Asian Pac. J. Health Sci., 2016; 3 (3):30-44 e-ISSN: 2349-0659, p-ISSN: 2350-0964

Effect of *Ipomoea staphylina* leaves on Streptozotocin- Nicotinamide Induced Type-II Diabetes in Wistar Rats

S M Firdous*, Anoop Singh

Department of Pharmaceutical Science, NIMS University, Shobha Nagar, Jaipur-Delhi Highway (NH-11C), Jaipur- 303121, Rajasthan, India

ABSTRACT

The aim of the present study was to evaluate the antidiabetic activity of Ipomoea staphylina (IS) leaves in Streptozotocin (STZ)-nicotinamide induced type-II diabetic in rats. Oral administration of ethanolic extract of IS leaves and its fractions at the doses of 100 mg/kg and 200 mg/kg was studied in glucose-loaded and STZ-nicotinamide induced diabetic rats. The effects of extract and its fractions on blood glucose, body weight, lipid profile, plasma enzymes (SGOT, SGPT and ALP), serum urea, creatinine, total protein, liver glycogen, and activities of SOD, CAT and GPx in diabetic rats were analyzed. The IS extract and its fractions significantly reduced the blood glucose level in glucose-loaded rats. After treatment with IS extract and its fractions (100 and 200 mg/kg) for 21 days there was a significant decrease in blood glucose, total cholesterol, triglycerides, LDL-C, VLDL-C, plasma enzymes (SGOT, SGPT and ALP), serum urea, creatinine and significant increase in body weight and total protein levels was observed in treated diabetic rats. The activities of antioxidant enzymes SOD, CAT and GPx were also inceased in diabetic mice after the treatment with IS extract and its fractions. Histological analysis showed improvement in the cellular architecture of pancreas, liver and kidney.

Key words: Antidiabetic, *Ipomoea staphylina* (IS), streptozotocin (STZ), nicotinamide, body weight, blood glucose, total cholesterol, triglycerides,creatinine

Introduction

Diabetes Mellitus (DM) is one of the most prevalent metabolic disorders characterized with increased blood sugar level and improper primary metabolism. It is characterized by alterations in the metabolism of carbohydrate, fat and protein, which are caused by a inappropriate secretion of insulin or insulin resistance or both [1]. Hyperglycemia lead to decrease in the number of glucose transporters and down-regulation of number of insulin receptors as well as defects of tissue insulin signal transduction. In addition, increase in the hepatic glucose output, which exceeds an increase of glucose utilization. Finally, the hyperglycemia condition manifest adverse effects on β-cell insulin secretion and thus insulin resistance occur [2-3]. The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. According to recent estimate, the greatest absolute increase in the number of people with diabetes will be in India and the total number of people with diabetes is projected to 79.4 million in 2030.It is expected that about 366 million people are likely to be diabetic by the year 2030 [4]. Many antidiabetic drugs are available for long term therapy are found to be associated with various toxicities and none of them gives long duration glycaemic control without causing any adverse side effects. Thus there is a growing interest in using natural plant sources having minimal side effects for the treatment of DM [5].*Ipomoea staphylina* (IS) is commonly found on hedges and bushes in the forests and waste lands. It is a perennial, woody and glabrous shrub with pink flowers. Traditionally Ipomoea staphylina is used for respiratory disorders. Traditionally genus Ipomoea is used as [6] purgative, dyspepsia, anthelmintic, bronchitis. A literature review reveals anti-inflammatory activity, 5-lipoxygenase, α-glucosidase

**Correspondence*
S M Firdous
Department of Pharmaceutical Science, NIMS University, Shobha Nagar, Jaipur-Delhi Highway (NH-11C), Jaipur- 303121, Rajasthan, India
E Mail: firdous.cology@gmail.com

and [6, 7] α-amylase inhibitory activity of *Ipomoea staphylina*. Bioactive chemical constituents reported from the leaves of Ipomoea staphylina [6] include Sitosteryl-3-O-β-D-glucoside and chiro deoxy inositol. Hence, the present study was aim evaluate the antidiabetic activitiy of *Ipomoea staphylina* leaves on streptozotocin-nicotinamide induced type-II diabetic in rats.

Materials and methods

Drugs and chemicals

STZ was procured from Hi- Media India, glibenclamide were obtained from Aventis Pharma, India. Total cholesterol, triglycerides, SGOT, SGPT, ALP, total protein, serum urea and creatinine kits were obtained from Span Diagnostics, India. All other chemicals chemicals were commercial products of analytical reagent grade.

Collection of plant material and extraction

Leaves of IS were collected from forest area of Karnataka near to Bangalore. The Ipomoea staphylina plant taxonomically identified and authenticated by Dr. K. Karthigeyan at Central National Herbarium, Botanic Garden, Howrah, where the voucher specimen is conserved under the reference number SMF-01.

The leaves of IS were cleaned and dried under shade at room temperature for several days and powdered. The powder was defatted with petroleum ether (60-80 GR) for 72 h and then the dried powder was extracted with ethyl alcohol to get a yield of 10.2 % w/w. The ethanolic extract was dispersed in distilled water and partitioned with ethyl acetate in a separating funnel till the colourless ethyl acetate fraction is obtained. Then the aqueous part is then partitioned with n-butanol to get the butanol fraction. Ethyl acetate and n-butanol fraction so obtained was concentrated by keeping in boiling water bath to get the solid residue. The dried extract and its fractions were stored in airtight container and placed in refrigerator [8].

Preliminary phytochemical screening

Preliminary phytochemical screening of ethanolic extract of IS leaves and its ethyl acetate and n-butanol fractions were performed for the presence of alkaloids, phenolics, flavonoids, saponins, proteins, carbohydrates and glycosides [9].

Animals

Healthy adult male Wistar Albino rats weighing 180-220 g maintained under controlled conditions of temperature (23±2°C) and humidity (50±5%) and a 12h light–dark cycle, were used for the experiment. They were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water ad libitum. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

Mice were kept overnight fasting prior to drug administration. Animals were received a single oral dose (2000 mg/kg, b.w.) of ethanolic extract of leaves of IS and its ethyl acetate and n-butanol fractions. After the administration of Ipomoea staphylina leaves extract and its fractions food was withheld for further 3-4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks [10].

Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test was performed in overnight fasted normal rats to assess the glucose tolerance. Rats were divided into eight groups of six each. Group I: Normal control rats were treated with vehicle (2.5 ml/kg of saline) alone; Group II: Rats were treated with treated with glibenclamide (10 mg/kg); Group III: Rats were treated with IS extract (200 mg/kg); Group IV: Rats were treated with IS extract (100 mg/kg); Group V: Rats were treated with ethyl acetate fraction of IS extract (200 mg/kg); Group VI: Rats were treated with ethyl acetate fraction of IS extract (100 mg/kg); Group VII: Rats were treated with n-butanol fraction of IS

extract (200 mg/kg); Group VIII: Rats were treated with n-butanol fraction of IS extract (100 mg/kg).Overnight fasted rats were fed glucose (2 g/kg) 30 min after the administration of extract and its fractions and and glibenclamide blood was collected at 0, 30, 60 and 120 min interval from orbital sinus for glucose estimation [11].

