Biomarkers and Genomic Medicine (2013) 5, 157–163

Available online at www.sciencedirect.com

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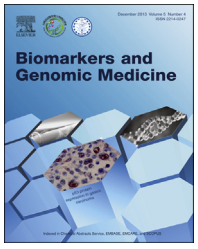

ORIGINAL ARTICLE

Preclinical evaluation of antihyperglycemic and antioxidant action of Nirmali (*Strychnos potatorum*) seeds in streptozotocin-nicotinamide-induced diabetic Wistar rats: A histopathological investigation

Shanti Bhushan Mishra [a,*], **Amita Verma** [b], **Madhavan Vijayakumar** [c]

[a] *Department of Pharmacognosy, United Institute of Pharmacy, Allahabad, India*
[b] *Department of Pharmaceutical Science, Faculty of Health Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India*
[c] *The Himalaya Drug Company, Bangalore, India*

Received 6 May 2013; received in revised form 20 July 2013; accepted 26 July 2013
Available online 20 September 2013

KEYWORDS
antidiabetic;
antioxidant;
seed extract;
streptozotocin;
Strychnos potatorum

Abstract This study aimed to evaluate the antidiabetic activity of *Strychnos potatorum* seeds in streptozotocin-nicotinamide–induced diabetes in experimental animals. Noninsulin-dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by an intraperitoneal injection (i.p.) of 60 mg/kg streptozotocin (STZ) and, after a 15-minute interval, 120 mg/kg of nicotinamide. *S. potatorum* extract 200 mg/kg or 400 mg/kg body weight was administered orally to the rats once daily for 21 days. The blood glucose level was assessed by a glucometer. The serum levels of cholesterol, triglycerides, and total lipid were determined by using diagnostic kits. Measurement of catalase (CAT), superoxide dismutase (SOD), glutathione–S-transferase (GST), reduced glutathione (GSH), and glutathione peroxidase (GPx) were determined to ascertain the antioxidant activity. A significant reduction in the blood glucose level was observed in diabetic animals treated with the different doses of the extract, compared to untreated diabetic rats. The treatment with the extract significantly increased the levels of GSH, GPx, GST, CAT, and SOD in the drug-treated group to levels comparable to the levels in the diabetic control group. The result of this study thus shows that 50%

* Corresponding author. Department of Pharmacognosy, United Institute of Pharmacy, Allahabad 211010, India.
E-mail address: shantipharma15@gmail.com (S.B. Mishra).

http://dx.doi.org/10.1016/j.bgm.2013.07.010

of the ethanolic extract at different doses possesses significant antidiabetic activity and potent antioxidant potential in diabetic conditions.

Introduction

In developing countries, the most common form of diabetes is noninsulin-dependent diabetes mellitus (NIDDM). The disorder affects more than 100 million people worldwide and by 2030 it is predicted that it will affect 366 million people.[1] There are several differences in the clinical presentation of NIDDM in tropical countries. Peripheral insulin resistance is a key feature of NIDDM and results from a combination of sedentary lifestyle, unhealthy dietary habits, and genetic predisposition.[2] Insulin resistance is also implicated in several life-threatening disorders, collectively referred to as the metabolic syndrome.[3]

Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects, and relatively low cost.[4] India has a rich history of using various potent herbs and herbal components for treating diabetes. Many Indian plants have been investigated for their beneficial use in treating different types of diabetes and have been reported in numerous scientific journals. *Strychnos potatorum* is a native plant of India and plentiful in the deciduous forests of West Bengal, Central India, and in South India up to 1200 meters. The ripe seeds are used for clearing muddy water. The clarification occurs because of the combined actions of colloids and alkaloids in the seeds. The seeds reportedly possess diuretic activity[5]; antidiarrheal activity[6]; hepatoprotective and antioxidant activity[7]; antiulcer and anti-inflammatory activity[8,9]; and antiarthritic activity.[10] In Madras, India the seeds are traditionally used in treating diabetes and gonorrhea.[11] A new triterpenoid, isomotiol, and alkaloids have been isolated from the bark of the plant.[12] The objective of this investigation was to ascertain the scientific basis for the antidiabetic potential of *S. potatorum* in streptozotocin-nicotinamide-induced diabetic rats.

