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Evaluation of *in vivo* antidiabetic activity of *Notonia grandiflora* Wall.

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The aim of this study was to determine the *in vivo* hypoglycaemic activity of ethyl acetate extract of *Notonia grandiflora* (EANG) in albino wistar rats. EANG was orally administrated to STZ (40 mg/kg, i.p, b.w) induced diabetic rats at the doses of 100 and 200 mg/kg b.w for 21 days. The effect of EANG on blood glucose, body weight, plasma insulin, urea, uric acid, creatinine, Hb, HbA1C, liver glycogen content, bilirubin level, liver enzymes (Serum glutamate pyruvate transaminases, serum glutamate oxaloacetate transaminases, alkaline phosphatase) were measured in the diabetic rats. Treatment of EANG significantly lowered the levels of blood glucose and glycosylated haemoglobin. It also restored body weight, liver glycogen content, and serum insulin level in diabetic rats in a dose-dependent manner. A significant reduction in the activity of liver function enzymes associated with diabetes and serum levels of renal parameters after treatment with EANG was observed, signifying the protective effects of EANG in diabetes-associated complications. Hence, it could be used as a safer complementary drug in the management of diabetes and associated complications.

Keywords: Antidiabetic activity, Ethyl acetate extract, *In vivo*, *Notonia grandiflora*, Streptozotocin.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycaemia (in untreated patients) either due to insulin resistance or destruction of pancreatic β-cells[1]. This condition is accompanied by an impairment of carbohydrate, lipid, and protein metabolism that can lead to premature death[2]. It may be asymptomatic or appear with most devastating symptoms like ketoacidosis or non-ketonic hyperosmolar state ultimately resulting in coma. Common symptoms of DM include extensive thirst, polyuria, weight loss, polyphagia, inability of body's healing capacity and blurring of vision etc[3]. Rapid increase in diabetes and the risk of Type 2 diabetes globally has made it an area of concern and research interest[4]. The prevalence of Type 2 diabetes mellitus (T2DM) was 462 million in 2017 with a worldwide incidence rate of 6059 cases per 100,000 people and is projected to increase to 7079 cases per 100,000 people by 2030. The incidence of Type 1 diabetes mellitus (T1DM) was 15 per 100,000 people and the prevalence was 9.5% in the world, which was statistically significant[5].

Globally, medicinal plants have been used as a source of medicine and 80–85% of populations rely on these medicinal plants using the extracts or their active components as traditional medicine to meet their primary health care needs. A number of active components were isolated from medicinal plants for direct use as drugs, or act as a lead compound or pharmacological agents. Among these are alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, and inorganic ions[6]. Traditional medicinal plants having antidiabetic properties can be used as drugs or simple dietary adjuvant to existing treatments of diabetes[7]. The hypoglycaemic activity of many plants/plant products has been evaluated and confirmed in animal models, as well as in human beings[8]. Metformin, for example, is an oral hypoglycemic agent isolated from medicinal plant *Galega officinalis* that was used historically in medieval Europe for the treatment of diabetes[6]. In India, several indigenous plant products have been reported to be used by the practitioners of the Ayurvedic system of medicine to treat diabetes[7].

Notonia grandiflora is a perennial succulent genus of Asteraceae-Senecioneae commonly known as

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"Common fleshy ragweed". Traditionally, the plant is named as Muyal Kathilai in Tamil. It is commonly found on bare, exposed slope and rocks of deciduous forests from plains to 1400 m[(Ref. 9)]. The plant has been found to possess pharmacological activities as antioxidant, analgesic and antinociceptive, anti-inflammatory[10], antimicrobial, antibacterial, antifungal, and antipyretic[11]. It really is utilized traditionally by the tribals in the treatment of joints pains, ear ache[12], gastric complaints[13], for pimples and hydrophobia, urinary disorders, infection, stones, diuretic, oedema[14], scabies and skin eruptions[15], wounds including sores and ulcers and scorpion bite[16]. The present study was designed to evaluate the *in vivo* antidiabetic effects of ethyl acetate extract of *N. grandiflora.*

