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AMELIORATIVE EFFECT OF MANGIFERA INDICA LEAVES ON STREPTOZOTOCIN INDUCED TYPE II DIABETES IN MALE ALBINO (WISTAR) RAT MODEL: ROLE OF ANTIDIABETIC AND ANTIOXIDANT EFFECTS

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Abstract
Diabetes is a chronic condition that affects the body mechanisms. It has a severe impact on the essential organs and causes a decrease in pancreatic enzymes (lipase and amylase). The objective of this research work was to investigate the antidiabetic and antioxidant effects of ethanolic leaves extract of *Mangifera indica* (*M. indica*) in type II diabetic male albino (Wistar) rat model. Streptozotocin (45 mg/kg) was used to induce diabetes in male rats. Glibenclamide (05 mg/kg), and sitagliptin (10 mg/kg) were used as standard drugs. Extract of *M. indica* at doses of 200 and 400 mg/kg respectively, were administered by oral route in diabetic rats daily up to 28 days. The extract of *M. indica* was assessed for its acute oral toxicity, biochemical parameters notably fasting blood glucose level and pancreatic enzymes; amylase and lipase, and its antioxidant effects in rats. Blood samples of rats were collected using cardiac puncture techniques for the biochemical analysis and fasting glucose levels were determined through strip method. Antioxidant potential of *M. indica* leaves extract in ethanol was assessed by using di-phenyl-picryl-hydrazyl radical scavenging assay. The result of an acute oral toxicity study revealed that the *M. indica* ethanolic leaves extract caused no behavioral changes and mortality in rats (LD50>6000 mg/kg). Extract of *M. indica* exhibited highly significant ($p<0.001$) antidiabetic effects by lowering the fasting blood glucose levels in diabetic rats and significantly ($p<0.001$) improved the levels of pancreatic enzymes (amylase and lipase) as compared with diabetes inducer group. *M. indica* leaves extract exhibited effective antioxidant activity.

Keywords: *Mangifera indica*, Animal model, Acute toxicity, Antidiabetic, Antioxidant

Introduction

Diabetes mellitus is a collection of metabolic disorders caused by a lack of insulin secretion and/or i nsulin resistance [1]. By 2025, the World Health Organization estimates, three hundred million individuals worldwide will suffer from diabetes [2]. Patients with diabetes for an extended period of time may develop peripheral neuropathy, nephropathy, and retinopathy [3-4]. At present, the primary treatments for type-II diabetes include antidiabetic medications likes; sulfonylureas, biguanides, dipeptidyl peptidase - 4 inhibitors and α-glucosidase inhibitors. The list of side effects from these medications is considerable. It remains consequently imperative to find safer and more effective drugs for diabetes [5].

Reactive oxygen species are examples of free radicals that the human body produces through a variety of endogenous processes, exposure to diverse physiochemical environments, or pathological states. These free radicals have been associated with the etiology of numerous diseases, such as diabetes, cancer, liver cirrhosis, and atherosclerosis [6]. In order to prevent mutilation from reactive oxygen species, antioxidants are playing an essential role [7]. It has been discovered that numerous plant products and extracts have strong antioxidant characteristics [8-10].

Traditional plant-based therapies for diabetes are used widely [11]. *Mangifera indica* (Mango), the most well-known tropical fruit in Asia, is an enormously evergreen tree belonging to the Anacardiaceae family [12-13]. Several research projects have documented the advantageous impact of *M. indica* on issues associated with diabetes. According to Parmar, rats given an atherogenic diet showed improvements in serum glucose, insulin, and cholesterol levels when exposed to a methanolic extract of *M. indica* peel [14]. Additionally, a number of polyphenols found in *M. indica* byproducts, including myricetin, epicatechin and epigallocatechin enhance the insulin signalling pathway and may have antidiabetic effects [15].

The botanicals are widely used to treat a variety of ailments, but less is known about their toxicity and safety issues, which are always a worry [16]. Poisoning is expressed as toxicity, which is the state of unfavorable effects triggered by the interaction of toxicants with cells. Toxic effects may occur prior to toxicant binding to essential organs such as the liver and kidneys [17]. The acute oral toxicity study was conducted on animals (rats) under the Organization for Economic Cooperation and Development (OECD) rules [18]. The evaluation of the acute oral toxicity of *M. indica* ethanolic leaves extract is important to ensure its safety. The present investigation was undertaken to study the anti-diabetic and antioxidant effects of the ethanolic extract of *Mangifera indica* on streptozotocin-induced diabetic rats.