Materials and methods

Plant material

Candidate plant material was collected from the local herbal drug market. The plant materials were authenticated by taxonomist Dr. A.K.S. Rawat, PhD. For future reference, the voucher specimen (CIF-RB-2-126-2) has been deposited in the departmental herbarium of the National Botanical Research Institute in Lucknow, India.

Preparation of the *S. potatorum* extract

The *S. potatorum* seeds (1 kg) were air-dried and then powdered. The powder was prepared by grinding the dried seeds in a blender. Five hundred grams of the seed powder was macerated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with 50% ethanol for 3 days (3 × 3 L) by using the cold percolation method and by centrifugation at 10,000 revolutions per minute (rpm). The extract was separated by filtration and concentrated on rotavapour (Buchi, New Castle, DE, USA), and then dried in lyophilizer (Heto Drywinner; Thermo Scientific, LLC, USA) under reduced pressure. This yielded 215.0 g of solid residue (i.e., a yield of 21.5% w/w).

Phytochemical screening

Phytochemical screening of the ethanolic extract was performed by standard procedures that revealed the presence of chemical constituents such as carbohydrates, phytosterols, alkaloids, flavonoids, and saponins.[13]

Experimental animals

Healthy adult Wistar albino rats of both sexes were used for the pharmacological studies. They were 2—3 months of age and weighed 200—250 g. The animals were housed in polypropylene cages, maintained under standard conditions of 12/12 hours of light/dark at 25°C ± 3°C and 35—60% humidity. They were fed with a standard rat pellet diet (Amrut, India) and water *ad libitum*. The Institutional Animal Ethical Committee of the United Institute of Pharmacy (Allahabad, India; No. 1451/PO/a/11/CPCSEA) approved the study.

Oral acute toxicity study

The lethal median dose (LD_{50}) determination was performed in mice by Organisation for Economic Co-operation and Development (*OECD*) *Guideline 423*.[14] A single dose of the extracts (i.e., 5 mg/kg, 50 mg/kg, 300 mg/kg, or 2000 mg/kg) in the appropriate quantity of water was administered orally by gavage to different groups of mice (three mice in each group). The animals were allowed free access to water and food. However, all the animals were deprived of food for 2 hours prior to dosing and 4 hours after dosing. For the first 12 hours, the animals were initially monitored continuously for any adverse effects for 4 hours and then monitored at 1-hour intervals. They were later monitored twice daily for any abnormal changes throughout the study period (which lasted 14 days). The lethal median dose (LD_{50}) of the 50% ethanolic extract of the *S. potatorum* seeds was 2000 mg/kg. One-tenth of the

maximum dose of the extract tested for acute toxicity was selected for pharmacological activity (i.e., 200 mg/kg and its double strength of 400 mg/kg of body weight [b.w.]).[15]

Experimental induction of diabetes

Streptozotocin (STZ) was freshly dissolved in citrate buffer (0.1 M at pH 4.5) and nicotinamide was dissolved in normal physiological saline and maintained on ice prior to use. All animals were allowed to adapt to their cages for 3 days, after which they were fasted overnight. Noninsulin-dependent diabetes mellitus was induced in the overnight-fasted rats by a single intraperitoneal injection of STZ (60 mg/kg b.w.). Fifteen minutes after the intraperitoneal administration of nicotinamide (120 mg/kg b.w.), all animals were allowed free access to food and water. Their blood glucose levels were measured 2 days after the STZ injection and were used as parameters to obtain matching pairs of rats with diabetes at a similar level of severity. Only rats with fasting blood glucose levels greater than 220 mg/dL were considered to be diabetic and were used in the experiment. The animals were randomly assigned to five different groups (i.e., Group I—Group V). Group I served as the control and contained six normal rats. All treatments started 3 days after the STZ injection.[16]

Experimental design

Five groups of rats were used to study the effect of 50% ethanolic extract of *S. potatorum*. Each group consisted of six rats and were as follows.

Group I. The control rats, which received vehicle normal saline solution.
Group II. Diabetic control rats, which received the vehicle normal saline solution.
Group III. Diabetic rats, which were treated with extract 200 mg/kg body weight.
Group IV. Diabetic rats, which were treated with extract at 400 mg/kg body weight.
Group V. Diabetic rats, which were treated with the standard drug glibenclamide at 600 μg/kg body weight.