Materials and Methods

Plant material and extract preparation

The fresh aerial parts (stems and leaves) of *N. grandiflora* were collected from Tirunellveli district of Tamil Nadu, India in the month of July 2017. It was identified and authenticated by Dr V. Chelladurai, Research Officer (Botany), Central Council of Research in Ayurveda and Siddha, Government Siddha Medical College, Palayamkottai, Tamil Nadu, India. The voucher specimen (MCPSR/N/001/2017) was deposited in the Herbarium of the Department of Pharmacognosy, Mookambika College of Pharmaceutical Sciences and Research, Muvattupuzha, Kerala.

The shade dried *N. grandiflora* aerial parts were powdered mechanically and stored in an air tight container. The extraction was carried out in a Soxhlet extractor for 8 h, sequentially with hexane, ethyl acetate, ethanol and water. The extract was concentrated by a rotary evaporator under 40°C and low pressure and finally dried to a constant weight. Dried extracts were kept at 20°C in air tight containers until further test were carried out[17].

Experimental animals

This research was carried out using albino wistar rats of 150-180 g weight range. The animals were obtained from the Central Animal House, Arulmigu Kalasalingam College of Pharmacy, Tamil Nadu, housed and maintained at normal room temperature. They were placed on standard commercial feeds and clean water *ad libitum* and allowed to acclimatize for 2 weeks before the work proceeded[18]. The study was approved by the Institutional Animal Ethics Committee of the Arulmigu Kalasalingam College of Pharmacy, Tamil Nadu (IAEC No: AKCP/IAEC/22/2019-2020).

Acute toxicity (LD_{50}) study

The acute oral toxicity study was conducted as per OECD guideline No. 423 for a period of 14 days. Female Albino rats were weighed and marked individually for proper observation purpose. They were then kept in individual cages at room temperature, *i.e.*, 22±2°C and 30% humidity. Food but not water was withdrawn from all animals overnight prior to administration of ethyl acetate extract of *N. grandiflora* and 3-4 h post administration. The extract was administered via oral route at doses of 5,50,300 and 2000 mg/kg. For each dosing level, 3 animals were used as recommended by guideline. Animals were monitored individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a study period of 14 days. The body weights and food intake of animals were noted. All observations were systematically recorded with individual records being maintained for each animal. Additional conditions like that of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma, and lethality were observed[19].

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of Streptozotocin STZ (40 mg/kg b.wt), freshly prepared in 0.1 M sodium citrate buffer (pH 4.5) after overnight fasting. Animals were fed with 20% glucose solution for 24 h to avoid initial drug-induced hypoglycaemia. The control animals received the sterile water as placebo. On the 4th day of STZ administration, blood glucose level was measured through Glucometer (one touch, Johnson & Johnson) and the rats with hyperglycemia (blood glucose range of above 250 mg/dL) were regarded as diabetic and were employed in the study[20].

Oral glucose tolerance test (OGTT)

Antihyperglycemic effect was studied in glucose overloaded hyperglycaemic rats. The animals were divided into various treatment groups (n=6). Glibenclamide (5 mg/kg) was used as the reference standard and the negative control group animals received only vehicle. The remaining groups were treated with 100 and 200 mg/kg of extract. The zero hour blood sugar level was determined from

overnight-fasted animals. After 30 min of the drug treatment, the animals were fed with glucose (2 g/kg) and blood glucose was determined after the half, one, and two hours of the glucose load[21].

Experimental design

In the STZ-induced diabetic animal model, the experimental animals were divided into five groups (I-nondiabetic control; II-diabetic control; III-administered hypoglycemic drug glibenclamide (5 mg/kg, p.o.), IV and V-received lower and higher daily doses of ethyl acetate extract of *N. grandiflora* at a rate of 100 and 200 mg/kg p.o., respectively) of six rats each. Ethyl acetate extract of *N. grandiflora* and glibenclamide were administered orally for 21 days.

