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Research Article

# HYPOGLYCEMIC ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *MANILKARA ZAPOTA* SEEDS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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**ABSTRACT**

Objective: To evaluate the hypoglycemic activity of aqueous and ethanolic extracts of Manilkara zapota seeds in streptozotocin induced diabetic rats.

Methods: 36 Wistar albino rats were selected and their baseline blood glucose and body weight were measured. Diabetes was induced by intra peritoneal injection of streptozotocin. Rats were divided into 6 groups with 6 animals in each. Control group received normal saline 10ml/kg, the standard group, glibenclamide 10mg/kg, and the four test groups received aqueous extract (MZAE) 200mg/kg, 400 mg/kg, ethanolic extract (MZEE) 200mg and 400mg/kg orally for 21 days. The blood glucose and body weight were measured before and after the experiment.

Results: The MZAE 400 mg and MZEE 200 mg of *Manilkara zapota* seeds were found to have hypoglycemic activity when compared with the control, but not statistically significant when compared with the standard. MZEE 200 mg had better hypoglycemic activity than aqueous extracts, and the gain in body weight was almost equal in the standard and MZEE 200mg/kg group.

Conclusion: Both the aqueous and ethanolic extracts of Manilkara zapota seeds have hypoglycemic effect. The body weight changes of the MZEE 200mg/kg group were similar to the standard group.

**Keywords:** Hypoglycemic activity, Manilkara zapota seeds, diabetes, Aqueous extract (MZAE), Ethanolic extract (MZEE).

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both [1]. India with over 63 million diabetics has been declared as the "Diabetic capital of the World". The International Diabetes Federation (IDF) estimated around 366 million people to have diabetes in 2011, and by 2030 this will have risen to 552 million [2]. Longstanding DM patients are susceptible to micro-vascular complications and macro-vascular disease. Treatment includes diet combined with exercise and pharmacotherapy**.**

Insulin replacement therapy is the treatment for patients with type 1 DM while oral anti diabetic agents along with diet and lifestyle modifications form the management of type 2 DM. The conventional oral anti diabetic agents are sulphonylureas, biguanides, α glucosidase inhibitors, meglitinides and thiazolidinediones. New and emerging therapies include Incretin-based therapies: glucagon-like peptide-1 receptor agonists (exenatide), dipeptidyl peptidase-4 Inhibitors (gliptins) and sodium glucose cotransport-2 (SGLT-2) inhibitors (gliflozins). It is evident that several groups of drugs with different mechanisms of action are available to treat type 2 DM. But no single drug can cause optimal glycemic control. It is mostly multidrug therapy along with diet and exercise which may also fail to control diabetes in some patients. Several medicinal plants have been used traditionally as well as in other systems in controlling DM. One among the medicinal plants is *Manilkara zapota*.

### Ethnobotanical description of *Manilkara zapota*

It belongs to the family, Sapotaceae**,** Genus: Manilkara, Species: zapota. Other names: *Manilkara zapotilla, Manilkara achras, Mimusopus manilkara, Achras zapota. Manilkara zapota* an *e*vergreen tree native to southern Mexico, has become a major commercial crop in India. Sapotaceae is a family of some 35–75 ill-defined genera and about 800 species. It is a large tree growing to a height of around 8 meters. It produces a dense crown and a characteristic branching system (sympodial), in which the young branches are arranged horizontally. The tree has an extensive root system. The bark is dark brown and deeply fissured. Leaves are spirally arranged and clustered at the shoot tips, simple, elliptic or oblong. Flowers are hairy outside, 6-8 mm long, greenish, solitary, with a brown pubescent peduncle. Fruit is brown, fleshy, ovoid to round, 3-8 cms long, containing 5 or more shiny blackish brown seeds.

### Phytochemical constituents

The plant contains several phytochemical constituents belonging to categories such as alkaloid (sapotin, saponin, achrassaponin), carbohydrate (saccharose, dextrose, levulose), glycoside, tannins, triterpenes and flavonoid etc. It also contains protein, ascorbicacid, phenols, carotenoids and minerals like iron, copper, zinc, calcium and potassium [3-5].

### Medicinal uses

Seeds have been proved to have diuretic [6], antibacterial [7] and anthelminthic activity[8]. Seed kernel oil was used as skin ointment. Bark extract has been evaluated for antimicrobial and anticancer activities [9].It is also traditionally used for the treatment of fever and pain. The leaves of the plant possess analgesic and anti inflammatory [10], antioxidant, antihyperglycemic and hypo cholesterolemic activities [11]. Roots are found to have hypoglycemic activity [12].

The phytochemical constituents of the leaves, root and seeds are almost similar. The leaves and root have already been proved to have anti hyperglycemic effect. The current study was undertaken to evaluate the antidiabetic activity of seed extracts of *Manilkara zapota*.

## MATERIALS AND METHODS

The I.A.E.C. approval was obtained before the initiation of the study (number IAEC5/Desp.No.42/ 09.11.12).

