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Research Article

Antidiabetic and Antioxidant Activities of *Ipomoea staphylina* Leaves in Streptozotocin (STZ) induced Diabetic Mice

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Abstract

The aim of the present study was to evaluate the antidiabetic and antioxidant effects of *Ipomoea staphylina* (IS) leaves in Streptozotocin (STZ) induced diabetic mice. 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 induced diabetic mice. The effects of extract and its fractions on 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 mice were analyzed. The IS extract and its fractions significantly reduced the blood glucose level in glucose-loaded mice. After treatment with IS extract and its fractions (100 and 200 mg/kg) for 28 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, total protein, and liver glycogen levels in treated diabetic mice. 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 liver and kidney cellular architecture.

Keywords: Antidiabetic, Antioxidant, *Ipomoea staphylina* (IS), Streptozotocin (STZ), Body weight, Creatinine

1.Introduction

Diabetes Mellitus (DM) is one of the most prevalent metabolic disorders characterized by increased blood glucose level and improper primary metabolism resulting from the defects in insulin secretion, insulin action, or both. It is one of the most common health problem worldwide, and the prevalence of this disease is rapidly increasing, leading to micrvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications.[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] Reactive oxygen species (ROS) produced during hyperglycemia, which plays a major role in complications of diabetes. Antioxidants from the natural sources act as free radical scavengers and lower the risk of diabetic complications.[3, 4] Though 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, 6]

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 purgative, dyspepsia, anthelmintic, bronchitis.[7] A literature review reveals anti-inflammatory activity, 5-lipoxygenase, α-glucosidase and α-amylase inhibitory activity of *Ipomoea staphylina*.[7,8] Bioactive chemical constituents reported from the leaves of *Ipomoea staphylina* include Sitosteryl-3-O-β-D-glucoside and chiro deoxy inositol.[7] Hence, the present study evaluates the antidiabetic and antioxidant activities of *Ipomoea staphylina* leaves on streptozotocin- induced diabetic mice.

2. Materials and methods

2.1. 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.

2.2. Collection of plant material and extraction

Leaves of *Ipomoea staphylina* 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 *Ipomoea staphylina* 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]

2.3. Preliminary phytochemical screening

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

2.4. Animals

In-breed Swiss albino mice weighing 22-30g 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).

2.5. 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 *Ipomoea staphylina* 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]

2.6. Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test was performed in overnight fasted normal mice to assess the glucose tolerance. Mice were divided into eight groups of six each.

Group I: Normal control mice were treated with vehicle alone; Group II: Mice were treated with treated with glibenclamide (10 mg/kg); Group III: Mice were treated with IS Extract (200 mg/kg); Group IV: Mice were treated with IS Extract (100 mg/kg); Group V: Mice were treated with ethyl acetate fraction of IS Extract (200 mg/kg); Group VI: Mice were treated with ethyl acetate fraction of IS Extract (100 mg/kg); Group VII: Mice were treated with n-butanol fraction of IS Extract (200 mg/kg); Group VIII: Mice were treated with n-butanol fraction of IS Extract (100 mg/kg).

Overnight fasted mice were fed glucose (2g/kg) 30 min after the administration of extract and its fractions and and glibenclamide blood was collected at 0, 30, 60 and 120min interval from orbital sinus for glucose estimation.[11]

2.7. Induction of diabetes and experimental design

Diabetes was induced in overnight fasted mice by single intra-peritoneal injection of 55 mg/kg of streptozotocin (STZ), freshly dissolved in in 0.1 M cold citrate buffer, pH 4.5. After five days of STZ administration, blood was collected and plasma glucose levels were determined. The animals confirmed as diabetic by the elevated plasma glucose levels (>200 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 mice treated with vehicle alone; Group II: Diabetic control mice treated with vehicle alone; Group III: Diabetic mice treated with glibenclamide (10 mg/kg); Group IV: Diabetic mice treated with IS Extract (200 mg/kg); Group V: Diabetic mice treated with ethyl acetate extract (100 mg/kg); Group VI: Diabetic mice treated with ethyl acetate fraction of IS extract (200 mg/kg); Group VII: Diabetic mice treated with ethyl acetate fraction of IS extract (100 mg/kg); Group VIII: Diabetic mice treated with n-butanol fraction of IS extract (200 mg/kg); Group IX: Diabetic mice treated with n-butanol fraction of IS extract (100 mg/kg).[11,12]

Treatment was given orally using an intragastric tube once daily for 28 days, continuously. On 28th 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.

