

EVALUATION OF ANTIDIABETIC POTENTIAL OF *NYMPHAEA NOUCHALI* BURM. F. SEEDS IN STZ - INDUCED DIABETIC RATS

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ABSTRACT

Objective: The aim of the present study was to investigate the antihyperglycaemic activity of hydroalcohol extract of *Nymphaea nouchali* seeds (NN) in streptozotocin (STZ) – induced diabetic rats.

Methods: Acute oral toxicity study was carried out in normal female rats. Male sprague dawley rats were rendered diabetic by STZ (50 mg/kg b.wt. i.p.). At a dose of 100 and 200 mg/kg of body weight, NN extract was administered orally to diabetic rats for 21 days. Metformin was used as a standard drug at a dose of 250 mg/kg b.wt. The antihyperglycaemic activity was determined by estimating various biochemical parameters.

Results: Oral administration of NN extract showed significant restoration of blood glucose level to normal. After 21 days of treatment, level of blood glucose, lipid profile (total cholesterol, triglyceride), hepatic and renal markers (SGOT, SGPT, γ GT, ALP, bilirubin, creatinine and BUN) significantly decreased when compared with the diabetic control. Concurrent histopathological studies of the heart and liver revealed normal histological pattern in the normal and extract-treated groups.

Conclusion: Lowering of blood glucose and other associated markers suggest that NN extract possess potent antidiabetic activity and hence confirms the traditional usage of this plant in the management of diabetes. However, histopathological examination of the pancreas suggests that this activity is brought by extrapancreatic mechanisms. Further, the exact mechanism of its action is yet to be elucidated.

Keywords: Antihyperglycaemic, *Nymphaea stellata*, Seeds, Streptozotocin, Blood glucose, Liver markers, Insulin sensitivity

INTRODUCTION

Diabetes is a group of metabolic disorder characterized by high levels of blood sugar (hyperglycaemia). It results from defects in insulin production (Type I) and / or insulin action (Type II) and impaired function in the metabolism of carbohydrates, lipids and proteins which leads to many long term health complications [1]. Diabetes is widely recognised as one of the leading causes of death and disability worldwide. In 2000, the WHO recorded 2.8% of the global population who have diabetes and it is expected to rise to 4.4% of the global population by 2030 [2]. Several oral hypoglycaemic agents are the primary forms of treatment for diabetes but they have prominent side effects and fail to significantly alter diabetic complications [3]. This is the main reason for increasing number of people seeking alternative therapies which are considered to be less toxic and free from side effects than the synthetic drugs. The burden of this disease is high not only because life-long treatment is necessary, but also due to prohibitive cost and unavailability of treatment in rural areas [4]. Hence, anti-diabetic compounds from natural origin can counter the high cost and poor availability of synthetic drugs in developing countries [5]. Thus, plant-based herbal drugs are emerging as primary components of holistic approaches to diabetes management [6]. Herbal anti-diabetics are found to have properties of releasing insulin from islets of langerhans and as an insulin sensitizer [7]. A great number of traditional medicinal plants have been reported to be useful in antidiabetic and antihyperlipidemic remedies worldwide, few of which have also established active compounds with its mechanism of action [8, 9].

One such medicinal plant is *Nymphaea nouchali* Burm. f. (synonym: *Nymphaea stellata* Willd.), an aquatic plant belonging to the family Nymphaeaceae. This is a well-known medicinal plant widely used in the Ayurveda and Siddha system of medicine for the treatment of diabetes, inflammation, liver disorders, urinary disorders, menorrhagia, blenorrhagia, menstruation problem, as an

aphrodisiac, and as a bitter tonic. The seeds are used in diabetes and also in cutaneous diseases [10]. With the plant having much beneficiary properties and having been traditionally claimed as an antidiabetic, not much work has been conducted in relation to the field of diabetes in the seeds of this plant. So far the ethanolic extract of leaves [11] and hydroalcoholic extract of flowers have been extensively studied for its antidiabetic activity [12]. Nymphayol, a steroid isolated from the flowers has been proved to be responsible for its antidiabetic activity [13]. The seeds have been investigated for its antioxidant [14] and antihepatotoxic activity [15]. The seeds are also reported to contain phenols, flavones, tannins, saponins and alkaloids [14]. Nymphasterol, a steroid has been isolated and identified from the seed of this plant [16].

