Control of hyperglycemia and retardation of cataract by mulberry (Morus indica L.) leaves in streptozotocin diabetic rats

B Andallu
Department of Home Science, Sri Sathya Sai Institute of Higher Learning, Anantapur 515001, India

and

N Ch Varadacharyulu
Department of Biochemistry, Sri Krishnadevaraya University, Anantapur 515 001, India

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Dried leaf powder of mulberry (M. indica L.) when given along with the diet at 25% level to streptozotocin induced diabetic male Wistar albino rats for 8 weeks, controlled hyperglycemia, glycosuria, albuminuria and retarded onset of retinopathy. Untreated diabetic rats showed hyperglycemia, glycosuria, albuminuria and developed lenticular opacity after 8 weeks of experimental period.

Diabetes mellitus is one of the oldest diseases known to mankind and yet with the tremendous scientific advances witnessed in this century, medicinal science can not claim that it knows all that needs to be known about this disease, including its management. This is the main reason for the persistent interest all-over the world to explore alternative remedies from the so called 'alternative systems' of medicine.

A large no. of plants were screened in India and else where for their hypoglycemic activity; mulberry is one of such plants having tremendous therapeutic applications but not exploited as a medicinal plant so far. Extracts of various parts of the plant, viz. methanolic extract of root bark, ethanol-insoluble extract of the leaves, aqueous extract of the leaves of shoot cultures and the leaves were reported to possess antidiabetic effect.

Retinopathy, neuropathy and nephropathy are identified in chronic diabetes failing which the disease is called impaired glucose tolerance. Therefore in this study the retardation of diabetic cataract, control over hyperglycemia, glycosuria and albuminuria by mulberry leaves have been investigated and reported.

Materials and Methods

Male Wistar albino rats (24) weighing 150-200g procured from germ free animal house of National Center for Laboratory Animal Sciences (NCLAS), Hyderabad, were housed in gridded cages in an air conditioned room where the congenial temperature of 25°±1°C and 12 hr light and dark cycle were maintained. The animals were allowed to acclimatize to the environment for 7 days and were divided into following 4 groups of 6 each:

Gr.1: Normal control
Gr.2: Normal rats treated with mulberry leaf powder
Gr.3: Diabetic control
Gr.4: Diabetic rats treated with mulberry leaf powder

The animals of groups 3 and 4 were rendered diabetic by a single ip injection of streptozotocin freshly prepared in 0.1M citrate buffer (pH 4.5) at a dose of 55mg/kg body wt. after an overnight fast. The animals of the groups 1 and 2 were injected with citrate buffer alone. The streptozotocin treated animals were given 5% glucose water for 24 hr following streptozotocin injection to prevent initial drug induced hypoglycemic mortality. After 72 hr of streptozotocin injection, blood was drawn from retro-orbital plexus of the rats and the fasting blood glucose levels were estimated by the method of Hugget and Nixon.

Fresh, young mulberry leaves (4th and 5th from the apex) collected from Regional Sericultural Research Station, Raptadu, Anantapur dist., were washed, shade dried, powdered and used. The leaves are rich in protein (23%), ash(15%) and fiber(13.8%) (dry wt. basis).

The feed for the animals was procured from the National Center for Laboratory Animal Sciences(NCLAS) in powder form to facilitate easy...
mixing of the mulberry leaf powder. The experimental animals of groups 1 and 3 were given standard diet whereas the animals of groups 2 and 4 were given experimental diet prepared by incorporating mulberry leaf powder in the standard diet at 25% level which was determined on the basis of dose response studies. Feed and water (boiled and filtered) were provided ad libitum in clean cups and feeding bottles respectively at 08:00 hrs daily for 8 weeks.

Fasting blood glucose levels were monitored at weekly intervals. At the end of 8 weeks of experimental period, fasting blood glucose, urinary glucose, glycosylated haemoglobin (HbA1c) serum and urinary albumin were determined. The data were statistically analysed by applying analysis of variance (ANOVA) to assess the significant differences among the variation of the groups.

**Results and Discussion**

The food consumption in diabetic control was increased significantly by 12% \((P<0.05)\) compared to normal control, which may be attributed to the polyphagic condition symptomatic of diabetes (Table 1).

