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Screening of *Madhuca indica* for Antidiabetic Activity in Streptozotocin and Streptozotocin – Nicotinamide induced Diabetic Rats

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Abstract: Diabetes is a chronic metabolic disorder characterized by hyperglycemia and polyurea and this is the leading disease in the world causing morbidity. Many allopathic medicines are available to treat diabetes, but treatment associates with many side effects which were compensated by replacing allopathic medicine with natural drugs. Many natural drugs shown significant antidiabetic activity but all those natural drugs are not commonly available which provoked us to initiate the present study of evaluation of Antidiabetic activity of *Madhuca indica*. In this present study we extracted *Madhuca indica* by using different solvents viz. methanol, Petroleum ether and water. Among these extracts Methanolic extract of *Madhuca indica* has shown significant anti diabetic activity against streptozotocin and streptozotocin – nicotinamide induced diabetic models in wistar rats.

Key words: *Madhuca indica*, Diabetes, Streptozotocin- nicotinamide.

Introduction

Diabetes mellitus is chronic metabolic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced [1]. It is estimated that 180 million people worldwide were suffering from diabetes and the number may probably double by the 2030. Reports from world health organization indicate that diabetes mellitus is one of the major killer of our time, with people in South East Asia and Western Pacific being most at risk [2]. India leads the world today with the largest number of diabetes patients in any given country. Several epidemiological studies confirm that diabetes is one of the most common non-communicable diseases globally, and it is the fourth or fifth leading cause of death in most developed countries [3].

The management of diabetes includes diet, exercise as well as oral hypoglycemic and insulin, these treatments do not effectively prevent the complications of diabetes and treatment with above said allopathic medication has several side effects. The obvious limitations of these management methods necessitate a search for help among arsenal of herbs available to human [4]. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic acitivity when assessed using presently available experimental techniques [5].

Madhuca indica commonly known as mahua belongs to the family Sapotaceae. Mahua is a large, shady, deciduous tree doting much of the central Indian landscape, both wild and cultivated. Mahua seeds are of economic importance as they are good source of edible fats [6]. The distilled juice of the flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, as well as bronchitis [7]. The leaves are applied as a poultice to relieve eczema. The aerial parts are used for treatment of inflammation [8]. The bark is a good remedy for itch, swelling, fractures and snake-bite poisoning, internally employed in diabetes mellitus. Previous phytochemical studies on *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides [9, 10].

Materials and Methods

Plant Material

The bark of *Madhuca indica* was collected and authentified by Dr. Madhav Chetty, Department of Botany, Venkateswara University; Tirupathi, India. Collected plant material was washed thoroughly with water and dried under shade. Dried pieces of bark were powdered in a grinder and the powder was extracted with different solvents such as methanol, petroleum ether and water by soxhelation process. The extracts were evaporated to dryness at a controlled temperature (45 ^{o}C).

Animals

Male wistar rats weighing 150-175 g were selected for the present study, procured two weeks prior to the study and maintained in institutional animal house, so that animals could acclimatize to the new environment. The institutional animal ethics committee permission was obtained before starting the experiments on the animals. All the chemicals used for the study were of analytical grade and Streptozotocin (STZ) was procured from sigma labs, Bangalore, India.

Acute oral toxicity studies

The aim of this study is to determine lethal dose. In this study the testing drug was administered in a single dose by oral route and the dose was increased in a graded manner. LD 50 for methanolic, petroleum ether and aqueous extracts were observed at 1000 mg/kg, 1500 mg/kg and 2000 mg/kg body weight, respectively. Therefore 1/10th of their preceding doses viz. 75 mg/kg, 125 mg/kg, 175 mg/kg body weight selected as testing doses.

Experimental studies

Streptozotocin induced diabetes mellitus[11]

Male wistar rats weighing 150-175 g were used for the study. After procuring, the animals were acclimatized for two weeks at normal laboratory condition. All animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages. Before conducting the experiment, ethical clearance was obtained from Institutional Animal Ethics Committee of Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad. Diabetes was induced in 16 h fasted male wistar rats by intraperitoneal injection of 65 mg/kg body weight of streptozotocin. Streptozotocin was dissolved in 50 mM cold sodium citrate buffer (pH 4.5) immediately before use. After 72 h, rats with marked hyperglycemic fasting blood glucose >200 mg/dL were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages.