Due to lack of sufficient literature on the role of *N.nouchali* seeds in diabetes, the current study was conducted to check various parameters to ascertain the antidiabetic activity of the seeds of *N.nouchali*. This study is focussed on evaluating the antihyperglycaemic effect of hydroalcohol extract of *N.nouchali* seeds in streptozotocin – induced diabetic rats.

MATERIALS AND METHODS

Plant material

Nymphaea nouchali (NN) seeds were collected from a pond in Kanyakumari District, India. Shade-dried seeds (500 g) were ground and extracted using 70% ethanol (1500 ml) in Soxhlet apparatus for 24 h. The extract was then evaporated to dryness in a rotary evaporator and the final brown-coloured, powdery crude extract was stored in air-tight container until used for the experiment.

Chemicals

Streptozotocin was obtained from Sigma chemicals, Bangalore, India. Kits to estimate glucose, triglyceride (TG), total cholesterol (TC), serum glutamate oxaloacetate transaminase (SGOT), serum

glutamate pyruvate transaminase (SGPT), γ -glutamyl transferase (γ -GT), alkaline phosphatase (ALP), bilirubin, creatinine and blood urea nitrogen (BUN) were purchased from Accurex Biomedical Pvt. Ltd., Mumbai. All other chemicals used were of analytical grade.

Experimental animals

Male Sprague Dawley rats (170-200 g) were used for the study. Animals were housed in groups (6 animals/cage) in polypropylene cages in a well-ventilated room (air cycles: 15/min; recycle ratio: 70:30) under an ambient temperature of $22\pm 3^\circ\text{C}$ and 40–65% relative humidity, with a 12-h light/dark artificial light cycle. They were provided with rodent feed (M/s. Provimi Animal Nutrition India Pvt. Ltd, India) and purified water *ad libitum*. Institutional Animal Ethics Committee (IAEC), Sri Ramachandra University, Chennai, India approved the study.

Acute oral toxicity study

Acute oral toxicity study was performed according to the OECD test guideline 423 – Acute toxic class method [17]. Young healthy adult Sprague Dawley female rats (140-180 g) were divided into two groups of 3 animals each. NN extract was administered once orally via gastric intubation at a dose level of 2000 mg/kg b.wt. Lethality and abnormal clinical signs were observed on the day of dosing and thereafter for 13 days. Body weight was recorded before dosing and thereafter once in a week till completion of the experiment. Gross pathological changes were also observed at the end of experiment.

Induction of diabetes

Diabetes was induced in rats by single intraperitoneal injection of STZ in citrate buffer (pH 4.5) at a dose of 50 mg/kg b.wt. After 72 h of induction, the animals which showed fasting blood glucose ≥ 250 mg/dl were considered diabetic and selected for the study.

Experimental design

The rats were divided into 5 groups of 6 rats each. The NN hydroalcoholic extract was suspended in 0.5% CMC and administered orally for 21 days.

Group 1: Normal control

Group 2: Diabetic control

Group 3: Diabetic + Metformin (250 mg/kg, p.o.)

Group 4: Diabetic + NN extract Low Dose (100 mg/kg, p.o.)

Group 5: Diabetic + NN extract High Dose (200 mg/kg, p.o.)

Biochemical analysis

Blood samples were withdrawn by retro-orbital puncture under light ether anaesthesia on the 0th, 7th, 14th and 21st day of the study for estimating blood glucose, TG and TC. At the end of 21 days treatment, the animals were euthanized and the levels of SGOT, SGPT, γ -GT, ALP, bilirubin, creatinine and BUN were determined using commercial kits following the manufacturer standard protocols in a semi-auto analyzer.

Histopathological examination

Organs were collected, blotted, freed from blood, fixed in 10% neutral buffered formalin for 48 h, trimmed and processed for paraffin embedment and 5 mm thickness of tissue sections were stained with haematoxylin and eosin for histopathological examination. Histological structures of liver, heart and pancreatic sections were examined using a light microscopy (Motic DMB1-2MP, China).

