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Evaluation of Antidiabetic Activity of Aqueous Extract of Mangifera Indica Leaves in Alloxan Induced Diabetic Rats

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ABSTRACT

Plants and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world; especially in countries where access to the conventional treatment of diabetes mellitus is inadequate. There have been several reports on the hypoglycemic activity of *Mangifera indica*(MI). Hence, the present study was undertaken to investigate the anti-diabetic effect of MI leaves extract alone and in combination with an oral hypoglycemic drug, gliclazide on alloxan induced diabetic rat models. Alloxan monohydrate (125mg/kg, I.P) was used to induce diabetes in albino rats weighing 200- 250 g. The fasted diabetic rats were divided in to 6 groups of 6 animals each. The control group received vehicle (5% gum acacia) only. Group 2 and 3received MI 200mg/kg, 400mg/kg respectively; Group 4 and 5 received Gliclazide 2mg/kg and 4mg/kg respectively and group 6 received MI leaves extract 200mg/kg + Gliclazide 2mg/kg. This study was conducted over a period of 15 days with oral administration of drugs and the plant extract which was started on the 6th day of alloxan treatment. The fasting blood glucose levels will be determined on day 0, 10, and 15th day by using glucometer. Data were statistically analyzed by ANOVA followed by Dunnet's multiple comparison test. All the test groups showed significant ($P < 0.0001$) decreased fasting blood glucose levels in alloxan induced diabetic rats treated with the aqueous extract of MI. However, the Combination of MI extract with gliclazide produced a significant ($P<0.0001$) decrease in blood glucose level which is higher than that produced by gliclazide alone. The decrease in blood glucose levels is probably due to reduction in the intestinal absorption of glucose. MI possesses significant hypoglycemic activity probably due to presence of flavonoids, tannins, steroids and trepenoids and the aqueous extract of leaves contain higher levels of phenol and flavonoids which exhibit greater antioxidant activity by lowering the diabetic complication.

Keywords: Type 2 Diabetes mellitus; albino rats; alloxan monohydrate; Mangifera indica(MI); Gliclazide; Hypoglycemia.

INTRODUCTION

Diabetes was described more than 2000 years ago. For the past 200 years, it has featured in the history of modern medicine. It is a syndrome characterized by disordered metabolism and inappropriate hyperglycemia due to either a deficiency of insulin secretion or due to a combination of insulin resistance and inadequate insulin secretion to compensate.[1]Diabetes is an "iceberg" disease. Type 2 DM is the prominent form of diabetes worldwide, accounting for 90% of cases worldwide. An epidemic of type 2 DM is under way in both developed and developing countries. According to World Health Organization (WHO) the prevalence of diabetes worldwide is 180 million and will reach the 300 million in 2025. [2]

In India, WHO estimated that there were 19.4 million diabetics in the year 1995 and it is likely to increase to 57.2 million by the year 2025.The revised figure are 80.9 million by year 2030. The prevalence rates have been increasing steadily since the ICMR study in1970 which had

reported the prevalence rate of 2.3% in the urban population and 1.5% in the rural population.[3]Current prevalence rates are 10% to 18% in adult urban Indian population and there is evidence that the prevalence of type-2 diabetes is increasing in rural population also. The prevalence of diabetes is rising because of increase in the life expectancy as well as a substantial increase in obesity and sedentary life style. The factors for this steep rise include genetic predisposition, urbanization, ethnicity, insulin resistance and central obesity.[4]Every 5th diabetic in the world is an Indian and every 5th and 10th Indian in metro city like Mumbai is diabetic.[5]

The goals of therapy for diabetes mellitus consist of glycemic control by diet, lifestyle modification, regular exercise, medication i.e., oral anti diabetic drugs and insulin therapy to treat associated conditions like dyslipidemia, hypertension, obesity, coronary heart disease and to screen for or manage complications of diabetes like retinopathy, cardiovascular disease, nephropathy, neuropathy and other complications.[6]

Plants and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world; especially in countries where access to the conventional treatment of diabetes mellitus is inadequate.[7]There has been several reports on the hypoglycemic activity of MI.[8,9]