The vehicles and the drugs were administered orally for three weeks by using an oral gavage tube daily. To measure the blood glucose level, blood samples were collected from the tail vein on Day 0, Day 7, Day 14, and Day 21. The blood glucose level was determined by a glucometer (SugarScan; Thyrocare Technologies Limited, Navi Mumbai, India). The values of the treated groups were compared to the values of the standard group that was treated with glibenclamide. The animals were then sacrificed by cervical dislocation. The liver, kidney, and pancreas were exposed and perfused with cold saline phosphate buffer (at pH 7.4) for histopathological examination. The blood-free liver and kidney were removed and separately homogenized in a glass Teflon homogenizer; Thomas Scientific, NJ, USA (10% w/v). Incubation was performed at 37°C under controlled conditions for biochemical estimation. The collected blood samples were immediately centrifuged at 2500 rpm for 15 minutes. The separated serum was collected in fresh serum tubes, which were tightly capped and then stored in a refrigerator (2—4°C). The effect of the test extracts on the levels of serum glucose, antioxidant enzymes, and lipid profile were assessed.

In vivo antioxidant activity in diabetic rats

Measurement of superoxide dismutase activity
Superoxide dismutase (SOD) was estimated, based on the method of Pari.[17] In this mixture, 2.5 mL of ethanol and 1.5 mL of chloroform (all reagents were chilled) were added and shaken for 1 minute at 4°C, and then centrifuged. The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 mL of 186 μM phenazine methosulfate (PMS), 0.3 mL of 30 μM nitroblue tetrazolium (NBT), 0.2 mL of 780 μM nicotinamide adenine dinucleotide (NADH); an appropriately diluted enzyme preparation; and water in a total volume of 3 mL. The reaction was initiated by the addition of NADH. The mixture was further incubated for 120 seconds at 37°C in a water bath. The reaction was stopped by adding 1 mL glacial acetic acid (17.4 M). A violet color developed. The solution was extracted in 4.0 mL of an n-butanol reagent blank. The activity was measured at 560 nm and the results are expressed as units (U) of SOD activity/mg protein. One unit of enzyme activity was defined as the enzyme concentration required to inhibit the chromogen production by 50% in 1 minute under the defined assay conditions.

Measurement of catalase activity
Decomposition of hydrogen peroxide (H_2O_2) in the presence of catalase was followed at 240 nm.[17] A 50 μM sample was added to buffered substrate (50 mM phosphate buffer, pH 7.0 containing 10 mM H_2O_2) to make a total volume of 3 mL, and the decrease in the absorbance was monitored at 37°C for 2.5 minutes at 15-second intervals. The activity was calculated by using the extinction coefficient of H_2O_2 of 0.041/(μmole•cm^{-2}) at 240 nm. Results are expressed as units (U) of catalase (CAT) activity/mg protein.

Determination of glutathione-S-transferase
The enzyme glutathione-S-transferase (GST) was measured in accordance with the Pari method.[17] Liver homogenate (0.1 mL), buffer (1.0 mL), double distilled water (1.7 mL), and chloro-dinitro benzene (CDNB) reagent (0.1 mL) were mixed. The mixture was incubated at 37°C for 15 minutes, and then 0.1 mL of GSH was added. The change in optical density was read at 340 nm from 0 minutes to 3.0 minutes in a Shimadzu UV-visible spectrophotometer; SHIMADZU CORPORATION, Kyoto, Japan. The activity of GST was expressed as μg/mg of protein.

Determination of reduced glutathione
The liver and kidney homogenates were mixed with 0.5 mL of 5% trichloroacetic acid (TCA) in 0.1 mM EDTA. Each sample was centrifuged at 2000 rpm for 10 minutes. The supernatant was then mixed with 2.5 mL of 0.1 M phosphate buffer (at pH 8). The color was developed by adding 100 μL of 0.01% 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The absorbance was measured at 412 nm by a spectrophotometer.[17]

Determination of glutathione peroxidase
A 0.2-mL sample each of 0.8 mM EDTA, 10 mM sodium azide, reduced glutathione, and H_2O_2; 0.4 mL of phosphate buffer; and 0.1 mL of enzyme (i.e., liver and kidney homogenates) were mixed and incubated at 37°C for 10 minutes. The reaction was arrested by adding 0.5 mL of 10% TCA. The tubes were centrifuged. Three milliliters of sodium hydrogen phosphate and 1.0 mL of DTNB were added to 0.5 mL of the supernatant. The color developed and was read at 412 nm immediately in a UV-visible spectrophotometer. Graded concentrations of the standard were similarly treated. Glutathione peroxidase activity in the liver homogenate is expressed as millimoles per milligram (mmol/mg) of tissue.[17]

