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PHARMACOLOGICAL EVALUATION OF HYPOGLYCAEMIC EFFECTS OF *MORINGA OLEIFERA* LEAVES IN WISTAR RATS

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Abstract:

The prevalence rate of diabetes mellitus continues to increase all over the world. Medicinal plants constitute an important source of potential therapeutic agents for diabetes. Moringa oleifera has anti-cancer, anti-inflammatory and some researchers reported its hypoglycemic potential. This study aimed to determine the antihyperglycemic effect of dried Moringa oleifera leaves powder of aqueous extract in STZ-induced diabetic male rats and on normal rats as well. Thirty rats were included and divided into 5 groups (6/group). The active ingredients of Moringa oleifera were determined through phytochemical analysis.

A significant increase in body weight was found in normal groups treated with Moringa oleifera leaves extract. A significant change was observed in blood glucose and biochemical parameters significant STZ-induced diabetic rats. These changes were reversed by treatment with dried leaves of Moringa oleifera extract. Moringa oleifera leaves or its aqueous extract can reduce reactive free radicals that might lessen oxidative damage and this might be due to rich presence of flavonoids which have antioxidant property.

Keywords- Moringa oleifera, STZ, Diabetes

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INTRODUCTION:

Diabetes is a complex multisystemic disorder characterized by a relative or absolute insufficiency of insulin secretion and disturbances in carbohydrate, protein and lipid metabolism[1]. The International Diabetes Federation has predicted that the number of individuals with diabetes will increase from 240 million in 2007 to 380 million in 2025 with 80% of the disease burden in low and middle-income countries[2]. Eth-nobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes due to their effectiveness, less side effects and relatively low cost. Most of the plants prescribed for Diabetes Mellitus are not edible and therefore, the studies on edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease[3]. It is the purpose of this experiment is to evaluate the effect of dried leaves powder or the ethanolic extract of *Moringa Oleifera* on blood glucose levels of Streptozotocin-Induced diabetics' rats.

Moringa oleifera belongs to the family of *Moringacaea*, a fast growing drought resistant tree but now distributed world wide in the tropic and sub tropics and is cultivated extensively in Central and South America, Africa, Indonesia, Mexico, Malaysia, the Philippines, and India. *Moringa oleifera* is an edible plant. Different parts of *Moringa* plant contain important minerals and a good source of protein, vitamins, beta-carotene, amino acids and various phenolics as zeatin, quercetin, sitosterol, caffeoylquinic acid and kaempferol and high concentrations of natural dietary antioxidants: Vitamins A, C and E[4-5].

Moringa oleifera provides high concentrations of four natural dietary antioxidants: Vitamins A, C, E and phenolics . Moringa contains 46 antioxidants which help cells to neutralize free radicals[7]. It is traditionally used for relieving spasm, for treatment of diarrhea, diuretic and stimulant in paralytic affliction, epilepsy and hysteria and treatment of diabetes mellitus, hepatotoxicity, rheumatism, venomous bites and also for cardiac stimulation . *Moringa oleifera* is very useful in regulating the thyroid hormone status in adult Swiss rats[8]. Its leaves are also used as nutritional supplement and growth promoters. The aim of the study was to assess the antidiabetic effect of *Moringa oleifera* leaf aqueous extract in rats against streptozotocin Induced diabetes[9].

MATERIALS AND METHODS:

Collection and authentication

Leaves of *Moringa oleifera* collected in the month of February from Government Nursery, Moinabad in Hyderabad. Then collected leaves were authenticated by P.V. Prasanna, Scientist G, Botanical Survey of India (BSI), Hyderabad. Freshly harvested *Moringa oleifera* leaves were washed in tap water and air dried under shade. After authentication plant parts were dried at optimum temperature for one week. The completely dried and crispy leaves were ground in a domestic corn-mill and packaged into black polyethylene bags for storage until they were ready to be used[10-11].

Plant Extract Preparation

About 200 g of leaves powder of *Moringa oleifera* was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at < 50°C to get the aqueous extract.

Phytochemical screening

A preliminary phytochemical screening of the leaves extract of *Moringa oleifera* was also done using the standard phyto chemical reagents and procedures as described[10].