Induction of diabetes and experimental design

Diabetes was induced in overnight fasted rats by single intraperitoneal injection of STZ (50 mg/kg), freshly dissolved in 0.1 M cold citrate buffer, pH 4.5 15 min after the intraperitoneal administration of nicotinamide (120 mg/kg). After a week in time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration, >250 mg/dl) that exhibited glycosuria and hyperglycemia were selected for the experiment. The animals were randomly assigned into nine groups of six animals in each group and received the following treatments: Group I: Normal control rats treated with vehicle (2 % gum acacia, 5 ml/kg/day) alone; Group II: Diabetic control rats treated with vehicle alone; Group III: Diabetic rats treated with glibenclamide (10 mg/kg); Group IV: Diabetic rats treated with IS Extract (200 mg/kg); Group V: Diabetic rats treated with ethyl acetate extract (100 mg/kg); Group VI: Diabetic rats treated with ethyl acetate fraction of IS extract (200 mg/kg); Group VII: Diabetic rats treated with ethyl acetate fraction of IS extract (100 mg/kg); Group VIII: Diabetic rats treated with n-butanol fraction of IS extract (200 mg/kg); Group IX: Diabetic rats treated with n-butanol fraction of IS extract (100 mg/kg) [12, 13]. Treatment was given orally using an intragastric tube once daily for 21 days, continuously. On 21st day, the animals were fasted for 12 h, blood was drawn from retro orbital vein under mild ether anaesthesia for various biochemical estimations. The animals were sacrificed by cervical decapitation. Liver and kidneys were dissected out, immediately rinsed in ice cold saline and stored for further biochemical estimations. Changes in the body weight were also determined at 0, 7th, 14th and 21st day.

Biochemical analysis

Serum glucose was measured by using a glucometer (Accu-Chek Active, India). Serum total cholesterol, total triglyceride, LDL-c, VLDL-c and HDL-c were estimated using standard Enzymatic (Span Diagnostics, India). The estimation of SGOT, SGPT , serum ALP, total protein, serum urea, and creatinine was done by using standard Enzymatic (Span Diagnostics, India). The liver and kidney homogenate, prepared in ice chilled 10% potassium chloride solution, was used to measure the levels and activities of lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Histopathological studies

Pancreas, liver and kidneys were instantly dissected out, excised and rinsed in icecold saline solution. A portion of liver and kidney were fixed in 10% neutral formalin fixative solution, were fixed in 10% formalin, dehydrated in alcohol and then embedded in paraffin. Microtome sections of 4–5 µm thickness were made by using a rotary microtome. The sections were stained with haematoxylin–eosin (H&E) dye to observe histopathological changes [14].

Statistical analysis

Results were expressed Mean ± SEM from six animals in each group. Comparison between the groups were made by using one way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Test with the help of INSTAT software. $p<0.05$ was considered as statistically significant.

Results

Acute oral toxicity

In LD50 studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

Effect of Ipomoea staphylina on oral glucose tolerance test (OGTT) in rats

After glucose loading, it was observed that normal control rats showed higher blood glucose level. Administration of ethanolic extract of IS leaves and its fractions (100 and 200 mg/kg, p.o.) significantly ($p<0.001$) lower the blood glucose levels at 30 mins and 60 mins as compared to normal control rats. The ethanolic extract of IS leaves (200 mg/kg, p.o.) also significantly reduced the blood glucose level at 120 mins. The ethyl acetate fraction (200 mg/kg, p.o.) also found to reduce the blood glucose level at 120 mins (Figure 1).

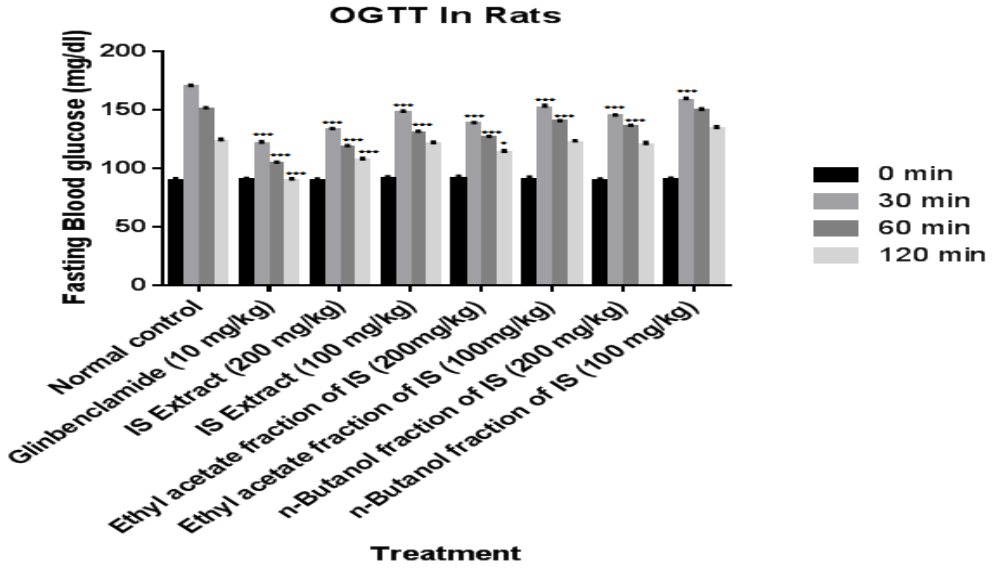

Figure 1: Effect of IS extract and its fractions on oral glucose tolerance test (OGTT) in rats

Effect of IS extract and its fractions on blood glucose level in STZ-nicotinamide induced diabetic rats

Figure 2 shows the effect of ethanolic extract of IS its ethyl acetate and n-butanol fractions on blood glucose levels of diabetic rats. It was found that a significant ($p<0.001$) increase in blood glucose level was observed in STZ-nicotinamide induced diabetic rats. After the daily treatment for 21 days showed significant ($p<0.001$) reduce in blood glucose levels with the doses of 100 and 200 mg/kg, p.o. of ethanolic extract of IS and its fractions and 10 mg/kg, p.o. of glibenclamide as compared to diabetic control group.

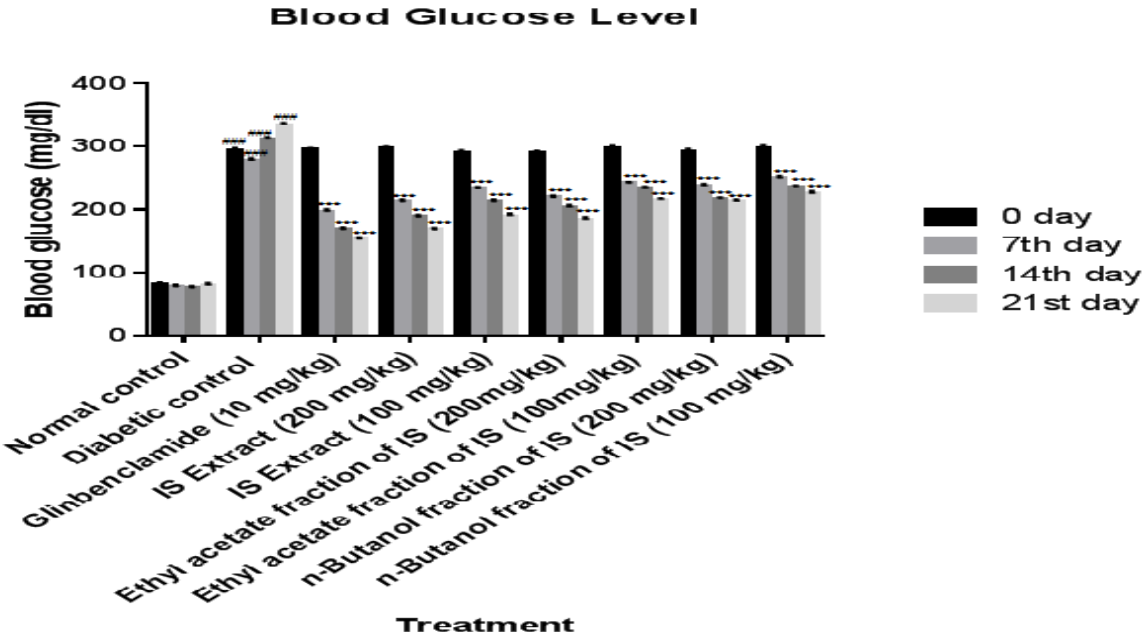

Figure 2: Effect of IS extract and its fractions extract and its fractions on blood glucose level in STZ-nicotinamide induced diabetic rats

