Effect of Diallyl Disulphide on Renal Glycated Proteins and Plasma Sialic Acid Levels in Alloxan Diabetic Rats

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Abstract – Diabetes mellitus (DM) induced hyperglycation of cellular and membrane proteins may result in altered ionic nature as well as an alteration in 3 dimensional structures of these molecules, thus resulting in a possible functional variation. Sialic acid (SA), a constituent of glomerular basement membrane (GBM) is a newly established potent indicator for the development of macro and microvascular complications in DM and its elevated levels are observed in DM patients with microalbuminuria and clinical proteinuria. Probably this elevation in SA may be due to increased SA release from the renal GBM due to hyperglycation. This biochemical alteration is the main initiating factor for the pathophysiology of diabetic complication, nephropathy. Diallyl disulphide (DADS), the principle compound of garlic oil, is well known for its anti-diabetic properties. Hence a study was undertaken to assess the anti-glycation properties of DADS and its usefulness in prevention of de-sialation of GBM, in alloxan diabetic kidneys, thereby to establish any beneficial effects of DADS in prevention of renal complications in DM. The current study showed a significant decrease (p<0.001) in kidney glycated proteins and plasma SA levels in DADS treated diabetic rats as compared to diabetic control rats. Hence it can be concluded that DADS helps in preventing glycation of renal proteins and de-sialation of GBM which may be useful in prevention of diabetic nephropathy.

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I. INTRODUCTION

On enzymatic glycosylation of cellular and membrane proteins will be normally proportional to available free glucose in the tissues (1). It can be expected that a consistent hyperglycemia in diabetic subject may induce hyperglycation of tissue proteins, membrane proteins or even membrane lipids. This probably may result in altered ionic nature as well as 3 dimensional structures of these molecules, thus resulting in a possible functional alteration. It has been observed by earlier workers that glycation of collagen of glomerular basement membrane (GBM) alters its structure and function, including changes in net charge (2-4).

Sialic acids (SA), a class of 9 membered ketoses, a common terminal sugar unit of the oligosaccharide of glycoproteins and glycolipids, which are components of GBM proteins. SA plays important role in maintaining negative charge of the renal glomerular basement membrane, one of the main regulators of glomerular permeability (5,6). It may be speculated that vascular permeability is regulated by SA moieties. This SA apparently enter the circulation by either shedding or cell lysis and are of considerable interest because of their potential diagnostic value in various conditions (7-9) including microangiopathies, observed in DM (10).

Garlic (Allium sativum) is one of the most commonly studied medicinal plant worldwide, for its antihyperglycaemic and antihyperlipidemic properties. Diallyl disulphide (DADS), the principle sulphur compound of steam-distilled garlic oil (11,12) is probably responsible for the anti-diabetic, anti-hyperlipidemic, anti-atherogenic as well as anti-carcinogenic actions of garlic (13-17).

Hence a study was undertaken to study the effects of DADS on renal protein glycation and its usefulness in prevention of de-sialation of GBM in alloxan diabetic rats.

II. MATERIALS AND METHODS

Alloxan and Diallyl disulphide (DADS) were procured from Sigma Chemical Company. Sialic acid (analytical grade) was obtained by the courtesy of Biochemistry Department, VM Medical College, Sholapur, India. All other chemicals employed were of analytical grade. Male albinos rats, weighing 200-250g randomly selected from Central Animal House, BMCH, Chitradurga, India, were used for the present investigation. The animals were maintained on a standard rat feed from Amrut rat feeds, Bangalore, supplied by Pranav Agro Industries, Pune, India. 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.) (18), in sterile normal saline. The animals were considered diabetic if their blood glucose were above 250mg/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 anaesthetised and were sacrificed. Blood was collected in heparinised tubes. Kidneys were dissected and their net weight was noted. Immediately the kidneys were processed as follows. One part of kidney was homogenised with 9 parts of cold Phosphate buffer (pH 7.4) and the extract was used for total proteins (19) and carbohydrate content of these protein [Glycated protein] (20). The free sugar content of phosphate buffer extract was estimated by Folin Wu method (19) and the value obtained was deducted from the total carbohydrate content of phosphate buffer protein to calculate glycated protein content. A part of whole blood was centrifuged at 3500 rpm for 6-8mins and the plasma was used for estimation of glucose (19) and sialic acid levels (21). Part of renal tissue was collected in buffered formalin and was processed for histopathological studies.

### III. Results

Results obtained in the present study are elaborated in Table 1.

### IV. Discussion

The most severe chronic complication of DM is nephropathy, which involves a definite alteration in GBM thickness as well as GBM composition. There are several reports indicating alterations in GBM in diabetes which is thought due to hyperglycation of GBM proteins (4) and possibly including renal lipids also. The results obtained in present study which is narrated in table 1 clearly indicates that there is a significant increase in protein glycation (p < 0.001) in alloxan diabetic rats as compared to normal rats. This hyperglycation of renal proteins including GBM proteins may lead to altered functioning of these proteins which may result in changed GBM functions leading to the renal complications.

