SUMMARY
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There has been a great demand for the discovery and supply of plant derived novel molecules / products which can be used as herbal medicines and / or therapeutic dietary supplements. Moreover, native preparations from leaves and other plant parts contain both dotes and antidotes to alleviate the undesirable effects if any, than isolated principles / drugs which may exert side / toxic effects often. Therefore, 88 % of global population turned to herbal medicines containing native preparations / dietary therapeutics.

Diabetes is a highly prevalent multifactorial disease. People of Indian origin are more prone to diabetes. Owing to the side effects, prohibitive cost of various drugs, and due to their limitations in exerting action against diabetic sequelae, temporary control over hyperglycemia, a multimodal therapeutic approach is warranted. Mulberry with its wide occurrence appears to open new avenues for the treatment of diabetes and related complications. Though mulberry leaves and other parts of the plant are known to exert antihyperglycemic effect in experimentally induced diabetic animals and humans, no systematic study was carried out on the related aspects. Further, the precise mechanism of action of antihyperglycemic effect of mulberry leaves is not completely understood. Hence, the present study was aimed at investigating the antihyperglycemic effect of mulberry leaves when administered through diet (MLP diet) and to understand the role of MLP diet in the control of various metabolic abnormalities and complications of diabetes.

The results of the present study clearly demonstrated the antidiabetic property and various other beneficiary effects of mulberry (Morus indica L. cv. Anantha) leaves when supplemented through diet. Hyperglycemia is the hallmark of diabetes. The results from this study indicated that MLP diet exerted antihyperglycemic effect selectively in experimentally induced diabetic rats and the degree of response varied with dosage with a maximal effect with control over glycosuria at 25% level of supplementation for 8 weeks. This effect appears to be brought about by the involvement of various specific (DNJ, Gal-DNJ, fagomine etc.) as well as nonspecific phytochemical profile (fibre, minerals, vitamins etc.) of mulberry leaves. Besides, MLP diet has relieved the animals from certain diabetic symptoms such as loss of
body weight and hyperphagia. The MLP diet could effectively counter the general abnormalities that are associated with metabolism in diabetic condition such as accumulation of pyruvate, lactate, free amino acids and free fatty acids by restoring these constituents partially to that of control values. Moreover, such partial restoration was also seen in the activities of some key glycolytic enzymes (hexokinase and lactate dehydrogenase in erythrocytes, liver and kidney), gluconeogenic enzymes (glucose 6 phosphatase, fructose 1,6 diphosphatase, phosphoenol pyruvate carboxy kinase in liver and kidney), amino transferases (serum and kidney) and acid and alkalkine phosphatases (serum, liver and kidney) in diabetic rats fed with MLP diet. Mulberry leaf principles already identified such as deoxynojirimycin (DNJ), galactopyranosyl-DNJ, Moran A, fagomine and some unidentified phytochemicals might have played a role in this regard for the observed effects.

The depleted antioxidant status and decreased activities of defense enzymes in liver and erythrocytes in diabetic rats were effectively reversed by MLP diet. Tissues of rats receiving MLP diet could escape from the oxidative stress and lipid peroxidative damage of plasma, tissues and membranes by the action of nutritive (β carotene, ascorbic acid, histidine etc.,) and phytochemical free radical scavengers (flavonoids, moracins, quercetin etc.,). Besides, enhancement of activities of defense enzymes and glycemic control achieved through MLP diet might have contributed for restoration. MLP diet could effectively restore the impaired mineral, electrolyte and vitamin levels in diabetic rats.

Lipid abnormalities and alterations in lipoprotein patterns were effectively countered by MLP diet. Results clearly demonstrate the hypolipidemic action exerted by the specific (β sitosterol, saponins etc.,) and nonspecific active principles such as fibre of mulberry leaves indicate the efficacy of mulberry leaf components because various widely used drugs and phytochemicals can achieve euglycemia but fail in rectifying diabetic lipid abnormalities. It is tempting to speculate that MLP diet could retard diabetic cataract development and also beneficially modulated the activities of enzymes of polyol pathway. In addition, MLP diet reduced the risk of lipid peroxidative damage of lens in diabetic rats in which enhanced GSH levels might have played a role.
MLP diet could increase plasma insulin levels of diabetic rats by successfully reversing streptozotocin induced diabetes as evidenced by decreased plasma glucose and HbA1c levels which were also attested by histopathological evidences of rat pancreatic islet section microphotographs indicating pancreoprotective effect exerted by mulberry leaf components. Results also indicated that mulberry leaf powder is superior to glibenclamide, a widely used hypoglycemic drug. Analysis of nutritional components of mulberry leaves revealed the usefulness of mulberry leaves as dietary supplements for they are naturally enriched with all the essential macro and micronutrients in addition to therapeutic principles.