MI (Anacardiaceae) is a tree, distributed in rural and semi urban parts of India. It is one of the most important tropical plants marketed in the world.[10]It is grown widely in different parts of Africa, especially in the southern parts of Nigeria, where it is valued for its edible fruits .There are traditional medicinal uses for the bark, roots and leaves of MI through the globe. MI is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains, and malaria. Phytochemical research from different parts of M.I has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols.[11-13]

This species is purposed to possess many therapeutic uses including analgesic, anti-inflammatory, antimicrobial, immunostimulant, antioxidant and antilipidemicapplications.[14-16]

The main disadvantage of current drugs (biguanides, sulfonylureas) is that they have to be given throughout the life and produce side effects.[17]Hence, the present study was undertaken to investigate the anti-diabetic effect of MI leaves extract alone and in combination with an oral hypoglycemic drug, gliclazide on alloxan induced diabetic rat models.

METHODS

Plant material and Preparation of extract

MI leaves available locally, were identified and used for the study. The leaves of MI were obtained after identification. The leaves were washed and shade dried, and after grinding in an electric grinder, the powder was soaked in equal amount of water and stirred intermittently and was left over night. The macerated pulp was dried at reduced temperature. This dry mass serve as aqueous extract of leaves of MI for experimentation.[3]

Animals

Adult albino rats (200-250 g) of either sex were used in this study; 36 rats were divided into 6 groups consisting of 6 animals each. All the drugs were administered to animals by mouth using polythene tubing sleeved on an 18-20 gauge blunted hypodermic needle. Animals were obtained from National Institute of Nutrition Hyderabad. The animals were stabilized for 1 week under standard conditions at temperature 25±1°C, 60±5% relative humidity and 12 hrs dark light cycles. They had been given free accesses to standard pellet diet and water ad libitum. Experiments were conducted according to the ethical norms approved by the Institutional Animal Ethics Committee guidelines of Navodaya Medical College, Raichur.

Drugs and chemicals

Alloxan monohydrate - obtained from sd fine-CHEM limited, Mumbai.
Gliclazide – Tablets 80 mg – obtained from medical store was used.
5% Gum acacia –Vehicle for administrating the fine suspension of gliclazide.

Induction of Diabetes Mellitus in rats

Alloxan monohydrate was used to induce diabetes mellitus. After an overnight fast, the rats

were injected single dose (125mg/kg) of freshly prepared 5% solution of alloxan monohydrate in 0.9% sodium chloride (normal saline) intraperitoneal. Following injection of alloxan, animals were observed for 24-48 hours for evidence of any allergic reaction, behavioral changes, convulsions and hypoglycemic symptoms. No untoward reaction was observed in any animal. The induction of diabetes was confirmed after the 5th day of alloxan treatment by estimation of elevated fasting blood glucose level. Only those rats with blood glucose level >150mg/dl was included in the study.

Experimental design for anti-diabetic activity

For Anti-diabetic study, the fasted diabetic rats will divided in to 6 groups of 6 animals each.

Group I : Diabetic control rats – received vehicle (5% gum acacia) only

Group II : Diabetic rats – received MI leaves extract 200mg/kg.

Group III : Diabetic rats – received MI leaves extract 400mg/kg.

Group IV : Diabetic rats – received Gliclazide 2mg/kg.

Group V : Diabetic rats – received Gliclazide 4mg/kg.

Group VI : Diabetic rats – received MI leaves extract 200mg/kg + Gliclazide 2mg/kg.

Treatment with drugs was started on the 6th day of alloxan treatment. All the drugs were given orally as a single dose in the morning. The fasting blood glucose levels were determined on day 0, 10, and 15.

Method of collection of blood

Blood sample for glucose estimation was collected from rat tail vein.[18]

Fasting blood glucose readings were recorded in all rats after an overnight fasting. Blood samples were obtained from rat tail vein, after applying xylene to make vein prominent. Blood glucose was estimated by glucose oxidase-peroxidase reactive strips and a glucometer.

