EFFECT OF MULBERRY LEAVES ON DIABETES

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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 (HbA_{1c}) and activities of serum enzymes viz., lactate dehydrogenase, acid and alkaline phosphatases, glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT).

KEY WORDS : 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 ± 1ºC 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

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water bottles respectively at specific timings (8 am daily). Control groups 1 and 3 received the standard diet while the experimental groups 2 and 4 received experimental diet prepared by incorporating mulberry leaf powder at 25% level, determined after preliminary trials. Animals of group 5 received standard feed, wherein the drug (glibenclamide, 0.5 mg/kg body wt. per day)(10) was mixed with a small amount of feed and the remaining feed was given after the consumption of the drug mixed feed.

Collection of blood

At the initial stage and after 60 days of experimental period, glucose was estimated in the blood collected from retro-orbital plexus after an overnight fast. It was also analysed for glycosylated hemoglobin and various enzymes.

Assay methods

The methods used to analyse various parameters are listed below:

Parameter	Method	
Fasting blood glucose		Huggest and Nixon (1957)[9]
Glycosylated hemoglobin		Eross et al. (1984)[11]
Lactate dehydrogenase	EC 1.1.1.27 -	Method of Henry et al (1960)[12]
Acid phosphatase	EC 3.1.3.2 -	Gutman and Gutman (1940)[13]
Alkaline phosphatase	EC 3.1.3.1 -	Method of Kind and Armstrong (1934)[12]
Glutamate pyruvate transaminase (GPT)	EC 2.6.1.2	Bergmeyer and Bernt (1974)[14]
Glutamate oxaloacetate transaminase (GOT)	EC 2/.6.1.1	"

RESULTS

Diabetes increases blood glucose, glycosylated hemoglobin(HbA_{1C}) and the activity of certain enzymes in the blood. Effect of administration of mulberry leaf powder and glibenclamide on blood glucose and glycosylated hemoglobin levels in diabetic rats is given in Table 1. Table 2 shows the effect on the activities of serum enzymes i.e lactate dehydrogenase, acid and alkaline phosphatases, and Table 3 depicts the effect on GOT and GPT.

DISCUSSION

Fasting blood glucose

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over production (excessive hepatic glycogenolysis and gluconeogenesis) as well as decreased utilization of glucose by the tissue [15].

Table 1 shows the initial and final fasting blood glucose and glycosylated haemoglobin levels of the different groups under investigation. A significant ($P<0.01$) elevation of 74% in blood glucose was observed in diabetic controls when compared with normal animals at the end of 60 days of experimental period. This increase indicates uncontrolled hyperglycaemia in STZ injected animals.

Table 1- Blood Glucose and Glycosylated Haemoglobin (HbA_{1c})

S.No.	Groups	Blood glucose (mg/dl)		HbA_{1c}
		Initial	Final	(%)
1.	Normal-control	91.2 +7.7	95.7 +5.5 (4)	2 .51 +0.38
2.	Normal-mulberry	91.7 +9.0	95.2 +10.7 (6)	2 .36 +0 .36 (6)
3.	Diabetic-control	266.2 +8.4 (74)	369.4** +12.9 (35)	3.72** +0.35
4.	Diabetic-mulberry	294.6 +8.3	187.9** +7.6 (50)	2.87** +0.17 (30)
5.	Diabetic-glibenclamide	291.75 +11.27	266.5** +11.3 (28)	3.51** +0.32 (6)

*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*

Fasting blood glucose levels in diabetic mulberry treated group were reduced significantly ($P<0.01$) by 50% when compared to diabetic control, while a 28% decrease was only noticed in glibenclamide treated group, which indicates that mulberry leaves are more effective in controlling hyperglycemia than the oral hypoglycemic drug-glibenclamide.

Curry leaves powder supplemented to type 2 diabetic subjects showed a significant ($p<0.01$) reduction (13%) in fasting blood sugar levels which was attributed to the presence of fibre [16]. Similarly, the hypoglycemic activity of mulberry leaves could be attributed to the high fibre content (13.85%) of mulberry leaves [17] and/or due to the presence of trigonelline bases [6] in mulberry leaves similar to that isolated from fenugreek and/or due to the presence of

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 (HbA_{1c})

HbA_{1c} is a good measure to indicate the average blood glucose concentration over the preceeding weeks while a single glucose determination gives a value which is true only at the time the blood sample is drawn [18]. HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} respectively. Therefore, prolonged intake of mulberry leaves may further reduce HbA_{1c} 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