Statistical analysis

Data were expressed as Mean \pm SEM of six replicates and subjected to one-way ANOVA followed by Tukey's multiple comparison tests using Graph Pad Prism 5.03 (Graph Pad Software, San Diego, CA, USA). Values were considered statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS

Acute oral toxicity

No treatment-related deaths, abnormal clinical signs or remarkable body weight changes were observed in all the experimental animals. No gross pathological observation was recorded in all the experimental animals. From the above tested condition, LD₅₀ of the test drug was found to be greater than 2000 mg/kg b.wt., and was found to be safe when administered once orally to fasted female Sprague Dawley rats.

Antidiabetic study

A marked decrease ($p < 0.001$) in body weight was observed in diabetic rats as compared to the normal rats (Table 1) after diabetes induction. After two weeks of treatment with NN extract, the body weight significantly increased ($p < 0.001$) compared to the diabetic control. Progress in weight gain of animals in drug-treated group was observed till the end of the study.

Table 1: Effect of NN extract on the body weight of STZ-induced diabetic rats

Group	Body weight (g)			
	Day 0	Day 7	Day 14	Day 21
I	170.00 \pm 5.51	181.17 \pm 5.32	188.67 \pm 3.45	194.33 \pm 3.21
II	142.67 \pm 5.01 ^{##}	131.00 \pm 2.24 ^{###}	124.50 \pm 2.48 ^{###}	119.50 \pm 2.40 ^{###}
III	142.33 \pm 5.74	150.00 \pm 4.29 [*]	155.67 \pm 3.89 ^{***}	164.83 \pm 2.81 ^{***}
IV	141.83 \pm 4.23	146.00 \pm 3.82	151.17 \pm 2.95 ^{***}	153.67 \pm 3.25 ^{***}
V	140.33 \pm 3.45	148.17 \pm 2.81 [*]	153.67 \pm 3.12 ^{***}	161.17 \pm 2.34 ^{***}

All values are Mean \pm SEM for six rats. #, ##, ### indicates $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively vs. group I; *, **, *** indicates $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively vs. group II

Table 2: Effect of NN extract on fasting blood glucose levels in STZ-induced diabetic rats

Group	Glucose level (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
I	82.57 \pm 1.88	87.12 \pm 0.08	85.43 \pm 4.09	86.93 \pm 2.50
II	267.67 \pm 5.63 ^{###}	270.05 \pm 6.58 ^{###}	269.93 \pm 6.60 ^{###}	270.18 \pm 6.54 ^{###}
III	269.33 \pm 5.60	194.89 \pm 5.58 ^{***}	154.61 \pm 5.62 ^{***}	104.90 \pm 3.45 ^{***}
IV	268.33 \pm 5.91	252.20 \pm 5.78	226.55 \pm 5.30 ^{***}	198.52 \pm 2.41 ^{***}
V	267.33 \pm 7.01	243.28 \pm 7.46 [*]	205.43 \pm 6.59 ^{***}	166.19 \pm 4.88 ^{***}

All values are Mean \pm SEM for six rats. #, ##, ### indicates $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively vs. group I; *, **, *** indicates $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively vs. group II

Table 3: Effect of NN extract on Total Cholesterol levels in STZ-induced diabetic rats

Group	Total cholesterol (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
I	55.15±3.10	57.65±1.15	55.47±3.03	58.49±1.45
II	126.68±6.37###	132.63±7.40###	130.37±5.50###	133.70±2.97###
III	130.93±4.00	113.16±2.76*	89.73±4.15***	68.05±2.13***
IV	131.40±3.48	123.41±2.93	112.61±3.01*	106.89±3.69***
V	131.52±4.07	119.94±2.14	100.38±2.42***	84.57±2.95***

All values are Mean ± SEM for six rats. #, ##, ### indicates p<0.05, p<0.01, p<0.001 respectively vs. group I; *, **, *** indicates p<0.05, p<0.01, p<0.001 respectively vs. group II

Table 4: Effect of NN extract on Triglyceride levels in STZ-induced diabetic rats

Group	Triglycerides (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
I	73.11±3.10	74.22±2.42	73.80±2.48	74.83±3.02
II	162.14±7.57###	164.11±4.10###	162.39±4.85###	164.34±6.99###
III	164.00±7.63	123.41±4.53***	96.87±4.03***	83.16±5.26***
IV	164.29±6.87	150.72±5.15	136.97±2.75***	123.65±2.98***
V	162.22±3.10	143.97±5.29*	125.02±3.65***	94.10±3.82***