Histopathology

At the end of the treatment period, the control and treated rats were sacrificed by cervical dislocation. Their pancreas was dissected. Part of the pancreatic tissue was immediately fixed in Bouin's fluid for 24 hours. The tissues was then washed in running tap water to remove the color of Bouin's fluid, and then dehydrated in alcohol in ascending and descending order. They were embedded in paraffin and cut at 5 μm sections in a rotary microtome. These sections were then deparaffinized in xylene, stained with hematoxylin-eosin, and mounted with Canada balsam. The histopathological slides were examined and photographs were captured with a digital stereomicroscope (Olympus, B061, USA).[18]

Statistical analysis

All statistical pharmacological analyses were performed by using GraphPad Prim version 3.03 software for Windows, GraphPad Software, Inc. La Jolla, CA, USA. The values are presented as the mean ± the standard deviation (SD). The data of six rats were analyzed by the Student *t* test and the analysis of variance (ANOVA) with *pos hoc* difference was analyzed by using the Newman-Keuls method.

Results

Antihyperglycemic activity

Table 1 shows the effect of the oral daily administration of 50% ethanolic extract (200 mg/kg and 400 mg/kg) on the blood glucose level in 21 days. By the end of treatment, the blood glucose level reduced from 238.36 mg/dL to 132.28 mg/dL in rats treated at a dose of 200 mg/kg ($p < 0.001$). For the oral administration of 400 mg/kg of the extract, the blood glucose level reduced from 256.43 mg/dL to 118.24 mg/dL at 21 days ($p < 0.001$). For glibenclamide (600 μg/kg), the blood glucose level reduced from 233.56 mg/dL to 94.68 mg/dL ($p < 0.001$). Table 2 shows the levels of biochemical parameters such as the levels of total cholesterol, triglycerides, and total lipids. In the STZ-nicotinamide-treated group, the cholesterol level was 72.83 mg/dL in the normal control group and 153.66 mg/dL in the diabetic control group ($p < 0.001$), and the triglyceride level was 70.16 mg/dL in the normal control group and 115.66 mg/dL in the diabetic control group ($p < 0.001$). By contrast, the 50% ethanolic extract of *S. potatorum* seeds in the groups treated at a dose of 200 mg/kg or 400 mg/kg once daily for 21 days prevented the diabetic condition in a dose-related manner. The results were moderately significant.

In vivo antioxidant activity

Table 3 presents the enzyme activities. Significant reductions in GSH, GPx, GST, SOD, and catalase were observed in the diabetic control rats in comparison to the normal control rats. There was a significant increase in GST, GPx, and CAT in the kidney and in the liver [GST, 6.59 ± 0.29 μmol CDNB-GSH conjugate/(min•mg^{-1} protein); GPx, 9.17 ± 0.88 μg glutathione consumed/(min•mg^{-1} protein); CAT, 70.22 ± 1.24 μmol of H_2O_2 consumed/(min•mg^{-1} protein)]. There was a significant increase in the liver GSH level (to 128.67 ± 2.54 nM of conjugated DTNB/mg protein), whereas there was no significant increase in the kidney GSH on the administration of the 400 mg/kg dose. The SOD level (6.22 ± 0.43 U min/mg Hb) in erythrocytes decreased on the

Table 1 The effect of 50% ethanolic extract of *Strychnos potatorum* on the serum glucose level in STZ-nicotinamide-induced diabetic rats.

Group	Treatment (dose)	0 day (mg/dL)	After 7 days (mg/dL)	After 14 days (mg/dL)	After 21 days (mg/dL)
I	Normal control	78.45 ± 12.41	76.12 ± 11.80	77.46 ± 10.32	78.86 ± 9.36
II	Diabetic control	248.22 ± 32.56***	227.24 ± 36.14***	229.46 ± 32.12***	212.32 ± 26.12***
III	Extract (200 mg/kg)	238.36 ± 2.64	209.36 ± 3.46	168.46 ± 3.26*	132.28 ± 3.14**
IV	Extract (400 mg/kg)	256.43 ± 2.25	162.46 ± 2.17	124.32 ± 2.43**	118.24 ± 1.22**
V	Glibenclamide (600 μg/kg)	233.56 ± 13.40	186.06 ± 12.76	134.51 ± 8.21**	94.68 ± 5.37**

The data represents the mean ± standard deviation for six rats per group.
* Indicates statistical significance, compared to the diabetic control group ($p < 0.05$).
** Indicates statistical significance, compared to the diabetic control group ($p < 0.001$).
*** Indicates statistical significance, compared to the normal group ($p < 0.001$).
STZ = streptozotocin.

