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Antidiabetic and anti-oxidant activity of *Moringa oleifera* leaf extract in streptozotocin induced diabetic rats

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Abstract

The effect of the aqueous extract of *Moringa oleifera* Lamk. leaves (MOLEt) in the treatment of diabetes along with its antioxidant activity in streptozotocin induced diabetic rats was determined in the current study. Oral administration of MOLEt @ 125 and 250 mg/kg for 20 days significantly prevented the STZ- induced hyperglycemia. Administration of the extracts at the dose of 250 mg/kg body weight/day resulted in a marked decrease in the levels of fasting blood glucose and urine sugar. The extract also produced a significant decrease in peroxidation products, viz., thiobarbituric acid reactive substances. The activity of reduced glutathione and glycogen content were found to be increased in the hepatic tissue of STZ- diabetic rats treated with MOLEt. STZ-diabetic rats treated with MOLEt significantly reversed all these changes to near normal. The MOLEt thus exhibits antidiabetic and anti-oxidative activity in STZ-induced diabetic rats.

Keywords: *Moringa oleifera*, streptozotocin, STZ-diabetic rats, anti-diabetic, thiobarbituric acid reative substances

Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Baquer *et al.*, 1998). Diabetes mellitus is a non-communicable disease considered to be one of the five leading causes of death worldwide (Murugesh *et al.*, 2006). About 100 million people around the world have been diagnosed with diabetes and by the year 2010, it is projected that 215 million people will have the disease (Zimmet, 1999). Insulin therapy affords effective glycemic control in IDDM patients, yet its short comings include ineffectiveness on oral administration, short shelf life, need for preservation in refrigeration, fatal hypoglycemia in the event of excess dosage, reluctance to take injection and above all, the resistance due to prolonged administration, which limits its usage. Similarly treatment of NIDDM patients with sulfonylureas and biguanides is always associated with side effects (Rang and Dale, 1991). Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine, to natural products that may be better treatments than currently used drugs (Rates, 2001). Hence search for a drug with low cost, more active and without side effect is being pursued in several laboratories around the World.

Moringa oleifera Lamk (Fam: Moringaceae) a perennial plant, is native to tropical Africa and is widely distributed in India, Ceylon and Madagascar (Eilert *et al.*, 1981). The medicinal value of different parts of the plant has long been recognised in folk medicine and is extensively used in the treatment of ascites rheumatism, venomous bites and as a cardiac and circulatory stimulant (Guenera *et al.*, 1996). *Moringa* species have long been recognized by folk medicine practitioners as having value in tumor therapy as well as vin other ailments (Hartwell 1967, 1971; Fahey *et al.*, 2004). Since no reports on scientific evaluation of these leaves in diabetes are available, in the current study, we have evaluated the antidiabetic activity of an aqueous extract of *Moringa oleifera* leaves in rats along with its antioxidant activity.

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Materials and Methods

Plant Material

Moringa oleifera is a small or medium sized, deciduous, perennial tree. The leaves of the plant were collected from the Dakshin Dinajpur district of West Bengal, India. It was identified by the Botanical Survey of India, Kolkata and a herbarium specimen was deposited to the North Bengal University herbarium.

Preparation of plant extracts

MOLEt (each 500g) were extracted separately with 1.5 L of water by the method of continuous hot extraction at 60^{0}C for 6 h and evaporated. The residual extract was dissolved in water and used in the study (Jain, 1968).

Animals

Male Wister albino rats (180-200g) were obtained from Ghosh Enterprise, Kolkata. The animals were grouped and housed in polypropylene cages and maintained under standard laboratory conditions (temperature 25±2^{0}C) with a 12-h/12-h dark and light cycle (Niyonzima and Vllietinck, 1993). All animals were maintained on a standard laboratory diet and tap water and had free access to food and water. All procedures described were reviewed and approved by the University Animals Ethical Committee (NBU).

Induction of experimental diabetes

A freshly prepared solution of Streptozotocin (55mg/kg. i.p.) in 0.1M citrate buffer, pH 4.5, was injected intra-peritoneally in the rats in a volume of 1ml/kg (Siddique

et al., 1987). After 48 h of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose of 200-300mg/dl) were taken for the experiment.