RESULTS

The present study was undertaken to investigate the anti-diabetic activity of aqueous extract of leaves of MI alone and in combination with conventional oral anti diabetic agent on fasting blood glucose levels in alloxan induced diabetic albino rats.

The study was conducted on 6 groups of 6 animals in each group of either sex of albino rats weighing between 200-250 g. The drugs were administered orally once daily for 15 days.

Effect of Mangifera indica on blood glucose levels in alloxan induced diabetic rats

In alloxan treated rats, the rise in blood glucose level reached its peak value on the 5th day and then remained stable throughout the study period.

Results of present study indicate that 15 days treatment with aqueous extract of MI (200mg/kg and 400 mg/kg) produced a highly significant ($P < 0.0001$) decrease in fasting blood glucose level. With 200mg/kg MI extract, the blood glucose levels were 136.0 ± 1.82 on 10th day and 123.5 ± 1.83 on 15th day as compared to fasting blood glucose level of 186.8 ± 4.52 on 10th day and 195.8 ± 4.37 on 15th day in diabetic control animals.

And with 400 mg/kg of MI extract, the fasting blood glucose levels were 131.0 ± 1.592 on 10th day and 116.0 ± 1.915 on 15th day as compared to fasting blood glucose level of 186.8 ± 4.52 on 10th day and 195.8 ± 4.37 on 15thday in control animals. Thus both 200mg/kg and 400 mg/kg of aqueous extract of MI showed dose dependent hypoglycemic effect by lowering the fasting blood glucose levels in comparison to the control group. (Table 1)

Effect of Gliclazide on blood glucose level in alloxan –induced diabetic rats

Gliclazide produced significant ($p<0.0001$) decrease in fasting blood glucose levels in alloxan induced diabetic rats. With 2mg/kg the fasting blood glucose levels were 129.3 ± 1.87 on 10th day and 110.3 ± 2.512 on 15th day and with 4 mg/kg, 121.7 ± 2.07 on 10th day and 99 ± 1.592 on 15th day as compared to fasting blood glucose levels of 186.8 ± 4.52 on 10th day and 195.8 ± 4.37 0n 15th day in diabetic control animals. (Table 1)

Effect of Mangifera indica in combination with gliclazide on blood glucose levels in alloxan–induced diabetic rats

Combination of aqueous extract of MI

(200mg/kg) with gliclazide (2mg/kg) orally for 15 days significantly enhanced the glucose lowering effect of gliclazide. The fasting blood glucose levels with combined treatment were 125.0 ± 2.39 on 10th day and 105.2 ± 2.315 on 15th day as compared to fasting blood glucose levels of 129.3 ± 1.87 on 10th day and 110.3 ± 2.51 on 15th day in gliclazide (2mg/kg) treated group.(Table 1)

DISCUSSION

The world is facing an explosive increase in the incidence of diabetes mellitus and cost effective complementary therapies are needed. Although insulin has become one of the most important therapeutic agents known to medicine, there is a continuing effort to find insulin substitute, secretogogues or sensitizers from synthetic or plant source for the treatment of diabetes.

Plants and their bioactive constituents are used for the treatment of diabetes mellitus; especially in countries where access to the conventional treatment of diabetes mellitus is inadequate. There have been several reports on the hypoglycemic activity of MI.

In the present study, we investigate the effect of aqueous extract of MI (200mg/kg & 400 mg/kg) alone and in combination with conventional oral

Table1: Effect Of Aqueous Extract Of Mangifera Indica Alone and In Combination With Gliclazide In Alloxan Induced Diabetic Rats.