Sialic acid contributes to the maintenance of the negative charge of the renal glomerular basement membrane (22). It is well established that vascular endothelium carries a high level of SA (23), and the vascular damage leads to its release into the circulation. It has been reported that serum SA levels are increased in diabetic patients with albuminuria (24) and further, several authors found the increased urinary concentration of SA in diabetic patients with microangiopathy (25). In addition, decrease in SA content in glomerulus is observed in human diabetes and also in alloxan diabetic rats (26). A relationship between serum SA levels and microvascular complications has been observed before, in diabetic patients with microalbuminuria and clinical proteinuria (24).

Protein hyperglycation, a common phenomenon observed in DM, is responsible for glycation of tissue and membrane proteins, resulting in misorientation and malfunction of these proteins. This glycation of membrane proteins do alter the 3 dimensional structure (4) as well as ionic nature of protein resulting in the altered orientation of its domains. Such an alteration in the GBM proteins may lead to exposure of the sialated portions of the membrane proteins, hence making them easily accessible for the action of sialidase enzyme. This may lead to removal of SA which in part may account for the increased plasma SA levels which is in agreement with the results given in table I. The observed elevated SA levels in alloxan diabetic rats is in agreement with earlier studies in diabetic nephropathy (24,27,28). A parallel raise in plasma SA levels along with increased glycation of renal proteins (ref. table I) indicates, the raised SA observed in alloxan diabetic rats might have araised from desialation of GBM proteins which may be due to glycation induced 3 dimensional alteration in GBM proteins. This is in agreement with the Studies of Alvaro C et al (29) and Lisette CF et al (30). This de-sialation of GBM proteins may alter their ionic nature, a decrease in negative charges leading to percolation of albumin which probably results in microalbuminuria, a predisposing factor observed prior to frank nephropathy.

A significant decrease in plasma SA levels (p<0.001) as well as a significant decrease in renal protein glycation (p<0.001) in DADS treated alloxan diabetic rats (group II rats) decisively indicates that DADS got a significant role in inhibiting protein glycation in alloxan diabetic rats. DADS, a disulphide, may be involved in sulphydryl exchange reactions with proteins or enzymes (31) similar to any other disulphide as follows:

\[
R_1\cdot S-S-R_1 + R_2 \cdot SH \rightarrow R_1\cdot S-S-R_2 + R_1\cdot SH
\]

Sialidase being a sulphydryl enzyme (32), might have undergone similar sulphydryl exchange reaction with DADS, that might have lowered the activity of sialidase enzyme, thereby retaining SA residues on GBM proteins, hence maintaining its shape as well as their negative ionic nature, thus preventing the percolation of albumin. This hypothesis is further supported by decreased plasma SA levels in the DADS treated diabetic rats, observed in present study (ref. table I) and supported by histopathological studies (ref.
figure 1, 2, 3), suggesting a usefulness of DADS in prevention of renal complication in alloxan diabetic rats.

Hence it can be concluded that DADS, a disulphide may decrease GBM protein glycation and prevents de-sialation of GBM, thus retaining the normalcy of GBM proteins resulting in delaying or decreasing diabetes induced renal changes. Thereby DADS may be useful in prevention of renal complication in alloxan diabetic rats. Further studies with respect to other animal models may certainly prove the therapeutic application of DADS in prevention of development of diabetic nephropathy.

REFERENCES Référence Referencias


Table 1 shows the plasma glucose levels, plasma sialic acid levels along with renal glycated proteins of normal rats (group I), alloxan diabetic control rats (group II) and DADS treated alloxan diabetic rats (group III):

<table>
<thead>
<tr>
<th></th>
<th>Plasma glucose mg/dl</th>
<th>Renal Total Proteins mg/g</th>
<th>Renal Glycated Protein %</th>
<th>Plasma Sialic acid mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n=6)</td>
<td>112.26 ± 19.6</td>
<td>145 ± 32.01</td>
<td>5.8 ± 1.7</td>
<td>66.12 ± 9.40</td>
</tr>
<tr>
<td>Group II (n=6)</td>
<td>623.66*** ± 102.08</td>
<td>120* ± 24.5</td>
<td>22.5*** ± 5.0</td>
<td>100.32*** ± 15.12</td>
</tr>
<tr>
<td>Group III (n=6)</td>
<td>565.00 ± 135.01</td>
<td>110 ± 22.36</td>
<td>13.1*** ± 3.0</td>
<td>84.82** ± 10.24</td>
</tr>
</tbody>
</table>

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

Fig. 1: Normal rat kidney — slide showing normal glomeruli indicated by arrows. (H&E X10).
Fig. 2: Alloxan diabetic rat kidney -- slide showing oedematous glomeruli indicated by arrows along with increased cellular infiltration and glycogen granules. (H&E X10).

Fig. 3: DADS treated alloxan diabetic rat kidney – slide showing normal glomerulus indicated by arrows. (H&E X10).