content (mg/100 g wet tissue)			SOD* in liver	Catalase in liver‡
	liver	kidney	heart		
1. Normal	396 ± 21	269 ± 15.2	420 ± 22	7.7 ± 0.2	65.8 ± 2.1
2. Diabetic	305 ± 19[a]	220 ± 12.9[b]†	293 ± 18[c]**	6.0 ± 0.18[d]	41.3 ± 2.0[e]**
3. Diabetic + SACS	377.2 ± 17	254 ± 4.5	380 ± 16	7.3 ± 0.18	62.0 ± 1.8
4. Diabetic + glibenclamide	325 ± 19[a]	216 ± 9.3[b]†	320 ± 24[c]	6.3 ± 0.2[d]	46.8 ± 1.9[e]
5. Diabetic + insulin	325 ± 19[a]	239.8 ± 9.8[b]	334 ± 20[c]	6.5 ± 0.17[d]	50.2 ± 2.0[e]

Level of significance, 0.05, is calculated as mentioned in the text.
[a, b]†, [c, d, e]Significantly lower as compared to normal and SACS treated groups.
[b]Significantly lower as compared to the normal;
**Significantly lower as compared to the insulin treated group also.
*Units/mg protein = enzyme concentration required to inhibit O.D. at 560 nm of chromogen production by 50% in one minute).
‡Values $\times 10^{-3}$ units/mg protein (unit = velocity constant/sec).

nificantly different at the 0.05 level are noted in the tables and in figure 1.

Results

The daily food consumption by different groups of rats was more or less the same (8–10 g/rat/day). However the changes in body weight gain for normal rats was 30 ± 3 g, that is 18% per month. The diabetic control group of rats which did not receive any hypoglycemic agent, lost 50 ± 4 g, that is 33%, after one month. The SACS, glibenclamide and insulin treated groups gained some weight; 8 ± 2 g; 9 ± 3 g and 10 ± 2 g respectively, that is 5–6%. There was no significant difference in the gain of body weight between the treated groups.

Results on blood, urine and serum values are given in table 1. Treatment of alloxan diabetic rats with SACS, glibenclamide or insulin significantly reduced the levels of blood glucose and glycosylated haemoglobin, while it significantly increased haemoglobin and serum insulin levels. The haemoglobin level was very close to normal. Insulin and glibenclamide showed a significantly higher effect in controlling blood sugar than SACS. The overall antidiabetic effects were in the order: insulin > glibenclamide > SACS.

In table 2, glutathione levels in the tissue and the activities of the free radical scavenging enzymes, SOD and catalase, in the liver, are given. In diabetic animals these levels were significantly decreased. On treatment, the levels were raised to varying extents. SACS treatment increased the GSH level in liver, kidney, and heart and the scavenging enzyme activities in liver; insulin treatment raised the GSH level in heart only, and the catalase activity in liver only. After glibenclamide treatment none of these parameters were improved significantly. The antioxidant activity of SACS was significantly higher than that of the other two drugs (table 2).

Figure 1 shows the variation in lipid peroxidation of the tissues. In the diabetic state, lipid peroxidation is significantly increased in the liver. However, in the kidney only MDA and conjugated dienes, and in heart only the latter, were found to be increased significantly.

In contrast in heart MDA and HPO were found to be decreased significantly in the diabetic condition. After treatment, the lipid peroxidation decreased significantly in all tissues, except the heart with respect to MDA. Between the treated groups there were significant variations in the beneficial effects of the drugs, and SACS was found to be the most effective. Glibenclamide invariably showed the least antioxidant effect and SACS showed the maximum effect, having a significant antiperoxide action in all tissues (see fig. 1). Therefore the beneficial effects of the drugs in controlling lipid peroxidation and maintaining GSH level in tissues are in the order SACS > insulin > glibenclamide.

The effects of SACS on insulin release from B cells of the pancreas are shown in table 3. In low glucose medium, concentrations of 50 µg/ml and above showed significant effects on insulin release as compared to the basal level, increasing with increase in SACS concentration from 50 to 100 µg/ml. In high glucose medium only

Table 3. Insulin secretagogue effects of SACS in vitro. Mean values ±S.D. of six experiments for each concentration of SACS in low and high glucose medium are shown. ANOVA followed by Tukey's HSD is used. Level of significance 0.05.

Conc. of SACS in µg/ml	2 mM glucose	20 mM glucose
	Insulin release µu/5 islets/hr	Insulin release µu/5 islets/hr
1. Basal	25 ± 3	90 ± 5
2. 25	30 ± 4	92 ± 5.5
3. 50	40 ± 5[a]	108 ± 6
4. 75	50 ± 4[b]	112 ± 5[d]
5. 100	72 ± 5[c]	118 ± 6[e]

[a, b, c]Significantly higher as compared to the basal value.
[b]Significantly higher as compared to the lowest test dose also.
[c]Significantly higher as compared to all other test doses.
[d, e]Significantly higher as compared to the basal value and the lowest test dose also.