Effect of IS extract and its fractions on body weight in STZ-nicotinamide induced diabetic rats

Average body weights of different animal groups at various intervals are shown in table 1. STZ-niconinamide induce diabetic produced significant ($p<0.001$) loss in body weight as compared to normal animals during the study. Diabetic control continued to lose weight till the end of the study while Glinbenclamide (10 mg/kg, p.o.) and ethanolic extract of IS and its ethyl acetate and n-butanol fractions (100 and 200 mg/kg, p.o.) showed significant ($p<0.001$) improvement in body weight compared to diabetic control at 21st days.

Table 1: Effect of IS extract and its fractions on body weight in STZ-nicotinamide induced diabetic rats

Groups	Treatment	Body Weight (gm)			
		0 day	7th day	14th day	21st day
I	Normal control	167.02± 2.90	176.10± 3.26	189.85± 2.25	192.28± 1.48
II	Diabetic control	168.27± 1.89	152.22± 2.00###	147.43± 1.93###	140.26± 1.94###
III	Glinbenclamide (10 mg/kg)	168.22± 2.38	173.52± 1.01***	178.39± 1.28***	180.75± 1.13***
IV	IS Extract (200mg/kg)	167.68± 2.56	170.93± 1.84***	174.75± 2.13***	179.75± 1.11***
V	IS Extract (100mg/kg)	164.53± 2.22	167.90± 1.34***	170.25± 1.21***	174.02± 1.93***
VI	Ethyl acetate fraction of IS (200mg/kg)	170.53± 1.79	175.04± 1.34***	178.15± 1.25***	181.25± 1.72***
VII	Ethyl acetate fraction of IS (100mg/kg)	165.21± 2.24	168.31± 2.00***	170.90± 1.02***	170.87± 2.09***
VIIII	n-Butanol fraction of IS (200mg/kg)	165.80± 1.73	167.79± 1.42***	170.11± 1.40***	172.03± 1.95***
IX	n-Butanol fraction of IS(100mg/kg)	166.58± 1.44	166.20± 1.02***	168.34± 1.54***	169.23± 1.48***

Values are given as mean ± SEM for groups of six animals each
p <0 .001 when compared with the normal control group
*** p <0 .001 when compared with the diabetic control group

Effect of IS extract and its fractions on lipid profile in STZ-nicotinamide induced diabetic rats

STZ-nicotinamide treatment resulted in significant ($p<0.001$) increase of TC, TG, LDL-C, VLDL-C and reduction of HDL-C levels as compared to the normal control rats. Treatment with ethanolic extract of IS and its ethyl acetate and n-butanol fractions (100 and 200 mg/kg, p.o.) showed significant ($p<0.001$) reduction in TC and TG. The extract and its fraction (100 and 200 mg/kg, p.o.) also significantly reduced the LDL-C and VLDL-C level compared to diabetic control. HDL-C level was increased significantly by the ethanolic extract of IS and its ethyl acetate fraction (200 mg/kg, p.o.). The n-butanol fraction increased the HDL-C level but not in a significant manner (Table 2).

Table 2: Effect of IS extract and its fractions on lipid profile in STZ-nicotinamide induced diabetic rats

Groups	Treatment	Lipid parameters (mg/dl)				
		TC	TG	HDL -C	LDL-C	VLDL-C
I	Normal control	73.10± 1.82	84.84± 2.66	34.72± 1.38	21.40±1.28	16.96± 0.53
II	Diabetic control	147.6± 2.63$^{###}$	173.8± 3.19$^{###}$	16.21± 1.00$^{###}$	96.63±2.98$^{###}$	34.77± 0.63$^{###}$
III	Glinbenclamide (10mg/kg)	87.42± 2.23***	97.10±2.10***	29.94± 2.08***	38.05±1.50***	19.48± 0.39***
IV	IS Extract (200mg/kg)	100.08± 2.47***	113.4± 2.26***	28.72± 1.73***	48.65±3.27***	22.68± 0.45***
V	IS Extract (100mg/kg)	125.89± 2.13***	142.54± 2.26***	22.37± 1.71	75.00±2.08***	28.50± 0.45***
VI	Ethyl acetate fraction of IS (200mg/kg)	113.18± 2.41***	121.04± 1.39***	25.03± 1.01**	63.93±2.56***	24.20± 0.27***
VII	Ethyl acetate fraction of IS (100mg/kg)	131.81± 3.29***	151.15± 1.65***	20.40± 1.04	81.17±2.52**	30.02± 0.49***
VIIII	n-Butanol fraction of IS(200mg/kg)	121.23± 1.65***	130.95± 2.50***	22.11± 1.17	72.92±2.52***	26.18± 0.50***
IX	n-Butanol fraction of IS(100mg/kg)	134.99± 2.08*	154.47± 1.16***	18.98± 1.28	85.10±0.98*	30.89± 0.23***

Values are given as mean ± SEM for groups of six animals each
$^{###}$p <0 .001 when compared with the normal control group
***p <0 .001, **p <0 .01 and *p <0 .05 when compared with the diabetic control group

Effect of IS extract and its fractions on SGOT, SGPT and ALP level in STZ-nicotinamide induced diabetic rats

In STZ-nicotinamide induced diabetic rats a significant increase in activities of SGOT, SGPT and ALP was observed. After treatment with ethanolic extract of IS and its ethyl acetate and n-butanol fraction (100 and 200 mg/kg, p.o.) the SGOT, SGPT and ALP activities were significantly reduced compared to diabetic rats (Table 3). The effect of ethanolic extract of IS and its ethyl acetate fraction (200 mg/kg) on SGOT, SGPT and ALP activities were more significant (p<0.001).

