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Effect of Diallyl Disulphide on Protein and Lipid Glycation, and Lipid Peroxidation in Brain of Alloxan Diabetic Rats

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Abstract- Non enzymatic glycosylation of proteins and lipids is the main initiating factor for the pathophysiology of chronic diabetic complications. This glycation is more prevalent in insulin independent tissues like brain, kidney, RBCs, etc. Diallyl disulphide (DADS), the principle compound of garlic oil, is well known for its antihyperglycemic, antihyperlipidemic, anticarcinogenic and antibiotic properties. Hence a study was undertaken to assess the anti-glycation properties of DADS, in alloxan diabetic brain tissue, thereby to establish any usefulness of DADS in prevention of central nervous system complications in diabetes mellitus like diabetic dementia or diabetic encephalopathy. The current study showed a significant decrease ($p < 0.001$) in glycated proteins, glycated lipids and total TBARS levels in brain tissue of DADS treated diabetic rats as compared to diabetic control rats. Hence it can be concluded that DADS helps in reducing glycation of brain proteins and lipids as well as lipid peroxidation and thus may be useful in prevention of CNS diabetic complications like diabetic encephalopathy.

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I. INTRODUCTION

Non enzymatic glycosylation of proteins and lipids will be normally proportional to available free glucose in the tissues. It can be expected that a consistent hyperglycemia in diabetic subjects may induce hyperglycation of tissue proteins and lipids, and this is high in tissues which are not dependent on insulin for glucose transport like kidney, brain, RBCs, optic lens, etc ¹⁻³. It is shown that the main initiating factor for the pathophysiology of chronic diabetic complications like diabetic nephropathy is non-enzymatic glycosylation of kidney proteins and lipids⁴. There are evidences for glycation to occurs in brain tissue of diabetic animals like studies of Miyazawa A⁵ have established increased lipid glycation in neurons of diabetic animals whereas studies of Jingsheng H⁶ have similarly established protein glycation in brain of diabetic rats. Few studies have shown that lipid glycation occurs faster than protein

glycation⁷. Since brain has rich content of lipids, lipid glycation is of significance in diabetes induced CNS complications. Glycation of proteins and lipids probably results in increased formation of advanced glycation end products (AGEPs) and advanced glycated lipid products (AGLPs), which leads to formation of various oxidants (like lipid peroxidation products, example Malonaldehyde, etc) resulting in tissue damage⁸⁻¹². These AGEPs and AGLPs are indicated in late diabetic CNS complications like Alzheimers disease¹³, diabetic dementia⁵ and diabetic encephalopathy¹⁴.

Among the various biological activities of the medicinal plants, the hypoglycaemic and hypolipidemic activities have been the most commonly studied. Garlic, (*Allium sativum* Linn) is well known for its antidiabetic, antihyperlipidemic, antiatherogenic as well as anticarcinogenic properties¹⁵⁻¹⁸. DADS, the principle sulphur compound of garlic is probably responsible for the above mentioned beneficial functions of garlic. Studies have shown that DADS crosses blood brain barrier^{19,20} and its use in various neurological disorders have been established^{21,22}.

Hence a study was undertaken to assess the anti-glycation properties of DADS on brain proteins and lipids in alloxan diabetic rats, thereby to establish the usefulness of DADS in prevention of CNS complications in diabetes mellitus like diabetic encephalopathy.

II. MATERIALS AND METHODS

Alloxan and Diallyl disulphide (DADS) were procured from Sigma Chemical Company. All other chemicals employed were of analytical grade.

Albino rats of both sexes, weighing 300-350g were randomly selected from Central Animal House, BMCH, Chitradurga and were used for the present investigation. The animals were maintained on a standard rat feed supplied from Amrut rat feeds, Bangalore. The experiments were conducted according to the norms approved by Ministry of Social Justice and empowerment, Government of India, and Institutional Animal Ethics Committee (IAEC) guidelines. The animals were fasted overnight and Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan (150mg/kg body wt.)²³, in sterile normal saline. The animals were considered diabetic if their blood

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glucose were consistently above 300mg/dl and urine showed consistent glucosuria. The treatment was started on 5th day after alloxan injection and was considered as first day of treatment. The rats were divided into three groups comprising six rats in each group as follows:

Group I: Normal rats – which were fed on 30 ml of normal saline per kg body weight, through gastric intubation, daily for 90 days.

Group II: Diabetic Control rats - which were fed on normal saline 30ml / kg body weight, through gastric intubation, daily for 90 days.