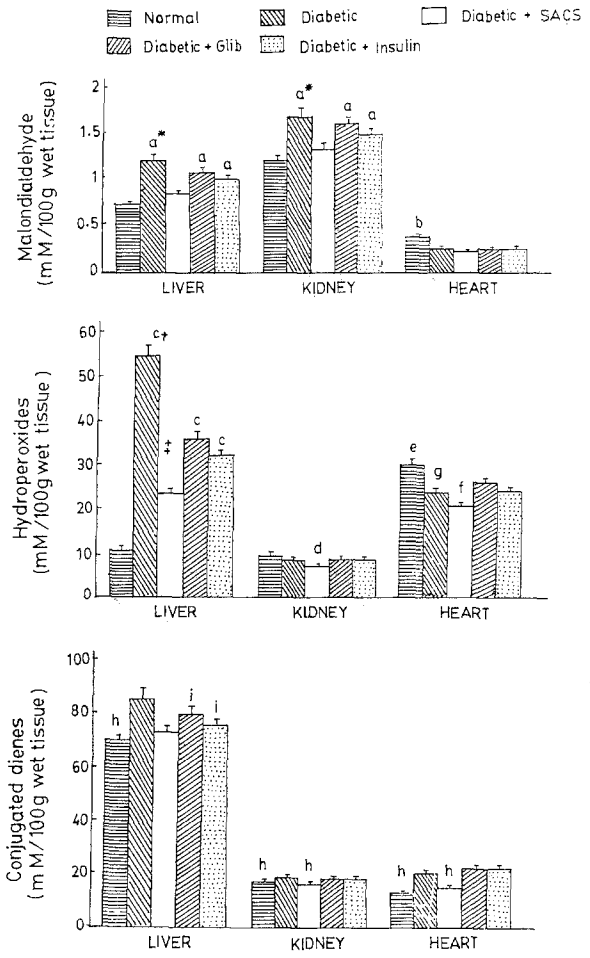

Figure 1. Mean values ±S.D. for 6 rats in each group are represented on the graphs. Group nos. are the same as in the text. Significant results are assessed as mentioned in the text and indicated by letters and other markings for the tissues concerned.

MDA

[a]Significantly higher as compared to normal and SACS treated groups.

*Significantly higher as compared to insulin treated group.

[b]Significantly higher as compared to other groups.

HPO

[c]Significantly higher as compared to normal and SACS treated groups.

†Significantly higher as compared to glibenclamide and insulin treated groups.

‡Significantly higher as compared to the normal.

[d]Significantly lower as compared to other groups.

[e]Significantly higher as compared to other groups.

[f]Significantly lower as compared to other groups.

[g]Significantly lower as compared to normal and glibenclamide treated groups.

Conjugated dienes

[h]Significantly lower as compared to other groups except for the liver value of SACS treated group.

[i]Significantly lower as compared to the diabetic control.

75 and 100 µg/ml concentrations of SACS increased insulin release significantly above the basal level. The insulin release in high glucose medium for each concentration of SACS was significantly higher as compared to the corresponding value in the low glucose medium.

Discussion

The results show that all three drugs used have a significant antidiabetic effect, but only SACS unequivocally showed a marked antiperoxide effect in the diabetic state. SACS is not toxic at the dose levels used. This dose is comparable to that of a natural antioxidant curcumin (diferuloyl methane; dose 250 mg/kg) isolated from *Curcuma longa* Linn, which was tested in mice by other workers[32]. It seems that the net effect of SACS is partly the result of insulin secretagogue effect, as evidenced by its in vivo and in vitro stimulation of insulin release, and partly due to biological actions independent of insulin, such as hypolipidemic[2] and antioxidant[33] activities, enabling it to stimulate SOD and catalase, as observed in the present study. In the latter activity both allyl and sulfoxide groups may have a role, as suggested by others[1,34].

The insulin secretagogue effects of organic sulfides[35], and also their insulin-independent actions[36], have been reported elsewhere. In one of our studies[2] we observed that SACS is a better hypolipidemic agent than glibenclamide and insulin, and this is mainly due to a strong inhibitory action of the sulfoxide aminoacid on lipogenic enzymes and an enhancing effect of the same on lipolytic enzymes in liver and adipose tissue[3]. The former effect of SACS may bring about a reduction in the synthesis of lipids and the latter effect may enhance the mobilization of lipid from depots, with the result that body lipids may be prevented from accumulating in tissues and blood. The other two drugs act on the above enzymes in just the opposite way. Therefore we can suggest that SACS achieves its insulin-independent therapeutic effects both by enhancing the antioxidant and lipolytic enzymes, and by suppressing certain others such as the lipogenic enzymes. The antidiabetic/insulinogenic action of SACS may perhaps be only a minor effect of this drug. Otherwise it would have acted just like the other two drugs. Unlike the other two drugs, SACS increased the GSH level in tissues, and it reflects the capacity of the tissues of SACS treated rats to dispose of free radicals, which are particularly harmful to heart tissues. Eventhough the stimulation of the scavenging enzymes SOD and catalase is minimal, this effect together with a substantial increase in tissue GSH might have brought about the very effective antioxidant action of SACS.

Ellagic acid, which possesses six phenolic hydroxyl groups and is a strong antioxidant, was reported to be effective at a very low dose level of 100 µmol/kg/day (30 mg/kg) against CCl_4-induced liver damage in rats[37]. In our experiment, SACS, which only has a sulfoxide functional group to act as an antioxidant, was found to be significantly active at 6–7 times this concentration. Moreover, to compare the two in terms of doses may not be fair. Because SACS is present in garlic, which is

a normal dietary component, its therapeutic value should be made use of, as it acts as an antioxidant and hypolipidemic agent. It protects the body from lipid peroxidation to a significant extent in a condition like diabetes, and this is noteworthy as antioxidants have also been reported to have a protective action on β cells[38] of pancreas.

Diabetics quite often suffer from myocardial infarction, mostly owing to hyperlipidemia and a high rate of lipid peroxidation, and they may benefit from the use of garlic in the diet, or SACS as a substitute. However, more detailed work is necessary to unravel the actions of SACS.

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