Animals used

Male and Female Wistar albino rats averagely weighing 120g were purchased from the Sai nath Agency, CPCSEA approved animal house in Hyderabad and housed in Institute animal house in plastic cages at 12 hours day and night cycle. The animals were allowed to get acclimatized to the animal house conditions for two weeks during which they were fed the control diet. The Research Protocol is approved by IAEC with no. IAEC/AUCOP/2020/2

Chemicals and diagnostic kits

ACCUCARE™ kits (Lab-Care Diagnostics, Mumbai, India) were used for conducting fasting blood sugar, cholesterol, high density lipoprotein, low density lipoprotein and triglyceride tests[11].

Instruments

Universal 320 centrifuge
Chemistry Analyzer
Haematology analyzer
Spectrophotometer[12]

Experimental design

The rats were selected and put into 4 groups of 6 animals each, kept 3 rats in per cage. Total no. of animals used will be 24 as follows:

Group-I: Normal Control - Animal received normal water
Group-II: Negative Control (streptozotocin 150 mg/kg.b.w.i.p in Tween 80)
Group-III: Standard metformin (5 mg/kg.b.w.p.o) + streptozotocin (150 mg/kg.b.w. i.p)
Group-IV: Test-I (Aqueous Extract of *Moringa oleifera* 300 mg/kg.b.w.p.o) + streptozotocin(150 mg/kg.b.w. i.p)
Group-V: Test-II (Aqueous Extract of *Moringa oleifera* 500 mg/kg.b.w.p.o) + streptozotocin(150 mg/kg.b.w. i.p)

Blood sampling

To carry out biochemical and haematological analyses the rats were sacrificed under anesthesia to collect the jugular vein blood[13]. For biochemistry samples gel clot activator and sodium fluoride tubes were used to collect blood and K_3 EDTA tubes were used to collect blood for the haematology samples. Samples were analyzed on the day of collection[14].

Statistical analyses

The antioxidant indices, biochemical, haematological and weight changes results were analyzed using one-way analysis of variance (ANOVA) and are presented as mean and the standard error of the mean (SEM)[15]. Dunnett's multiple comparison tests was used to establish any statistical significance between control and experimental groups. Statistical significance was set at $p < 0.05$.

RESULTS:

Table no- 1 Results of Phytochemical screening of the aqueous extract of *Moringa oleifera*

Chemical Constituents	Petroleum extract	Aqueous extract
Carbohydrates	+	+
Glycosides	+	+
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Fixed oils	–	–
Steroids	+	+
Saponins	+	+
Gums & mucilage	+	–
Proteins & free amino acids	+	+

- - - Absent + Present

Changes in Mean Animal Body Weights

Animals in the diabetic control group had significantly reduced body weight over the duration of treatment compared to the normal control group which had a relatively stable body weight throughout the period. *Moringa oleifera* treated groups had an upward trend in weight gain of about 50 % compared to the diabetic controls. The metformin, had relatively lower weight gains compared to *Moringa oleifera* extract.

TIME (week)

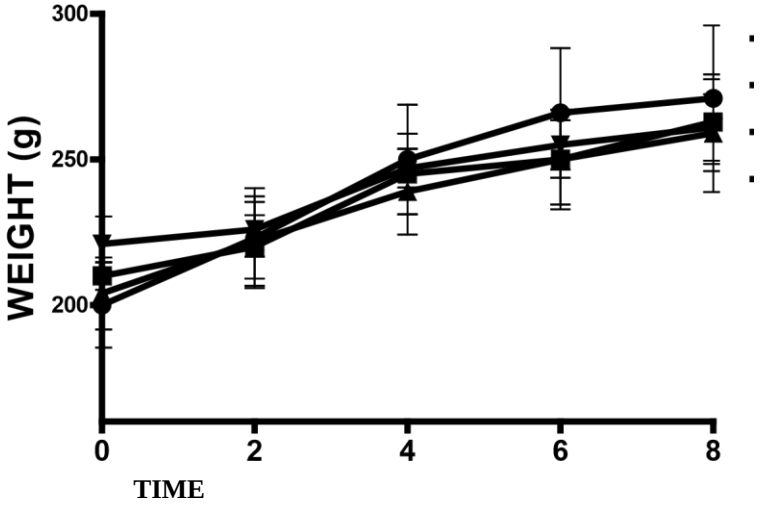


Figure no. 1 Weight changes observed at 7 days of feeding in rats.