### Preparation of seed extract

Fresh seeds were washed with distilled water thoroughly to remove traces of contaminants and shade dried for one month. After complete drying, the seeds were powdered and used for extract preparation. Ethanolic extract was prepared by soaking the seed powder in ethanol. After every 24 hours, fresh ethanol was added and crude extract was separated after 72 hours [8]. Aqueous extract was prepared by

soaking the ground seed powder in distilled water. Both the extracts were filtered and the filtrate was allowed for complete evaporation of the solvent on water bath. Ethanolic extract was dissolved in 0.9% NaCl. Aqueous extract was centrifuged at -4 degrees at 4000 rpm for 10 minutes. The supernatant was dissolved in 0.9% NaCl.

**Animals**

36 adult male Wistar albino rats weighing 150-200 grams were selected for the study. The animals were procured from "Central Animal House, Chettinad Hospital and Research Institute", Chennai. The animals had 12+/-1hr light dark cycle to maintain day and night rhythm throughout the experimental period. The animals were housed in large polypropylene cages. The animals received a balanced commercially available pelleted rat feed and provided with clean water.

**Induction of diabetes**

All the rats were fasted overnight before the induction of diabetes. The basal fasting blood glucose and baseline body weight were measured. Blood glucose levels were estimated using an electronic glucometer (One Touch Horizon, sensitivity-mg $dL^{-1}$) drawing blood from the tip of the tail. Diabetes was induced by intra peritoneal injection of streptozotocin (dissolved in 0.1M sodium citrate buffer pH4.5). 2 doses of 30mg/kg of streptozotocin was given at one week interval [13]. After the injection they had free access to food and water. The development of diabetes was confirmed after 72 hours of last dose of Streptozotocin injection. The animals having fasting blood glucose level more than 200mg/dl were considered diabetic and used for the experimentation. The treatment was started soon after confirming diabetes. The treatment was continued for 21 days.

**Drugs and chemicals**

Streptozotocin was procured from Sigma Chemical Co. (St. Louis, MO, USA) and Glibenclamide was purchased from Sanofi Aventis Company (Mumbai, India). Both were of standard analytical grade.

**Experimental design:** Animals were divided into 6 groups with 6 animals in each.

**Group 1 - Control** -Normal saline, 10ml/kg

**Group 2 - Standard** -Glibenclamide, 10mg/kg

**Group 3 -** MZAE 200mg/kg

**Group 4 -** MZAE 400mg/kg

**Group 5 -** MZEE 200 mg/kg

**Group 6 -** MZEE 400mg/kg

The doses of 200 mg and 400 mg/kg body weight for aqueous and ethanolic extracts were selected based on previous study [14]. All the drugs were given orally for 21 days.

The effect of aqueous and ethanolic extracts of *Manilkara zapota* (L.) on blood glucose and body weight were studied and compared with control and standard drug Glibenclamide.

**Statistical analysis**

Data were expressed as mean ± SD. Statistical analysis was done using one way ANOVA.

**RESULTS**

The base line blood glucose and body weight were measured before the start of the study,72 hours after the last streptozotocin injection (post induction), $7^{th}$,$14^{th}$ and $21^{st}$ day. Of the 36 rats which were given injection streptozotocin 33 developed diabetes. The remaining 3 rats did not develop diabetes and they were excluded from the study (1each in groups 4, 5 and 6).The MZEE 400mg was not found to be safe in our study and four out of 5 animals in this group died. Hence the effect on blood glucose and body weight of this group could not be evaluated.

MZAE 400 mg and MZEE 200 mg of Manilkara zapota seeds had significant hypoglycemic activity when compared with the control. But the hypoglycemic activity when compared with the standard was not found to be significant. The ethanolic extract in the dose 200 mg had better hypoglycemic activity than the aqueous extracts (Table 1). There was a statistically significant increase in body weight of the standard and MZEE 200 mg group when compared with the control. The gain in body weight was almost equal in the standard and MZEE 200mg group (Table 2).

**DISCUSSION**

The ideal drug for diabetes should be effective in maintaining optimal glycemic control, preventing long term complications and also free from serious adverse effects. No single drug can satisfy these criteria and management of diabetes is always multidrug therapy combined with diet, exercise and weight reduction. Exogenous insulin therapy is indicated mainly for the management of type 1 diabetes. But of late the requirement of insulin in type 2 diabetes is increasing due to treatment failure developed to the oral antidiabetic drugs (OAD). Both primary and secondary failure to OAD has been reported. Primary failure denotes unresponsiveness to OAD from the beginning. In secondary failure patients respond to therapy initially at least for a month and acquire resistance subsequently. Overall 3-30% OAD failure has been reported [15,16].The causes for resistance to oral antidiabetic drugs include patient related factors like poor patient compliance, lack of dietary restrictions, stress, obesity and lack of exercise. The disease related factors responsible for OAD failure are insulin resistance as well as cellular resistance. In addition to therapy failure the adverse effects of insulin as well as OADs cause great concern.