An insignificant decrease (3%) in food consumption was exhibited by diabetic group treated with mulberry when compared with diabetic control, which may be considered as a positive sign in controlling polyphagia.

No significant difference was found in normal control and normal treated with mulberry with respect to food consumption which indicates that consumption of food in the experimental animals is not effected by the incorporation of mulberry leaves at 25% level in the diet as the leaves were reported to be nutritious and palatable.

A tremendous decrease in body weight was seen in diabetic control when compared with normal animals \((P<0.05)\) which is due to increased muscle wasting in diabetes. In diabetic rats treated with mulberry, an increase in body weight was seen when compared with untreated \((P<0.05)\) which shows that mulberry treatment controlled muscle wasting i.e., reverted the gluconeogenic condition.

**Fasting blood glucose and urinary glucose** — The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues.

Weekly blood glucose levels for a period of 8 weeks in different groups under investigation indicate maintenance of blood glucose in normal rats and normal rats treated with mulberry; uncontrolled hyperglycemia in STZ rats and glycemic control in STZ rats treated with mulberry (Fig. 1).

Initial and final fasting blood glucose and urinary glucose levels of the different groups under investigation revealed a significant elevation \((P<0.01, 274\%)\)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Food consumption (g/week)</th>
<th>Change in body weight (g/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>20.43 ±0.85*</td>
<td>-10.46±1.34*</td>
</tr>
<tr>
<td>Normal + mulberry</td>
<td>19.80 ±0.86*</td>
<td>-11.65±1.17*</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>18.32 ±0.77</td>
<td>+19.31±0.37</td>
</tr>
<tr>
<td>Diabetic + mulberry</td>
<td>17.58 ±1.15</td>
<td>+15.19±0.67*</td>
</tr>
</tbody>
</table>

Comparison between groups: 1 and 2; 1 and 3; 3 and 4

*\(P < 0.05\)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>95.05±9.77</td>
<td>—</td>
</tr>
<tr>
<td>Normal + mulberry</td>
<td>98.15±14.71</td>
<td>—</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>266.23±8.43</td>
<td>++++</td>
</tr>
<tr>
<td>Diabetic + mulberry</td>
<td>283.50±8.26</td>
<td>++++</td>
</tr>
</tbody>
</table>

Comparison between groups: 1 and 2; 1 and 3; 3 and 4

** ** \(P < 0.01\)

Urine sugar (-) indicates nil and (++++) indicates more than 2%
in blood glucose in diabetic controls when compared
with normal animals at the end of 8 weeks of experi-
mental period (Table 2). This increase indicates un-
controlled hyperglycemia in STZ injected animals.

Fasting blood glucose levels in diabetic mulberry
treated group were reduced significantly (P<0.01) by
69% when compared to diabetic control group which
indicates that mulberry leaves are very effective in
controlling hyperglycemia. Similar observation was
made by Henri Leclerc in M. nigra treated diabetic
subjects15.

No significant difference in the blood glucose lev-
es of normal control and normal treated groups indi-
cates that mulberry leaves maintain glucose homeo-
stasis in normal conditions.

Glycosuria was observed in diabetic controls
throughout the experimental period while it controlled
gradually and no urinary glucose was noticed after
eight weeks of feeding of mulberry which is in accor-
dance with control of blood glucose in mulberry
submitted group (Table 2).

The present observation (antidiabetic) of hypogly-
cemic influence of mulberry leaves concurs with the
observation of Kelkar et al.4 who found a beneficial
effect of single dose of aqueous extract of shoot cul-
ture of Morus indica L in STZ induced diabetic rats
while the present study reports influence of long term
feeding of mulberry leaf powder on STZ induced dia-
betes.

The hypoglycemic response of mulberry leaves
could be attributed to the high fibre content (13.85%)
of mulberry leaves6 and/or due to the presence of
trigonelline bases15 in mulberry similar to that isolated
from fenugreek and/or due to the presence of Moran
A, which showed good glycemic response in alloxan
diabetic mice6.