Treatment

In the experiment, a total of 20 rats (12 diabetic surviving rats, 8 normal rats) were used. The rats were divided into 5 groups of 4 rats each. Group-1 and Group -2 treated as control, Group-3 treated as diabetic control. After 48 h of STZ induction diabetic rats treated with the MOLEt were grouped into Group-4 to Group-5.

Group-1: Control untreated rats receiving distilled water.

Group-2: Control rats receiving 0.1M citrate buffer (pH 4.5).

Group-3: STZ treated diabetic rats.

Group-4: STZ treated diabetic rats treated with MOLEt (125mg/kg body weight) in dist water using intragastric tube twice a day for 20 days.

Group-5: STZ treated diabetic rats treated with MOLEt (250mg/kg body weight) in dist water using intragastric tube twice a day for 20 days

The body weight gain, fasting blood glucose and urine sugar of all the rats were determined at regular intervals during experimental period.

After 20 days, all the rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected in tubes containing sodium fluoride for the estimation of fasting blood glucose. Urine was collected from the treated and untreated albino rats after 24 h of fasting livers were removed immediately, rinsed in ice chilled normal saline and patted dry and weighed.

Biochemical studies

Qualitative determination of urine sugar

Glucose was detected in the urine by the method of Benedict *et al.* (1908). Benedict's reagent (5ml) was taken in a test tube and 8 drops of urine was added to it. Tubes were boiled for 1-2 min and then cooled slowly. The solutions were filled with greenish/yellow/red and no precipitate depending upon the quantity of glucose present. Greenish precipitate would indicate very small amount of glucose. The solution remained clear where no glucose was there.

Estimation of blood glucose

Fasting blood glucose was quantified by the method of Nelson and Somogyi (1945) Absorbances were read at 500 nm and were quantified using a standard curve of glucose.

Quantitative estimation of glycogen

Glycogen was hydrolyzed to glucose by the method of Raghuramula *et al.* (2003) and the glucose thus formed was estimated by Nelson and Somogyi's method (1944, 1945).

The liver was taken out rapidly from the animal and the excess blood removed by blotting between folds and filter paper and immediately put into a weight stopper test tube containing 30% KOH and weight again. The amount of alkali was then adjusted to get 2ml per g of liver. The tissue was digested in a boiling water bath for 1 hr. The filtrate was cooled in ice cold water. Two volumes of 95% ethanol were then added and the mixture heated just to boiling. Spurting was avoided. This was left to stand overnight in the cold. The tubes were centrifuged and the precipitate dissolved in 5-10 ml warm water. The glycogen was re-precipitated with 2 volumes of 95% ethanol. The precipitate was centrifuged and washed several times with 60% ethanol. Two ml of 2 N H_2SO_4 per g of initial liver weights was added and hydrolyzed in a boiling water bath for 3-4 h. The solution was neutralized with NaOH using Phenol red as indicator. Volume was noted and filtered. Glucose was determined in that aliquot. The factor 0.93 used to convert glucose to glycogen.

Determination of thiobarbituric acid reactive substances (TBARS)

TBARS in tissues was estimated by the method of Ohkawa *et al.* (1979). After collection of blood samples the rats were killed and livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weight. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation. The levels of lipid peroxides were expressed as moles of thiobarbatituric acid reactive substances (TBARS)/mg protein.

Determination of reduced glutathione (GSH)

GSH in tissues was estimated by the method of Ellman *et al.* (1959). After killing the rats livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A part of homogenate after precipitating proteins with Trichloro acetic acid (TCA) was used for estimation of glutathione. The amount of GSH in the sample was calculated using the standard curve generated from known GSH.

Statistical analysis

The collected data were subjected to statistical analysis by standard procedures of Standard Error, CD and Student's t' test.

Results and Discussion

Initially, the rats were injected with STZ to induce hyperglycemia. After 2 days of STZ induction rats were found to be suffering from strong hyperglycemia. There was marked reduction in the body weight of the STZ treated rats, along with an increase in food craving and thirst. Subsequently, the rats were treated with extracts of the test plants and parameters associated with hyperglycemia such as fasting blood glucose, urine sugar, glycogen content in liver tissue, TBARS and GSH of liver tissue, were tested. After 20 days of treatment with test plants most of the rats recovered from hyperglycemia and became healthy.