Material and Method

Plant material collection

M. indica leaves were taken from cultivated trees in the Department of Botany at the University of Karachi. Plant leaves were identified by plant taxonomist as voucher specimens for the herbarium; G H. NO. 95717.

Reagents and Instruments

Ethanol (Analytical grade 99.99%), Streptozotocin (BioShop Canada, Lot No. 5K40517), Standard drugs (glibenclamide and sitagliptin), Ethanolic extract of *M. indica* (200 and 400 mg/kg), Alpha amylase kit (Cat. No. 5.17160.0001), Lipase kit (Cod. 38297).

The following instruments were used in the research work; Rotary evaporator (EYELA N-1000, Tokyo Rikakikai Co., Tokyo, Japan), Glucometer (Exactive vital), Eppendorf centrifuge machine (5810 R), Automatic selectra ProM chemical analyzer (ELITech Group - Clinical Systems).

Extraction of *Mangifera indica* leaves

The leaves of *M. indica* were taken and washed for the removal of dust particles. Then, dried under the shed, and then grated. The grated leaves were ground and then extracted with ethanol. Ethanol

was removed from plant extract by using a rotary evaporator to produce a thick gummy residue of dark greenish appearance [19-20].

Acute oral toxicity study

Acute oral toxicity, the LD50 was carried out by the OECD guideline 420 (Fixed dose Method) [21]. Albino male rats were weighed 180-200±05 grams and selected by random sampling technique. Normal healthy rats were alienated into two groups of animals (control & treated groups). Prior to the experiment, the rats were fasted for four-hours and were kept in standard conditions [22]. Control group received distilled water and extract groups received single once dose of ethanolic leaves extract of *M. indica* at doses of 2000 and 6000 mg/kg through oral route and observed the signs of toxicity like behavioral changes, augmented respiratory rate, nervous imbalance and mortality within initially 06 hours, 24 hours and followed by 14 days [23].

Animals for studies of antidiabetic and antioxidant effects

Male albino (Wistar) rats, weighing between 180-200±5 grams, were acquired by the animal house of Baqai Institute of Pharmaceutical Sciences (BIPS), Baqai Medical University (BMU), Karachi, Pakistan. A standard diet and unlimited water were given to albino rats, who were housed in a 12-hour light-dark cycle at ambient temperature of within 25 ± 2°C and a relative humidity of 55 - 65% [24].

Experimental design

Albino male rats were alienated into six groups of 12 rats. Drugs were orally administered for 28 days according to the following schedule: Control group (Group A) received distilled water. Diabetic inducer group (Group B) received streptozotocin at 45 mg/kg [25]. Streptozotocin induced type II diabetic rats (Group C and D) were given standard drugs glibenclamide at 05 mg/kg [26] and Sitagliptin 10 mg/kg [27], respectively. Group E and F (streptozotocin induced type II diabetic rats) received ethanolic leaves extract of *M. indica* 200 mg/kg and 400 mg/kg, respectively.

Ethical consideration

The research work was permitted by reference # BMU-EC/02-2021 from the board of studies (BASR), Baqai Institute of Pharmaceutical Sciences (BIPS), Baqai Medical University (BMU), Karachi, Pakistan.

Anti-hyperglycemic effects in streptozotocin induced diabetic rats

The streptozotocin liquified in citrate buffer (pH 4.5) was once injected intraperitoneally to induce diabetes in a group of rats that had fasted overnight. After 3-5 days of streptozotocin administration, the blood glucose levels of each rat were monitored. Rats with plasma glucose levels ≥200 mg were diagnosed as diabetic, and treatment was started [25]. During 28 days of treatment, checked fasting glucose level i.e., 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day, through the tail vein technique and glucometer was utilized to check the blood glucose level [28].