Table 2 The effect of 50% ethanolic extract of *Strychnos potatorum* on the total cholesterol, triglyceride, and lipid levels in the blood serum after 21 days.

Groups	Treatment (dose)	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	Total lipids (mg/dL)
I	Normal control	70.16 ± 4.81	72.83 ± 4.74	84.83 ± 5.14
II	Diabetic control	115.66 ± 3.94****	153.66 ± 5.24****	148.83 ± 4.29****
III	Extract (200 mg/kg)	108.12 ± 2.65	104.47 ± 1.36***	134.36 ± 1.18*
IV	Extract (400 mg/kg)	88.19 ± 1.26***	78.65 ± 1.98***	126.54 ± 2.48**
V	Glibenclamide (600 μg/kg)	75.66 ± 4.92***	81.83 ± 7.77***	93.33 ± 5.73***

The data represents the mean ± standard deviation for six rats per group.
* Indicates statistical significance, compared to the diabetic control group ($p < 0.05$).
** Indicates statistical significance, compared to the diabetic control group ($p < 0.01$).
*** Indicates statistical significance, compared to the diabetic control group ($p < 0.001$).
**** Indicates statistical significance, compared to the normal group ($p < 0.001$).

induction of diabetes, but significantly increased on treatment with 50% ethanolic extract, thereby restoring the SOD level to nearly that of the normal control group. The results were comparable to that of glibenclamide. The activities of these enzymes decreased significantly in the diabetic control rats in comparison to the normal control rats. The oral administration of the extract (at 200 mg/kg or 400 mg/kg body weight) for 3 weeks significantly reversed these enzymes to near-normal values.

Histopathological studies

Microscopically examined pancreas section of the control group showed normal islets, the architecture was preserved, and the acini were lined by round to oval cells with moderate cytoplasm and small round to oval nuclei (Fig. 1A). However, in the diabetic control group, the islets showed depleted cells, the architecture was preserved, and the acini were lined by round to oval cells with moderate cytoplasm and small round to oval nuclei (Fig. 1B). In the 200 mg/kg extract-treated group, the architecture was partially effaced, the islets were normal, and there was a mild and diffuse infiltrate of lymphocytes within the stroma (Fig. 1C). In the 400 mg/kg extract-treated group, the architecture was normal, the islets showed depletion of the acinar cells, and there was no evidence of inflammation (Fig. 1D); however, in the standard-treated group, there was a mild infiltrate of lymphocytes at some foci and the

Table 3 The effect of *Strychnos potatorum* extract on antioxidant enzyme activity in STZ-nicotinamide-induced diabetic rats.

Parameters	GSH (nM of DTNB conjugated/ mg protein)	GST [μmol of CDNB-GSH conjugate formed/ min•mg^{-1} protein)]	GPx [μg glutathione consumed/ (min•mg^{-1} protein)]	CAT [μmol of H_2O_2 consumed/ (min•mg^{-1} protein)]	SOD [U min/(mg•Hb^{-1})] in erythrocytes
Normal control					
Liver	129.67 ± 2.66	6.75 ± 0.97	9.38 ± 0.91	71.25 ± 2.17	6.34 ± 0.19
Kidney	118.77 ± 2.31	6.88 ± 0.34	7.33 ± 0.14	38.33 ± 1.22	
Diabetic control					
Liver	74.23 ± 1.51*****	3.42 ± 0.18*****	5.19 ± 0.88*****	38.67 ± 1.92*****	3.41 ± 0.22*****
Kidney	46.17 ± 2.49*****	2.66 ± 0.27	4.99 ± 1.01****	20.55 ± 1.75****	
Extract-treated (200 mg/kg)					
Liver	107.97 ± 1.97*	4.55 ± 0.63**	6.11 ± 0.74**	56.41 ± 3.69**	4.97 ± 0.62**
Kidney	71.22 ± 3.14 (NS)	3.69 ± 0.71*	5.22 ± 0.44*	25.69 ± 1.20*	
Extract-treated (400 mg/kg)					
Liver	128.67 ± 2.54***	6.59 ± 0.29***	9.17 ± 0.88***	70.22 ± 1.24***	6.22 ± 0.43***
Kidney	91.33 ± 2.89 (NS)	5.11 ± 0.33**	6.97 ± 0.76***	36.57 ± 1.41***	
Glibenclamide-treated (600 μg/kg)					
Liver	130.56 ± 1.41***	5.12 ± 0.11**	7.24 ± 0.61**	65.31 ± 2.56***	5.18 ± 0.31**
Kidney	95.17 ± 2.55 (NS)	3.76 ± 0.91**	5.66 ± 0.58*	35.24 ± 1.81***	