2.8. Biochemical analysis

Serum glucose was measured by using a glucometer (Accu-Chek Active, India). Serum total cholesterol, [13] total triglyceride, [14] LDL-c, VLDL-c [15] and HDL-c [16] were estimated using standard Enzymatic (Span Diagnostics, India). The estimation of SGOT, SGPT [17], serum ALP, [18] total protein, [19] serum urea, [20] and creatinine [21] was done by using standard Enzymatic (Span Diagnostics, India). Glycogen content in liver was measured according to spectrophotometric determination of glycogen with o-toluidine reagent.[22]

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). [23-26]

2.9. Histopathological studies

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.[27]

2.10. Statistical analysis

Results were expressed Mean $\pm$ 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.

3.Results

3.1. 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 behaviour 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.

3.2. Effect of *Ipomoea staphylina* on oral glucose tolerance test (OGTT) in mice

After glucose load, it was observed that normal control mice showed higher blood glucose level. Administration of ethanolic extract of IS leaves and its fractions (100 and 200 mg/kg) significantly ($p<0.001$) lower the blood glucose levels compared to normal control mice.

3.3. Effect of *Ipomoea staphylina* on blood glucose level in STZ-induced diabetic mice

Table 2 shows 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 28 days showed significant ($p<0.001$) decrease in blood glucose levels with the doses of 100 and 200 mg/kg of ethanolic extract of IS and its fractions and 10 mg/kg of glibenclamide as compared to diabetic control group.

3.4. Effect of *Ipomoea staphylina* on body weight in STZ-induced diabetic mice

Average body weights of different animal groups at various intervals are shown in Table 3. STZ produced significant 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) and ethanolic extract of IS and its ethyl acetate and n-butanol fractions (100 and 200 mg/kg) showed significant improvement in body weight compared to diabetic control at 28th days.

Table 1. Effect of *Ipomoea staphylina* on oral glucose tolerance test (OGTT) in mice

Groups	Treatment	Fasting Blood glucose(mg/dl)			
		0 min	30 min	60 min	120 min
I	Normal control	93.23±1.76	164.15±1.90	148.88± 2.38	130.24±2.01
II	Glinbenclamide (10 mg/kg)	91.77±3.19	114.22±1.67***	99.29±0.84***	83.76±1.24***
III	IS Extract (200 mg/kg)	89.22±2.39	123.38±2.01***	110.05±1.61***	90.46±1.76***
IV	IS Extract (100 mg/kg)	88.11±3.22	136.31±2.08***	125.55±1.87***	102.03±1.25***
V	Ethyl acetate fraction of IS (200 mg/kg)	90.24±2.39	127.09±1.37***	111.48±1.80***	99.70±2.22***
VI	Ethyl acetate fraction of IS (100mg/kg)	91.01±2.21	140.25±1.24***	128.83±1.87	102.25±1.87
VII	n-Butanol fraction of IS (200 mg/kg)	89.82±1.50	130.38±1.17***	122.74±1.90***	98.26±2.21***
VIII	n-Butanol fraction of IS (100 mg/kg)	88.96±2.20	149.05±1.39***	127.23±1.35***	104.07±2.17***

*Values are given as mean ± SEM for groups of six animals each *** p<0.001 when compared with the normal control group*

Table 2. Effect of *Ipomoea staphylina* on blood glucose level in STZ-induced diabetic mice

Groups	Treatment	Blood glucose(mg/dl)				
		0 day	7th day	14th day	21th day	28th day
I	Normal control	96.01±2.11	94.06±2.86	94.68±3.11	94.49±3.63	95.84±2.26
II	Diabetic control	213.49±2.33###	269.89±4.68###	301.03±2.17###	317.94±3.20###	307.27±4.36###
III	Glinbenclamide (10 mg/kg)	208.10±3.27	163.14±3.36***	151.31±3.37***	134.09±3.03***	126.54±2.66***
IV	IS Extract (200 mg/kg)	207.94±3.39	169.11±4.24***	158.46±1.71***	146.13±3.10***	137.43±2.85***
V	IS Extract (100 mg/kg)	209.86±2.83	192.52±3.623***	188.75±3.91***	180.89±1.83***	167.77±1.35***
VI	Ethyl acetate fraction of IS (200 mg/kg)	206.35±3.89	173.29±2.10***	165.21±1.23***	164.83±1.53***	142.02±3.72***
VII	Ethyl acetate fraction of IS (100mg/kg)	208.64±4.19	188.30±4.25***	181.61±2.47***	166.33±1.52***	156.75±1.76***
VIII	n-Butanol fraction of IS (200 mg/kg)	210.10±2.48	183.30±2.10***	177.66±1.52***	167.04±2.85***	166.83±2.00***
IX	n-Butanol fraction of IS (100 mg/kg)	211.03±3.48	205.42±1.65***	191.68±39***	184.25±1.92***	178.02±1.43***