Table 3: Effect of IS extract and its fractions on SGOT, SGPT and ALP level in STZ-nicotinamide induced diabetic rats

Groups	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (mg/dl)
I	Normal control	81.10±2.05	62.38±2.65	113.34±2.57
II	Diabetic control	138.54±3.11$^{###}$	110.4±3.56$^{###}$	196.81±3.13$^{###}$
III	Glinbenclamide (10mg/kg)	90.75±2.14***	76.76±3.29***	135.09±1.57***
IV	IS Extract (200mg/kg)	97.14±1.66***	82.13±3.38***	140.51±2.65***
V	IS Extract (100mg/kg)	120.28±3.05***	91.96±2.17***	161.29±2.84***
VI	Ethyl acetate fraction of IS (200mg/kg)	107.17±2.40***	88.92±1.86***	148.06±2.04***
VII	Ethyl acetate fraction of IS(100mg/kg)	124.29±1.96**	100.53±2.59	176.56±1.72***
VIIII	n-Butanol fraction of IS(200mg/kg)	120.26±2.13***	94.27±1.50**	169.62±2.42***
IX	n-Butanol fraction of IS (100mg/kg)	131.45±2.92	99.32±3.18	180.98±2.67**

Values are given as mean ± SEM for groups of six animals each

p <0 .001 when compared with the normal control group
*** p <0 .001 and ** p <0 .01 when compared with the diabetic control group

Effect of IS extract and its fractions on serum creatinine, blood urea, blood urea nitrogen and total protein level in STZ-nicotinamide induced diabetic rats

The effect of ethanolic extract of IS and its ethyl acetate and n-butanol fractions (100 and 200 mg/kg, p.o.) on serum creatinine, blood urea, blood urea nitrogen (BUN) and total protein level in STZ-nicotinamide induced diabetic rats is shown in table 4. In diabetic rats a significant ($p<0.001$) increase in the levels of serum creatinine, blood urea and blood urea nitrogen was observed when compared to normal rats. Diabetic rats treated with ethanolic extract of IS and its fractions (100 and 200 mg/kg, p.o.) showed significant ($p<0.001$) reduction in the levels of serum creatinine level when compared with diabetic rats. Blood urea and blood urea nitrogen (BUN) were also significantly reduced by the ethanolic extract of IS and its ethyl acetate fraction (200 mg/kg, p.o.) when compared with diabetic rats. Diabetic rats showed a significant ($p<0.001$) decrease in serum total protein which was increased significantly with treatment of ethanolic extract of IS and its fractions.

Table 4: Effect of IS extract and its fractions on serum creatinine, blood urea, blood urea nitrogen and total protein level in STZ-nicotinamide induced diabetic rats

Groups	Treatment	Serum Creatinine (mg/dL)	Blood Urea (mg/dL)	Blood Urea Nitrogen (mg/dl)	Total Protein (g/dl)
I	Normal control	0.64± 0.02	30.70± 1.69	14.33± 0.79	6.86± 0.11
II	Diabetic control	2.04± 0.03###	50.26± 2.74###	23.46± 1.28###	3.06± 0.10###
III	Glinbenclamide (10 mg/kg)	0.81± 0.02***	34.29± 2.31***	15.77± 1.01***	5.34± 0.14***
IV	IS Extract (200 mg/kg)	0.89± 0.02***	37.39± 2.42**	17.46± 1.13**	5.15± 0.09***
V	IS Extract (100 mg/kg)	1.32± 0.01***	40.64± 2.17	18.97± 1.01	4.37± 0.14***
VI	Ethyl acetate fraction of IS (200 mg/kg)	0.97± 0.02***	34.49± 1.70*	17.97± 0.79*	4.93± 0.08***
VII	Ethyl acetate fraction of IS (100 mg/kg)	1.41± 0.02***	42.33± 1.52	19.76± 0.74	4.19± 0.06***
VIII	n-Butanol fraction of IS (200 mg/kg)	1.18± 0.03***	40.66± 2.03	18.98± 0.95	4.41± 0.10***
IX	n-Butanol fraction of IS (100 mg/kg)	1.46± 0.03***	44.71± 2.51	20.87± 0.95	3.82± 0.05***

Values are given as mean ± SEM for groups of six animals each
p <0 .001 when compared with the normal control group
***p <0 .001, **p <0 .01 and *p <0 .05 when compared with the diabetic control group

Changes in hepatic LPO, GPx, CAT and SOD of normal and STZ-nicotinamide induced diabetic rats after 21 days of treatment with IS extract and its fractions

In diabetic rats, a significant ($p<0.001$) rise in the activity of LPO was observed in the liver. Treatment with ethanolic extract of IS and its ethyl acetate and n-butanol fractions (200 mg/kg, p.o.) showed significant decrease in liver LPO level. The activities of GPx, CAT and SOD level in liver were also significantly decrease ($p<0.001$) in diabetic rats. Treatment with the ethanolic extract of IS and its fractions were significantly increased the liver GPx and CAT level. The extract and its ethyl acetate fraction but not n-butanol fraction was found to increase the SOD level in the liver (Table 5).

Table 5: Changes in hepatic LPO, GPx, CAT and SOD of *STZ-nicotinamide* induced diabetic rats after 21 days of treatment with IS extract and its fractions

Groups	Treatment	LPO (ηm of MDA / mg of protein)	GPx (µMole of the oxidise GSH/min/mg of protein)	Catalase (µm H_2O_2/mg of protein)	SOD (U/mg of protein)
I	Normal control	9.85±1.03	15.85±0.87	13.16±0.89	16.62±0.66
II	Diabetic control	38.10±1.63$^{###}$	6.53±1.06$^{###}$	5.60±0.60$^{###}$	7.13±0.58$^{###}$
III	Glinbenclamide (10 mg/kg)	16.28±1.08***	13.02±1.19***	12.03±1.05***	13.47±0.84***
IV	IS Extract (200mg/kg)	20.81±1.30***	12.26±0.99**	11.29±0.78**	12.05±0.68***
V	IS Extract (100mg/kg)	29.75±1.58**	8.84±0.86	8.33±0.96	9.64±0.62
VI	Ethyl acetate fraction of IS (200mg/kg)	25.16±1.33***	10.34±1.11	10.35±1.09*	11.37±1.05**
VII	Ethyl acetate fraction of IS (100mg/kg)	28.93±1.07***	8.64±0.77	8.06±0.90	8.30±0.65
VIII	n-Butanol fraction of IS (200mg/kg)	27.23±1.62***	9.68±0.62	9.80±0.53*	9.93±0.58
IX	n-Butanol fraction of IS (100mg/kg)	31.72±1.25*	8.20±0.81	7.39±0.64	7.97±0.70

Values are given as mean ± SEM for groups of six animals each
$^{###}$ $p<0.001$ when compared with the normal control group
$^{***}p<0.001$, $^{**}p<0.01$ and $^{*}p<0.05$ when compared with the diabetic control group

Changes in kidney LPO, GPx, CAT and SOD of normal and STZ-incotinamide induced diabetic rats after 21 days of treatment with IS extract and its fractions

Table 6 shows the activities of LPO, GPx, CAT and SOD in the kidney of normal and diabetic rats. A significant ($p<0.001$) increase in the activity of LOP and reduction in the activities of GPx, CAT and SOD were seen in diabetic rats. Treatment with ethanolic extract of IS and its ethyl acetate and n-butanol fractions (100 and 200 mg/kg, p.o.) significantly ($p<0.001$) lowered the LOP level. The ethanolic extract of IS and its ethyl acetate fraction significantly increased the GPx, CAT and SOD levels. But the n-butanol fraction failed to show significant increase in GPx, CAT and SOD levels.