Group III: Diallyl disulphide (DADS) treated Diabetic rats – which were fed on DADS (100mg/ kg body weight) prepared in normal saline, given as 30ml / kg body weight suspension, through gastric intubation, daily for 90 days.

On completion of the stipulated period, the rats were anaesthetized by anaesthetic ether and were sacrificed by cervical dislocation. Blood was collected in heparinized tubes from internal jugular vein. Whole brain was dissected and net weight was noted. Immediately the brain was processed as follows. One part of whole brain was homogenized with 9 parts of cold Phosphate buffer (pH 7.4) using Potter Elvehjem homogeniser and the extract was used for estimation of total proteins²⁴ and carbohydrate content of these protein [Glycated protein]²⁵. A second part of brain was homogenized with 9 parts of Chloroform methanol (1:1 v/v) mixture using Potter Elvehjem homogeniser and the extract was used for total lipids²⁶ and carbohydrate content of this lipids [Glycated lipids]²⁵. And another part of whole brain was homogenized with 9 parts of trichloroacetic acid (10%) and extract was used for the estimation of thiobarbituric acid reactive substances (TBARS) levels²⁷. Whole blood was employed for glycated hemoglobin estimation²⁵. A part of whole blood was centrifuged at 3500 rpm for 6-8mins and the free separated plasma was used for glucose estimation²⁸. The free sugar content of phosphate buffer extract was estimated by Folin Wu method²⁹ and the value obtained was deducted from the total carbohydrate content of phosphate buffer protein to calculate glycated protein content.

The results were expressed as mean $\pm$ SD. Statistical analysis was done by using student's *t* test.

III. RESULTS

The results obtained are given in table 1 and 2. Table 1 gives the glycated Hb levels, plasma glucose levels, body weight and ratio of brain to body weight in normal rats (group I), alloxan diabetic rats (group II), as well as in DADS treated alloxan diabetic rats (group III).

As seen from the table, there is a significant increase in plasma glucose levels ($p < 0.001$), glycated hemoglobin levels ($p < 0.001$) body weight and ratio of brain to body weight ($p < 0.001$) in group II as compared

to group I rats. A significant decrease is seen in the above parameters ($p < 0.001$) in group III rats as compared to group II rats. Further no significant alteration is observed in plasma glucose levels in group III rats as compared to group II rats.

Tables 2 shows the levels of brain tissue total proteins, glycated brain proteins, brain tissue total lipids and glycated brain lipids in group I, group II and group III rats. A significant raise in glycated brain proteins ($p < 0.001$), glycated brain lipids ($p < 0.001$) and brain total lipids ($p < 0.001$) were observed in group II rats as compared to group I rats whereas a significant decrease in brain total proteins ($p < 0.05$) was observed in group II as compared to group I. A significant decrease in glycated brain proteins ($p < 0.001$), glycated brain lipids ($p < 0.001$) and brain total lipids ($p < 0.001$) is observed in DADS treated diabetic rats (group III) as compared to diabetic control rats (group II).

IV. DISCUSSION

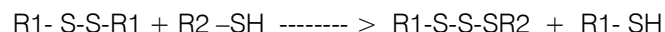
In the present study, administration of alloxan (150mg/kg body weight) induced hyperglycemia in the albino rats as evidenced by elevated plasma glucose levels and glycated hemoglobin levels in group II rats (refer table 1). The levels of glycated hemoglobin have been shown to be an important parameter of chronic glycemic control in diabetes. The decrease in body weight of diabetic rats is due to increase in the protein catabolism mainly in skeletal muscles that helps to channel amino acids for gluconeogenesis, decrease in protein uptake as well as insulin deficiency induced lipolysis³⁰.

There is substantial epidemiological evidence that, besides the long-term complications of diabetes mellitus, which include accelerated atherosclerosis, retinal microvascular damage, renal failure caused by glomerular injury, and peripheral neuropathy, the disease also has multiple effects on the central nervous system. Diabetic patients have at least twice the risk of stroke³¹ and may show performance deficits in a wide range of cognitive domains³². The mechanisms underlying this gradually developing end-organ damage, known as diabetic encephalopathy, are only partially understood and can involve vascular changes and direct damage to neuronal cells by glucose^{33, 34}. Although the high level of glucose in the brain cortex of diabetic rats has been questioned³⁵, it has recently been reported that glucose levels increase by up to three times in the hippocampus of diabetic rats compared with controls³⁶. Emerging evidence suggests that increased glycation leads to the overproduction of superoxide by the respiratory chain and consequent oxidative stress play a role in the pathogenesis of diabetes complications¹⁴.

