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ANTIDIABETIC EFFECT OF *IPOMOEA STAPHYLINA* LEAVES IN ALLOXAN INDUCED RATS

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ABSTRACT

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The aim of the present study was to evaluate the antidiabetic activity of *Ipomoea staphylina* (IS) leaves in alloxan induce 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 alloxan 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 and total protein 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. Histological analysis showed improvement in the cellular architecture of pancreas, liver and kidney.

Keywords: Antidiabetic, *Ipomoea staphylina* (IS), alloxan, body weight, blood glucose, total cholesterol, triglycerides, creatinine

INTRODUCTION

Diabetes Mellitus (DM) is one of the most prevalent metabolic disorders characterized by increase blood sugar level and alterations in the metabolism of

carbohydrate, fat and protein due to inappropriate secretion of insulin or insulin resistance or both [1]. 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 [2].

Antidiabetic drugs are available for long term therapy. Those drugs are 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 [3].

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 [4] purgative, dyspepsia, anthelmintic, bronchitis. A literature review reveals anti-inflammatory activity, 5-lipoxygenase, α-glucosidase and [4, 5] α-amylase inhibitory activity of *Ipomoea staphylina*. Bioactive chemical constituents reported from the leaves of Ipomoea staphylina [4] include Sitosteryl-3-O-β-D-glucoside and chiro deoxy inositol. Hence, the present study was undertaken to evaluate the antidiabetic effect of *Ipomoea staphylina* leaves in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals

Alloxan was procured from Loba Chemie, India. Glibenclamide were obtained from Aventis Pharma, India. Blood glucose, total cholesterol, triglycerides, SGOT, SGPT, ALP, total protein, serum urea and creatinine kits were obtained from Span Diagnostics, India. All other chemicals were commercial products of analytical reagent grade.

Collection of plant material and extraction

Leaves of *Ipomoea staphylina* (IS) plant were collected from forest area of Karnataka near to Bangalore and the 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 [5].

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 sanitized 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 [6].

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 [7].

Induction of diabetes and experimental design

Diabetes was induced in overnight fasted rats by single intraperitoneal injection of 150 mg/kg of alloxan monohydrate, was prepared in 0.9% w/v of NaCl. To prevent fatal hypoglycemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 hr followed by supply of 5% glucose solution bottles in their cages for next 24 h. After 48 h of alloxan administration, blood was collected and plasma glucose levels were determined. The animals confirmed as diabetic by the elevated plasma glucose levels (>180 mg/dl) were used 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) [8].

Treatment was given orally using an intragastric tube once daily for 21 days, continuously. On 21st day, the animals were fasted for 12 h and blood was drawn from retro orbital vein under mild ether anaesthesia for various biochemical estimations. The animals were sacrificed by cervical decapitation. Pancreas, 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

The estimation of blood glucose,

total cholesterol, total triglyceride, LDL-c, VLDL-c and HDL-c SGOT, SGPT, ALP, total protein, serum urea, and creatinine was done by using standard Enzymatic kits (Span Diagnostics, India).

Histopathological studies

Pancreas, liver and kidneys were instantly dissected out, excised and rinsed in ice-cold 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.

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 IS extract and its fractions on oral glucose tolerance test (OGTT) in rats

After glucose load, 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 compared to normal control rats (Figure 1).

