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Evaluation of anti-hyperglycemic and anti-hyperlipidemic effects of *Naravelia Zeylanica* in streptozotocin- induced diabetic rats

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Abstract

Naravelia zeylanica DC (Ranunculaceae), a woody climber, have been used from ancient times to treat various ailments like rheumatoid arthritis, skin diseases, wound and ulcer. The present study was designed to investigate the anti-hyperglycemic activity of methanolic extract of *Naravelia zeylanica* (NZYM) using experimental diabetic model. Diabetes was induced in wistar rats by a single dose of streptozotocin (55 mg/kg i.p.) (STZ) and treated with NZYM at doses of 100 and 200 mg/kg for 45 days. Glibenclamide (5 mg/kg b.w.) was used as standard drug. Blood glucose and body weight were monitored at regular intervals and the levels of serum insulin, lipid and the carbohydrate metabolic enzyme in the liver were measured at the end of the study. Oral administration of NZYM and glibenclamide significantly reduced the blood glucose level ($p<0.05$), with increased serum insulin and significant alteration in lipid profiles and liver carbohydrate enzymes ($p<0.05$) after 45 days. Furthermore, the biochemical parameters correlated with the histopathological changes in the pancreas of STZ-induced diabetic rats, which structurally proved the efficacy of NZYM. The findings suggest that NZYM possess anti-hyperglycemic activity and anti-hyperlipidemic properties and restored STZ-induced pancreatic damage in diabetic rats. NZYM might therefore have a beneficial effect in treatment of diabetes mediated through regulation of carbohydrate metabolic enzyme activities.

Keywords: Anti-hyperglycemic, Glibenclamide, *Naravelia zeylanica,* streptozotocin, Ranunculaceae.

Introduction

Diabetes mellitus is a multi-factorial endocrine metabolic disorder characterized by deficiency in insulin or defects in insulin action in regulation of blood glucose or both. Because of its high prevalence, it is found to be the third mortal disease of mankind, preceding cancer and cardiovascular diseases [1]. Current treatment strategy for the control of diabetes includes diet, exercise, oral hypoglycemic agent and insulin therapy. In recent years, much attention has been paid to research on anti-diabetic potential of medicinal plants, since the commercially available anti-diabetic drugs have various side effects [2]. The identification of novel molecules which act in mechanistically distinct way compared to existing drug targets is of increasing demand [3].

Medicinal plants serve as the principle source of raw materials for various ailments since centuries. Several plants were scientifically proved for various pharmacological activities [4]. Medicinal plants have been used traditionally in Indian system of Ayurveda, which provides a valuable source of oral hypoglycemic compounds for the development of new therapeutic strategies [5]. Few bioactive molecules extracted from medicinal plants have been documented for insulinomimetic or insulin secretagogue effects such as *Saraca asoca* [6], *Selaginella tamariscina* [7], *Scoparia dulcis* [8] and *Gymnema* montanum [9].

Naravelia zeylanica (NZY) (Ranunculaceae) is an indigenous plant distributed in hilly areas. In Indian system of medicine ayurveda, it has been used in the treatment of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers [10]. The phytoconstituents of NZYM include tannins, flavonoids, phytosterols, alkaloids and terpenoids [11]. Various parts of NZY have been reported to possess various biological activities and proved to have anti-helminthic, anti-ulcer, anti-inflammatory activity [12], anti-microbial activity [13], anti-arthritic activity and anti-oxidant activity [14].

Therefore, based on the ethanopharmacological importance, the present study was focused on the evaluation of anti-hyperglycemic and anti-hyperlipidemic activity of NZYM in STZ-induced animal model.

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Materials and Methods

Plant collection, identification and extraction

The plant was collected from the hills of Munnar, Iddikki districts, Kerala, India. Botanical identification was performed by Prof. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, India (Ref. no. PARC/2011/914). The dried whole plant powder (100g) of NZY was extracted sequentially using various organic solvents in increasing order of polarity (hexane, dichloromethane, ethyl acetate and methanol) at room temperature and was concentrated using reduced pressure [15].

Reagents and chemical

Streptozotocin and Glibenclamide were obtained from ProLab marketing Pvt. Ltd. (New Delhi). Rat insulin ELISA kit was purchased from eBioscience (Austria). The chemicals utilized for the study were obtained from Hi Media (Mumbai) and were of analytical grade.