S.No.	Groups	LDH (IU/l)	Acid phosphatase (KA units/dl)	Alkaline phosphatase (KA units/dl)
1.	Normal-control	344.8 ±7.2	7.1 ±4.8	64.42 ±5.6
2.	Normal-mulberry	374.9 ±5.4 (9)	28.5 ±4.0 (5)	68.0 ±7.0
3.	Diabetic-control	714.7** ±13.6 (110)	68.4** ±4.8 (60)	139.3** ±11.7 (116)
4.	Diabetic-mulberry	460.8** ±9.5 (36)	41.8** ±4.6 (39)	90.0** ±6. (35)
5.	Diabetic-gliben-clamide	561.9** ±7.5 (27)	48.9** ±4.6 (29)	137.4 ±5.7 (2)

*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 sulphoxide 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.

Acid and alkaline phosphatases

Increased activities of phosphatases in diabetes may affect the transport of metabolites across the membrane due to alteration in dephosphorylation reactions. Enhanced levels of phosphatases cause increased intracellular inorganic phosphate, which further affects the efficiency of ionic pumps which is reflected in decreased activities of Na^+-,K^+-ATPases in diabetes(26).

Table 2 represents the activities of acid and alkaline phosphatases in the groups under investigation. In uncontrolled diabetes, the acid phosphatase level was significantly ($p<0.01$) increased by 60% causing decreased functioning of ionic pumps and increased inorganic phosphate levels in blood .

Mulberry leaves treatment decreased the enhanced levels of acid phosphatase (ACP) observed in uncontrolled diabetes significantly ($p<0.01$) by 39% while the activity of the enzyme in glibenclamide treated group was decreased significantly ($p<0.01$) but not as much as with mulberry leaves treatment. No significant difference was observed in the activity of aforesaid enzyme in normal treated and untreated animals.

The activity of alkaline phosphatase in the various groups represented in Table 2 showed an incredible increase in diabetic control by 116% ($p<0.01$) when compared to normal control.

During diabetes, an increase in serum alkaline phosphatase (ALP) has been observed and this has been reported to be the soluble form of intestinal ALP leached from intestine and translocated to the circulation (27) and a decrease in the activity of ALP was observed during the insulin treatment (28).

Mulberry leaves treatment brought down such elevated levels of ALP significantly ($p<0.01$) by 35% while glibenclamide failed to control elevated levels of the enzyme in diabetic animals. The effect of mulberry leaves on ACP and ALP observed in the present study, is in accordance to a study on the effect of S-allyl cysteine sulphoxide isolated from garlic on alloxan diabetic rats (10).

The decreased activity of ALP in mulberry treated group could be due to certain compounds present in mulberry which undergo exchange reactions with the titrable –SH groups of enzymes and proteins in the body spontaneously and inhibit the enzyme activity as reported by Ahmed and Sharma(29).

No significant difference was noted in the activity of both the enzymes in normal treated with mulberry with untreated ones.

Table 3- Activities of Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) in Serum

S.No.	Groups	GOT (IU/L)	GPT (IU/L)
1.	Normal-control	53.8 ±5.2	23.2 ±3.9
2.	Normal-mulberry	55.3 ±5.7 (3)	21.4 ±3.5 (7)
3.	Diabetic-control	94.6** ±4.4 (79)	79.6** +4.2 (243)
4.	Diabetic-mulberry	82.3** +4.5 (13)	42.9** +3.4 (46)
5.	Diabetic-glibenclamide	94.0 ±5.2 (1)	62.3** ±3.7 (22)

*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.*

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)

Accelerated gluconeogenesis, negative nitrogen balance and muscle wasting are among the hallmarks of uncontrolled diabetes (30). There is a catabolism of branched amino acids and alanine release by skeletal muscle (31). Glutamate is an obligate precursor of alanine and glutamine production by muscles. The later two amino acids comprise more than 50% of all the amino acids released by the muscle, alanine being the preferred amino acid precursor of gluconeogenesis in the liver and glutamine in the kidney (32).

It is evident from Table 3 that the activity of GOT and GPT were enormously elevated ($P<0.01$) by 79% and 243% respectively in uncontrolled diabetes from that of normals, indicative of enhanced gluconeogenesis in uncontrolled diabetes.

Mulberry leaves treatment significantly ($P<0.01$) decreased the activity of GOT by 13% that of GPT

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 treated and control animals.

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