The data represents the mean ± standard deviation for six rats per group.
* Indicates statistical significance, compared to the diabetic control group ($p < 0.05$).
** Indicates statistical significance, compared to the diabetic control group ($p < 0.01$).
*** Indicates statistical significance, compared to the diabetic control group ($p < 0.001$).
**** Indicates statistical significance, compared to the normal group ($p < 0.01$).
***** Indicates statistical significance, compared to the normal group ($p < 0.001$).
CAT = catalase; CDNB = chloro-dinitro benzene; DTNB = 5,5'-dithiobis-(2-nitrobenzoic acid); GPx = glutathione peroxidase; GSH = glutathione; GST = glutathione-S-transferase; H_2O_2 = hydrogen peroxide; Hb = hemoglobin; SOD = superoxide dismutase; STZ = streptozotocin; U = units.

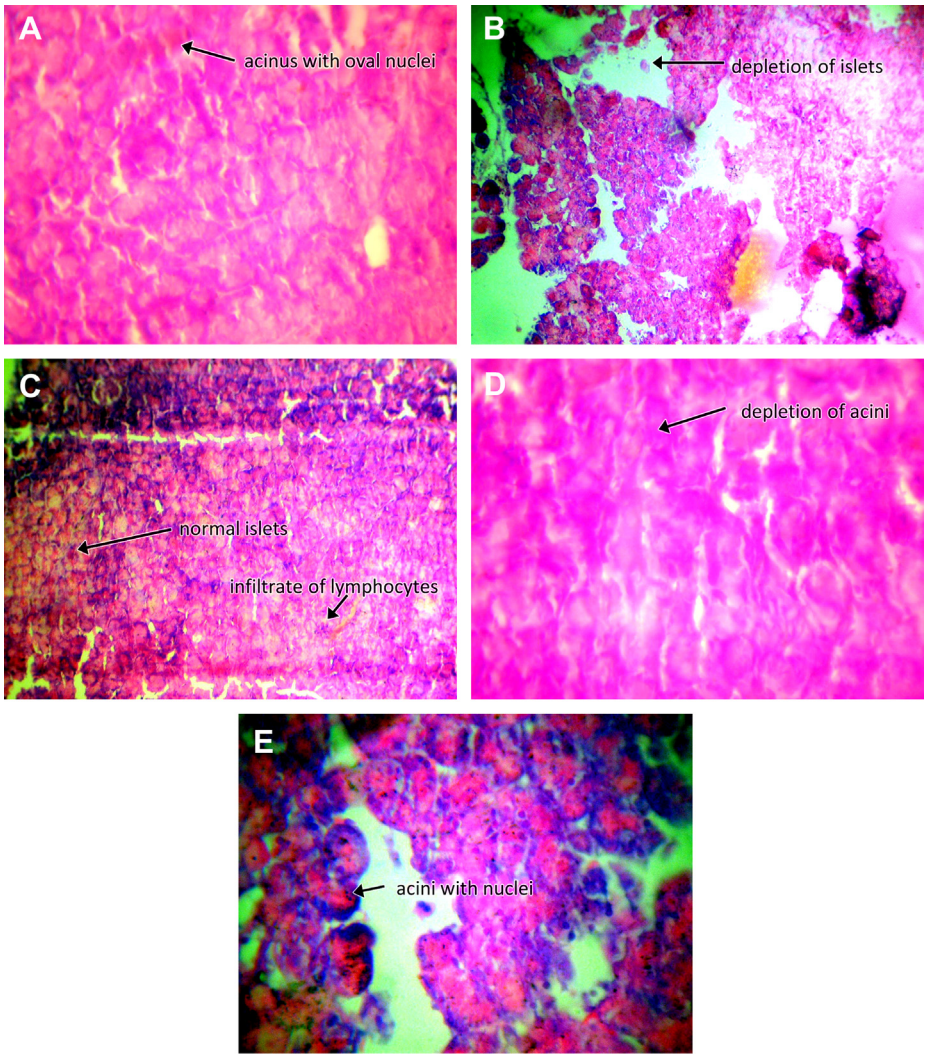

Figure 1 Pathologic findings. (A) The islets of Langerhans are normal and the architecture is preserved. (B) The islets show the depletion of cells. (C) The islets are normal but have a mild and diffuse infiltrate of lymphocytes within the stroma. (D) The islets show depletion of the acinar cells, but there is no evidence of inflammation. (E) Some foci show mild infiltration of lymphocytes. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. (Hematoxylin and eosin stain; original magnification, 200×.)

acini were lined by round to oval cells with moderate cytoplasm and small round to oval nuclei (Fig. 1E).

Discussion

There are numerous pathways and mechanisms involved in the manifestation of diabetes, which cannot be significantly cured by a single drug or by a drug acting on a single target or receptor. The medicinal preparations in conventional medicines contain a variety of herbal and nonherbal constituents that act on an array of targets by various modes and mechanisms. The STZ-nicotinamide injection caused diabetes mellitus, which may have resulted from the destruction of beta cells of the islets of Langerhans of the pancreas—followed by insulin deficiency—and may have led to a variety of derangements in metabolic and regulatory processes.[19] Our investigations indicate the efficiency of the extract in maintaining blood glucose levels in STZ-nicotinamide-induced diabetic rats. The administration of an aqueous ethanolic extract of *S. potatorum* to diabetic rats moderately decreased the blood glucose levels. A possible mechanism by which the 50% ethanolic extract exerts its hypoglycemic action in diabetic rats may be by potentiating the plasma insulin effect either by increasing the pancreatic secretion of insulin from the existing beta cells or by increasing the release of insulin from its bound form. A marked