Animal studies

All animal studies were carried out with the approval from the Institutional Animal Ethical Committee (52/IAEC/2011) at SRM University, Tamil Nadu, India.

Induction of experimental diabetes

Diabetes was induced in overnight fasted Albino Wistar rats weighing 150-200g by a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 55mg/kg b.w. in freshly prepared 0.1M cold citrate buffer (pH 4.5) [16]. Fasting blood glucose was measured periodically after induction. Blood glucose levels above 250 mg/dL were considered as diabetic and used for the study.

Experimental groups and treatment

Male wistar rats (150 – 200g) were purchased from King's Institute, Chennai. The animals were maintained under laboratory condition in the animal house, with five rats per cage at 18±2ºC in a light controlled room (12hrs dark/12hrs light) and were provided commercial pellet diet with free access to water *ad libitum*.
After 1 week, rats were randomly divided into six groups with five animals each.

Group I: Control rats (5% CMC);
Group II: Control rats + NZYM (200 mg/kg b.w.);
Group III: Diabetic rats (STZ- induced);
Group IV: STZ- induced+ NZYM (100 mg/kg b.w.);
Group V: STZ- induced+ NZYM (200 mg/kg b.w.);
Group VI: STZ- induced+ Glibenclamide (5mg/kg b.w.) [16]

NZYM was dissolved in 5% CMC and administered orally for 45 days. Blood glucose level and body weight was monitored at regular intervals throughout the experimental period. At the end of the study, the animals were starved overnight and then sacrificed by cervical decapitation and the blood samples were collected [17]. The serum was separated by centrifugation and the biochemical parameters were analyzed by measuring the absorbance using UV spectrophotometer (Ultraspec2100 *pro*, Amersham Bioscience) according to manufacturer's protocol (Span Diagnostics Ltd., India).

Estimation of Glucose and Serum Insulin

The blood was collected by pricking the tail vein [18] using surgical blade and the blood glucose level was determined using Accu-Check Active blood glucose monitoring system (Roche Diagnostics India Pvt, Ltd, Mumbai). The serum insulin levels were quantified using commercial enzyme-linked immunosorbant assay kit (eBioscience, Austria). The assay was performed as per manufacturer's protocol using rat insulin as standard. The insulin levels were expressed as nanograms per milliliter.

Oral Glucose tolerance test (OGTT)

Experimental animals underwent an oral glucose tolerance test after 12 hrs fasting at 45th day [19]. Glucose at a dose of 2g/kg b.w was administered orally [8] and the blood glucose was recorded at 0, 30, 60, 90, 120 min using Accu-Check Active blood glucose monitoring system (Roche Diagnostics India Pvt, Ltd, Mumbai).

Estimation of serum physiological parameters

Total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined from the serum of experimental animals using standard kits (Span Diagnostics Ltd, India).

Estimation of Key enzymes of Carbohydrate Metabolism

The liver homogenate (in 0.1M Tris-HCl (pH 7.4)) was used to measure the activities of glucokinase (E.C.2.7.1.2) [20], glucose-6-phosphatase (E.C.3.1.3.9) [21] and glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) [22]. The glycogen content was estimated by anthrone method [23] and the protein content was measured by Bradford's method [24].

Histopathology of Pancreas

Animals were euthenized with mild ether anesthesia and dissected. Pancreas was excised, washed with saline and a small portion of the organ was quickly fixed in 10% formalin [6]. The tissue was processed, embedded and sectioned using microtome. Thin sections of pancreas (5 micron) were mounted on slide and stained with Hematoxylin & Eosin. The slides were then examined and photographed.

Statistical analysis

All the data were expressed as mean ±SEM and evaluated by one way analysis of variance (ANOVA) followed by a Bonferroni´s post-hoc test. *p<0.05* were considered as statistically significant.

Results

Effect of NZYM on body weight and blood glucose

The effects of the oral administration of NZYM on body weight and blood glucose in normal and diabetic rats were represented in Table 1 & 2. STZ-induced diabetic rats exhibited significant loss in the body weight and elevated blood glucose level as compared to the control rats. The treatment of NZYM at both the doses showed a significant (*p<0.05*) increase in body weight and reduction in elevated blood glucose level compared to the diabetic rats. Interestingly, the administration of NZYM at low dose (100 mg/kg b.w.) showed maximal reduction in blood glucose level (*p<0.05*) than high dose (200 mg/kg b.w), and found to be on par with glibenclamide in normalizing the elevated blood glucose level.