All values are Mean ± SEM for six rats. #, ##, ### indicates p<0.05, p<0.01, p<0.001 respectively vs. group I; *, **, *** indicates p<0.05, p<0.01, p<0.001 respectively vs. group II

Table 5: Effect of NN extract on hepatic and renal markers in STZ-induced diabetic rats

Group	SGOT (IU/l)	SGPT (IU/l)	GGT (IU/l)	ALP (IU/l)	Bilirubin (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)
I	84.00±3.88	20.90±2.27	2.24±0.35	382.71±3.60	0.88±0.16	0.65±0.09	24.01±1.40
II	175.91±5.50###	55.14±5.60###	4.75±0.65##	866.50±8.98###	1.70±0.03###	2.44±0.18###	43.17±0.87###
III	125.88±7.40***	32.83±4.73**	2.66±0.60*	554.26±7.26***	1.23±0.07***	0.70±0.09***	31.83±2.01***
IV	154.63±3.00*	44.58±2.36	3.02±0.09	708.89±8.49***	1.08±0.01**	1.48±0.10***	39.99±0.60
V	136.77±2.59***	39.12±1.05*	2.73±0.11*	604.33±9.67***	1.04±0.01***	0.86±0.04***	36.27±1.32**

All values are Mean ± SEM for six rats. #, ##, ### indicates p<0.05, p<0.01, p<0.001 respectively vs. group I; *, **, *** indicates p<0.05, p<0.01, p<0.001 respectively vs. group II

The effect of repeated oral administration of NN extract on blood glucose levels in STZ-diabetic rats is presented in Table 2. The NN extract at doses of 100 and 200 mg/kg caused significant reduction (p<0.001) of blood glucose levels compared to diabetic control and was related to the duration of treatment. Maximum reduction was observed on the 21st day. The NN extract showed results comparable to that of the standard metformin.

The level of serum TC increased significantly (p<0.001) in all the diabetic groups on the 0th day when compared with the normal rats. Treatment with 200 mg/kg NN extract and metformin showed a significant reduction (p<0.001) in the elevated TC level from the second week of the study (Table 3). Similarly, there was also a rise in the level of serum TG in diabetic animals. NN extract significantly (p<0.001) brought down the TG level to normal (Table 4).

The efficacy of NN extract on hepatic and renal markers was analysed on the 21st day (Table 5). At 200 mg/kg of the drug, there was a significant reduction (p<0.001) in the SGOT, ALP, bilirubin and creatinine levels when compared with the diabetic control group. The level of BUN also showed significant reduction (p<0.01) in the treated groups as compared with the untreated diabetics, while levels of SGPT and GGT significantly reduced (p<0.05) compared to the diabetic control.

The histopathological observations support the results obtained from serum enzyme assays. Histology of liver, heart and pancreas sections of normal control animals showed well-preserved cell structure (Figure 1). But the sections of diabetic control showed abnormalities with multifocal hepatocellular hypertrophy and atrophy of 50-60% islet cells (Figure 2). On treatment with 200 mg/kg of NN extract, the histological architecture of liver and heart showed more or less normal pattern, while the pancreas showing still a reasonable degree of abnormalities (Figure 3).

DISCUSSION

Diabetes and its complications is becoming the third leading cause of death after cancer and cardiovascular diseases. Many serious side effects of insulin therapy and oral hypoglycaemic drugs necessitate the search for newer effective and safer class of compounds to overcome diabetic problems [18]. In recent years, herbal products have started to gain importance as a source of antidiabetic medicines. It has been estimated that more than 1000 plant species are used as folk medicine for treating diabetes though most lack scientific evidence [19].