Table 6: Changes in kidney LPO, GPx, CAT and SOD of STZ-nicotinamide induced diabetic rats after 21 days of treatment with IS extract and its fractions

Groups	Treatment	LPO (ηm of MDA / mg of protein)	GPx (µMole of the oxidise GSH/min/mg of protein)	Catalase (µm H_2O_2/mg of protein)	SOD (U/mg of protein)
I	Normal control	12.04±1.09	11.03±1.10	15.64±0.96	13.86±1.04
II	Diabetic control	41.03±1.80$^{###}$	4.85±0.83$^{###}$	7.03±0.82$^{###}$	7.16±0.61$^{###}$
III	Glinbenclamide (10 mg/kg)	13.82±1.16***	9.78±1.03**	13.92±0.81***	12.21±0.70**
IV	IS Extract(200mg/kg)	19.22±1.15***	9.32±1.24**	12.67±1.12***	11.98±0.79*
V	IS Extract (100mg/kg)	25.73±0.89***	6.12±0.56	9.37±0.78	9.07±0.81
VI	Ethyl acetate fraction of IS (200mg/kg)	22.27±1.38***	8.61±0.72*	13.04±0.99***	10.72±0.84*
VII	Ethyl acetate fraction of IS(100mg/kg)	28.33±1.40***	5.61±0.38	8.07±0.61	8.82±0.56
VIII	n-Butanol fraction of IS(200mg/kg)	25.78±0.67***	6.86±0.46	9.83±0.80	8.87±0.74
IX	n-Butanol fraction of IS (100mg/kg)	30.53±1.02***	5.08±0.40	7.42±0.67	7.73±0.43

Values are given as mean ± SEM for groups of six animals each
$^{###}$p <0 .001 when compared with the normal control group
***p <0 .001, **p <0 .01 and *p <0 .05 when compared with the diabetic control group

Effect of IS on serum insulin level in STZ-nicotinamide induced diabetic rats

In STZ-nicotinamide induced diabetic rats the serum level of insulin was significantly decreased. Treatment with the ethanolic extract of IS and its ethyl acetate and n-butanol fractions (100 and 200 mg/kg, p.o.) for 21 days increased the serum level of insulin significantly (Figure 3).

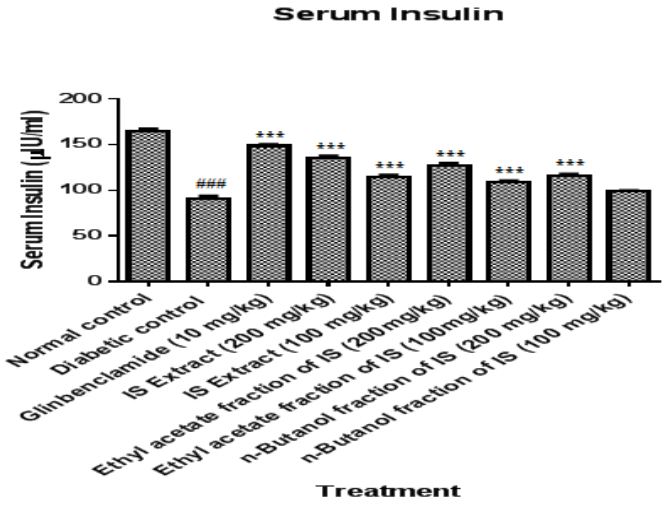

Figure 3: Effect of *Ipomoea staphylina* (IS) extract and its fractions on serum insulin in *STZ-nicotinamide* induced diabetic rats

Effect of ethanolic extract of leaves of IS and its fractions on histopathological analysis of pancreas, liver and kidney

The section of pancreas of normal control rats showed normal pancreatic acini and islets of langerhans. The section of pancreas of STZ-nicotinamide induced diabetic rats showed atrophy, degenerative changes in pancreatic acini, infiltration of inflammatory cells and loss of cells in islets of langerhans. Diabetic rats treated with glibenclamide (10 mg/kg, p.o.) and the extract of leaves of IS and its fractions (200 mg/kg, p.o.) showed almost normal cellular architecture with less atrophy, degenerative changes in pancreatic acini, infiltration of inflammatory cells and less loss of cells in islets of langerhans (Figure 4). The liver of the STZ-nicotinamide induced induced diabetic rats showed hypertrophy of hepatocytes, kupffer cells, hepatocellular necrosis and vacuolization with loss of nuclei. Diabetic rats treated with glibenclamide (10 mg/kg, p.o.) and the extract of leaves of IS and its fractions (200 mg/kg, p.o.) showed an improvement of the hepatoellular architecture with normal nucleus and cytoplasm with less necrosis of hepatocytes (Figure 5).The kidney of normal control rats in STZ-nicotinamide induced diabetic model showed the intact tubules and glomeruli whereas STZ-nicotinamide induced induced diabetic rats was found to cause degenerating tubules with desquamated epithelial cells in the lumen, misshapen tubules, tubulitis and glomerular congestion. Diabetic rats treated with glibenclamide (10 mg/kg, p.o.) and the extract of leaves of IS and its fractions (200 mg/kg, p.o.) showed the almost normal cellular architecture with intact tubules and glomeruli (Figure 6).

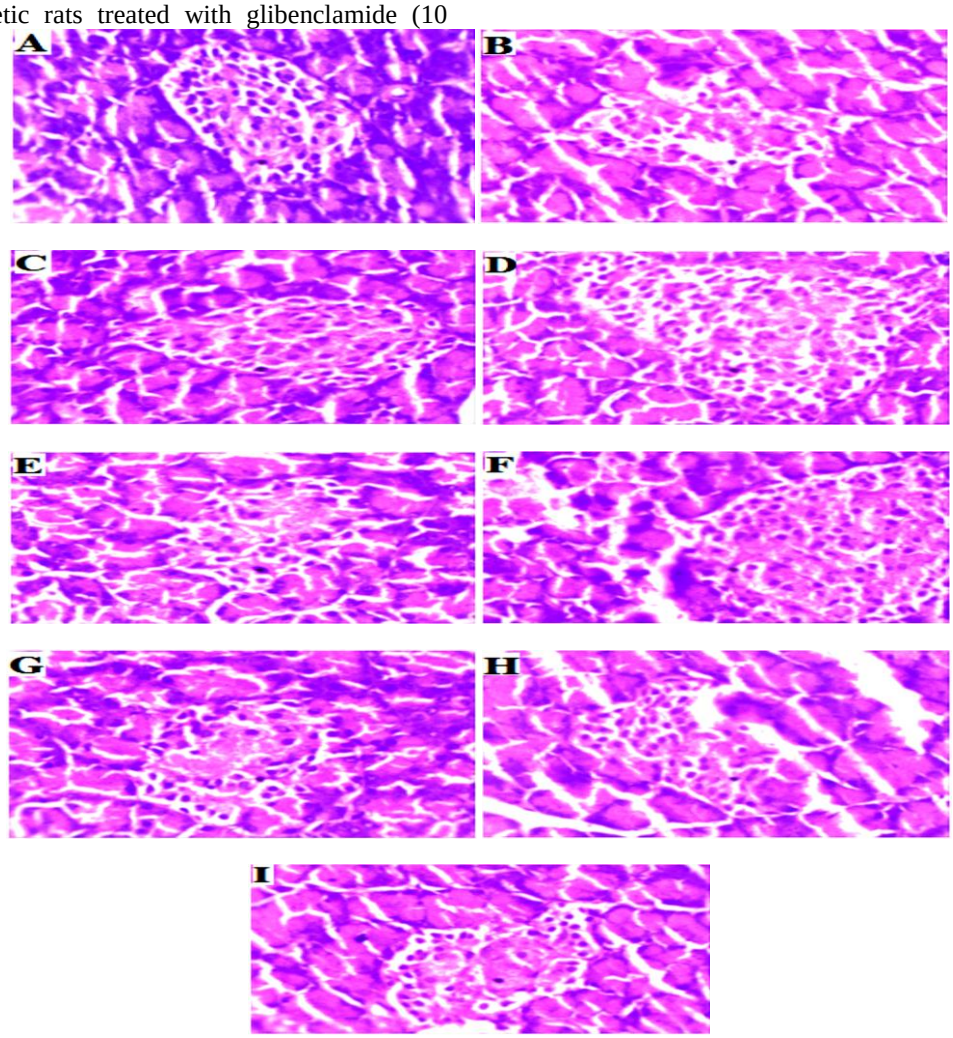

Figure 4: Histology of pancreas in STZ-nicotinamide induced diabetic rats after 21 days of treatment