All treatments were given orally after the 4th day of STZ administration for 21 days. The body weight was recorded initially and after the end of treatment whereas blood glucose level was measured by Glucometer (one touch, Johnson & Johnson) on 1, 4, 8, 12, 15, 18, and 21 days of the study. After the last day of treatment, rats were sacrificed by cervical dislocation. The blood samples were collected, centrifuged, and the serum was stored for various biochemical measurements. The pancreas, liver, and kidney were excised immediately, washed in ice-cold saline and stored at −20°C until analysed for further studies.

Biochemical estimations

Estimation of blood glucose and body weight

The glucose level and body weight were estimated on days 1, 4, 8, 12, 15, 18, and 21 after the treatments. Blood glucose was estimated by O-toluidine method[18]. Precisely 0.1 mL of the blood was mixed with 1.9 mL of 10% trichloroacetic acid solution to precipitate and then centrifuged. Then 1 mL of supernatant was mixed with 4 mL of O-toluidine reagent and was kept in the boiling water bath for 15 min and cooled. The absorbance was read at 620 nm. Data were expressed as mg/dL.

Estimation of haemoglobin, glycosylated haemoglobin (HbA1c) and plasma insulin

Haemoglobin in the blood was determined by the method of Drabkin and Austin[22]. Glycosylated haemoglobin (HbA1c) was estimated using the method of Nayak and Pattabiraman[23] and plasma insulin was assayed by the solid phase system amplified sensitivity immunoassay using reagent kits obtained from Medgenix-INS-ELISA, Biosource, Europe S.A., Belgium[24].

Estimation of hepatic and renal parameters

Hepatic glycogen was extracted with 30% KOH, precipitated with alcohol, and quantified by the colourimetric anthrone method[25]. The biochemical parameters of liver such as SGPT, SGOT, ALP, and Total bilirubin were determined[26-28]. The renal parameters such as creatinine, urea, and uric acid was determined[29-31].

Histopathological assessment

Histopathological examinations were performed on the pancreas of the rats. They were fixed in 10% formalin, dehydrated in graded ethanol concentrations (50-100%), cleared in toluene and embedded in paraffin. Sections (4-6 μM thick) were prepared and then stained with Hematoxylin and Eosin (H-E) dye for photomicroscopic observation under light microscope at high power magnifications (x 400 objectives). The stained section was observed. The cell architecture in the liver was observed under high power objective in a microscope[32].

Statistical analysis

The results of experiments performed in at least six independent experiments are displayed as mean±S.E.M. Multiple comparisons were assessed by one-way ANOVA, followed by Tukey's post hoc test, with the significance level set at P <0.05 using SPSS software (SPSS for Windows, version 16.0, Chicago, IL).

Results and Discussion

Extraction

The powdered plant materials were successively extracted with hexane (68°C), ethyl acetate (76-78°C), ethanol (78.37°C) and water for eight hours. The extracts were then concentrated by a rotary evaporator under 40°C and low pressure and finally dried to constant weight. Dried extracts were stored at 20°C in air tight containers until further test were conducted. It was found that water gave highest yield of extraction among other solvents for extracting aerial parts of *N. grandiflora*. The extraction yield for water was 18.78 wt.%, trailed by ethanol 12.40 wt.%, ethyl acetate 9.67 wt.% and hexane 4.52 wt.% respectively.

Acute toxicity

The acute toxicity study of ethyl acetate extract of *N. grandiflora* was evaluated as per OECD guideline No. 423. The ethyl acetate extract was orally fed to the rats at the dose level of 5, 50, 300, and

2000 mg/kg, respectively. The test showed no mortality even at maximum dose of 2000 mg/kg body weight (b.wt.). Hence, 100 and 200 mg/kg doses were selected for further study.