Many studies have shown that garlic and its compounds exhibit diverse biological activities like anti-

tumorigenic, anti-atherosclerosis, detoxification, anti-inflammatory, antioxidant etc.^{21,37,38}. Also, garlic oil-derived organosulfur compounds such as diallyl trisulphide, diallyl disulphide, and diallyl sulphide provide significant protection against carcinogenesis, and this protection is likely related with their antioxidant properties³⁹. Moreover, the lipophilic characteristics of these compounds allow crossing the blood-brain barrier as follows: diallyl sulfide crosses the blood-brain barrier easier than diallyl disulphide > diallyl trisulfide > S allylcysteine^{20,22}.

DADS, the principle sulphur compound of garlic oil is well known to possess hypoglycemic, hypolipidemic action^{15,16} as well as anti-glycation activity^{4,40}. It is known that DADS may enhance the half life of insulin probably by decreasing the activity of insulinase enzyme by a sulphydryl exchange reaction⁴¹. The results given in table 2 indicates, the glycated protein and lipid levels in brain are significantly decreased in DADS treated diabetic rats as compared to diabetic control rats suggesting that DADS may interfere in the non-enzymatic glycation process. This in part may be due to increased glucose oxidation or due to decreased gluconeogenesis, hence resulting in lesser availability of glucose, thus lowering glycation, as DADS has been suggested to possess hypoglycaemic action. DADS is a disulphide, may be involved in sulphydryl exchange reactions with proteins or enzymes^{42,43} similar to any other disulphide as follows:



Such non-enzymatic glycation in tissue proteins and probably in tissue lipids may induce an alteration in three dimensional structure of tissue proteins and thereby making the protein thiol (-SH) groups vulnerable for oxidative damage⁴⁴. DADS decreases tissue protein glycation as well as tissue lipid glycation, thereby may decrease sulphydryl protein/lipid oxidation and hence preventing the possible tissue damage. This is evidenced by a decrease in brain tissue TBARS levels in DADS treated diabetic rats as compared to alloxan diabetic control rats (refer table II).

V. CONCLUSION

The present study suggests that DADS reduces glycation of brain protein and lipids as well as lipid peroxidation in alloxan diabetic brain tissue thus may be effective in prevention of CNS complications in diabetes mellitus like diabetic encephalopathy, diabetic dementia, etc.

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Table 1 : showing the plasma glucose levels, glycated hemoglobin levels as well as body weight and brain to body weight ratio in Group I, Group II and (Goup III) rats

	Plasma glucose (mg/dl)	Glycated Hb (%)	Body weight (Gms)	Brain wt / body weight ratio
Group I (n=6)	112.26 $\pm$ 19.6	3.9 $\pm$ 1.2	323.81 $\pm$ 55.65	0.0062 $\pm$ 0.004
Group II (n=6)	623.66*** $\pm$ 102.08	16.2*** $\pm$ 1.5	217.85**** $\pm$ 31.40	0.0087* $\pm$ 0.005
Group III (n=6)	565.00 $\pm$ 135.01	12.5*** $\pm$ 1.9	210.16 $\pm$ 50.32	0.0089 $\pm$ 0.004

Note: 1. Number in parentheses indicate the number of animals in each group.
 2. The values are expressed as their mean $\pm$ SD
 3. Significance level * p < 0.05; ** p < 0.01; *** p < 0.001

Table 2 : showing brain tissue total proteins, glycated proteins, total lipids, glycated lipids and brain tissue total TBARS levels in Group I, Group II and Group III rats

	Brain Total Proteins (mg/g)	Brain Glycated Protein (%)	Brain Total Lipids (mg/g)	Brain Glycated Lipids (%)	Brain Tissue TBARS (μ mol/g)
Group I (n=6)	85 $\pm$ 21.21	8.26 $\pm$ 0.98	62.18 $\pm$ 4.09	6.49 $\pm$ 2.22	7.12 $\pm$ 1.67
Group II (n=6)	75 $\pm$ 15.43	9.97**** $\pm$ 0.98	78.30**** $\pm$ 12.66	27.98**** $\pm$ 5.06	13.17**** $\pm$ 2.13
Group III (n=6)	75 $\pm$ 15.43	9.12** $\pm$ 1.37	77.57 $\pm$ 6.54	17.66**** $\pm$ 1.59	9.34**** $\pm$ 1.88

Note: 1. Number in parentheses indicate the number of animals in each group.
 2. The values are expressed as their mean $\pm$ SD
 3. Significance level * p < 0.05; ** p < 0.01; *** p < 0.001

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