(A) Normal control, (B) Diabetic control, (C) Diabetic+Glinbenclamide (10 mg/kg, p.o.), (D) Diabetic+IS extract (200 mg/kg, p.o.), (E) Diabetic+IS extract (100 mg/kg, p.o.), (F) Diabetic+Ethyl acetate fraction of IS extract (200 mg/kg, p.o.), (G) Diabetic+Ethyl acetate fraction of IS extract (100 mg/kg, p.o.), (H) Diabetic+n-bulanol fraction of IS extract (200 mg/kg, p.o.) and (I) Diabetic+ n-butanol fraction of IS extract (100 mg/kg, p.o.)

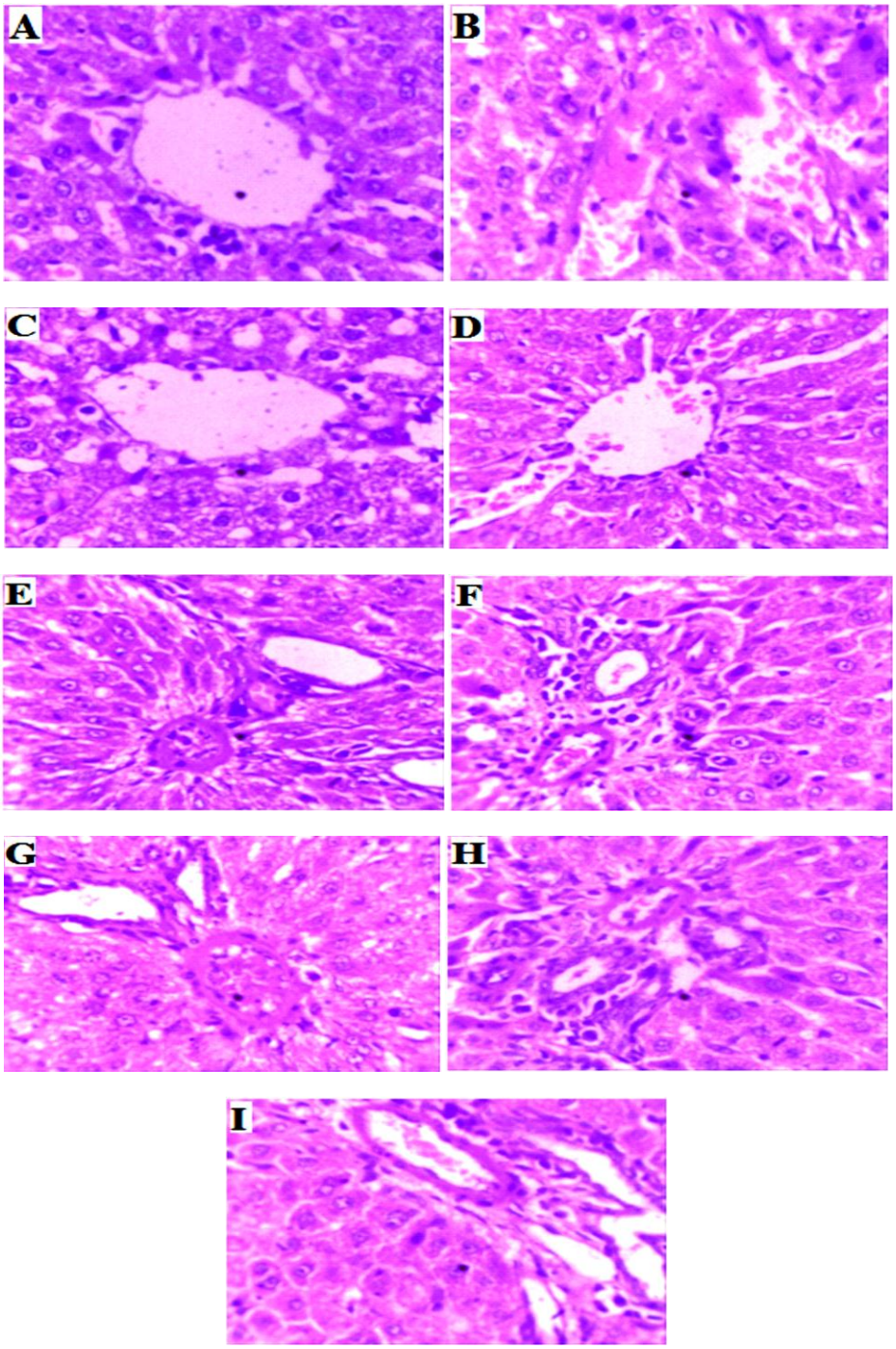

Figure 5: Histology of liver in experimental STZ-nicotinamide induced diabetic rats after 21 days of treatment

(A) Normal control, (B) Diabetic control, (C) Diabetic+Glinbenclamide (10 mg/kg, p.o.), (D) Diabetic+IS extract (200 mg/kg, p.o.), (E) Diabetic+IS extract (100 mg/kg, p.o.), (F) Diabetic+Ethyl acetate fraction of IS extract (200 mg/kg, p.o.), (G) Diabetic+Ethyl acetate fraction of IS extract (100 mg/kg, p.o.), (H) Diabetic+n-bulanol fraction of IS extract (200 mg/kg, p.o.) and (I) Diabetic+ n-butanol fraction of IS extract (100 mg/kg, p.o.)