The present study was hence carried out to evaluate the antidiabetic effect of hydroalcoholic extract of *N.nouchali* seeds on streptozotocin-induced diabetic rats. The extraction used in the study provided an efficient method for extracting the active principles that could have brought the possible hypoglycaemic effect. Many plant chemical constituents have been reported earlier as to be responsible for bringing antidiabetic effect [20]. Acute oral toxicity studies performed showed no gross pathological abnormalities which provided NN extract direct relevance for protecting human and animal health. The data presented in the study demonstrated that 100 mg/kg was the minimum dose that produced an effective glucose lowering effect. The phytochemical analysis showed that the major constituents were tannins and other phenolic compounds [14], which could have been responsible for its hypoglycaemic effect. More recently, phenolic compounds [21, 22] and tannins [23] have been reported as active principles and as being involved in glucose metabolism regulation.

STZ was used as an agent to induce diabetes mellitus in this study which acts by selectively causing cytotoxicity on pancreatic β -cells [24]. Thus it affects the endogenous insulin release and subsequent

increase in blood glucose level [25] by rapid depletion of β -cell and is generally dependent upon the degree of β -cell destruction. It is a difficult task to regenerate the integrity and function of β -cells once they are destroyed. However, a number of herbal compounds have been reported to have this effect on chemically-induced diabetic rats [26]. Under our experimental conditions, Sprague Dawley rats treated with 50 mg/kg STZ underwent a strong hyperglycaemia

(250-275 mg/dl) that remained unchanged until the study was complete due to incomplete β -cell destruction. The NN extract at the tested dose of 100 and 200 mg/kg showed a comparable activity with the standard metformin.

Metformin decreases hyperglycaemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis) [27].