**Table 1: Mean blood glucose (mg/dl)**

| Group | Basal | Post induction | Day 7 | Day 14 | DaY 21 |
|---|---|---|---|---|---|
| Control(n=6) | 98.5±3.6 | 272.3±50.2 | 304.6 ±68.30 | 357.5± 69.14 | 401.7± 34.74 |
| Standard(n=6) | 96.5±9.4 | 407.1±99.1* | 311.6±61.41* | 240 ±59.39* | 160 ±41.99* |
| MZAE 200(n=6) | 97.5±10.7 | 283.5±63.5 | 324± 26.23 | 314± 44.97 | 353.2± 82.21 |
| MZAE 400(n=5) | 106±7.7 | 334±137 | 240± 51.19* | 275.6±55.41* | 269.2±73.49* |
| MZEE 200(n=5) | 93±4.1 | 355.8±100.9 | 219.6±56.31* | 249.2±41.11* | 211.7±39.16* |

Data were expressed as Mean ±SD *indicates P value of <0.05 which is statistically significant compared with the control

**Table 2: Mean body weight (g)**

| Group | Basal | Post induction | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|---|
| Control(n=6) | 213.3±21.4 | 210.8±22.5 | 201.6±23.7 | 197.8±24.2 | 186.8±25.2 |
| Standard(n=6) | 208.16±23.3 | 200.5±25.3 | 210.5±17.2* | 216±20.3* | 221±15.6* |
| MZAE 200(n=6) | 204±12.7 | 191.3±9.3* | 191.5±20.6 | 194.6±30.3 | 200.4±29 |
| MZAE 400(n=5) | 210.6±18.6 | 210.8±16.9 | 196±20.3 | 203±40.8 | 214±39.4 |
| MZEE 200(n=5) | 207.4±18.6 | 205.4±21.7 | 201±27.2* | 207±26.8* | 221.2±27.5* |

Data were expressed as Mean ±SD *indicates P value of <0.05 which is statistically significant compared with control.

Hence finding alternative approach to the overall management of diabetes is a continuous need. Several medicinal plants are being screened for antidiabetic activity. Among these plants one of the few widely grown in-house plants is Manilkara zapota. Its leaves and roots are reported to have antidiabetic activity. Hence this study was conducted to find out whether the seed extract of Manilkara zapota has antidiabetic activity in streptozotocin induced diabetes in rats.

The results of the study have shown that both the aqueous and ethanolic extracts of the seeds have hypoglycemic activity. Between the two extracts the ethanolic extract was found to have better hypoglycemic effect than the aqueous extract. The ethanolic extract 400 mg was not found to be safe. The standard group treated with glibenclamide had significant reduction in blood glucose. The control group showed a gradual rise in blood glucose.

All groups showed a reduction in the body weight 72 hours after injection of streptozotocin due to the onset of diabetes. Subsequently there was a progressive increase in body weight in all the groups except the control. There was a statistically significant increase in body weight of the standard and MZEE 200 group when compared with the control. Increase in body weight may be due to recovery from diabetes following treatment.

It can be inferred that 400 mg of aqueous extract and 200 mg of ethanolic extract had hypoglycemic effect. But the extent of glucose reduction was higher with glibenclamide. The gain in body weight after treatment was similar with MZEE 200mg and glibenclamide. The MZEE 200mg produced an effect similar to that of Glibenclamide. But MZEE 400mg was not found to be safe. Hence MZEE seems to have narrow therapeutic index. The important phytochemical constituents present in Manilkara zapota seed are saponin, sapotin, achrassaponin and the bitter principle sapotinine. Saponins are known to have anti diabetic activity and has been proved to have significant hypoglycemic effect in streptozotocin-induced diabetic rats [17]. Allium sativum, Eugenia jambolana, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Trigonella foenum graecum and Tinospora cordifolia are some commonly used plants containing saponins and proved to have antidiabetic activity[18-24].

The probable mechanisms attributed to the actions of saponin are stimulating insulin release [25], improving insulin sensitivity [26], promoting glucose uptake [27], which is similar to sulfonylureas. In addition Saponins have other biological activities such as antioxidant, anticarcinogenic, antiulcerogenic, antidiarrheal, hypocholesterolemic, anticoagulant, hypoglycemic, hepato-protective, neuroprotective and anti-inflammatory activities [28]. The antioxidant property of saponin may also have contributed to hypoglycemic activity.

## CONCLUSION

The present study has shown that Manilkara zapota seed extracts have hypoglycemic activity. MZAE 200mg and 400mg were found to be safer and MZAE 400mg was more effective than 200mg. The hypoglycemic effect of MZEE 200mg was better than the aqueous extracts, though not comparable with glibenclamide. The increase in body weight of MZEE 200mg was also equal to that of glibenclamide. Hence the preparation of this extract has the potential to be used as one of the drugs in multi drug anti diabetic therapy. Further studies have to be done to confirm the mechanism of action and its use in DM along with other drugs.

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**CONFLICT OF INTEREST:** Nil.

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