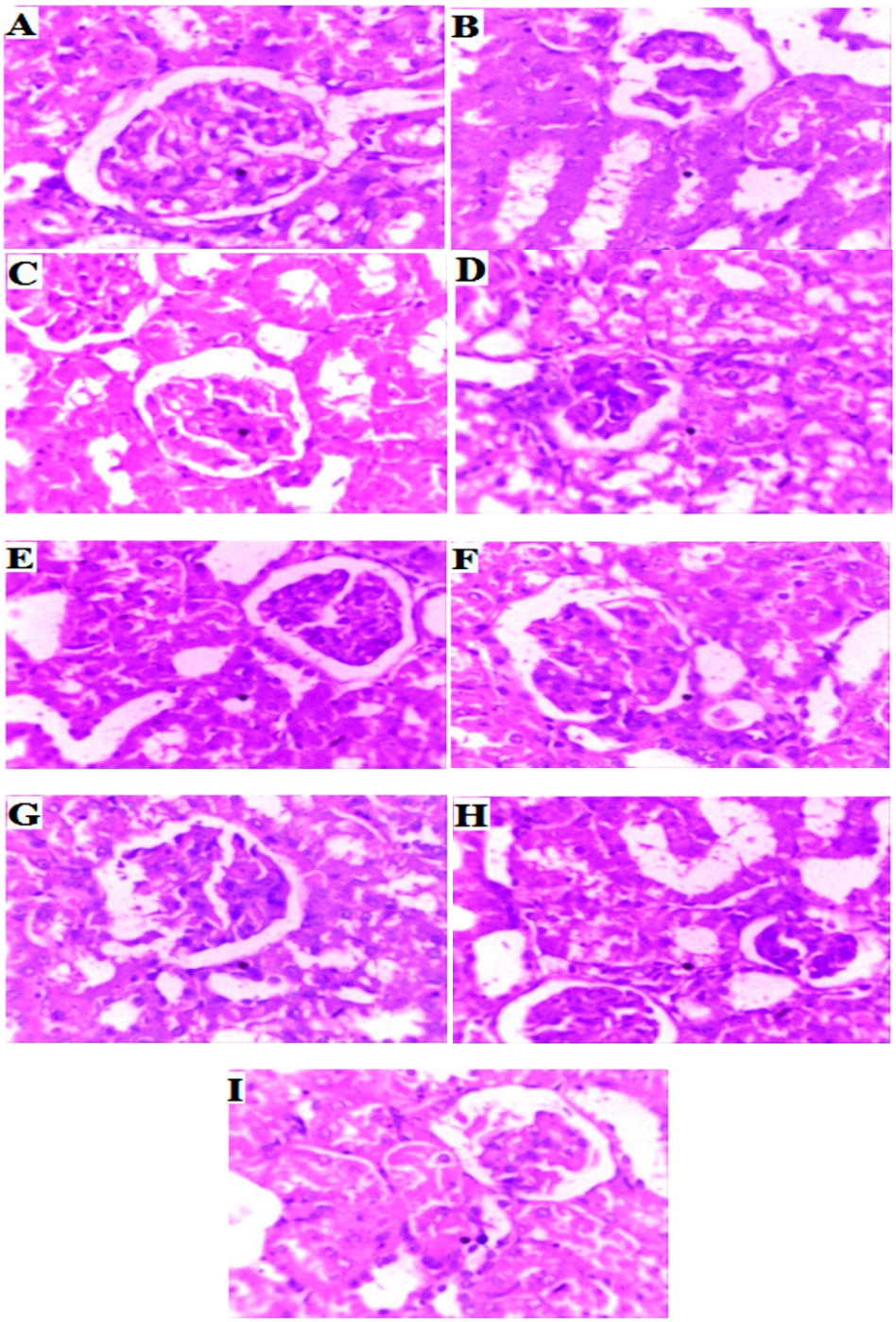

Figure 6: Histology of kidney in STZ-nicotinamide induced diabetic rats after 21 days of treatment

(A) Normal control, (B) Diabetic control, (C) Diabetic+Glinbenclamide (10 mg/kg, p.o.), (D) Diabetic+IS extract (200 mg/kg, p.o.), (E) Diabetic+IS extract (100 mg/kg, p.o.), (F) Diabetic+Ethyl acetate fraction of IS extract (200 mg/kg, p.o.), (G) Diabetic+Ethyl acetate fraction of IS extract (100 mg/kg, p.o.), (H) Diabetic+n-bulanol fraction of IS extract (200 mg/kg, p.o.) and (I) Diabetic+ n-butanol fraction of IS extract (100 mg/kg, p.o.)

Discussion

Streptozotocin (STZ) is 1-methyl-l-nitrosourea attached to the carbon-2 position of glucose that causes β-cell necrosis and induces experimental diabetes in many animal models. It causes DNA strand breaks that induce the activation of poly-ADP-ribose synthetase followed by lethal nicotinamide adenine dinucleotide (NAD) depletion [15]. Nicotinamide dinucleotide (NA) causes activation of the poly ADP ribose synthase to repair the damaged DNA and protecting the decrease in the level of NAD and proinsulin thereby partially reversing the inhibition of insulin secretion to prevent the aggravation of experimental diabetes [16, 17]. This condition shows a number of features which are similar with type 2 diabetic mellitus (T2DM). Hence, based on this point of view, the hypoglycaemic activity of ethanolic extracts IS and its fractions were carried out on glucose-loaded hyperglycemic and streptozotocin-nicotinamide induced type 2 diabetic rats. In the present study, the IS extracts and its fractions showed significant decrease in blood glucose various intervals in oral glucose tolerance test (OGTT). In streptozotocin-nicotinamide induced type 2 diabetic mellitus, oral administration of ethanolic extract of IS and its fractions for 21 days showed a significant reduction in blood glucose level. The possible mechanim by which the extract and its fractions reduced the blood glucose level in diabetic rats might be due to stimulation of surviving β-cells leading to increase in insulin secretion. Decrease in body weight in streptozotocin-nicotinamide induced type 2 diabetic rats was observed during the study. This notable decrease in the body weight in diabetic rats might be because of protein wasting or degradation of structural proteins. The loss of protein is probably due to the unavailability of carbohydrates for energy metabolism [18]. Diabetic rats treated with ethanolic extract of IS and its fractions showed an improvement in body weight as compared to the diabetic control rats which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis.In diabetes mellitus a reflective changes in the serum cholestrol, triglycerides and lipoprotein occur which increase the risk of coronary heart disease [19]. Diabetic rats were treated with ethanolic extract of IS and its fractions showed reduction in the level of total cholesterol, triglycerides, LDL-C and VLDL-C and improved the level of HDL-C. From this study, we can conclusively state that the ethanolic extract of IS and its fractions could modulate blood lipid abnormalities. It is hypothesized that increase in SGOT, SGPT and ALP are predictors of diabetes. Increased activities of SGOT, SGPT and ALP are a common sign of liver disease. In streptozotocin-nicotinamide induced type 2 diabetic, rats increase in serum SGOT, SGPT and ALP level may be due to the leakage of these enzymes from liver cytosol into blood stream as a result of the hepatotoxic effect of STZ (20). Treatment with ethanolic extract of IS and its fractions decreased the levels of SGOT, SGPT and ALP in diabetic animals, which indicates that the extract tends to prevent liver damage in diabetes by maintaining integrity of plasma membrane, thereby suppressing the leakage of enzymes through membrane.Insulin deprivation in diabetic state causes a profound increase in protein catabolism. In this present study, fall in plasma total protein and rise in serum urea, creatinine and blood urea nitrogen (BUN) levels were observed in diabetic rats. Urea and creatinine in the serum are significant markers to detect the renal dysfunction, might be due to increased protein catabolism in the body. Accumulation of urea nitrogen in experimental diabetes may due to the enhanced breakdown of both liver and plasma proteins [20]. The decrease in serum urea and creatinine levels on treatment with ethanolic extract of IS and its fractions indicated that the extract and its fractions prevented the progression of renal damage in diabetic rats.Generation of free radicals in diabetes mellitus reacts with lipids causing lipid peroxidation, resulting in the release of products such as malondialdehyde, hydroperoxide and hydroxyl radicals. The oxidative stress in diabetes decreases the antioxidant status. SOD, CAT and GPx are enzymatic antioxidants plays an important role in protecting cells from being exposed to oxidative damage by direct elimination of reactive oxygen species (ROS). They reduce superoxide anion ($O2^{-}$) and hydrogen peroxide generation which further reduce the generation of hydroxyl radicals and thus, reduce the initiation and propagation of lipid peroxidation and decrease the LPO level [21]. Treatment with ethanolic extract of IS and its fractions significantly reduced levels of lipid peroxidation in liver and kidney and increased the activity of SOD, CAT and GPx in both liver and kidney. STZ causes diabetes by the rapid depletion of β-cells and thereby brings about a