The observed beneficial effects of mulberry leaves are postulated to be brought about by the net resultantance of interactions of various identified specific principles (DNJ, GAL-DNJ, fagomine, moran A, quercetin, isoquercitrin, moracins etc.,) and unidentified nonspecific compounds. Besides, its nutrient composition also played a role as diabetic therapy is indispensably associated with therapeutic nutrition. The presence of antioxidants and other miscellaneous compounds such as phytates, saponins, polyphenols might have contributed to a large extent for the observed beneficiary effects exerted by mulberry leaves. The salient features of the present study are:

♦ Maximal antihyperglycemic effect in STZ-diabetic rats at 25% level of mulberry leaf powder supplementation through diet.
♦ Correction of abnormalities and derangements in hematological parameters, erythrocyte indices, blood biochemical parameters i.e metabolites, electrolytes, minerals and vitamins by mulberry leaf powder supplementation.
♦ Antioxidant role exhibited by mulberry leaf powder supplementation.
♦ Rectification of zig zags in the activities of various glycolytic and gluconeogenic enzymes by mulberry leaf powder supplementation.
♦ Hypolipidemic action and retardation of cataract by mulberry leaf powder diet therapy.
♦ Protection of pancreatic β cells in STZ-diabetic rats by mulberry leaf powder diet.
♦ Presence of various nutrients, fibre, phytates, saponins and polyphenols in mulberry leaves.
Publications


Papers under communication


ABSTRACT
Mulberry leaves, rich in protein, fibre, minerals and vitamins C, contain trigonelline bases, glycoprotein Moran A, which have been found to possess antidiabetic effect. Inclusion of the dried leaf powder at 25% level in the diet of diabetic rats for a period of 60 days, significantly decreased blood glucose, glycosylated haemoglobin (HbA1c) and activities of serum enzymes viz., lactate dehydrogenase, acid and alkaline phosphatases, glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT).

KEYWORDS : Mulberry leaves : Diabetes mellitus : Hypoglycemic effect; Serum transaminases.

INTRODUCTION
Mulberry (Morus indica L.) leaves, the sole feed for silk worm, possess many medicinal properties. The leaves were reported to possess hypoglycemic [1,2], hypotensive [3] antipyretic and antiinflammatory [4] effects. These effects of mulberry leaves have been ascribed to a glycoprotein Moran A [5], trigonelline bases [6] Moranoline [7] and Morin [8]. Present paper deals with a comparative study on the effect of mulberry leaves with that of a standard drug-glibenclamide on blood glucose, glycosylated hemoglobin levels and on the activity of certain enzymes in the blood in diabetic rats.

MATERIALS AND METHODS
Fresh, young (4th and 15th) mulberry leaves (Morus indica L.) were procured in bulk from Regional Sericultural Research Station, Rapthadu, Anantapur district, Andhra Pradesh. They were washed thoroughly under running tap water, shade dried for three days and powdered in an electric mixer.

Experimental animals
Male Wistar albino rats (30 in number) with body weights ranging from 130 to 150 grams, procured from the germ free animal house of National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, were used as experimental animals and were maintained as per the specifications of National Centre for Laboratory Animal Sciences. The animals were distributed into five groups according to similar weights with six animals in each group:

Group 1 – Normal control
Group 2 – Normal experimental- normal rats treated with mulberry leaf powder
Group 3 – Diabetic control
Group 4 – Diabetic experimental- diabetic rats treated with mulberry leaf powder
Group 5 – Diabetic experimental- diabetic rats treated with oral hypoglycaemic drug- glibenclamide

Induction of diabetes
Diabetes was induced by a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 55mg/kg body weight in 1ml of freshly prepared 0.1M citrate buffer (pH 4.5) after an overnight fast. The control rats were injected with citrate buffer alone. The animals were given 5% glucose water for 24 hours following STZ injection to prevent initial drug induced hypoglycemic mortality. After 72 hours of administration of injection, fasting blood glucose levels were determined by the method of Hugget and Nixon [9] in these STZ injected rats in the blood drawn from retro-orbital plexus. Rats with blood glucose levels above 225 mg/dl were distributed into the three diabetic groups: viz, diabetic control, diabetic treated with mulberry mixed diet and diabetic group treated with oral hypoglycemic drug.

Animal housing facility
All the animals were housed in grilled cages in an air-conditioned room wherein a congenial temperature of 23 ± 1°C and the twelve hours light and dark cycle were maintained.

Feeding procedure
The animal feed, used in the experiment was procured from the National Centre for Laboratory Animal Sciences in the powder form to facilitate easy and uniform mixing of mulberry leaf powder in the feed. Water was boiled; filtered and the pH was made slightly acidic to prevent microbial growth. Feed and water were provided ad libitum in clean cups and
Moran A [5] and/or due to Moranoline [7].

There was no significant difference in the blood glucose levels of normal control and normal treated groups indicating that mulberry maintains glucose homeostasis in normal conditions also even after administration of mulberry leaves at 25% level.

Glycosylated Hemoglobin (HbA₁c)

HbA₁c 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 drawn [18]. HbA₁c is formed progressively and irreversibly over a period of time and is stable till the life of the RBC and is unaffected by diet, insulin or exercise on the day of testing [19].