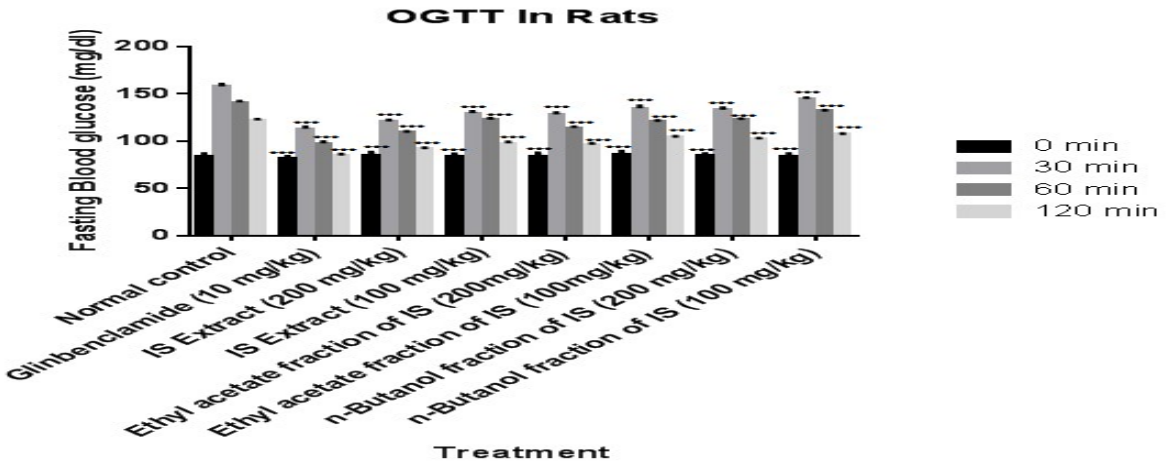

Figure 1: Effect of *Ipomoea staphylina* (IS) extract and its fractions on oral glucose tolerance test (OGTT) in rats

Effect IS extract and its fractions on blood glucose level in alloxan-induced diabetic rats

Table 1shows the effect of ethanolic extract of IS its ethyl acetate and n-butanol fractions on blood glucose levels of diabetic animals. After the daily treatment for 21 days showed significant ($p<0.001$) decrease 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 of glibenclamide as compared to diabetic control group.

Table 1: Effect of *Ipomoea staphylina* (IS) extract and its fractions on blood glucose level in alloxan-induced diabetic rats

Groups	Treatment	Blood glucose (mg/dl)			
		0 day	7th day	14th day	21st day
I.	Normal control	97.33± 2.21	93.81± 3.67	94.90± 2.20	94.19± 1.83
II.	Diabetic control	199.69± 4.46###	256.63± 3.17###	303.79± 3.26###	282.44± 3.38###
III.	Glinbenclamide (10 mg/kg)	198.98± 3.92	157.48± 2.43***	144.09± 1.52***	125.69± 1.91***
IV.	IS Extract (200 mg/kg)	195.77± 5.44	165.41± 1.67***	154.53± 1.41***	142.16± 3.00***
V.	IS Extract (100 mg/kg)	200.52± 3.52	213.31± 1.53***	199.48± 2.15***	185.83± 3.05***
VI.	Ethyl acetate fraction of IS (200 mg/kg)	196.15± 4.64	184.96± 1.53***	171.59± 2.083***	157.22± 2.79***
VII.	Ethyl acetate fraction of IS (100 mg/kg)	194.59± 4.67	220.07± 3.31***	202.11± 1.31***	194.97± 1.55***
VIII.	n-Butanol fraction of IS (200 mg/kg)	196.66± 5.56	197.83± 2.65***	186.76± 1.38***	170.16± 1.87***
IX.	n-Butanol fraction of IS (100 mg/kg)	197.30± 4.27	228.32± 2.81***	207.50± 2.27***	187.25± 3.01***

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 body weight in alloxan-induced diabetic rats

Average body weights of different animal groups at various intervals are shown in table 2. Alloxan produced significant loss in body weight as compared to normal rats 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

improvement in body weight compared to diabetic control at 21st days.

Table 2: Effect of *Ipomoea staphylina* (IS) extract and its fractions on body weight in alloxan-induced diabetic rats

Groups	Treatment	Body Weight (gm)			
		0 day	7th day	14th day	21st day
I.	Normal control	152.65± 1.21	160.84± 1.21	173.83± 0.84	184.00± 0.85
II.	Diabetic control	151.69± 1.03	123.75± 1.65###	108.80± 1.08###	106.50± 1.13###
III.	Glinbenclamide (10 mg/kg)	152.14± 1.33	135.39± 1.33***	141.39± 1.41***	150.59± 1.79***
IV.	IS Extract (200mg/kg)	150.85± 1.34	131.59± 0.90**	136.07± 1.11***	144.33± 1.38***
V.	IS Extract (100mg/kg)	151.98± 1.31	128.20± 1.46	130.58± 1.38***	138.77± 2.04***
VI.	Ethyl acetate fraction of IS (200mg/kg)	153.46± 1.13	130.07± 1.23*	135.28± 0.95***	143.12± 1.82***
VII.	Ethyl acetate fraction of IS (100mg/kg)	152.87± 1.70	128.82± 0.84	132.08± 1.53***	139.20± 1.11***
VIII.	n-Butanol fraction of IS (200mg/kg)	154.78± 1.15	129.67± 1.24*	133.39± 1.43***	139.80± 1.98***
IX.	n-Butanol fraction of IS (100mg/kg)	153.06± 2.00	126.93± 1.05	131.59± 1.55***	136.84± 1.33***