Table: 1 Effect of NZYM on body weight in control and diabetic rats

Groups	Body weight in grams							
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Control	166.5±5.5	164±5.65	164.5±4.5	193.5±2.5	195±3.00	212±1.5	216±3.00	218.5±4.5
Control + NZYM (200mg/kg b.w.)	162.5±3.5	158±2.8	161±3	191.5±2.5	193.5±2.5	201±3	211±2	216±2.5
Diabetic	182.5±3.5	173.5±3.18	138.5±1.5*	135.5±4.5*	126.5±5.5*	119±3.46*	108±5.50*	109±3.51*
Diabetic + NZYM (100mg/kg b.w.)	184.5.±4.85	180.5±1.148**	186.5±4.57**	197.25±4.30**	205.5±4.71**	208.67±4.17**	210.33±6.8**	212.67±4.05**
Diabetic + NZYM (200mg/kg b.w.)	182±4.72	179.33±3.55*	183±2.96**	199.667±6.33**	194.33±4.702**	188.66±2.90**	203.67±2.603**	209.33±3.75**
Diabetic + Glibenclamide	181.33±2.90	175±2.62**	175.67±4.63**	180.33±2.90**	208±6.027**	210.67±2.027**	212±2.64**	214±2.64**

Values are expressed as mean ±SEM. (n=5), *, *p<0.05,* compared with the control group, ***, p<0.05,* compared with the diabetic group.

Table: 2 Effect of NZYM on fasting blood glucose level in control and diabetic rats

Groups	Fasting blood glucose level mg/dL							
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Control	100±3.00	103.5±1.5	81±1.414	79±2.00	78.5±0.5	99±3.00	85.5±3.5	81.5±2.5
Control + NZYM(200mg/kg)	84.5±1.5	90±6.05	101.5±4.94	97±1.08	104±1.5	101±2.07	103±4.13	99.5±2.5
Diabetic	104.5±3.9	382±3.00*	351±4.24*	381.5±3.5*	397.5±1.5*	389.33±2.84*	391±3.00*	392.5±3.5*
Diabetic + NZYM (100mg/kg)	109.75±5.55	352±1.87**	219.25±2.5**	180.75±4.25**	122.75±2.09**	102±3.21**	101±2.081**	95±1.527**
Diabetic + NZYM (200mg/kg)	102.8±4.3.32	351.67±1.76**	226.33±3.21**	232.33±2.02**	202.33±2.72**	132±1.15**	106±4.58**	98.33±1.85**
Diabetic + Glibenclamide	108.33±2.84	354.67±2.02**	230.33±3.51**	182±3.78**	121±4.358**	118.33±4.4**	99.33±3.52**	96.33±4.33**

Values are expressed as mean ±SEM. (n=5), *, *p<0.05* compared with control group, ***, p<0.05,* compared with the diabetic group.

Effect of NZYM on oral glucose tolerance (OGTT)

The glucose tolerance test was conducted on diabetic rats administered with NZYM and the results were represented in Figure 1. Both the control and diabetic rats showed sharp increase in blood glucose levels at 60 min. The NZYM treated rats exhibited significant reduction in blood glucose levels from 60 to 150 min when compared with the diabetic rats *(p<0.05)*. In diabetic rats, the blood glucose levels reached the maximum level at 60 min after oral glucose ingestion and the levels remained high till 150 min. The effect of NZYM (100 mg/kg b.w.) treatment significantly *(p<0.05)* increased the glucose tolerance from 60 to 150 min. The effect was similar to that of glibenclamide treated diabetic rats.