Oral glucose tolerance test (OGTT)

Administration of glucose (2 g/kg) showed a sharp rise in blood glucose level in normal rats at 30 mins after glucose load and then began to decrease. As compared to the normal control, the rats treated with extracts and standard, the blood glucose level reached peak level at 30 mins and returned to normal at the end of 120 mins.

Administration of ethyl acetate extract of *Notonia grandiflora* EANG showed significant lowering in blood glucose values when compared to normal control rats (Fig. 1). Oral glucose tolerance test could be concluded as dose of 200 mg/kg showed maximum improvement in glucose tolerance.

Body weight and blood glucose changes in type 2 diabetic rats

Body weights of streptozotocin injected rats were found to be significantly reduced (P <0.01) as compared to normal rats. Induction of diabetes by STZ leads to loss of body weight due to increased muscle wasting and loss of tissue proteins. The failure of diabetic animals to gain weight during the course of time is due to continuous excretion of glucose because of the defect in peripheral uptake and impairment of liver's capacity to synthesize glycogen[33]. Body weights of all the animals were measured on day 1st and 21st day (Table 1). A significant increase in body weight in diabetic rats was observed after EANG 100 and 200 mg/kg and in glibenclamide administration when compared to diabetic control rats.

The diabetic rats showed an elevated fasting blood glucose levels when compared to normal rats. The normal control rats continued to maintain their basal blood glucose levels throughout the experiment period. The fasting blood glucose levels of the diabetic rats were elevated throughout the study, while the ethyl acetate extracts of *N. grandiflora* treated groups at the dose of 100 and 200 mg/kg exhibited significant lowering of blood glucose levels on 8th, 12th, 15th, 18th, and 21st day (Table 2). The results of the study have shown a significant difference between the initial and final fasting blood glucose levels of extracts treated groups at the dose of 100 and 200 mg/kg and glibenclamide treated diabetic rats.

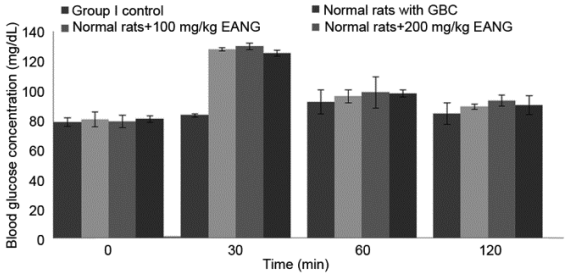

Fig. 1 — Effect of ethyl acetate extract of *Notonia grandiflora* on the blood glucose level of oral glucose loaded rats.

Table 1 — Effect of EANG on changes in body weight in rats

Groups	Body weight (g)	
	1st day	21st day
Group I control	178.56±22.48	196.72±24.54
Group II diabetic control	180.56±24.46	149.90±25.15 [a]
Group III STZ+GBC	181.70±25.32	207.80±23.86*
Group IV STZ+100 mg/kg EANG	180.35±25.16	195.76±24.28*
Group V STZ+200 mg/kg EANG	185.45±23.52	208.10±21.48*

Values are given as mean±S.E (n=6).
[a]P <0.01 when compared to normal control, *P <0.05 when compared to STZ control group

Table 2 — Effect of ethyl acetate extract on fasting plasma glucose level in rats

Groups	Fasting blood glucose level (mg/dL)						
	Day 1	Day 4	Day 8	Day 12	Day 15	Day 18	Day 21
Group I control	79.14±24.78	81.28±20.34	82.06±22.16	83.74±23.52	83.18±21.48	82.70±24.62	82.14±27.68
Group II diabetic control	243.18±22.54	314.92±35.54	327.88±40.82	316.14±36.02	306.26±41.86	299.84±38.62	288.98±24.22
Group III STZ+GBC	240.28±24.35**	293.25±33.24***	213.12±30.66***	168.36±38.72***	151.02±29.38***	124.40±21.76***	110.64±26.15***
Group IV STZ+100 mg/kg EANG	240.48±26.76	303.52±28.56*	299.24±29.38**	264.73±31.45***	229.86±25.58*	169.38±22.10***	160.40±21.72***
Group V STZ+200 mg/kg EANG	242.68±20.30	300.48±26.78**	263.86±26.20***	201.02±28.24*	169.74±22.42***	138.12±20.75***	112.56±23.46***