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 lipid profile in alloxan-induced diabetic rats

Alloxan 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) showed significant reduction in TC, TG, LDL-C, VLDL-C and elevation of HDL-C levels compared to diabetic control (Table 3).

Table 3: Effect of *Ipomoea staphylina* (IS) extract and its fractions on lipid profile in alloxan-induced diabetic rats

Groups	Treatment	Lipid parameters (mg/dl)				
		TC	TG	HDL -C	LDL-C	VLDL-C
I.	Normal control	107.33± 1.66	120.66± 1.68	47.83± 1.70	35.30± 2.82	24.13± 0.33
II.	Diabetic control	205.66± 1.82###	179.23± 1.22##	29.16±1.66# ##	137.36± 2.68###	38.13 ± 2.98###
III.	Glinbenclamide (10 mg/kg)	123.16± 2.35***	135.14± 3.58***	45.04± 1.81***	51.12± 2.95***	27.00± 0.71***
IV.	IS Extract (200mg/kg)	124.50± 1.64***	138.12± 2.03***	42.28± 0.93***	54.90± 1.33***	27.6 ± 0.39***
V.	IS Extract (100mg/kg)	159.33± 2.74***	161.50± 1.47***	33.66± 1.02	93.36± 3.39***	32.30± 0.29*
VI.	Ethyl acetate fraction of IS (200mg/kg)	135.21± 2.30***	148.38± 1.91***	37.13± 2.08*	68.40±4.15***	29.67± 0.38***
VII.	Ethyl acetate fraction of IS (100mg/kg)	169.18± 1.38***	160.31± 2.56***	35.11± 1.67	102.00± 2.59***	32.06± 0.51**
VIII.	n-Butanol fraction of IS	152.00± 1.86***	157.00± 2.17***	34.30± 1.48	86.30± 3.39***	31.4± 0.43**

	(200mg/kg)					
IX.	n-Butanol fraction of IS (100mg/kg)	178.50± 2.61***	169.33± 2.61	30.75± 0.79	115.50± 1.95***	33.80± 0.47

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 alloxan-induced diabetic rats

In alloxan 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 when compared to diabetic rats (Table 4).

Table 4: Effect of *Ipomoea staphylina* (IS) extract and its fractions on SGOT, SGPT and ALP level in alloxan-induced diabetic rats

Groups	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (mg/dl)
I.	Normal control	77.05±4.00	46.94±2.93	124.89±2.73
II.	Diabetic control	129.33±3.16###	99.65±2.66###	224.74±4.05###
III.	Glinbenclamide (10mg/kg)	83.18±2.85***	67.16±2.65***	164.89±2.74***
IV.	IS Extract (200mg/kg)	90.63±1.80***	69.03±1.93***	162.33±2.93***
V.	IS Extract (100mg/kg)	115.97±3.16	86.32±2.14**	205.94±2.08**
VI.	Ethyl acetate fraction of IS (200mg/kg)	96.60±1.07***	70.91±1.86***	174.86±3.09***
VII.	Ethyl acetate fraction of IS (100mg/kg)	123.97±3.09	89.78±2.44	209.67±2.50
VIII.	n-Butanol fraction of IS (200mg/kg)	101.75±2.02***	78.22±3.16***	194.57±2.51***
IX.	n-Butanol fraction of IS (100mg/kg)	125.96±4.96	91.11±1.53	214.34±2.75

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 alloxan-induced diabetic rats

The effect of ethanolic extract of IS and its ethyl acetate and n-butanol fraction (100 and 200 mg/kg, p.o.) on serum creatinine, blood urea, blood urea nitrogen (BUN) and total protein level in alloxan induced diabetic rats is shown in Table 5. 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 reduction in the levels of serum creatinine, blood urea and blood urea nitrogen, 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 5: Effect of *Ipomoea staphylina* (IS) extract and its fractions on serum creatinine, blood urea, blood urea nitrogen, total protein and liver glycogen level in alloxan-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	0.45± 0.023	26.90± 1.95	12.56± 0.91	7.67± 0.20
II	Diabetic control	1.96±	53.14±	24.31±	3.37±