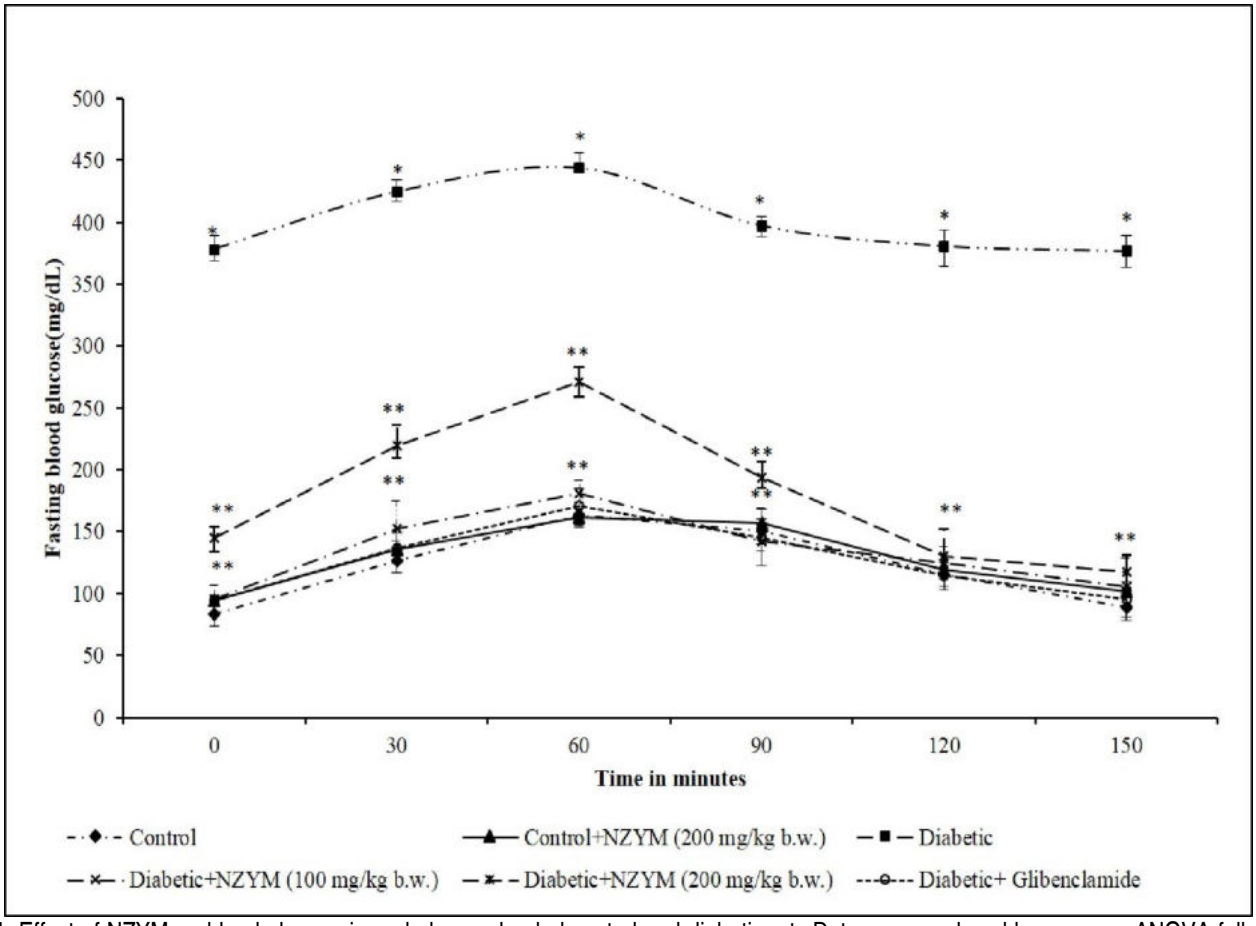

Figure 1: Effect of NZYM on blood glucose in oral glucose loaded control and diabetic rats.Data were analysed by one-way ANOVA followed by post-hoc test to indicate difference among groups. Values are expressed as mean ±SEM (n=5). **p<0.05 compared with the control group, *p<0.05, compared with the diabetic group. NZYM- methanolic extract of *Naravelia zeylanica*

Effect of NZYM on serum insulin level

As shown in Figure 2, administration of NZYM (100 and 200 mg/kg b.w) showed a marked increase in insulin levels, when compared with the diabetic group that exhibited significant decrease in serum insulin levels *(p<0.05)*. There was no significant change in insulin level between normal and NZYM treated control rats. Among the two doses, 100mg/kg b.w of NZYM showed the maximum modulation in blood glucose and serum insulin levels on par with glibenclamide treated diabetic rats.