Recently studies have clearly demonstrated the

Table 1 : Effect of MOLEt extracts on changes in body weight in normal and experimental rats

Group	Treatments	Body Weight (g)			
		First day	After 2 days of STZ induction	Days after plant extract treatment 10	20
	Normal (only vehicle distilled water)	173.5 ± 4.73	175.0 ± 4.83	188.7 ± 5.54	202.5 ± 5.86
II	Citrate buffer treated	166.0 ± 4.99	167.0 ± 5.00	179.5 ± 4.50	191.5 ± 3.50
III	STZ (diabetic Control)	171.0 ± 1.15	167.6 ± 0.66	156.66 ± 0.88	147.0 ± 0.57
IV	STZ + MOLEt (125mg/kg)	175.0 ± 2.00	177.5 ± 1.50	184.5 ± 1.50	191.5 ± 1.22
V	STZ + MOLEt (250mg/kg)	171.5 ± 0.50	172.5 ± 0.50	182.5 ± 2.50	194.5 ± 4.70

Each value represents mean; ± =SE; MOLEt = *Moringa oleifera* leaves extract. Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$

Table 2 : Qualitative detection of sugar in urine of diabetic and treated rats

Groups	Treatment	Urine sugar*
I	Normal	Nil
II	Citrate Buffer	Nil
III	Diabetic Control	+++
IV	MOLEt (125mg/kg)	+
V	MOLEt (250mg/kg)	Nil

MOLEt:*Moringa oleifera* leaves extract; *Nil:Sugar not detected; +:low sugar; and ++:high sugar in urine

importance of medicinal plants in the treatment of experimental diabetes, where oxidative stress induced β-cell death (Kinloch *et al.*, 1999; Sandhya *et al.*, 2000).

The effect of MOLEt on the body weight of diabetic and normal rats have been demonstrated in Table 1. Decrease in bodyweight due to derangement of metabolic pathways is a common feature in diabetes (Al-Shamaony *et al.*, 1994). In the present study the body weight of the diabetic rats decreased from 171g to 147g after the 20 days treatment with STZ. The body weight of MOLEt treated group (125mg/kg and 250mg/kg respectively) increased from 175.0g and 171.5g to 191.5g and 194.5g respectively after the 20th day's treatment. Similar result found after oral administration of the methanol fraction of *Salacia reticulata* twice daily to the diabetic animals gained the body weight (Rubin Kumara *et al.*, 2005).

A conventional method for the determination of sugar level in urine is qualitative estimation. Changes in the urine sugar of different treated groups have been shown in Table 2. The results of the test were positive (+++) to the diabetic rats and amount of sugar were nil to the MOLEt (250mg/kg) treated groups. Least amounts (+) of sugar were found in the MOLEt (125mg/kg) treated groups.

The levels of blood glucose in normal and experimental rats were determined at different time intervals till 20 days.. Fasting blood glucose levels in the control rats remained unchanged during the course of the experiment. There was a significant ($p< 0.05$) increase in blood glucose in diabetic rats after two days of STZ administration. The rats having blood glucose level more than 200 were considered for the study. In the study, MOLEt reduced the fasting blood glucose level significantly ($p< 0.05$) on the 20th day of treatment.Results have been presented in Fig.1 and Table 3. Venkateswaran and Pari (2002) also found that the diabetic rats showed a significant increase in blood glucose. In their experiment it was observed that the administration of *Coccinia indica* leaves extract in diabetic rats restored the level of blood glucose to near normal levels.

Diabetes mellitus is associated with a marked decrease in the level of liver glycogen (Pugazhenthi *et al.*, 1991). The effects of MOLEt on Glycogen content are shown in the Table 4. The level of glycogen content decreased (21.17 mg/100g) significantly ($p< 0.001$) in the STZ-diabetic rats as compared to control (40.64 mg/100g). Treatment with MOLEt (250mg/kg and 125mg/kg) significantly ($p< 0.001$) increase the glycogen and brought them near to normal level. Pari and Latha (2004) have shown that the hepatic and skeletal muscle glycogen content was reduced significantly in diabetic control.