Table 1 indicates a significance (p<0.01) increase (3.5%) in glycosylated haemoglobin levels in diabetic control group. On treatment with mulberry leaves the level dropped by 30% (p<0.01) of that of diabetic control group, which is almost equal to the levels of normal control group. Glibenclamide group showed a significant decrease of 6% (p<0.01) when compared to diabetic control, but to a lesser extent when compared to diabetic group treated with mulberry leaves.

The present study indicates that hyperglycemia can enhance protein glycation. The increased HbA₁c levels in the diabetic control group indicate that erythrocytes are more prone to oxidative stress in diabetes. The longer the exposure of erythrocytes to hyperglycemia, the shorter is its life span.

The glycosylated hemoglobin lowering effect of mulberry leaves treatment is better than insulin therapy reported by Tilvis et al., (20) for a period of four weeks; D-400, a herbo-mineral formulation (21) and fenugreek seeds (22) which showed a reduction of 27, 23, and 12.5% in HbA₁c respectively. Therefore, prolonged intake of mulberry leaves may further reduce HbA₁c levels and probably help in achieving better glycemic control.

Enzymes in serum

Lactate dehydrogenase (LDH)

There is a significant increase in the activity of lactate dehydrogenase in diabetes, which could be due to excessive accumulation of pyruvate. This excessive pyruvate is converted to lactate for which LDH is needed and therefore the activity of LDH may be increased due to less insulin availability in diabetes (23).

Table 2- Activities of Lactate Dehydrogenase, Acid and Alkaline Phosphatases in Serum

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>LDH (IU/I)</th>
<th>Acid phosphatase (KA units/dl)</th>
<th>Alkaline phosphatase (KA units/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal-</td>
<td>344.8</td>
<td>7.1</td>
<td>64.42</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>+7.2</td>
<td>+4.8</td>
<td>+5.6</td>
</tr>
<tr>
<td>2.</td>
<td>Normal-</td>
<td>374.9</td>
<td>28.5</td>
<td>68.0</td>
</tr>
<tr>
<td></td>
<td>mulberry</td>
<td>+5.4</td>
<td>+4.0</td>
<td>+7.0</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Diabetic-</td>
<td>714.7**</td>
<td>68.4**</td>
<td>139.3**</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>+13.6</td>
<td>+4.8</td>
<td>+11.7</td>
</tr>
<tr>
<td></td>
<td>(110)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic-</td>
<td>460.8**</td>
<td>41.8**</td>
<td>90.0**</td>
</tr>
<tr>
<td></td>
<td>mulberry</td>
<td>+9.5</td>
<td>+4.6</td>
<td>+6.0</td>
</tr>
<tr>
<td></td>
<td>(36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Diabetic-</td>
<td>561.9**</td>
<td>48.9**</td>
<td>137.4</td>
</tr>
<tr>
<td></td>
<td>gliben-</td>
<td>+7.5</td>
<td>+4.6</td>
<td>+5.7</td>
</tr>
<tr>
<td></td>
<td>clamide</td>
<td>(27)</td>
<td>(29)</td>
<td>(2)</td>
</tr>
</tbody>
</table>

Values are mean ±SD of 6 animals in each group. Figures in parentheses indicate percent increase/decrease. Comparison between groups : 1 and 2; 1 and 3; 3 and 4; 3 and 5; & 4 and 5. ** P < 0.01

Table 2 indicates the activity of lactate dehydrogenase in serum of all the five groups under investigation. An enormous increase of 110% was noticed in LDH activity in diabetic control when compared with that of normal control which indicates increased gluconeogenesis in uncontrolled diabetes, involving increased conversion of alanine to pyruvate which is evidenced by increased levels of pyruvate and lactate resulting in lactic acidosis (24). Such an elevation in pyruvate levels requires the activity of LDH to convert pyruvate to lactate due to less insulin availability in diabetes (25).

Mulberry leaves treatment decreased (36%, p<0.01) LDH activity indicating control over gluconeogenesis. Glibenclamide treatment also decreased the activity of the enzyme but to a lesser extent (27%) when compared to mulberry treatment.

The influence of mulberry leaves on the activity of LDH was similar to the effect of Coccinia indica which caused a 33% decrease in the enzyme activity (25) and higher than the effect of S-allyl cysteine sulphone isolated from garlic which showed a 13% fall in the activity of the same enzyme when fed to alloxan diabetic rats (10).

There was a significant decrease in LDH activity in normal animals treated with mulberry leaves as compared to untreated animals.
by 46% when compared to untreated diabetic animals indicating that mulberry leaves controlled the rate of gluconeogenesis in diabetes. Glibenclamide treatment could not decrease the activity of GOT, but could decrease the activity of GPT, but to a lesser extent when compared to mulberry leaves treatment. This denotes that glibenclamide could not effectively control gluconeogenesis. No significant difference was noticed in the activities of these enzymes in normal control and treated animals.

REFERENCES