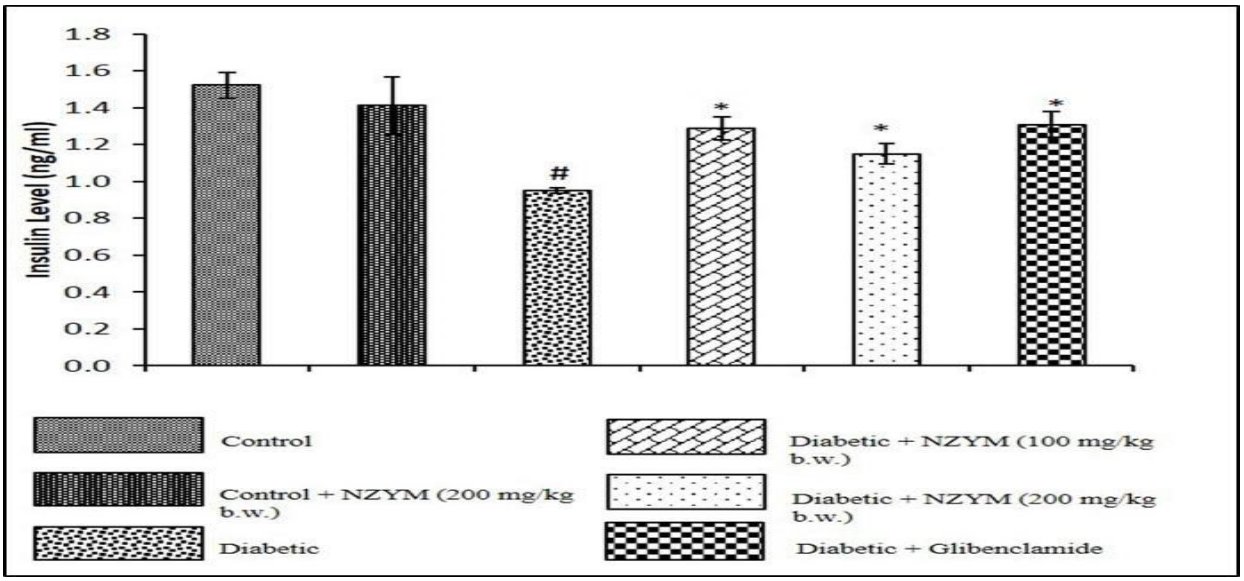

Figure 2: Effect of NZYM on serum insulin level in control and diabetic rats. Data were analysed by one-way ANOVA followed by post-hoc test to indicate difference among groups. Values are expressed as mean ±SEM (n=5). *p<0.05 compared with the control group, **p<0.05 compared with the diabetic group.

Effect of NZYM on serum lipid profile

The effect of NZYM on serum lipid profile of control and experimental groups were represented in Table 3. In this study, profound alterations of the lipid profile were observed in diabetic rats. Both the doses of NZYM showed significant (*p<0.05*) reduction in the elevated total cholesterol, triglycerides, LDL and increased HDL cholesterol (*p<0.01*) compared to diabetic group. The low dose showed significant modulation *(p<0.05)* in lipid profile when compared to high dose of NZYM. Administration of NZYM and glibenclamide to diabetic rats showed significant reduction in VLDL levels than diabetic rats *(p<0.05)*.

Table: 3 Effect of NZYM on serum lipids parameters in control and diabetic rats

Groups	TC (mg/dL)	TG (mg/dL)	HDL(mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	69.97±0.36	35.835±0.49	39.785±0.06	22.4±0.39	7.165±0.101
Control + NZYM (200mg/kg b.w.)	66.49± 1.82	31.23±0.77	36.41±0.54	23.83±2.21	6.24±0.15
Diabetic	160.58±0.35*	97.655±0.66*	21.07±0.080 *	118.2±1.11*	20.93±0.67*
Diabetic + NZYM (100mg/kg b.w.)	73.358±0.31**	33.1±0.27**	44.88±0.58**	21.67±0.11**	6.615±0.054**
Diabetic + NZYM (200mg/kg b.w.)	94.67±0.34**	44.36±0.24**	29.85±0.33**	38.965±1.36**	8.865±0.049**
Diabetic + Glibenclamide	68.26±0.71**	44.465±0.46**	33.055±0.81**	27.605± 0.216**	8.89±0.092**

Values are expressed as mean ±SEM. (n=5). *, *p<0.05* compared with the control group, * *, *p<0.05* compared with the diabetic group.