Values are given as mean±S.E (n=6).
*P <0.05, **P <0.01,***P <0.001 when compared to STZ control group

There was an improvement in glucose tolerance with the reduction of plasma glucose level in the study which indicates the glucose metabolism capacity of ethyl acetate extract. The results are in accordance with the previous *in vitro* antidiabetic study of ethyl acetate extracts of *N. grandiflora*, with its probable mechanisms of action such as stimulation of glucose uptake via insulin-dependent pathways in skeletal muscle and/or insulin-independent pathways in hepatocytes, as well as the inhibition of intestinal alpha amylase and alpha glucosidase in preventing rise in postprandial glucose level[34]. The observed hypoglycaemic effect may be also due to either regeneration of β-cells or enhanced insulin sensitivity to the target tissues.

Further, to assert the mode of action, the insulin levels were estimated along with the histology of the pancreas. The diabetic rats showing reduced insulin levels and the degranulated and dilated islets were restored after treatment with ethyl acetate extracts and glibenclamide (Table 3).

Estimation of Hb and HbA1c

Decrease in Hb in diabetic control group, indicating the presence of hypochromic, microcytic anaemia in STZ diabetic rats[35]. The level of HbA1c is monitored as a reliable index to consider glycaemic is in control in diabetes. High glycosylated haemoglobin level signifies poor glycaemic control and responsible for the development of diabetic complications viz. vascular dysfunction, neuropathy and diabetic nephropathy[36]. Haemoglobin level decreased and HbA1c increased significantly in diabetic control rats and these values were reversed by treatment with ethyl acetate extracts of *N. grandiflora* and glibenclamide. Higher doses of ethyl acetate extract produced more reduction HbA1c level. This action represents that *N. grandiflora* has an ability to prevent the development of diabetes associated complications.

Effect of ethyl acetate extracts of *N. grandiflora* on hepatic parameters

The liver glycogen level was found to be low in diabetic control rats when compared to normal rats. Insulin activates the glycogen synthase system[37]. The significant increase in the glycogen level (P <0.05) on treatment with ethyl acetate extracts (100 and 200 mg/kg b.wt) may be due to the reactivation of glycogen synthase system by *N. grandiflora* (Table 4).

Bilirubin is excreted by the liver, therefore, interference with the normal liver function affects its rate of conjugation and excretion. Thus, a high level of bilirubin is used as indices for liver function and bile excretion status[38]. In the present study, bilirubin level was found to be decreased from 3.52±1.62 mg/dL (diabetic control) to 0.40±0.20 mg/dL (Glibenclamide treated groups), 0.42±0.56 mg/dL in ethyl acetate extracts of *N. grandiflora* (200 mg/kg), suggesting the enhancement of liver functions by the extracts as well as standard drug glibenclamide.

Studies have shown that STZ induces CYP2E1 dependent oxidative stress and causes the release of various liver microsomal enzymes including SGOT, SGPT, and serum ALP in the blood that indicates liver damage or condition of T2D disease[39].