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 when compared with the diabetic control group*

Table 3. Effect of *Ipomoea staphylina* on body weight in STZ-induced diabetic mice

Groups	Treatment	Blood Weight (gm)				
		0 day	7th day	14th day	21th day	28th day
I	Normal control	23.83±0.79	24.16±0.94	26.5±0.84	27.83±0.79	31.00±1.37
II	Diabetic control	26.16±1.15	24.33±1.47	21.00±1.31#	18.83±0.98###	16.83±0.87###
III	Glinbenclamide (5mg/kg)	25.26±1.47	22.66±1.22	21.33±0.61	23.88±1.14	25.00±0.96***
IV	IS Extract (200 mg/kg)	24.50±1.25	23.00±1.29	21.16±1.16	22.83±1.07	23.33±1.14**
V	IS Extract (100 mg/kg)	25.16±1.13	22.66±0.98	20.16±0.98	21.66±0.91	22.83±1.07**
VI	Ethyl acetate fraction of IS (200 mg/kg)	24.83±1.44	22.83±1.40	21.00±0.66	22.00±1.48	23.16±1.35**
VII	Ethyl acetate fraction of IS (100mg/kg)	27.50±0.76	24.33±0.80	22.66±0.66	22.83±0.65	24.16±0.74***
VIII	n-Butanol fraction of IS (200 mg/kg)	26.16±0.94	23.33±0.71	22.00±0.85	22.66±0.80	24.33±0.76***
IX	n-Butanol fraction of IS (100 mg/kg)	24.33±1.56	21.5±1.38	20.16±1.24	20.83±1.35	22.00±1.23*

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

3.5. Effect of *Ipomoea staphylina* on lipid profile in STZ-induced diabetic mice

STZ 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 mice. Treatment with ethanolic extract of IS and its ethyl acetate and n-butanol fractions (100 and 200 mg/kg) showed significant ($p<0.001$) reduction in TC, TG, LDL-C, VLDL-C and elevation of HDL-C levels compared to diabetic control (Table 4).

Table 4. Effect of *Ipomoea staphylina* on lipid profile in STZ-induced diabetic mice

Groups	Treatment	Lipid Parameters (mg/dl)				
		TC	TG	HDL -C	LDL-C	VLDL-C
I	Normal control	82.08±211	107.1±3.49	44.5±1.64	22.3±3.02	21.41±0.69
II	Diabetic control	176.7±2.69$^{###}$	203.90±2.97$^{###}$	24.99±1.53$^{###}$	110.9±3.24$^{###}$	40.77 ±0.59$^{###}$
III	Glinbenclamide (10mg/kg)	99.77±2.79***	113.6±3.18***	43.26±1.78***	33.77±3.22***	22.72±0.63***
IV	IS Extract (200 mg/kg)	112.8±2.08***	119.4±3.02***	40.87±2.36***	48.05±2.84***	24.05 ±0..53***
V	IS Extract (100 mg/kg)	148.7±2.17***	158.8±2.69***	34.07±1.12*	82.85±2.08***	31.77±0.53***
VI	Ethyl acetate fraction of IS (200 mg/kg)	122.1±3.12***	124.1±3.49***	39.38±2.01***	57.92±1.56***	24.82±0.69***
VII	Ethyl acetate fraction of IS (100mg/kg)	154.81±1.62***	161.82±4.31***	32.69±1.94	89.43±2.37***	32.68±0.77***
VIII	n-Butanol fraction of IS (200 mg/kg)	137.7±2.32***	142.9±2.96***	30.60±2.65	78.60±2.50***	25.58±0.59***
IX	n-Butanol fraction of IS (100 mg/kg)	163.7±2.88*	176.2±1.94***	26.26±1.34	102.1±3.94	35.24±0.38***

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<0.05 when compared with the diabetic control group*

3.6. Effect of *Ipomoea staphylina* on SGOT, SGPT and ALP level in STZ-induced diabetic mice

In STZ induced diabetic mice 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) the SGOT, SGPT and ALP activities were significantly reduced compared to diabetic mice (Table 5).