Effect of NZYM on carbohydrate metabolic enzymes

The effect of NZYM on carbohydrate metabolic enzymes of control and experimental rats were tabulated in Table 4. The activity of glucokinase and glucose-6-phosphate dehydrogenase significantly decreased (*p<0.05*) while the glucose-6- phosphatase levels were increased in diabetic rats. These enzyme activities were reverted to near normal by the administration of NZYM (both doses) was on par with glibenclamide treated rats. The activities of glucose-6-phosphatase were decreased in NZYM treated rats. But decrements in these metabolic enzyme activities were observed in high dose (200 mg/kg) treated rats which was equivalent to that of diabetic rats. All these enzyme levels were found to be normalized in liver after NZYM treatment and remained on par with glibenclamide. The glycogen content in liver was significantly decreased *(p<0.05)* in diabetic rats as compared with control. Administration of NZYM at both the doses resulted in considerable increase in liver glycogen levels. Among the two doses, the low dose (100 mg/kg b.w.) showed a significant (*p<0.05*) increase in glycogen level.

Table: 4 Effect of NZYM on carbohydrate metabolic enzymes in control and diabetic rats

Groups	Glucokinase (U/mg Protein)	Glucose 6 phosphate dehydrogenase (U/mg Protein)	Glucose 6 phosphatase (U/mg Protein)	Glycogen(mg/g of tissue)
Control	248.07±4.6	7.49±0.34	5.74±1.26	24.72
Control + NZYM(200mg/kg)	237.57±3.56	6.66±0.39	7.04±2.12	22.46
Diabetic	104.41±2.87*	1.59±0.36*	27.69±2.86*	10.35*
Diabetic +NZYM (100mg/kg)	175.82±7.49**	2.98±0. 08**	14.70±0.98**	20.32**
Diabetic+NZYM (200mg/kg)	142.45±7.85**	2.62±0. 072	20.24±1.26	17.25**
Diabetic + Glibenclamide	210.70±6.17**	5.59±0. 89**	11.04±1.07**	21.22**

Values are expressed as mean ±SEM. (n=5), **; p<0.05* compared with control group, ** *; p<0.05* compared with the diabetic group.

Histopathology and microscopic examination of pancreas

The histopathological observation revealed the alterations in the pancreas of STZ-induced diabetic rats as depicted in Figure 3. The pancreas of control and NZYM treated control rats (drug control) showed normal acinar pattern with well-structured islets (Figure 3 A&B). The pancreas of diabetic rats showed smaller islets with necrosis and inflammatory infiltrate with edema due to streptozotocin injury (Figure 3C). Diabetic rats treated with NZYM and glibenclamide showed increased number of islets on comparison with the diabetic rats, revealing the protective effect on the islets (Figure 3D, E&F respectively).