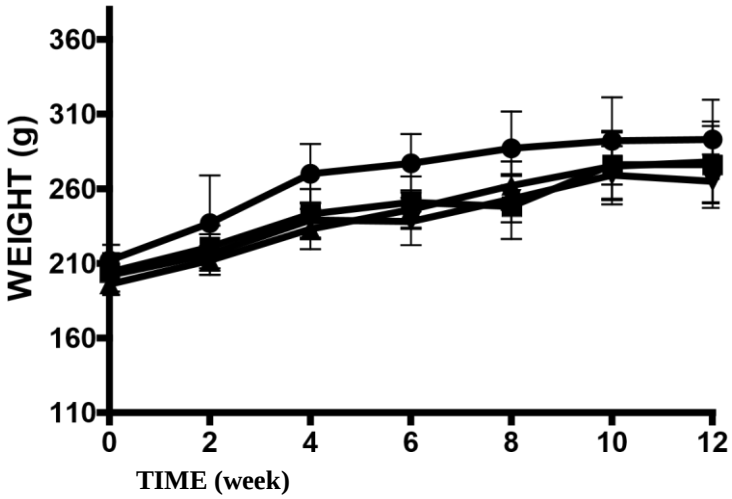


Figure no.2 Weight changes observed for 14 days of feeding in rats.

Biochemistry

Fasting blood glucose (FBS)

The fasting blood glucose levels as seen in Table for a 7-day and 14-day feeding period were nonsignificant. In relation to the control, the FBS levels were lowered as percentage *Moringa oleifera* increased.

Table -2 Fasting blood sugar at day 7 of feeding

Parameter		Group			
	I	II	III	IV	V
FBS (*Moringa oleifera* ml/L)	4.0±0.1	5.7±0.2	4.9±0.2	5.1±0.2[b]	4.9±0.3

The results are presented as mean ± SEM, n = 6.

Table no. 3 Fasting blood sugar at day 14 of feeding

Parameter		Group			
	I	II	III	IV	V
FBS (*Moringa oleifera* ml/L)	3.9±0.2	5.9±0.1	3.5±0.1	4.6±0.2	4.1±0.3 [b]

The results are presented as mean ± SEM, n = 6. P-values significant from control; a = p<0.05, b = p<0.001

Lipid profile

Tables no.4 show the measurements of total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglyceride for the groups at 7 and 14 days respectively. The results of lipid profile reveal significant changes occur after treatment with *Moringa oleifera.*

Lipid profile at day 7 of feeding

Table no-4

Parameter		Group			
	I	II	III	IV	V
Total Cholesterol HDL-C LDL-C Triglyceride	1.0±0.3 1.1±0.2 0.1±0.2 0.3[a]	1.7±0.1 1.2±0.1 0.2±0.1 0.6±0.1	1.8±0.1 1.2±0.1 0.5±0.1 0.4±0.1[a]	1.8±0.1 1.2±0.1[b] 0.4±0.2 0.4±0.1[a]	1.5±0.1 1.2±0.1 0.2±0.1 [b] 0.4[a]

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = p<0.05, b = p<0.001

Lipid profile at day 14 of feeding

Table no-5

Parameter		**Group**			
	I	II	III	IV	V
Total Cholesterol HDL-C LDL-C Triglyceride	2.0±0.3 0.8±0.2 1.0±0.2 0.3±0.1	2.1±0.2 0.9 1.0±0.2 0.5±0.1	2.2±0.2 0.9 [b] 1.1±0.2 [b] 0.5±0.1	2.1±0.2 1.0±0.1 0.9±0.2 0.6±0.2	2.5±0.1 1.0±0.1[b] 1.2±0.1 0.5±0.1 [b]

The results are presented as mean ± SEM, n = 5. P-values significant from control; a = p<0.05, b = p<0.001

Effect on *Moringa oleifera* on Pancreas

Islets are present and conspicuous (having a large diameter) with no evidence of injury. Pancreas of normal control rat showed high cell density in several multifocal islet of Langerhans. The cells have hyperchromatic rounded to ovoid nucleus and eosinophilic cytoplasm (H&E x400) (Figure 3).

Pancreas of diabetic is control *Moringa oleifera* that islets of Langerhans were stay not present and the cells of islets had been replaced by fibrous tissues (fibroblast) occurring in the foci that were previously occupied by islet.

A few lobules of the pancreas had been invaded by fibrous tissue that replaced the exocrine pancreas. This lesion was initiated by pancreatic necrosis that triggers fibrosis to replace the necrotized tissue as seen in diabetic controls (Figure 4).