Pancreatic enzymes: Amylase and Lipase

After 28 days of treatment with extract of *M. indica* at different doses, the blood samples were taken in a vacutainer containing gel-tubes via cardiac-puncture technique [29-30]. The serum was centrifuged at 3000 rpm for 15 minutes [31]. Pancreatic amylase was evaluated by using alpha amylase kit assay (CNP-G3, enzymatic, kinetic) according to automatic selectra ProM analyzer. Pancreatic lipase was measured through lipase kit assay (Kinetic colorimetric) according to automatic selectra ProM analyzer.

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The objective of the DPPH radical scavenging assay is to quantify a stable free radical that is violet in color. After incorporating the DPPH solution with an antioxidant (standard gallic acid), the yellowish color DPPH was seen. After that, different concentrations of *M. indica* ethanolic leaves extract were incorporated into the DPPH solution, and the absorbance at 517 nm was measured [32]. Gallic acid served as a standard control radical scavenger. The IC50 of ethanolic extract of *M. indica* was then calculated and compared with standard radical scavenger.

$$\textbf{Percentage inhibition} = [(Ac-As)/Ac]\times 100$$

Where Ac and As represented the absorbance of the control and sample solutions at 517 nm, respectively.

Statistical Interpretation

SPSS version 21 one-way ANOVA test was used to estimate the data. The descriptive statistics (Mean±SEM) were calculated. At $p<0.05$, differences were considered significant.

Results

Acute oral toxicity study

The effect of ethanolic leaves extract of *M. indica* on the appearance and the general behavioral pattern of rats are revealed in table 1. In an acute oral toxicity study, the single administration of ethanolic leaves extract of *M. indica* 2000 and 6000 mg/kg was done. The behavioral patterns of rats were observed first 06 hours, 24 hours and followed by 14 days after the administration of ethanolic extract. Rats in both groups control and treated were normal and didn't show significant vicissitudes in behavior, hair loss, conscious, postural abnormalities, meal consumption and skin effects. In the extract group, fearfulness and piloerection were observed after administration of 6000 mg/kg dose during the first 06 hours, but it then became normal and this might be due to the stress of receiving the high oral dose of the extract.

Table 1. Acute oral toxicity of ethanolic leaves extract of *Mangifera indica* at doses of 2000 and 6000 mg/kg

Observations	Control group			Treated groups					
	Distilled water			Ethanolic extract (2000 mg/kg)			Ethanolic extract (6000 mg/kg)		
	06 hours	24 hours	14 days	06 hours	24 hours	14 days	06 hours	24 hours	14 days
Skin	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fur	Normal	Normal	Normal	Normal	Normal	Normal	PE	Normal	Normal
Restlessness	NO	NO	NO	NO	NO	NO	NO	NO	NO
Eyes	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Touch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Loss of appetite	NO	NO	NO	NO	NO	NO	NO	NO	NO
Fearfulness	NO	NO	NO	NO	NO	NO	Obs.	NO	NO
Tremors	NO	NO	NO	NO	NO	NO	NO	NO	NO
diarrhea	NO	NO	NO	NO	NO	NO	NO	NO	NO
Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality (%)	Nil			Nil			Nil		