reduction in insulin release. Reduced (or absent) secretion of insulin, often coupled with reduced sensitivity to its action causes diabetes mellitus [22]. In streptozotocin-nicotinamide induced type 2 diabetic rats decrease in serum insulin was observed. Treatment with ethanolic extract of IS and its fractions significantly improved the serum level of insulin. The histology study of pancreas showed atrophy, degenerative changes in pancreatic acini, infiltration of inflammatory cells and loss of cells in islets of langerhans in diabetic rats. Teatment with ethanolic extract of IS and its fractions reduced atrophy, degenerative changes in pancreatic acini, infiltration of inflammatory cells and loss of cells in islets of langerhans. Histological studies of liver of the diabetic rats revealed that the ethanolic extract of IS and its fractions significantly reduced hypertrophy of hepatocytes, hepatocellular necrosis and vacuolization with loss of nuclei. The kidney histological exposed improved architecture with intact tubules and glomeruli in the diabetic rats treated with ethanolic extract of IS its fractions. It has been reported in our previous study that *Ipomoea staphylina* leaves are rich in phenolic and flavonoid content [23]. Phenolic compounds and flavonoids are known for hypoglycaemic and antioxidant properties [24]. Thus, the antidiabetic potential of the *Ipomoea staphylina* leaves may be due to the presence of phenolic compounds and flavonoids.

Conclusion

This study shows that the ethanolic extract of IS leaves and its fractions has beneficial effects on STZ-nicotinamde induced type-II diabetes in rats. The extract and its fractions improved the blood glucose level and restored the altered total cholesterol, triglycerides, serum enzymes (SGOT, SGPT and ALP), total protein levels. It also enhanced the activities of endogenous antioxidant enzymes SOD, CAT and GPx in the liver and kidneys. The ethanolic extract of IS leaves and its ethyl acetate fraction have shown more significant effect on STZ induced diabetic in mice than the n-butanol fraction. Thus, further studies are needed to explore the antidiabetic mechanisms of ethanolic extract and its ethyl acetate fraction of IS leaves.

References

1. Umar A, Ahmed QU, Muhammad BY, Dogarai BB, Soad SZ. Antihyperglycemic activity of the leaves of Tetracera scandens Linn. Merr. (Dilleniaceae) in alloxan induced diabetic rats. J Ethnopharmacol 2010; 1:140-45
2. Gerich JE.Clinical significance, pathogenesis, and management of postprandial hyperglycemia. Arch Intern Med 2003; 163: 1306–1316
3. Kecskemeti V, Bagi Z, Pacher P, Posa I, Kocsis E, Koltai MZ. New trends in the development of oral antidiabetic drugs. Cur Med Chem 2002; 9: 53–71
4. Rahman AU, Zaman K. Medicinal plants with hypoglycaemic activity. J Ethnopharmacol 1989; 26: 1-55
5. Veerapur VP, Prabhakar KR, Kandadi MR, Srinivasan KK, Unnikrishnan MK. Antidiabetic effect of Dodonaea viscosa aerial parts in high fat diet and low dose streptozotocin-induced type 2 diabetic rats: A mechanistic approach. Pharm Biol 2010;48:1137-48
6. Reddy DP, Kota R, Renuka S, Anarthe SJ, Raghavendra NM. Isolation, characterization of phytoconstituents and pharmacological screening of Ipomoea staphylina. Asian J Pharm Clin Res 6(1);2013:30-33.
7. Firdous SM, Koneri R. In vivo and in vitro antiinflammatory activity of leaves of Ipomoea staphylina. Int J Pharm Pharm Sci 2012;4(5):339-43
8. Firdous SM, Koneri R. In vivo and in vitro antiinflammatory activity of leaves of Ipomoea staphylina. Int J Pharm Pharm Sci 2012;4(5):339-43
9. Kokate CK.Preliminary phytochemical screening, practical pharmacognosy. 1st ed. New Delhi: Vallabh Prakashan 1986; p. 111
10. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economics co-operation, development, Paris, June, 2000
11. Shirwaikar A, Rajendran K. Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin–nicotinamide induced type-II diabetes mellitus. J Ethnopharmacol 2006;112:1-6
12. Palsamy P, Subramanian S. Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocin-nicotinamide induced experimental diabetic rats. Biomedicine & Pharmacother, 2008; 62: 598-605
13. Shirwaikar A, Rajendran K, Barik R. Effect of aqueous bark extract of *Garuga pinnata* Roxb. In streptozotocin-nicotinamide induced type-II diabetes mellitus. J Ethnopharmacol, 2006; 107: 285–290.
14. Firdous SM, Sravanthi K, Debnath R, Neeraja K. Protective effect of ethanolic extract and its ethylacetate and n-butanol fractions of Sechium

edule fruits against carbon tetrachloride induced hepatic injury in rats. Int J Pharm Pharm Sci 2011;4(1):354-5

15. Vira´g L, Szabo´ C. The therapeutic potential of poly (ADP-ribose) polymerase inhibitors. Pharmacol Rev, 2002; 54: 375-429

16. Anderson T, Schein PS, McMenamin MG, Cooney DA. Streptozotocin diabetes: correlation with extent of depression of pancreatic islet nicotinamide adenine dinucleotide. J Clin Invest, 1974; 54: 672-677

17. Shima K, Zhu M, Kuwajima M. A role of nicotinamide-induced increase in pancreatic beta-cell mass on blood glucose control after discontinuation of the treatment in partially pancreatectomized OLETF rats. Diabetes Res Clin Pract, 1998; 41: 1-8

18. Brodsky IG. Nutritional effects of dietary protein restriction in insulindependent diabetes mellitus. J Nutr, 1998; 128: 337S-3379S

19. Betteridge J. Lipid disorders in diabetes mellitus. In: Pickup JC, Williams G. Text book of diabetes. 2nd ed. Blackwell Science, London, 1997: 55.1-55.31 (s)

20. El-Demerdash FM, Yousef MI, El-Naga NI. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol, 2005; 43: 57-63

21. L Pari, R Saravanan. Beneficial effect of succinic acid monoethyl ester on erythrocyte membrane bound enzymes and antioxidant status in streptozotocin–nicotinamide induced type 2 diabetes. Chemico-Biol Interact, 2007; 169: 15–24.

22. Insulin [Internet], 2001 Aug 1 [Update 2014 Sep 4; cited 2014 Sep 5]. Available from: http://en.wikipedia.org/wiki/Insulin

23. Bag AK, Firdous Mumtaz SM. Hepatoprotective and nephroprotective activity of hydroalcoholic extract of *Ipomoea staphylina* leaves. Bangladesh J Pharmacol, 2013; 8(3): 263-268

24. Babu PV, Liu D, Gilbert ER. Recent advances in understanding the antidiabetic actions of dietary flavonoids. J Nutr Biochem 2013; 24(11): 1777-1789.

Source of Support: Nil
Conflict of Interest: None