Table 5. Effect of *Ipomoea staphylina* on SGOT, SGPT and ALP level in STZ-induced diabetic mice

Groups	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (mg/dl)
I	Normal control	69.90±3.05	54.80±3.72	141.49±3.89
II	Diabetic control	153.08±4.99$^{###}$	104.82±2.47$^{###}$	249.94±2.72$^{###}$
III	Glinbenclamide (10mg/kg)	83.98±5.52***	63.16±2.34***	161.06±2.55***
IV	IS Extract (200 mg/kg)	95.32±2.66***	75.35±3.06***	183.36±2.72***
V	IS Extract (100 mg/kg)	127.44±3.37***	89.35±2.89**	230.90±2.97**
VI	Ethyl acetate fraction of IS (200 mg/kg)	103.29±2.46***	80.69±2.19***	187.13±3.81***
VII	Ethyl acetate fraction of IS (100mg/kg)	134.22±2.61*	88.25±2.37**	230.69±3.50**
VIII	n-Butanol fraction of IS (200 mg/kg)	124.35±2.73***	85.14±1.73***	208.39±3.74***
IX	n-Butanol fraction of IS (100 mg/kg)	139.35±3.14	97.61±1.88	237.99±4.35

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<0.001 when compared with the diabetic control group*

3.7. Effect of *Ipomoea staphylina* on serum creatinine, blood urea, blood urea nitrogen, total protein and liver glycogen level in STZ-induced diabetic mice

The effect of ethanolic extract of IS and its ethyl acetate and n-butanol fraction (100 and 200 mg/kg) on serum creatinine, blood urea, blood urea nitrogen (BUN), total protein and liver glycogen level in streptozotocin induced diabetic mice is shown in Table 6. In diabetic mice a significant ($p<0.001$) increase in the levels of serum creatinine, blood urea and blood urea nitrogen was observed when compared to normal mice. Diabetic mice treated with ethanolic extract of IS and its fractions (100 and 200 mg/kg) showed significant reduction in the levels of serum creatinine, blood urea and blood urea nitrogen, when compared with diabetic mice. Diabetic mice showed a significant ($p<0.001$) decrease in serum total protein and liver glycogen which was increased significantly with treatment of ethanolic extract of IS and its fractions.

Table 6. Effect of *Ipomoea staphylina* on serum creatinine, blood urea, blood urea nitrogen, total protein and liver glycogen level in STZ-induced diabetic mice

Groups	Treatment	Serum Creatinine (mg/dl)	Blood Urea (mg/dl)	Blood Urea Nitrogen (mg/dl)	Total Protein (g/dl)	Liver Glycogen (mmol/L)
I	Normal control	0.55±0.030	24.27±1.67	11.33±0.78	7.08±0.17	5.14±0.16
II	Diabetic control	2.17±0.75$^{###}$	56.55±1.84$^{###}$	26.40±0.86$^{###}$	3.17±0.19$^{###}$	2.14±0.70$^{###}$
III	Glinbenclamide (10mg/kg)	0.72±0.020***	31.62±1.76***	14.76±0.82***	6.12±0.14***	4.64±0.13***
IV	IS Extract (200 mg/kg)	1.04±0.038***	36.85±1.82***	17.20±0.85***	5.84±0.11***	4.24±0.06***
V	IS Extract (100 mg/kg)	1.76±0.033***	40.90±3.72***	19.04±1.75***	5.01±0.21***	3.11±0.04***
VI	Ethyl acetate fraction of IS (200 mg/kg)	1.21±0.019***	39.19±1.34***	18.22±0.66***	5.23±0.07***	3.97±0.03***
VII	Ethyl acetate fraction of IS (100mg/kg)	1.89±0.023***	45.08±1.25**	21.04±0.58**	4.40±0.09***	3.07±0.16***
VIII	n-Butanol fraction of IS (200 mg/kg)	1.66±0.050***	43.75±1.79**	20.48±0.85**	4.98±0.04***	3.30±0.09***
IX	n-Butanol fraction of IS (100 mg/kg)	1.99±0.040	50.89±2.11	23.51±1.05	3.92±0.34	2.69±0.09*

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*

3.8. Changes in hepatic LOP, GPx, CAT and SOD of normal and diabetic mice after 28 days of treatment with *Ipomoea staphylina*

In diabetic mice, a significant rise (p<0.001) in activity of LPO was observed in the liver. Treatment with ethanolic extract of IS and its fractions (100 and 200 mg/kg) 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 mice. Treatment with the ethanolic extract of IS and its fractions were significantly increased the liver GPx, CAT and SOD level (Table 7).