For the metformin-treated rats, the islet of Langerhans had fewer cells when compared with normal control and the cells occurred within a matrix of hyalinized fibrous connective tissue. The cells are smaller (atrophied) than in normal control rat group (Figure 5).

The islets of the pancreas of the 300 mg/kg *Moringa oleifera* extract treated rats are prominent, numerous and had a high cell density, Langerhans that were large, numerous and frequently observed. Further *Moringa oleifera*, these histopathological changes were closely comparable to those in the pancreas of normal rats (Figure 6).

The pancreas of 300 mg/kg *Moringa oleifera* extract treated rats had islets of Langerhans that were frequently observed but much smaller than in normal control rats. The cells were viable with round to ovoid surrounded by eosinophilic cytoplasm. The cell *Moringa oleifera* morphology was similar, but cell density was slightly less than for the normal control (Figure 7).

The summary of the histological examination of the pancreas of various animal treatment groups is reported below

Summary for histological examination of the pancreas Treatment

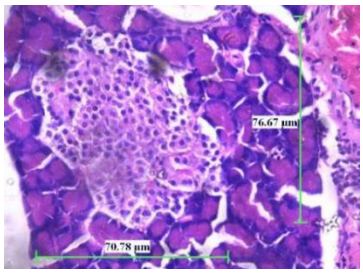
Normal control- Many prominent islets that have histologically normal viable cells.

Diabetic control- No islet seen, only one site that is a remnant of islet. No islet cells present. They have been replaced by fibrous connective tissue cells (fibroblasts).

Metformin- Islets are few and not prominent, they occur as small islands located far and between. The cells are viable.

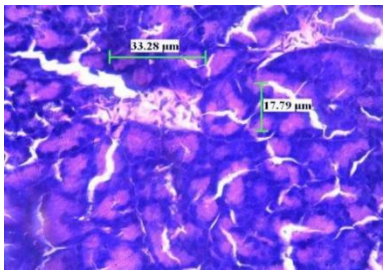
***Moringa oleifera* Aq. extract (300mg/kg)-** Many prominent islets that have viable cells

***Moringa oleifera* Aq. extract (500mg/kg)-** Small scattered islets that have viable cells



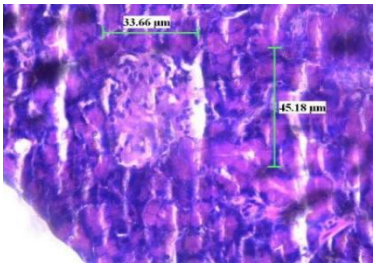
A. Normal control

Fig no-3



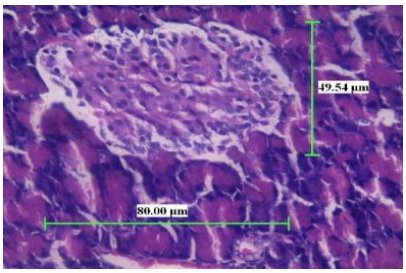
B. Negative control

Fig no-4



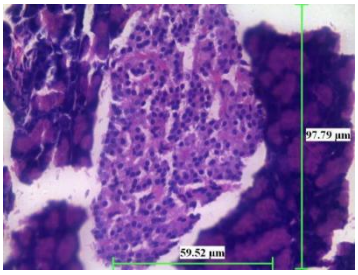
B. Diabetic + Metformin

Fig no-5



D. Test-I

Fig no-6



E. Test- II

Fig no- 7

DISCUSSION:

Diabetes is a multifactorial disorder, and as such several driving factors could be responsible for its manifestation. It is, therefore, imperative to exploit solutions across every possible mechanism in order to develop novel interventions to reduce global incidence of diabetes[16].

The global incidence of diabetes is increasing alarmingly and there is, therefore, an urgent need to explore novel and innovative intervention strategies. Current treatment therapies of diabetes have been successful in providing a solution to the hyperglycemic condition in diabetes patients[17].

Moringa olifera has high nutritional value and is a good source of protein, vitamins, β-carotene, amino acids and various phenolics which agree with. The leaf powder is also an interesting dietary supplement for pregnant and lactating women[18-19].

Moringa leaves are natural sources of calcium and multivitamins with high bioavailability. Phytochemical screening Result of the preliminary

phytochemical screening of *Moringa olifera* leaves extract revealed the presence of flavinoids, tannin, anthraquinone, cardiac glycosides alkaloids, triterpenoids, saponins, reducing sugars and phenolic acids[20].