Blood glucose concentration in mg/dl Groups	Treatment	0th day	10th day	15thday
I	Diabetic rats with gum acacia 5%	189.5± 7.108	186.8± 4.520	195.8 ± 4.370
II	Diabetic rats with MI – 200mg/kg	182.5± 8.049	136.0 ± 1.826*	123.5 ± 1.839*
III	Diabetic rats with MI – 400mg/kg	197.5 ± 6.692	131.0 ±1.592*	116.0 ± 1.915*
IV	Diabetic rats with Gliclazide 2mg/kg	194.3± 5.258	129.3± 1.874*	110.3± 2.512*
V	Diabetic rats with Gliclazide 4mg/kg	192.3± 5.625	121.7± 2.076*	99 ± 1.592*
VI	Diabetic rats with Gliclazide 2mg/kg + MI 200 mg/kg	194.7± 6.275	125.0± 2.394*	105.2 ± 2.315*
F value		0.7303	88.77	185.8
P value		0.6063	< 0.0001 *	< 0.0001*

*All values are expressed as mean ± SEM (n=6) p<0.0001 on 10th day and 15th day (ANOVA followed by Dunnet's multiple comparison test).

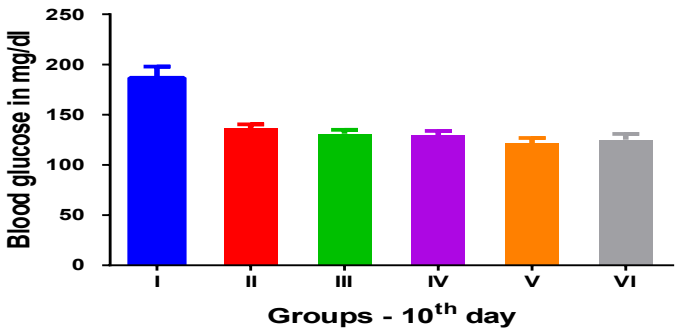
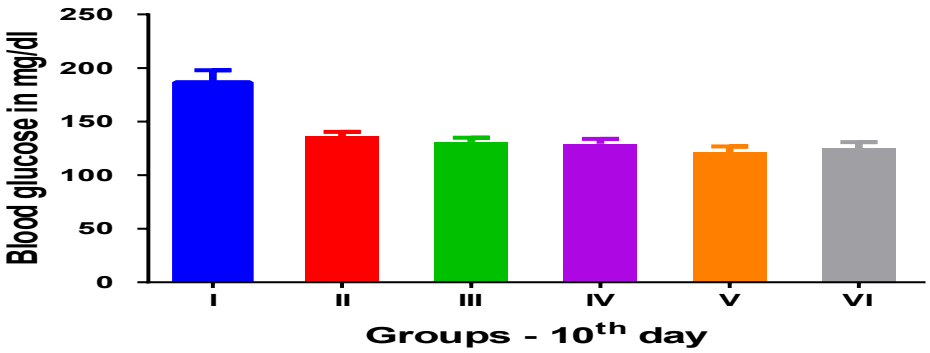


Fig. 1: Effect Of Aqueous Extract Of Mangifera Indica Alone And In Combination With Gliclazide in Alloxan Induced Diabetic Rats on 10th day



I- Diabetic rats with gum acacia 5%; II- Diabetic rats with MI 200mg/kg; III-Diabetic rats with MI 400mg/kg; IV- Diabetic rats with Gliclazide 2mg/kg; V- Diabetic rats with Gliclazide 4 mg/kg; VI- Diabetic rats with Gliclazide 2mg/kg +MI 200mg/kg

Fig. 2 : Effect Of Aqueous Extract Of Mangifera Indica Alone And In Combination With Gliclazide In Alloxan Induced Diabetic Rats on 15th day

anti-diabetic agent on fasting blood glucose level in alloxan induced diabetic albino rats. Following the administration of alloxan, it gets concentrated in the islet cells and in the liver, where it is reduced to dialuric acid. This acid is unstable in aqueous solution and undergoes oxidation back to alloxan, accompanied by generation of O_2^-,hydrogen peroxide and hydrogen radical. The liver contains high superoxide dismutase (SOD), catalase and glutathione peroxidase activity, which can scavenge these free radicals. On the contrary, the islet cells have low concentration of these enzymes and are vulnerable to the cytotoxic effects of the free radical. It is reported that increase in islet cell SOD activity can prevent or decrease alloxan toxicity.