Glycosylated haemoglobin (HbA1c)—Glycosylated
haemoglobin is a good measure to indicate the average
blood glucose concentration over the preceding
weeks while a single glucose determination gives a
value which is true only at the time the blood sample
is drawn16. Hb A1c is formed progressively and irre-
versibly over a period of time and is stable till the life
of the RBC and is unaffected by diet, insulin or exer-
cise on the day of testing17.

Table 3 indicates a significant (P<0.01) increase
(48%) in glycosylated hemoglobin levels in diabetic
control group and this Hb A1c level is in accordance
with earlier reports18,19. Treatment with mulberry for 8
weeks dropped the levels by 23% (P<0.01) from that
of diabetic control group, which is almost equivalent
to the levels of normal control group. Besides, mul-
berry treatment showed a 6% reduction in HbA1c levels
in normals treated with mulberry.

The increased HbA1c levels in the diabetic control
group indicate that erythrocytes are more prone to
oxidative stress in diabetes. The abnormal hemoglo-
bins are associated with a reduction in red cell life span20.

The glycosylated hemoglobin lowering effect of
mulberry treatment is slightly lesser than insulin ther-
apy for 4 weeks reported by Tilvis et al.21, equal to
that of D-400, a herbomineral formulation19 and bet-
ter than that of fenugreek seeds 22 which showed a
reduction of 27, 23, and 12.5% in HbA1c levels
respectively. Therefore, prolonged intake of mulberry
may further reduce HbA1c levels and probably helps
in achieving a better glycemic control.

Serum albumin—Excessive breakdown of body
protein in conjunction with either inadequate supply
or defective utilization observed in uncontrolled dia-
betes may be accompanied by hypoalbuminemia17.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycosylated hemoglobin (%)</th>
<th>Serum albumin (mg/dl)</th>
<th>Urine albumin (mg/24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.51±0.38</td>
<td>4.66±0.20</td>
<td>2.10±0.35</td>
</tr>
<tr>
<td>Normal + mulberry</td>
<td>2.36±0.34</td>
<td>4.67±0.24</td>
<td>1.98±0.20</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3.72±0.35**</td>
<td>2.50±0.39**</td>
<td>22.01±0.38**</td>
</tr>
<tr>
<td>Diabetic + mulberry</td>
<td>2.87±0.17**</td>
<td>3.62±0.19**</td>
<td>9.05±0.15**</td>
</tr>
</tbody>
</table>

Comparison between groups: 1 and 2; 1 and 3; 3 and 4
** P < 0.01

Fig. 1—Weekly blood glucose (mg/dl) levels in different groups of rats
Table 3 represents a significant decrease (46%) in serum albumin concentration in diabetic condition while mulberry treatment significantly improved ($P<0.01$, 45%) albumin levels in the blood which indicates control over the break down of body protein by mulberry leaves. The values of serum albumin after treatment, however, were still below the normal concentration.

*Urinary albumin*—Excretion of albumin in urine is observed in diabetes mellitus. In the present study, albuminuria was noticed in diabetic controls throughout the period. The diabetic animals excreted 11 fold excess albumin when compared to normal rats while the amount of albumin excretion was significantly lower (59%) in mulberry treated diabetic animals, although it is still much higher than normal.

*Diabetic retinopathy*—Diabetic retinopathy is primarily a disease of retinal vascularisation which may later involve the retina and vitreous.

In the present study, development of cataract in diabetic controls was noticed after 8 weeks of experimental period (Fig. 2). This effect was due to high blood sugar sustained for 8 weeks in untreated hyperglycemic rats which invariably lead to the formation of lenticular opacity, while no traces of development of cataract were observed in mulberry treated diabetic rats (Fig. 3) which corresponds with the controlled hyperglycemia in this group. This is in accordance with the observation of Srivastava where in aqueous extract of *Momordica charantia* was orally administered to alloxan diabetic rats for 2 months.

Mulberry treatment significantly countered some of the abnormalities of experimentally induced diabetes. In addition, retarded the development of cataract. It did not show much influence on normal animals which indicates discriminative and adaptive capacity of mulberry leaves.

**Acknowledgement**

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**References**

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