The growth curves and charts for rats did not show any inferior or backward growth meaning that all the animals cells were growing normally.

In Figure 7 , it was realized that all the weight changes at weekly or daily interval were not significant. However, it was observed in the 14 days that the experimental groups had low body weights with respect to the controls. It is possible that the *Moringa oleifera* was responsible for this weight effect[21].

The lipid profile when performed gives an idea of an individual's risk of developing cardiovascular diseases. The total cholesterol, HDL-C, LDL-C and triglyceride are used to assess this risk[22].

Another reason that can explain the insignificance in weight changes is the fact that the animals had lean body mass. This was evident in the lipid profile (Table no-4- Table no-5) measurements; where the change in total cholesterol was insignificant.

Fasting blood sugar levels are used to help diagnose diabetes mellitus and hypoglycemia. A randomly timed test for glucose is usually performed for routine screening and nonspecific rating of carbohydrate metabolism.

The only significant change for an overnight fast was in Table no-2 & 3, where a decrease occurred for *Moringa oleifera* after 14 days feeding. This is an indication that the *Moringa oleifera* may be a hypoglycaemia agent when used for a long period of time. This suggests that while providing nutritional benefits it can lower blood sugar too for some people[23].

Cholesterol is a sterol compound synthesized exogenously in the liver from dietary fats and endogenously within the cells. It is present in all body tissues and is a major component of bile salts, steroid *Moringa oleifera*, low-density lipoproteins (LDLs), brain and nerve cells, cell membranes, and some gallstones[24].

Cholesterol is transported in the blood by the low density lipoproteins to the peripheral blood and high density lipoproteins from the peripheral blood to the liver. When the levels of cholesterol in the blood is high (hypercholesterolemia), especially in combination with low levels of HDL, it is known to increase a person's risk of atherosclerosis and heart disease[25].

In the results, it can be seen that no significant changes were recorded for total cholesterol concentrations. The *Moringa oleifera* was able to maintain the relative total cholesterol concentrations and as such effect will be desirable for preventing unhealthy disease increases. High total cholesterol is a risk factor for developing cardiovascular diseases.

High-density lipoprotein is a type of cholesterol carried by alpha-lipoprotein. HDL is believed to help protect against the risk of coronary artery disease (CAD) and has been shown to be inversely related to the risk of coronary heart disease[26]. The *Moringa oleifera* appears not to have any direct influence on HDL-C metabolism. This probably suggests that it may not increase the HDL-C production and its protective effect against CAD which will be negligible. The result did not show any significant changes in HDL-C for duration of feeding[27].

When very low-density lipoproteins (VLDL) are degraded, intermediate density lipoproteins (IDL) are produced which are taken up by the liver and converted to lowdensity lipoproteins (LDLs). LDLs can be oxidized and are atherogenic and thus associated with an increased risk of arteriosclerotic heart and peripheral vascular disease[28] . This parameter did not record any significant changes, especially, decrease which pro *Moringa oleifera* good for cardiovascular health.

Dietary triglycerides are carried as part of chylomicrons through the lymphatic system and bloodstream to adipose tissue, where they are released for storage. Triglycerides are also synthesized in the liver from fatty acids and from protein and glucose when these substrates are above the body's current needs and then stored in adipose tissue[29]. They may be later retrieved and formed into glucose through gluconeogenesis when needed by the body. From the Table no-5 there were significant decreases in its concentration with respect to the control[30].

Some studies state that increased fasting triglyceride in the blood is a risk factor for coronary heart diseases, diabetes and pancreatitis. The decreases therefore suggest that *Moringa oleifera* in this regard may prevent the raising of triglycerides to dangerous levels [31].

The role of *Moringa oleifera* in lipid (especially total cholesterol, HDL-C and LDL-C) metabolism was not observed though the growth chart (Figure no-1, Figure no-2) could suggest weight control ability of the experimental compared to the controls. The ratio of HDLC/LDL-C was over twice in Table no-2 than those in Tables. Nonetheless, its ability to significantly reduce triglyceride level in the short term makes it a good ingredient as part of the diet when cardiovascular health is concerned.

CONCLUSION:

The present study showed that the use of *Moringa oleifera* in the diet as a nutritional supplement has shown to be relatively safe, non toxic to the pancreas. It is able to reduce blood glucose levels over a long-term of use in normal rats, which implies it should be used cautiously since it may be able to cause hypoglycaemia.

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Conflict of interest

The authors declare no conflict of interest.

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