The result of our study showed that aqueous extract of MI, administered orally for 15 days, significantly ($p<0.0001$) decreased fasting blood glucose levels in alloxan induced diabetic rats. Combination of MI extract with gliclazide produced a significant ($p<0.0001$) decrease in blood glucose level which is higher than that produced by gliclazide alone.

The results of present study correlated with the earlier work. The 50% ethanol extract of the leaves of MI has been reported to produce a significant hypoglycemic effect at a dose of 250 mg/kg, both in normal and streptozotocin- induced diabetic animals. The stimulation of ² – cells to release insulin was thought to be part of mechanism of action.[19]The extract leaves of MI also produced anti diabetic activity in using normoglycemic glucose– induced hyperglycemia and streptozotocin induced diabetic mice.[20]The aqueous extract of MI (400mg/kg), administered for 21days has been reported to reduce the concentration of glucose, cholesterol and triglycerides in alloxan induced diabetic rats, without any toxic effect on the liver, as the biochemical markers of liver damage i.e.; AST, ALP and ALT were seen in lower concentration.[21]

Recent studies have shown that methanolic extract of MI leaves inhibit the DPPIV (dipeptidyl peptidase IV) and enhances the GLP- 1 (Glucagon Like Peptide) for type 2 DM and thus could be a good lead for further development as a new anti-diabetic agent.[22]A clinical study conducted in type 2 (Non-insulin dependent diabetes mellitus – NIDDM)

patients, showed that MI leaves (powdered part, aqueous or alcohol extract) combined with oral hypoglycemic agents lowered glucose to normal levels in patients whose diabetes was not controlled with these agents or in those patients in whom these agents produced adverse effects on dose increment, which supports the need for the study.[23]

It has been reported that MI extract had powerful antioxidant activity because of its high total phenols and total flavonoids content. These antioxidants activities might be the factor responsible for lowering diabetic complications observed in the streptozotocin induced diabetic rats.[24]

In brief, MI extract increases peripheral utilization of glucose; increases hepatic and muscle glycogen content; promotes ² cell repair and regeneration; increases C peptide levels; it has antioxidant properties and protects ² cell from oxidative stress; it exerts insulin like action by reducing the glycated hemoglobin levels, normalizing the micro albuminuria and modulating lipid profile. Thus, minimizes long term diabetes complication.[25]

The tender leaves of mango tree are traditionally considered as useful medicine of diabetes. Generally in ethno medical practices, about 15 g of fresh young mango leaves are kept overnight in about 250 ml of water and are grind in the next morning before filtering. The aqueous filtrate is given to the diabetic patient in the morning to control early diabetes. Sometimes instead of fresh leaves dried leaves powder is also used as a diabetic medicine.[26]Therefore this extract of MI can be added to the natural products used for treating diabetes as neutraceutical or functional foods. And can be safely co-administered with a conventional oral hypoglycemic agent for better Glycemic control thereby preventing the development of complications of diabetes mellitus at bay.

CONCLUSION

Thus from our study, it is concluded that MI when administered in alloxan induced diabetic rats has shown the following effects: MI has decreased the fasting blood glucose levels to normal in alloxan induced diabetic rats. The dose dependent lowering of the fasting blood glucose level is observed with extract. The best control on fasting blood glucose levels were observed with combination of MI extract and gliclazide.

In conclusion, the result of the present study shows that MI extract brings back the fasting blood glucose levels to normal in alloxan - induced diabetic rats i.e. shows hypoglycemic activity and the chemical compounds responsible for this effect is flavonoids, tannins, steroids and trepenoids and the aqueous extract of the leaves contains higher level of phenols and flavonoids which exhibit greater antioxidant activity there by lowering the diabetic complications.

Thus, instead of increasing the dose of the conventional oral hypoglycemic agent, which would result in more side effects, a combination of the low dose oral hypoglycemic agent with low dose or even higher dose of MI leaves extract, which not only serves to control the blood glucose levels but also has many advantages as mentioned above, can be used in the treatment of diabetes mellitus.

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