increase in the level of total cholesterol has been observed in untreated diabetic rats. Under normal circumstances, insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzyme, thereby causing hypertriglyceridemia. The significant control of the levels of serum lipids in the extract-treated diabetic rats may be directly attributed to improvements in insulin levels with extract therapy. The induction of diabetes with STZ is associated with the characteristic loss of body weight, which results from increased muscle

wasting and loss of tissue proteins.[20] The treatment of prediabetic animals with nicotinamide improves diabetic metabolic alteration, most likely by counteracting beta cell dysfunction and loss associated with oxidative stress.[21,22] However the exact mechanism of the action of nicotinamide in diabetes remains under investigation.

One consequence of hyperglycemia is increased metabolism of glucose by the sorbitol pathway. Besides this, other pathways, such as fatty acid and cholesterol biosynthesis also compete for NADPH with GSH. The decrease in GSH level in the liver during diabetes is probably because of its increased utilization by the hepatic cells which could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes.[23] We have also observed a decrease in GSH in the liver and the kidney. The activities of GPx and GST decreased significantly in diabetic rats. GPx (a selenium-containing enzyme) and GST catalyze the reduction of hydrogen peroxide to nontoxic compounds.

The administration of the extract or glibenclamide increased the activity of GPx and GST in diabetic conditions. The enzymers enzymes SOD and catalase are two major scavenging enzymes that remove the toxic-free radical *in vivo*. Reduced SOD activity in erythrocytes and reduced catalase activity in the liver and kidney have been observed during diabetes, and this may result in several deleterious effects because of the accumulation of superoxide radicals and H_2O_2.[24]

In conclusion, the results presented in this study suggest the following: (1) *S. potatorum* possesses antidiabetic and antioxidant activity; (2) one or more antidiabetic compounds in the plant extract improve the physiology of rats affected by type 2 diabetes; and (3) bioactive constituents responsible for improving the physiology of type 2 diabetic rats need to be isolated and characterized to contribute to better treatment of NIDDM.

Acknowledgments

The authors genuinely thank Professor Alok Mukerjee PhD, Principal of the United Institute of Pharmacy (Allahabad, India) for providing the animal housing facilities to perform the animal studies.

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