		0.063$^{###}$	**1.99$^{###}$**	**0.93$^{###}$**	**0.111$^{###}$**
III	**Glinbenclamide (10 mg/kg)**	**0.69± 0.022*****	**36.05± 1.95*****	**16.84± 0.90*****	**6.48± 0.13*****
IV	**IS Extract (200 mg/kg)**	**0.80± 0.140*****	**38.31± 2.01*****	**17.89± 0.94*****	**5.63± 0.14*****
V	**IS Extract (100 mg/kg)**	**1.56± 0.026*****	**44.99± 1.22**	**21.00± 0.57**	**4.44± 0.13*****
VI	**Ethyl acetate fraction of IS (200 mg/kg)**	**1.09± 0.022*****	**40.18± 2.48*****	**18.76± 1.16*****	**5.19± 0.10*****
VII	**Ethyl acetate fraction of IS (100 mg/kg)**	**1.73± 0.039**	**48.86± 2.01**	**22.98± 1.05**	**4.46± 0.13*****
VIII	**n-Butanol fraction of IS (200 mg/kg)**	**1.44± 0.050*****	**42.05± 2.15****	**19.36± 1.00****	**4.54± 0.17*****
IX	**n-Butanol fraction of IS (100 mg/kg)**	**1.88± 0.030**	**51.53± 1.45**	**24.06± 0.68**	**4.08± 0.11***

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 histopathology of pancreas, liver and kidney

Section of pancreas from normal control rats showed pancreatic acini with normal morphology. The section of pancreas of diabetic control showed atrophy and degenerative changes in pancreatic acini with 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 and degenerative changes in pancreatic acini with infiltration of inflammatory cells and loss of cells in islets of langerhans (Figure 2).

The liver of the alloxan 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 3).

The kidney of normal control rats in alloxan induced diabetic model showed the intact tubules and glomeruli whereas alloxan treated group was found to cause degenerating tubules with desquamated epithelial cells in the lumen, distorted tubular shape, 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 4).