The rats were divided into six groups consisting of six each.

Group 1: Administered vehicle serves as normal control.

Group 2: Administered streptozotocin (65 mg/kg i.p.) serves as diabetic control

Group 3: Diabetic rats treated with methanolic extract (75 mg/kg, p.o. once daily)
Group 4: Diabetic rats treated with petroleum ether extract (125 mg/kg, p.o. once daily)
Group 5: Diabetic rats treated with aqueous extract (175 mg/kg, p.o. once daily)
Group 6: Administered reference standard, Insulin (6 IU/kg, i.p.)
The animals were treated with respective drugs for 30 days. On the next day, blood was withdrawn by puncturing retro orbital plexus, serum was separated and serum glucose, cholesterol, triglycerides (TGs) and HDL levels were estimated.

Streptozotocin – Nicotinamide induced diabetes mellitus [12]

Diabetes was induced in 16 h fasted male wistar rats by combined intraperitoneal injection of streptozotocin (STZ) 65 mg/kg and nicotinamide (NIC) 230 mg/kg. Streptozotocin was dissolved in 50 mM cold sodium citrate buffer (pH 4.5) immediately before use and Nicotinamide was administered 15 min before administration of STZ. After 72 h, rats with marked hyperglycemic fasting blood glucose >200 mg/dL were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages.

The rats were divided into six groups consisting of six each.
Group 1: Administered vehicle serves as normal control.
Group 2: Administered streptozotocin (150 mg/kg i.p.) and nicotinamide (360 mg/kg
i.p.) Serves as diabetic control
Group 3: Diabetic rats treated with methanolic extract (75 mg/kg, p.o. once daily)
Group 4: Diabetic rats treated with petroleum ether extract (125 mg/kg, p.o. once daily)
Group 5: Diabetic rats treated with aqueous extract (175 mg/kg, p.o. once daily)
Group 6: Administered reference standard, Metformin (200 mg/kg, p.o)

The animals were treated with respective drugs for 30 days. On the next day, blood was withdrawn by puncturing retro orbital plexus, serum was separated and serum glucose, cholesterol, triglycerides (TGs) and HDL levels were estimated.

Statistical Analysis: The values were expressed as mean ±SEM for 6 animals. The results were subjected to statistical analysis by using one – way ANOVA followed by Tukey –Kramer test to calculate the significance difference among the groups. $P<0.05$ was considered as significant.

Table 1: Effect of different extracts of *Madhuca indica* on serum glucose, cholesterol, triglycerides and HDL in streptozotocin induced diabetic rats

Groups	Treatment	Glucose (mg/dL)	Cholesterol (mg/dL)	TGs (mg/dL)	HDL (mg/dL)
G1	Control	86.5± 1.688	56.17± 2.358	65.17± 1.797	41.33± 1.022
G2	Diabetic Control	262± 3.568^{+++}	127.33± 2.246^{+++}	77.67± 1.333^{+++}	30.67± 0.954^{+++}
G3	Methanolic MI (75 mg/kg, p.o. once daily)	164.83± 2.774***	84± 2.033***	64.33± 1.801***	39.17± 0.833***
G4	Pet. Ether MI (125 mg/kg, p.o. once daily)	249± 1.291*	120.17± 1.973^{ns}	75.17± 1.42^{ns}	31.17± 0.601^{ns}
G5	Aqueous MI (175 mg/kg, p.o. once daily)	248± 2.828*	123.83± 1.956^{ns}	74.5± 1.088^{ns}	31.83± 0.703^{ns}
G6	Standard Insulin	118.17± 3.341***	64.67± 1.801***	63.17± 1.302***	40.67± 1.202***

Values expressed as Mean ± SEM for six animals
$^{+++}P<0.001$, $^{++}P<0.01$, $^{+}P<0.05$ when compared to normal control group
$^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$ when compared to diabetic control group

Table 2: Effect of different extracts of Madhuca Indica on serum glucose, cholesterol, triglycerides and HDL in streptozotocin- nicotinamide induced diabetic rats