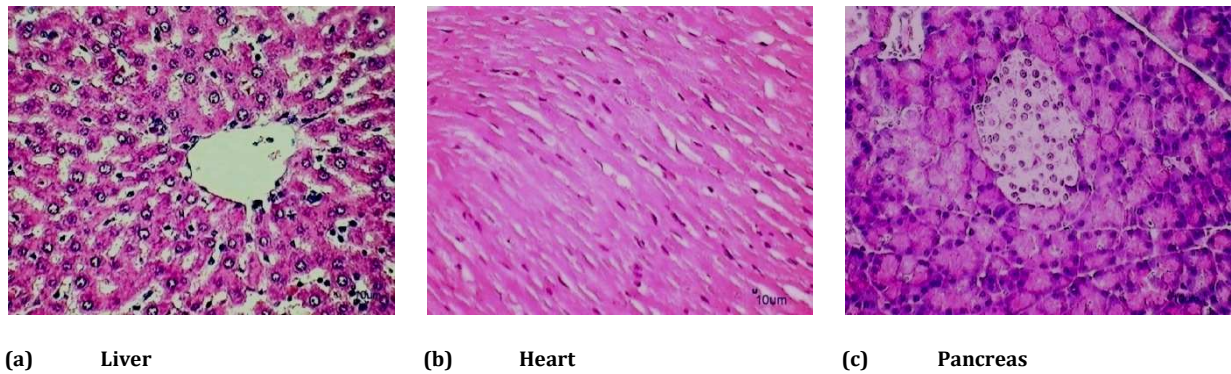


Fig. 1: Histopathology of normoglycaemic rats

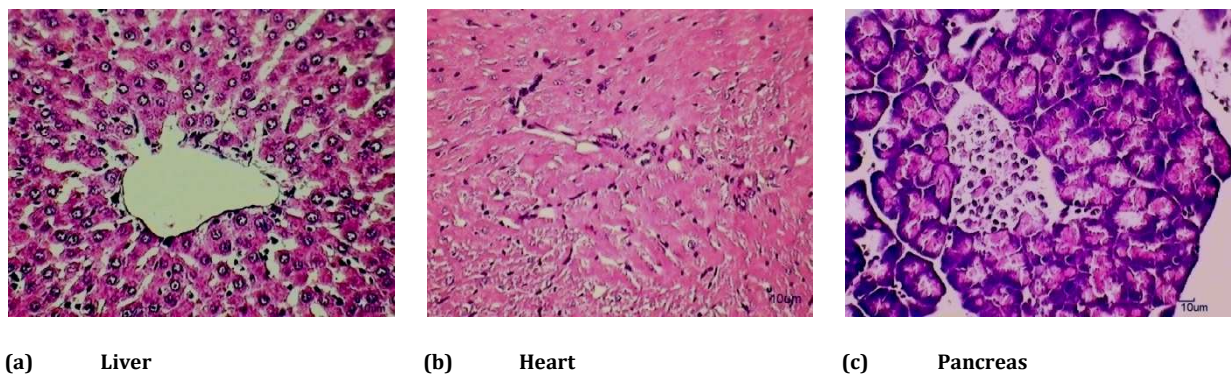


Fig. 2: Histopathology of hyperglycaemic rats

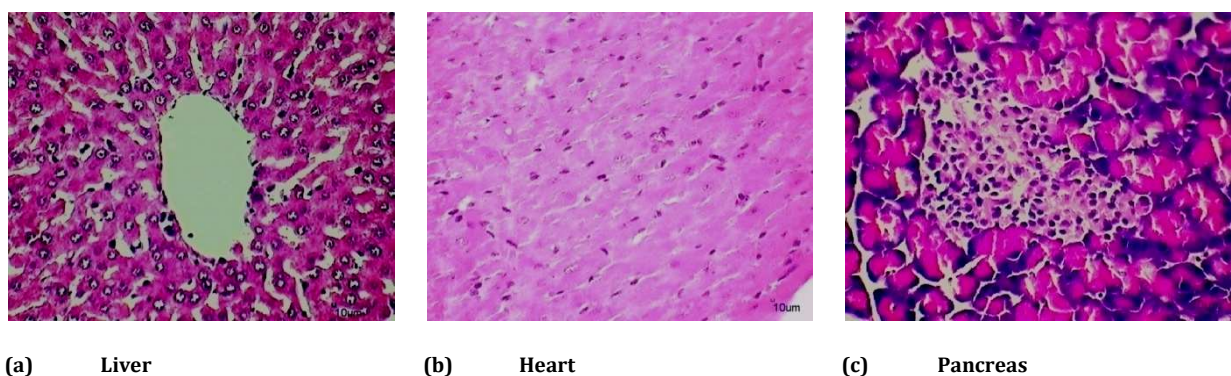


Fig. 3: Histopathology of hyperglycaemic + NN extract (200 mg/kg) treated rats

Besides body weight, lipid profile, hepatic and renal markers are well-known manifestations indicating the progression of diabetic state. Treatment with NN extract for 21 days caused a significant body weight gain and a gradual decrease in serum cholesterol and triglyceride levels. There was also a considerable decrease in the elevated hepatic enzyme levels such as SGOT, SGPT and ALP which

can be correlated to its hepatoprotective activity [28]. Serum biochemical investigation report showed that creatinine, bilirubin and BUN which are good indices of liver and kidney damage were significantly reduced by the extract and therefore may be presumed that NN extract protect cellular damage [29]. Since pancreatic tissue on histopathological analysis did not show obvious improvement

and still had atrophy of 50% β -cells, the NN extract would have mediated glucose uptake in the peripheral tissues by increasing insulin sensitivity with the available insulin or would have initiated non-insulin mediated glucose uptake [30, 31]. Studies have been reported to show phenolic compounds from natural origin to stimulate glucose transport in adipocytes and muscle cells [32, 33].

Moreover, glucose in chronic excess causes toxic effects on structure and function of many organs due to the formation of reactive oxygen species [34]. Oxidative stress directly correlates with the early onset of vascular complications and the progression of peripheral insulin resistance in diabetes. The protective effect of NN extract on various organs suggest that it penetrates cells and protects them against oxidative stress induced under hyperglycaemic conditions at a much lower concentration [35] and prevent further advancement of the disease.

Taken as a whole, the results presented here lead us to the conclusion that NN extract is involved in antidiabetic activity *in vivo* and would be useful in the treatment of diabetes. The active compounds tannins and phenols in the hydroalcoholic extract could have ameliorated the diabetic condition by acting synergistically.

CONCLUSION

In conclusion, from the data obtained in the present study we can conclude that the hydroalcoholic seed extract of *Nymphaea nouchali* possesses antihyperglycaemic properties. Additionally, the extract could prevent various complications of diabetes as well as aid in renal and hepatoprotective action. Therefore, this medicinal plant could be considered as a potential and alternative approach for the treatment of diabetes. However, further pharmacological investigation is in progress to evaluate the precise mechanism of its anti-diabetic action.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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