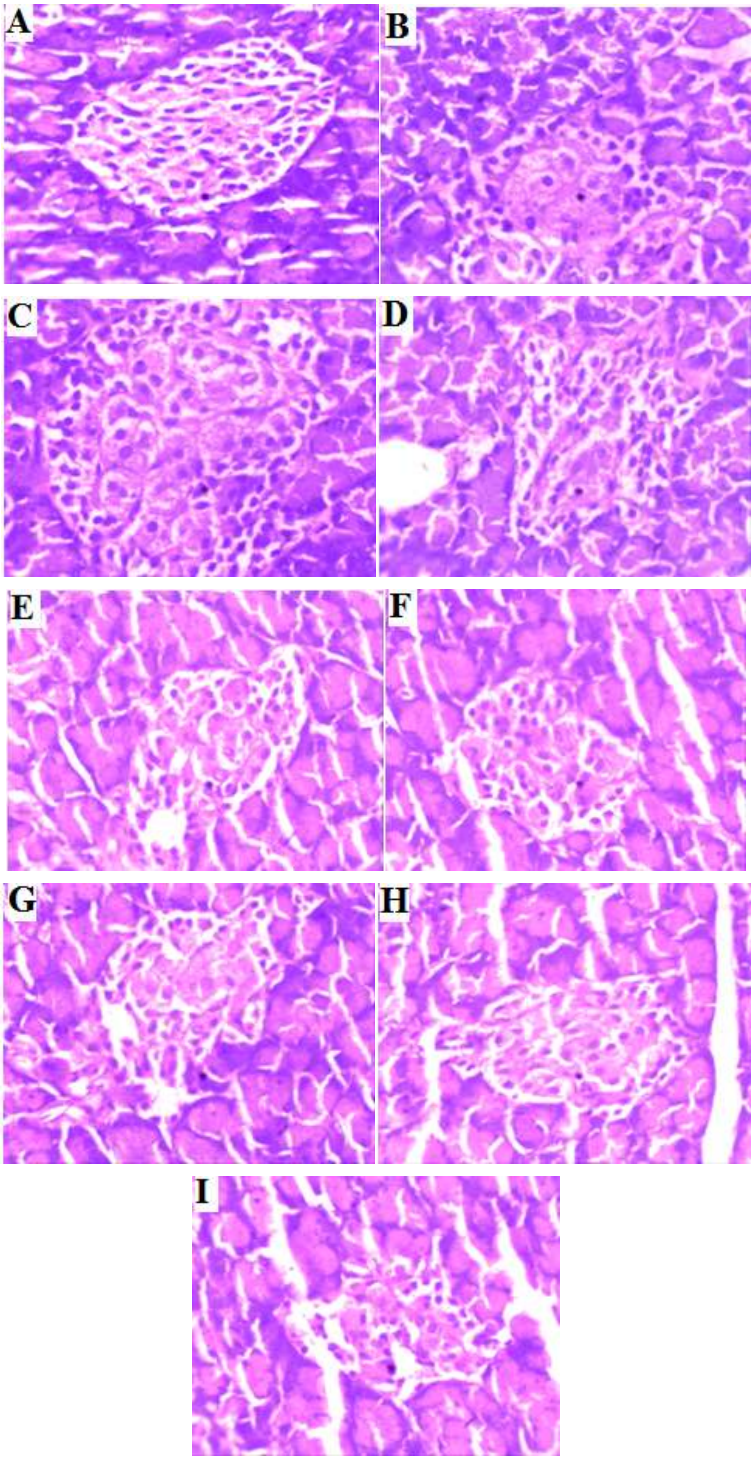

Figure 2: Histology of pancreas in alloxan-induced diabetic rats after 21 days of treatment (A) Normal control, (B) Diabetic control, (C) Diabetic+Glinbenclamide (10 mg/kg, p.o.), (D) Diabetic+IS extract (200 mg/kg, p.o.), (E) Diabetic+IS extract (100 mg/kg, p.o.), (F) Diabetic+Ethyl acetate fraction 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.)