Groups	Treatment	Glucose (mg/dL)	Cholesterol (mg/dL)	TGs (mg/dL)	HDL (mg/dL)
G1	Control	86.17± 1.579	59.5± 1.708	64.67± 2.060	42.33± 1.085
G2	Diabetic Control	250.17± 3.877^{+++}	86.67± 2.028^{+++}	79.33± 1.333^{+++}	31.67± 0.882^{+++}
G3	Methanolic MI (75 mg/kg, p.o. once daily)	165.17± 3.673***	122± 1.571***	68.16± 1.740***	38.17± 0.946***
G4	Pet. Ether MI (125 mg/kg, p.o. once daily)	245.17± 1.922^{ns}	119± 2.217*	74.67± 1.382^{ns}	29.83± 0.703^{ns}
G5	Aqueous MI (175 mg/kg, p.o. once daily)	239.67± 3.148^{ns}	62.5± 2.432**	72.5± 0.992*	29± 0.966^{ns}
G6	Metformin (200 mg/kg, p.o)	127.5± 4.349***	62.5± 2.432***	68.83± 1.014***	41.33± 1.116***

Values expressed as Mean ± SEM for six animals
$^{+++}$P<0.001, $^{++}$P<0.01, $^{+}$P<0.05 when compared to normal control group
***P<0.001, **P<0.01, *P<0.05 when compared to diabetic control group

Results

Table 1 summarizes the effects of STZ and treatments on serum glucose, cholesterol, TGs and HDL levels. From the biochemical evidences it is clearly understood that the STZ has raised the serum glucose, cholesterol, TGs and decreased the HDL levels. Among the treatment group's methanolic extract of *Madhuca indica* and standard drug significantly reversed the parameters which were associated with induction of STZ where as the treatment with pet. ether and aqueous extracts did not significantly reverse the STZ induced parameters.

Table 2 summarizes the effects of STZ-NIC and treatments on serum glucose, cholesterol, TGs and HDL levels. From the biochemical evidences it is clearly indicating that the combination of STZ and NIC has effectively induced the diabetes which is marked by the elevated serum glucose, cholesterol, TGs and decreased the HDL levels. After the treatment with different extracts of *Madhuca indica* and standard drug has reversed the altered parameters but among the treatment groups only methanolic extract of *Madhuca indica* and standard drug significantly reversed the altered parameters.

Discussion

Methanolic extract of *Madhuca indica* have significantly decreased the serum glucose level in streptozotocin and STZ-NIC induced diabetic rats. *Momordica charantia*, *Gymnema sylvestre*, *Ocimum sanctum* etc plants have also shown antihyperglycemic activity in streptozotocin induced diabetes. The mechanisms of both allopathic medicines and the traditional herbal medicines to lower blood glucose are [13].

- To stimulate beta -cell of pancreatic islet to release insulin
- To resist the hormones which rise blood glucose levels
- To increase the sensitivity of insulin receptor site to insulin
- To decrease the leading-out of glycogen
- To enhance the use of glucose in the tissue and organ
- To clear away free radicals, resist lipid peroxidation and
- Correct the metabolic disorder of lipid and protein

Oral administration of methanolic extract of *Madhuca indica* showed significant regression of the diabetic state and restored the deranged serum glucose, cholesterol, triglycerides and HDL parameters in STZ and STZ-NIC induced diabetes. This observation may indicate that *Madhuca indica* enhances insulin release from destroyed pancreatic β-cells. Diabetic rats treated with methanolic extract of *Madhuca indica* significantly decreased the serum cholesterol and triglyceride level since insulin is a major hormone regulating lipid metabolism. *Madhuca indica* facilitated stimulation of insulin secretion in STZ induced rats will help to overcome lipid metabolism abnormalities and increase of glucose uptake in the presence of insulin suggests the possibility of increased binding of insulin to receptors in STZ-NIC induced rats.

Conclusion:

From the above results, it can be conclude that methanolic extract of *Madhuca indica* has significantly decreased the elevated blood glucose, cholesterol, TG's and increased the HDL levels in diabetic rats, and hence *Madhuca indica* may be effectively active against Diabetes mellitus.

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