The effect of TBARS in the liver of STZ-diabetic rats on the treatment with MOLEt is depicted in the Table 4. The STZ-diabetic rats showed a significant increase in TBARS (1.84 mM/100g) when compared with normal ((d H_2O-0.81 mM/100g and citrate buffer-0.79

Table 3: Effect of MOLEt extract on changes in fasting blood glucose in normal and experimental rats

Group	Treatments	Initial	Final
I	Normal (only vehicle distilled water)	76.11 ± 1.05	77.75 ± 2.89
II	Citrate buffer treated	76.85 ± 0.68	76.85 ± 1.13
III	Diabetic Control	76.40 ± 0.81	337.07 ± 6.36
IV	MOLEt (125mg/kg)	75.95±0.37	137.97±1.73
V	MOLEt (250mg/kg)	77.49±0.28	82.02±1.84

MOLEt = *Moringa oleifera* leaves extract. Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$ as compared with normal.

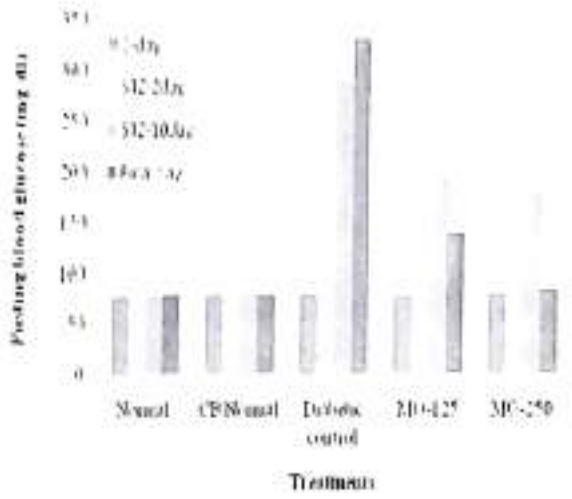

Fig.1: Fasting blood glucose in normal and diabetic induced rats after different time intervals

mM/100g) in liver. Thiobarbituric acid reactive substance levels were decreased in the MOLEt treated groups when compared with the normal rats. There was a significant ($p< 0.001$) reduction in the activity of TBARS in the liver of rats with the treatment of MOLEt (250mg/kg and 125mg/kg) when compared with normal rats.

Kamalakkannan and Stanely (2004) investigated the antidiabetic and antioxidant activity of *Aegle marmelos* in streptozotocin-induced diabetic rats. The diabetic rats showed a significant increase in TBARS and hydroperoxides in liver and kidney. Oral administration of *Aegel marmelos* fruit extract maintained the tissue TBARS and hydroperoxides to near normal status.

The concentrations of GSH in tissues in experimental diabetic rats are shown in Table 4. It was seen that the reduced glutathione level in the liver of STZ-diabetic rats were significantly ($p< 0.01$) decreased. Administration of MOLEt (250mg/kg and 125mg/kg) increased the levels of GSH in the liver ($p< 0.01$) during diabetes. Pari and Latha (2002) demonstrate the levels of GSH in normal and experimental animals while they have worked on the antidiabetic activity of *Cassia auriculata* flowers. Their study showed that GSH level was significantly lower in diabetic rats than in normal rats. Administration of *Cassia auriculata* flower extracts at a0.45g/kg body weight and glibenclamide increased significantly the GSH levels as compared with the levels in diabetic rats.

The results of the current investigation of *Moringa oleifera* leaf extracts showed significant antidiabetic and anti-oxidant activity. It is not known which of the recorded groups of biologically active compounds are responsible for this observed hypoglycemic effect. Neither is the mechanism of action clearly understood. However, this work has clearly brought out the importance of common plants in medicine, which if used properly, can be as good as any other forms of medicine. Several lines of action are still open for further research which can pinpoint the actual component(s) involved and their specific modes of action.

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Table 4: Effect of MOLEt on glycogen, TBARS and reduced glutathione in normal and experimental rats

Groups	Treatment	Glycogen (mg/100g)	TBARS (mM/100g)	Reduced glutathione (mM/100g tissue)
I	Control (distilled water treated)	40.65 ± 2.58	0.82 ± 0.03	45.33 ± 1.76
II	Citrate buffer control	41.40 ± 2.77	0.79 ± 0.05	46.00 ± 1.52
III	Diabetic Control	21.18 ± 2.66	1.84 ± 0.12	25.66 ± 1.85
IV	MOLEt (125mg/kg)	30.16±2.58**	1.23± 0.01**	32.00 ± 1.15*
V	MOLEt (250mg/kg)	40.20±1.39**	0.91± 0.05**	39.33 ± 0.87*

MOLEt = *Moringa oleifera* leaves extract. * $p < 0.01$ as compared with diabetic control, **$p< 0.001$ as compared with diabetic control

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