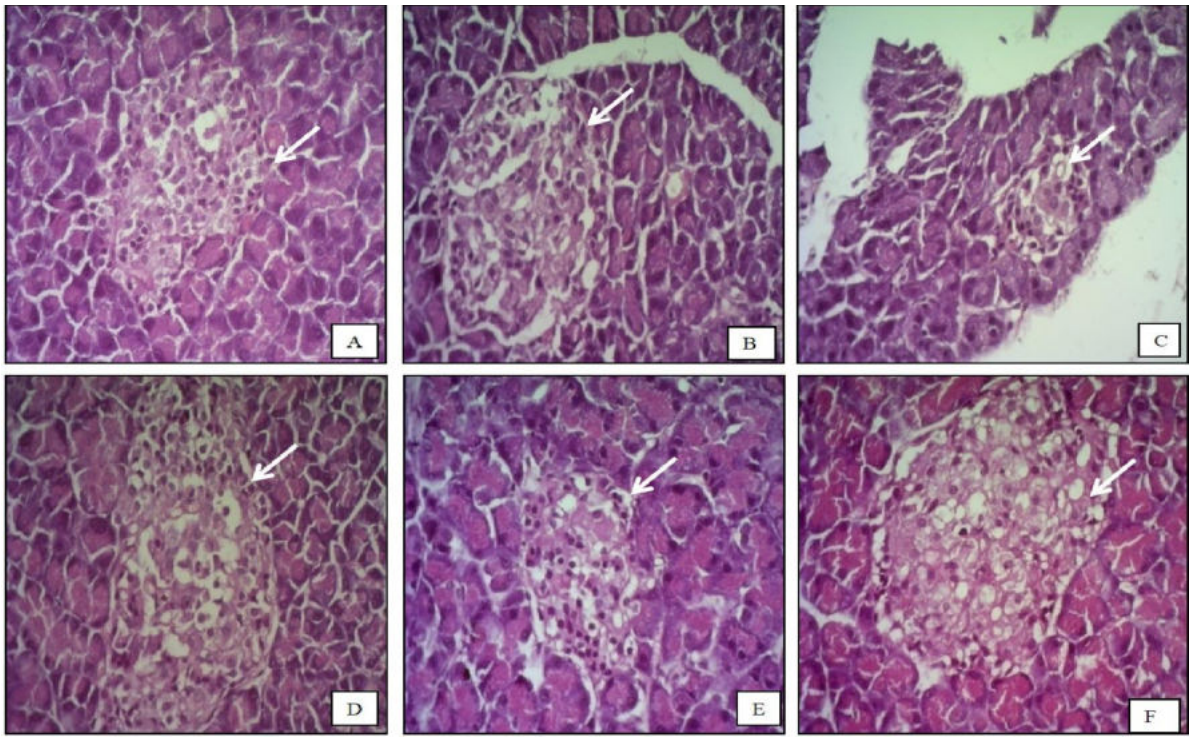

Figure 3: Histopathology of the pancreatic section. (A) control rats shows normal pancreatic histoarchitecture with prominent cytoplasm and darkly stained β-cells, (B) control rats treated with NZYM (200mg/kg b.w.) shows normal pancreatic histoarchitecture, (C) diabetic rats showing decreased β-cells mass with shrunken islets. Intracellular space and cellular debris with severe necrosis are visible, (D) diabetic rats treated with low dose of NZYM shows pancreatic islets with increased β-cell mass and rejuvenation of histoarchitecture. (E) diabetic rats treated with high dose NZYM showing normal pancreatic islets with increased β-cell mass and mild vacuolization with intracellular space, (F) diabetic rats treated with Glibenclamide showing normal pancreatic histoarchitecture with increased number of β-cells. (Hematoxylin and eosin stain; magnification 40 x, scale bar 50µm). Arrows indicate islets.

Discussion

The present study was envisioned to evaluate the anti-diabetic efficacy of NZYM in streptozotocin-induced diabetic rats. STZ-induced diabetes served as one of the well characterized animal model for experimental diabetes for its selective pancreatic beta cell cytotoxicity [25]. Glibenclamide was used as standard anti-diabetic drug to compare the efficacy of variety of hypoglycemic agents [9]. Administration of NZYM produced a marked decrease in blood glucose level at both doses after 45 days of treatment to diabetic rats, but no considerable effect on fasting blood glucose level was observed with respect to the NZYM treated control rats. Similar findings have been reported with other medicinal plants such as *Codariocalyx motorius* [6], *Sphaeranthus indicus* [16] which clearly explained the anti-diabetogenic action of the medicinal plants used. In our study, the low dose (100 mg/kg b.w.) exhibited a marked glycemic change than the high dose (200 mg/kg b.w.). Our findings coincide with earlier studies conducted on methanolic stem bark of *Adansonnia* digitata [26] at low dose (100 mg/kg b.w.) were found to be effective in glycemic change. Similarly, administration of aqueous extract of *Scoparia dulcis* [9] at 200 mg/kg b.w. (low dose) produced a marked anti-hyperglycemic effect than 400 mg/kg b.w. (high dose). Several reports have been documented with the diabetic rats administered with polyherbal extracts exhibiting the same pattern. This must be due to the effect of low dose that could be accredited to a greater extent in bringing down the blood glucose level to normal. In this context, number of other medicinal plants have been documented [27] with the similar pattern in exerting the effect.

A severe loss in body weight is one of the characteristic features in the progression of diabetes in STZ–induced animal model which might be due to degradation of structural proteins [28] and this was also observed in the present study. Administration of NZYM and glibenclamide showed an improvement in the body weight to a certain extent in diabetic rats indicating that control over muscle wasting resulted from glycemic control, whereas decreased body weight in diabetic control rats. The medicinal plants exhibiting hypoglycemic activity act through multiple mechanisms by improving insulin sensitivity, augmenting glucose – dependent insulin secretion and stimulating the regeneration of islets of langerhans in pancreas of STZ-induced diabetic rats [16].