***NO = Not observed; Obs. = Observed; PE = Piloerection**

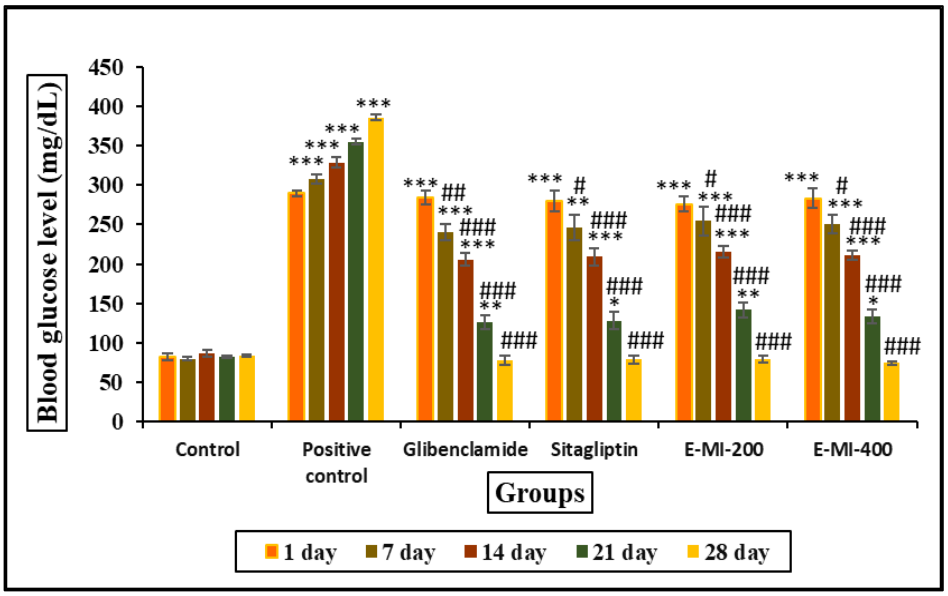
Anti-hyperglycemic effects

Effect of standard drugs on blood glucose levels of albino rats

Glibenclamide and sitagliptin produced highly significant ($p<0.001$) reduction in fasting glucose levels in streptozotocin induced diabetic rats. With glibenclamide, the fasting blood glucose levels were 284.2±8.81 (Mean±SEM) on 1st day and 77.6±5.62 on 28th day and with sitagliptin, the fasting blood glucose levels were 280.2±13.41 (Mean±SEM) on 1st day and 78.8±5.31 on 28th day as compared to fasting blood glucose level of 290±3.47 (Mean±SEM) on 1st day and 386±3.17 on 28th day in diabetic positive control group and same pattern of activity as comparison with control group (Graph 1).

Effect of ethanolic leaves extract of *Mangifera indica* on blood glucose levels of albino rats

After 28 days of treatment with ethanolic extract of *M. indica* (200 mg/kg and 400 mg/kg) produced highly significant ($p<0.001$) reduction in fasting glucose levels in streptozotocin induced diabetic rats. With 200 mg/kg *M. indica* extract, the blood glucose levels were 276.2±9.13 (Mean±SEM) on 1st day and 79.4±4.37 on 28th day as compared to fasting blood glucose level of 290±3.47 (Mean±SEM) on 1st day and 386±3.17 on 28th day in diabetic positive control group. While 400 mg/kg of *M. indica* extract, the fasting blood glucose levels were 283.2±12.33 (Mean±SEM) on 1st day and 74.4±2.20 on 28th day as compared to fasting blood glucose level of 290±3.47 (Mean ± SEM) on 1st day and 386±3.17 on 28th day in diabetic positive control group. In light of this, the ethanolic extract of *M. indica* at 200 mg/kg and 400 mg/kg demonstrated dose dependent hypoglycemic impact by reducing fasting blood glucose levels as compared to the positive control group and exhibiting the same pattern of activity as compared with the control group (Graph 1).



Graph 1. Blood Glucose Level: Comparison between control, positive control, standard (glibenclamide and sitagliptin) and treated groups (ethanol 200 and 400 mg/kg): Plasma glucose levels at weekly bases after oral administration of glibenclamide, sitagliptin and *M. indica* ethanolic leaves extract (200 & 400 mg/kg) in type II diabetic rat model.

E-MI = Ethanolic extract

Level of significance $p<0.05$*, $p<0.01$**, $p<0.001$***, respectively = all groups compared with control group

Level of significance $p<0.05$#, $p<0.01$##, $p<0.001$###, respectively = all groups compared with positive control group