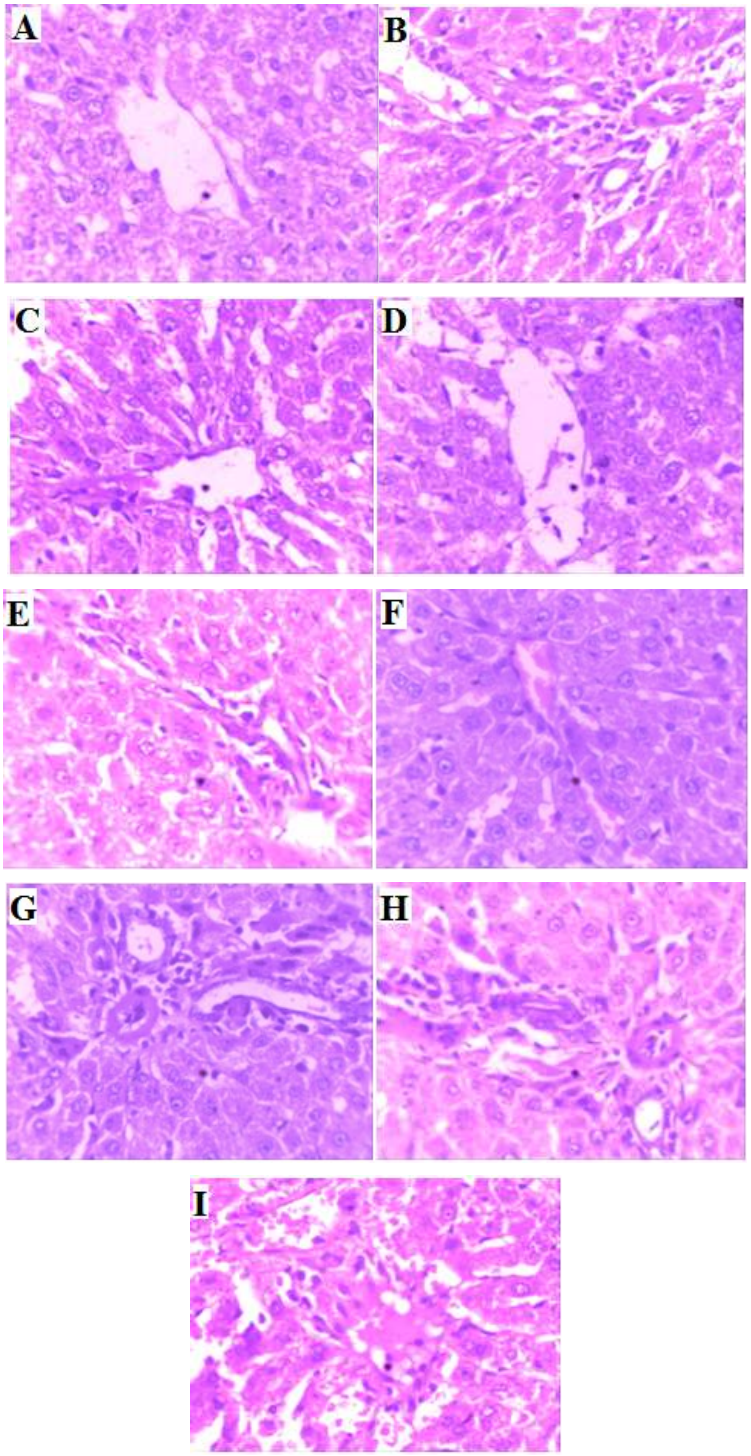

Figure 3: Histology of liver in alloxan-induced diabetic rats after 21 days of treatment
(A) Normal control, (B) Diabetic control, (C) Diabetic+Glinbenclamide (10 mg/kg, p.o.), (D) Diabetic+IS extract (200 mg/kg, p.o.), (E) Diabetic+IS extract (100 mg/kg, p.o.), (F) Diabetic+Ethyl acetate fraction of 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.)