The anti-hyperglycemic effect of NZYM was further substantiated by assessing the OGTT in glucose –loaded diabetic rats. From the glucose tolerance test, it was evident that blood glucose level reached a peak at 60 min and returned to fasting values after 120 min in both normal and extract treated diabetic rats as depicted in Figure1. In diabetic rats, the blood glucose level remained high in all the time points even after 120 min due to severe hyperglycemic condition. Administration of NZYM effectively maintains the blood glucose homeostasis without causing a hypoglycemic state which was evident from the drug control group (Group II). The effect of NZYM was significant with the dose of 100 mg/kg b.w. ($p<0.05$) which coincide with the results of fasting blood glucose level observed for 45 days. This proves that the low dose has pronounced anti-hyperglycemic activity. The possible mechanism by which NZYM exhibit its anti-hyperglycemic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as proved by the significant increase in insulin level in the diabetic rats treated with NZYM . The low dose (100mg/kg b.w.) could be accredited to a greater extent for the improvement in insulin level, which was significantly brought down in STZ-induced rats.

Diabetic dyslipidemia constitutes an important cardiovascular risk factor which is marked by STZ- induced diabetic rats by elevated triglyceride, cholesterol, low density lipoprotein (LDL) and decreased high density lipoprotein (HDL). These conditions were reversed in NZYM treated diabetic rats, suggesting its beneficial role in preventing diabetes related complications. Glucokinase, a key glycolytic enzyme plays an important role in the maintenance of glucose homeostasis is known to be decreased in the diabetic condition [29]. Administration of NZYM to diabetic rats resulted in significant reversal ($p<0.05$) in the glucokinase activity causing an increase in glycolysis and utilization of glucose for energy production. The reduction of glucose-6-phosphate dehydrogenase activity in liver of STZ-induced diabetic rats obstructs glucose utilization through pentose phosphate pathway which is controlled by insulin [30]. Administration of NZYM to diabetic rats significantly improves this enzyme activity in liver tissue which enlightens another possible way for anti-diabetic activity. The activities of glucose-6-phosphatase are increased in liver during diabetic condition. Treatment with NZYM to diabetic rats has markedly normalized the activity of glucose-6-phosphatase as shown in table 4. Several reports have been documented for anti-hyperglycemic activity with respect to the modulation in carbohydrate metabolizing enzymes [31]. The level of serum insulin was found to be increased in diabetic rats treated with NZYM which could be the possible reason for the significant reduction in the level of gluconeogenic enzymes.

In diabetic condition, the normal function of liver to synthesis glycogen was found to be impaired due to the inactivation of glycogen synthetase system [32]. Administration of STZ caused a decrease in glycogen content in liver which is due to enhanced glycogenolysis and insulin deficiency in diabetes. Therefore the normal capacity of the liver to synthesize glycogen is impaired. A significant increase in the liver glycogen by administration NZYM to diabetic rats restored the glycogen content demonstrating the possible role in the regulation of glycogen metabolism. This clearly suggests the anti-hyperglycemic activity of the extracts in diabetic rats might be due to the improvement in glycogenesis.

STZ plays an important role in the development of experimental diabetes causing structural damage to the pancreatic tissue [33]. In the diabetic rats, treatment with NZYM resulted in reverting the pancreas to normal architecture remarkably. The increase in the number of beta cells in the islets showed that they were regenerated. A decrease in the number of β-cells, nuclear

shrinkage and pycnosis has been observed in the islets of STZ – induced diabetic rats as evident from Figure 3C.

Berberine is one of the phytoconstituent present in *Naravelia zeylanica* [34]. The HPLC chromatogram confirms the presence of berberine in NZYM (data not shown). The hypoglycemic and insulin-sensitizing property of berberine is well documented [35]. Hence, berberine present in NZYM might be responsible for the anti-hyperglycemic activity. However further purification of the active extract is required to substantiate the above statement.

Conclusion

This study validates the anti-hyperglycemic and anti-hyperlipidemic effect of NZYM in diabetic rats and the effect was found to be on par with the glibenclamide. However further research is required to gain better understanding of the exact mechanism of NZYM underlying its anti-hyperglycemic and anti-hyperlipidemic property.

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Authors' contribution

RA identified the research concept, carried out *in vivo* work and drafted the manuscript.

SP carried out glycemic and lipid profiling. SS analyzed the results, supervised, edited the manuscript and reviewed the entire work.

Disclosure

The authors declare no conflicts of interests.

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