Pancreatic enzymes

Effect of *Mangifera indica* on amylase level

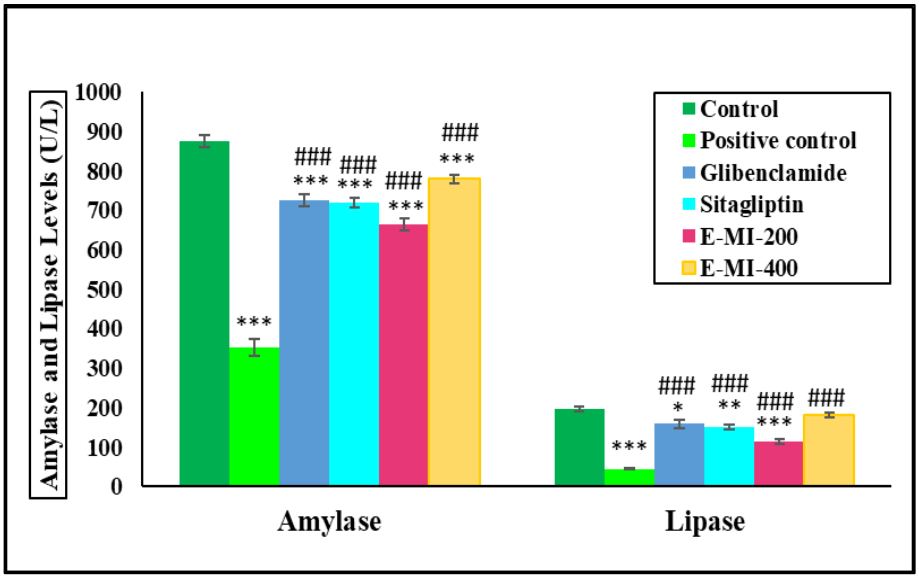
The pancreatic enzyme (amylase) levels were highly significant ($p<0.001$) increased in ethanolic leaves extract of *M. indica* (treated group) of doses 200 & 400 mg/kg were 665±15.54 (Mean±SEM) and 780±10.80 respectively, as compared to pancreatic enzyme amylase level of 352.50±20.56 in diabetic positive control group and same pattern of activity as compared with control group (Graph 2). Thus, both 200 mg/kg and 400 mg/kg of ethanol extract of *M. indica* showed dose dependent effect.

The pancreatic amylase levels of glibenclamide and sitagliptin (standard groups) were 725±14.43 (Mean±SEM) and 720±11.54 respectively, both standard groups results showed significant ($p<0.001$) increased in pancreatic enzyme amylase levels in diabetic rats as compared to positive control group (352.50±20.56) (Graph 2).

Effect of *Mangifera indica* on lipase level

The pancreatic enzyme (lipase) levels were highly significant ($p<0.001$) increased in ethanolic leaves extract of *M. indica* (treated group) of doses 200 & 400 mg/kg were 115±5.40 (Mean±SEM) and 181.25±6.88 respectively, as compared to the pancreatic enzyme lipase level of 45.50±2.25 in diabetic positive control group and same pattern of activity as compared with control group (Graph 2). Thus, both 200 mg/kg and 400 mg/kg of ethanol extract of *M. indica* showed dose dependent effect.

The pancreatic lipase levels of glibenclamide and sitagliptin (standard groups) were 160±10.80 (Mean±SEM) and 150±6.45 respectively, both standard groups results showed significant ($p<0.001$) increased in pancreatic enzyme amylase level in diabetic rats as compared to positive control group (45.50±2.25) (Graph 2).



Graph 2. Effect of control, positive control, standard (glibenclamide and sitagliptin) and treated groups (*Mangifera indica* ethanolic leaves extract) on pancreatic enzymes (amylase and lipase) in albino male rats

E-MI = Ethanolic extract

Level of significance $p<0.05$*, $p<0.01$**, $p<0.001$***, respectively = all groups compared with control

Level of significance $p<0.001$### = all groups compared with positive control group