Table 3 — Effect of ethyl acetate extracts of *Notonia grandiflora* on plasma insulin, Hb and HbA1c

Groups	Insulin (U/mL)	Haemoglobin (g/dL)	Glycosylated haemoglobin (mg/g of Hb)
Group I control	18.24±0.32	13.15±0.30	0.43±0.05
Group II diabetic control	5.36±0.30 [a]	6.75±0.38 [a]	1.20±0.04 [a]
Group III STZ+GBC	17.56±0.44[*]	12.24±0.30[*]	0.51±0.07[*]
Group IV STZ+100 mg/kg EANG	14.12±0.40[**]	10.26±0.34[**]	0.62±0.06[**]
Group V STZ+200 mg/kg EANG	17.46±0.52[**]	12.52±0.36[*]	0.54±0.08[*]

Values are given as mean±S.E (n=6).
[a]P <0.01 when compared to normal control, [*]P <0.05, [**]P <0.01, when compared to STZ control group

Table 4 — Effect of ethyl acetate extracts of *Notonia grandiflora* on serum biomarkers of liver

Groups	Bilirubin mg/dL	Glycogen (mg/100 g tissue)	SGOT (U/L)	SGPT (U/L)	ALP (IU[b]/L)
Group I control	0.30±0.05	55.08±1.48	170.12+0.34	96.78+0.48	78.96±4.35
Group II diabetic control	3.52±1.62 [a]	16.15±1.56 [a]	309.34+1.32 [b]	203.23+0.92 [b]	137.52±6.78[a]
Group III STZ+GBC	0.40±0.20[*]	50.10±1.34[*]	192.46+0.30[***]	115.30+0.88[***]	86.26±5.22[*]
Group IV STZ+100 mg/kg EANG	1.42±3.68[*]	42.34±1.20[*]	218.54+0.56[**]	180.74+0.52[*]	93.32±5.38[*]
Group V STZ+200 mg/kg EANG	0.42±0.56[**]	49.55±1.32[*]	193.68+1.23[**]	167.46+3.25[*]	86.90±5.94[*]

Values are given as mean ± S.E (n=6).
[a]P <0.01, [b]P <0.001 when compared to normal control, [*]P <0.05, [**]P <0.01,[***]P <0.001 when compared to STZ control group.

Restoration to normal level shows recovery from damage. Treatment with ethyl acetate extracts of *N. grandiflora* and glibenclamide normalized the hepatic marker enzyme. Both low and high doses of the ethyl acetate extract significantly improved the level of all three enzymes; however, the effect of the high dose was significant to a higher extent.

Effect of ethyl acetate extracts of *Notonia grandiflora* on renal parameters

The elevation of serum urea, uric acid, and creatinine are significant markers related to renal dysfunction in diabetic hyperglycaemia. The protein glycation in diabetes may lead to muscle wasting and increased release of purines, the main source of uric acid as well as the activity of xanthine oxidase[40]. In this work, diabetic control showed a significant increase in creatinine, uric acid, and urea levels compared to control animals demonstrating renal failure. Diabetic rats treated with ethyl acetate extracts of *N. grandiflora* showed the reversal of these parameters to near normal levels indicating protective effects on kidneys (Table 5).

Histopathology of pancreases

In Fig. 2, the photomicrographs of pancreatic section staining with hematoxylin and eosin were shown. In the pancreatic sections of the normal control group, the islet boundaries were clear. No necrosis or fatty degeneration was observed in normal rats. In pancreatic sections of the diabetic control group, completely destructed cells were observed. Besides ballooning, necrosis also occurred. Fatty layer degeneration, loss of normal structure, and normal cellular integrity was also observed in the pancreatic section of the diabetic control rats. Due to necrosis, irregular gap junctions appeared and coagulation occurred. In the test group of low doses (100 mg/kg body weight), in most portions, cellular integrity was normal with slight fatty degeneration. In the test group of high dose (200 mg/kg b. wt), cellular integrity was normal to a great extent. In the standard treated group (5 mg/kg b. wt), cellular integrity was normal, no blood clotting or necrosis was noticed.