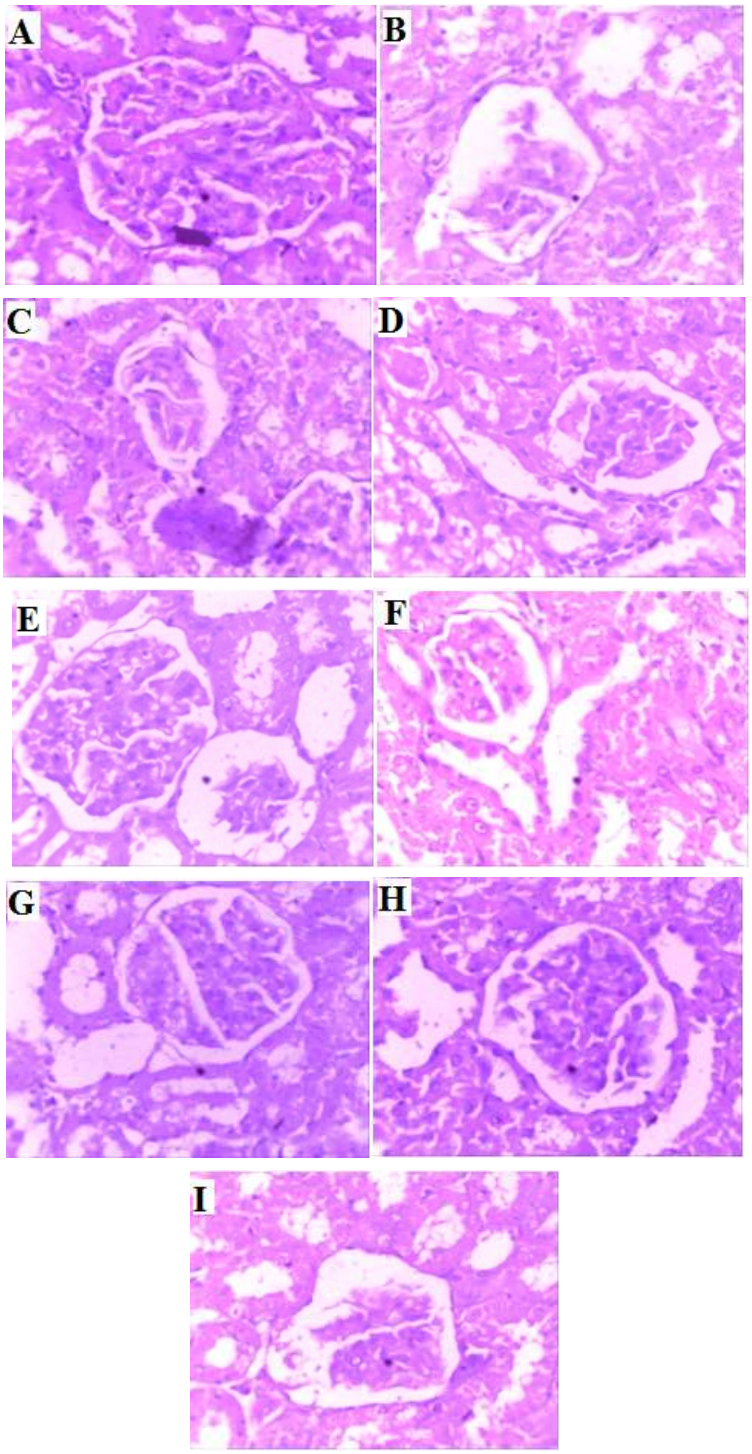

Figure 4: Histology of kidney in alloxan-induced diabetic rats after 21 days of treatment (A) Normal control, (B) Diabetic control, (C) Diabetic+Glinbenclamide (10 mg/kg, p.o.), (D) Diabetic+IS extract (200 mg/kg, p.o.), (E) Diabetic+IS extract (100 mg/kg, p.o.), (F) Diabetic+Ethyl acetate fraction of 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

Alloxan is an oxygenated pyrimidine derivative betacytotoxin and is known to induce diabetes in a wide variety of animal species through generation of free radicals which cause DNA

fragmentation of pancreatic cells. Thus, damage the pancreatic β-cells resulting in a decrease in endogenous insulin release, which paves the ways for Diabetes mellitus with the decreased utilization of glucose by the tissue [9, 10]. Based on this point of view, the hypoglycaemic activity of ethanolic extracts IS and its fractions were carried out on glucose-loaded hyperglycemic and alloxan induced 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 alloxan induced hyperglycemia, oral administration of ethanolic extract of IS and its fractions for 21 days showed a significant reduction in blood glucose level. The possible mechanism 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 alloxan induced diabetes is common. This may be due to increased muscle wasting and due to loss of tissue proteins or degradation of structural proteins [11]. 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.

It is well known that in uncontrolled diabetes mellitus is associated with profound alterations in the plasma cholestrol, triglycerides and lipoprotein and with an increased risk of coronary heart disease [11]. High level of total cholesterol and triglycerides is one of the major factor for coronary heart disease and arthrosclerosis [12]. 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 increased 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.

Elevated activities of SGOT, SGPT and ALP are a common sign of liver disease, and are more frequently observed among people with diabetes, than in the general population [13]. In this present study, marked elevations in SGOT, SGPT and ALP in diabetic control rats signify the hepatocellular damage [11]. In alloxan induced diabetic the liver hepatocellular necrosis occurs, cause an increase in the activities of SGOT, SGPT and ALP in plasma. 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.

Decrease in plasma total protein and elevation in serum urea, creatinine and blood urea nitrogen (BUN) levels were observed in alloxan induced diabetic rats. Increased levels of urea and creatinine in the serum, which are considered significant markers of renal dysfunction, might be due to increased protein breakdown and renal dysfunction. Accumulation of urea nitrogen in experimental diabetes may due to the enhanced breakdown of both liver and plasma proteins [14]. 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.

The histology study of pancreas showed that diabetic rats treated with ethanolic extract of IS and its fractions reduced pancreatic acini, infiltration of inflammatory cells, islet necrosis and degenerated islet cell mass loss. Histological studies of liver of the diabetic rats revealed that the ethanolic extract of IS and its fractions significantly reduced hypertrophy of hepatocytes and hepatocellular necrosis. 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 [15]. Phenolic compounds and flavonoids are known for hypoglycaemic and antioxidant properties [16]. 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 alloxan 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, serum creatinine, and urea and BUN levels. The ethanolic extract of IS leaves and its ethyl acetate fraction have shown more significant effect on diabetic rats than n-butanol fraction. Thus, further studies are require to explore the antidiabetic mechanisms of ethanolic extract and its ethyl acetate fraction of IS leaves.

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