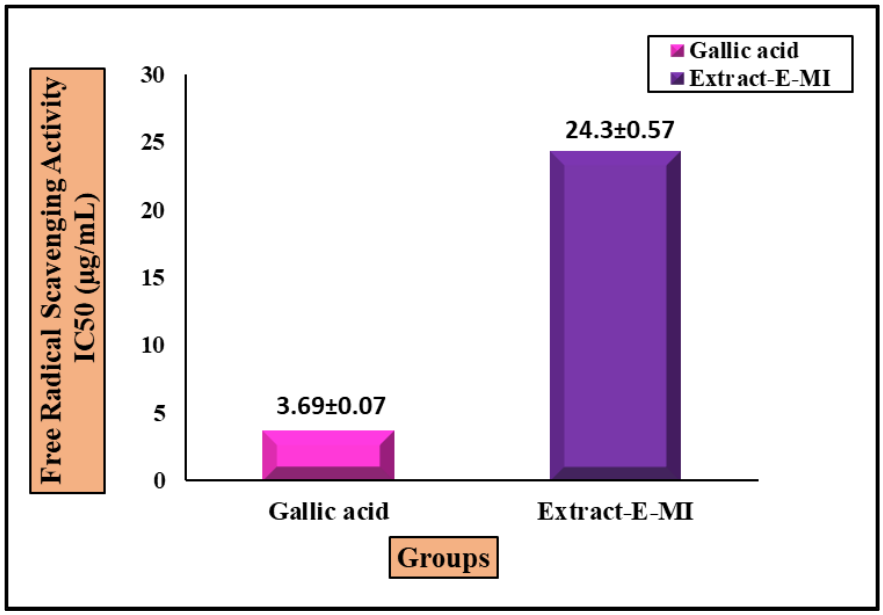
Antioxidant effects

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The extract was allowed to react with the stable free radical DPPH in order to assess its free radical scavenging potential. In the 517 nm range, the deep violet-colored DPPH radical exhibits intense absorption. The free radical assay of *M. indica* ethanolic leaves extract against DPPH were illustrated in table 2. The lower IC50 value indicates higher inhibition of free radicals. The antioxidant results confirmed that the extract of *M. indica* was depicted to have effective antiradical scavenging capacity 81.3% at a concentration of 500 µg/ml, as compared with standard gallic acid (95.3%) (Table 2). Results revealed that *M. indica* extract has effectively scavenged the free radicals with IC50 value of 24.3±0.57 µg/mL. Reference standard gallic acid has scavenged the free radicals with IC50 value of 3.69±0.07 µg/mL and it was found to be potent (Graph 3). Thus, the effective antioxidant effects of *M. indica* extract might contribute to mitigating the progress of free radicals associated with diabetes.

Table 2. Scavenging of DPPH radical by gallic acid and ethanolic leaves extract of *Mangifera indica*

	Concentration	Percentage Inhibition	IC_{50}
Ethanolic leaves extract *M. indica*	**(µg/ml)**	**(%)**	**(µg/ml)**
	100	**17.1**	**24.3±0.57**
	250	**40.6**	
	500	**81.3**	
Gallic acid			**3.69±0.07**



Graph 3. Free radical scavenging effects of ethanolic leaves extract of *Mangifera indica:* The bar graph illustrates the IC50 value of the ethanolic extract of *M. indica* and gallic acid.

Values shown as Mean±SEM
E-MI = Ethanolic extract

DISCUSSION

Acute oral toxicity study gives initial insight into a material's level of toxicity. In acute oral toxicity, low dose (2000 mg/kg/b.w.) and high dose (6000 mg/kg/b.w.) of the ethanolic leaves extract of *M. indica* administered single dose orally in rats. The results of the present study did not show any

adverse effects or mortality either at the low or high doses. Thus, extract of *M. indica* considered non-toxic at the acute level and consequently, the oral lethal dose (LD50) of the ethanolic leaves extract of *M. indica* is more than 6000 mg/kg/b.w. Previous study reported no toxicity of *M. indica* at level of 4.64 g/kg (LD50>4.64g/kg) [33]. Earlier studies of *M. indica* leaves extract revealed no acute toxicity at a maximum dose of 18.4 g/kg [34].

Diabetes mellitus is a chronic health condition that is connected with a number of consequences such as atherosclerosis, myocardial infarction, neuropathy, and nephropathy [35]. In this study, the determination was to assess the consequence of *M. indica* on antihyperglycemic activity, pancreatic enzymes and antioxidant activity in streptozotocin induced diabetic rats. Streptozotocin used universally to induce diabetes in experimental animals [36]. It induces diabetes by devastation of beta cells on Langerhans by alkylation of DNA [37].

In this study, glibenclamide and sitagliptin were used as standard drugs. Glibenclamide is the worldwide standard treatment for type II diabetes mellitus, and it works by increasing insulin release from pancreatic beta cells [38]. Sitagliptin is also used to treat diabetes, and it stimulates insulin release by stabilizing the GLPP-1 AND GIP (Incretins), both of which boost glucose-dependent insulin secretion, demonstrating significant glucose lowering efficacy with a lower risk of hypoglycemia [39].