Table 5 — Effect of ethyl acetate extracts of *Notonia grandiflora* on urea, uric acid and creatinine in the plasma of control and diabetic rats

Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Group I control	25.26±2.10	1.17±0.18	0.91±0.05
Group II diabetic control	38.04±2.56[a]	2.18±0.16[a]	2.30±0.20[a]
Group III STZ+GBC	26.34±2.38[**]	1.28±0.10[**]	1.08±0.18[**]
Group IV STZ+ 100 mg/kg EANG	30.48±2.78[*]	1.68±0.14[*]	1.48±0.12[*]
Group V STZ+ 200 mg/kg EANG	26.96±2.96[*]	1.30±0.10[*]	1.10±0.16[*]

Values are given as mean±S.E (n=6).
[a]P <0.01, [b]P <0.001 when compared to normal control, [*]P <0.05, [**]P <0.01 when compared to STZ control group.

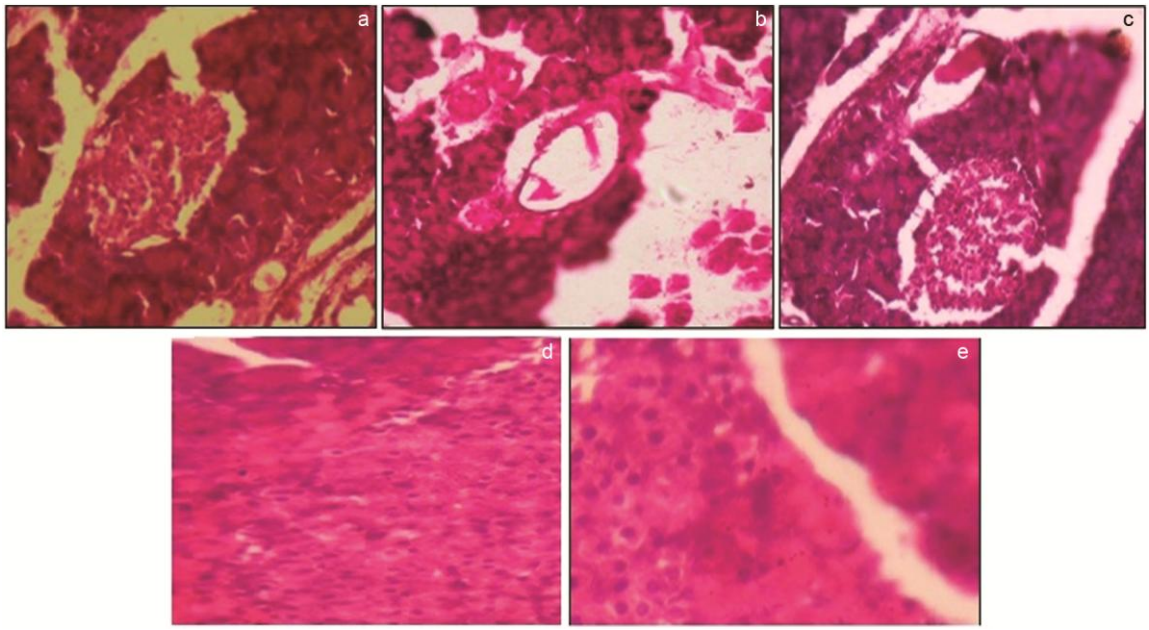

Fig. 2 — Representative photomicrographs of Pancreas, a) Normal control, b) Diabetic control, c) Standard-Glibenclamide, d) EANG 100 mg/kg, e) EANG 200 mg/kg; (Hematoxylin-Eosin stain; Magnification: 10x).

Histopathological studies of the pancreas in the diabetic and treated groups substantiated the cyto-protective action of extract.

Conclusion

The present study provides a scientific confirmation of the antidiabetic activity of ethyl acetate extracts of *N. grandiflora* in STZ-induced diabetic rats. Therefore, it can be concluded that the *N. grandiflora* might be used safely as an adjunct therapy in the management of diabetes and its associated complications. Furthermore, it is suggested to carry out long-term research work to recognize and isolate the active moiety responsible for antidiabetic property.

Conflict of interest

The authors declare that there are no conflicts of interest.

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