Ethanolic leaves extract of *M. indica* was effective in type II diabetic rats when 28 days treatment was given to the diabetic induced rats. Muruganandan stated that mangiferin displayed antidiabetic activity by decreasing fasting plasma glucose levels in STZ diabetic rats and improving glucose tolerance [40].

The results of the study revealed that the ethanolic leaves extract of *M. indica* presented significant antihyperglycemic effects in type II diabetic model of albino male rats. When the ethanolic extract of *M. indica* leaves was orally administered at doses of 200 mg/kg and 400 mg/kg body weight respectively, produces potent hypoglycemic effect in type II albino rats but 400 mg/kg dose showed more significant ($p<0.001$) results as compared with 200 mg/kg dose. Ethanolic extract of *M. indica* presented equivalent pattern of activity as compared with glibenclamide group and sitagliptin group (standard groups). Positive control group have significant ($p<0.001$) results as compared with control group. Treated and standard groups have almost similar results at 28^{th} day of treatment. The acquired results are supported by the findings of other researchers [33, 41]. Perpetuo reported that diabetic rats ingesting *M. indica* flour had lower blood sugar levels. According to the author, increased glycogen levels could have caused animals' blood glucose levels to decrease [42].

The results of the study showed that the levels of amylase and lipase (pancreatic enzymes) were increased significantly ($p<0.001$) as compared with the positive control group. The amylase and lipase levels reduced significantly ($p<0.001$) in the positive control group (streptozotocin) as compared with the control group which expressed that diabetes is successfully induced in experimental rats. Ethanolic extract of *M. indica* showed the same pattern of activity as compared with standard groups (glibenclamide and sitagliptin). Treated and standard groups have highly significant ($p<0.001$) amylase and lipase values as compared with diabetic inducer group.

Many studies have shown various bioactive components in *M. indica* leaves, including gallic acid, catechin, and quercetin [43]. These chemicals could be the cause of the extract's hypoglycemic action. Because of their poor absorption characteristic, flavonoids such as myricetin, quercetin, and isoquercitrin have been evaluated for their noncompetitive inhibition of glucose and fructose transport on GLUT-2 by functioning as a strong luminal inhibitor [44]. Simultaneously, gallic acid might improve glucose absorption activity and GLUT-4 translocation [45]. It has been shown that mangiferin and its metabolite norathyriol have antidiabetic properties through a variety of pathways, including reducing blood glucose levels, enhancing glucose utilization, and improving insulin sensitivity by upregulating AMP-activated protein kinase phosphorylation [46].

Antioxidants may lower oxidative stress in diabetes, and as observed, antioxidants have been given in diabetes to reduce the long-term problems. It has been stated that oxidative stress-induced free radical production in cells may cause diabetes, particularly type II diabetes [47]. The three

mechanisms of increased oxidative stress associated with diabetes include metabolic stress, autoxidative glycosylation, and non-enzymatic glycosylation [35].

The action of antioxidants on DPPH is speculated to be due to their ability to donate hydrogen [48]. Free radical scavenging by DPPH is a well-known mechanism by which antioxidants reduce lipid peroxidation [49]. The outcomes of the current study demonstrated that the ethanolic leaves extract of *M. indica* has effective DPPH scavenging activity (81.3%) as compared with standard gallic acid (95.3%). Nevertheless, the free radical scavenging activity of *M. indica* extract was effective with IC50 value of 24.3±0.57 µg/mL. The outcomes revealed that the extract had proton donating potential, which might be used as a primary antioxidant as well as a scavenger or inhibitor of free radicals.

Antioxidants may play a significant part in the treatment of diabetes mellitus [50]. By consuming more natural antioxidants, might be able to reduce oxidative stress, which has been associated with the pathogenesis of diabetes mellitus, and maintain a tolerable antioxidant level. Among secondary metabolites, flavonoids are the most significant class of bioactive compounds [47].

Conclusion

Herbal plants are a rich source of possible bioactive components for diabetes therapy and management. *M. indica* leaves extract have potential hypoglycemic and antioxidant effects, as demonstrated in this work. It can be concluded that *M. indica* leaves are beneficial in the processing industry and a rich source of bioactive compounds.

Conflict of interest: No conflicts of interest are disclosed by the authors.

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