Table 7. Changes in hepatic LOP, GPx, CAT and SOD of normal and diabetic mice after 28 days of treatment with *Ipomoea staphylina*

Groups	Treatment	LPO (ηm of MDA / mg of protein)	GPx(µMole of the oxidise GSH/min/mg of protein)	CAT (µm H_2O_2/mg of protein)	SOD (U/mg of protein)
I	Normal control	11.51±1.33	9.62±0.52	8.40±0.41	11.03±0.93
II	Diabetic control	50.89±1.55$^{###}$	3.10±0.24$^{###}$	2.91±0.12$^{###}$	2.68±0.49$^{###}$
III	Glinbenclamide (10mg/kg)	20.68±1.22***	6.67±0.21***	5.88±0.46	7.92±0.48***
IV	IS Extract (200 mg/kg)	25.11±1.46***	7.11±0.26***	6.59±0.20***	7.20±0.30***
V	IS Extract (100 mg/kg)	33.55±1.75***	5.35±0.16***	4.55±0.13***	4.91±0.35*
VI	Ethyl acetate fraction of IS (200 mg/kg)	31.58±2.20***	6.17±0.22***	5.85±0.13**	6.48±0.27***
VII	Ethyl acetate fraction of IS (100mg/kg)	37.30±1.81***	5.11±0.18***	4.25±0.20*	3.37±0.18
VIII	n-Butanol fraction of IS (200 mg/kg)	38.62±1.10***	5.54±0.18***	5.17±0.20***	5.32±0.16**
IX	n-Butanol fraction of IS (100 mg/kg)	42.32±1.81*	4.24±0.38	4.21±0.16*	2.97±0.37

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

3.9. Changes in kidney LOP, GPx, CAT and SOD of normal and diabetic mice after 28 days of treatment with *Ipomoea staphylina*

Table 8 shows the activities of LOP, GPx, CAT and SOD in the kidney of normal and diabetic mice. 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 mice.

Treatment with ethanolic extract of IS and ethyl acetate fraction (100 and 200 mg/kg) significantly increased the GPx, CAT and SOD levels. But the n-butanol fraction failed to show significant increase in GPx, CAT and SOD levels. The LOP level was also significantly decreased after the treatment of ethanolic extract of IS and its fractions (100 and 200 mg/kg).

Table 8. Changes in kidney LOP, GPx, CAT and SOD of normal and diabetic mice after 28 days of treatment with *Ipomoea staphylina*

Groups	Treatment	LPO (ηm of MDA / mg of protein)	GPx(µMole of the oxidise GSH/min/mg of protein)	CAT (µm H_2O_2/mg of protein)	SOD (U/mg of protein)
I	Normal control	9.83±0.82	12.11±0.83	10.63±0.59	20.92±1.17
II	Diabetic control	28.12±1.27$^{###}$	4.14±0.24$^{###}$	4.11±0.46$^{###}$	9.18±0.81$^{###}$
III	Glinbenclamide (10mg/kg)	17.25±0.65***	7.04±0.38***	5.80±0.44	14.63±0.63***
IV	IS Extract (200 mg/kg)	14.85±0.79***	9.88±0.39***	6.86±0.29***	16.42±0.86***
V	IS Extract (100 mg/kg)	18.11±0.63***	6.20±0.19*	4.98±0.30	12.06±0.57
VI	Ethyl acetate fraction of IS (200 mg/kg)	17.60±0.84***	7.41±0.19***	6.13±0.36*	13.18±0.91*
VII	Ethyl acetate fraction of IS (100mg/kg)	20.54±0.54***	5.76±0.24	4.75±0.28	11.37±0.67
VIII	n-Butanol fraction of IS (200 mg/kg)	19.10±0.77***	5.96±0.32	4.85±0.26	11.92±0.73
IX	n-Butanol fraction of IS (100 mg/kg)	24.28±1.09	4.51±0.34	4.29±0.28	10.08±0.46

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

3.10. Effect of ethanolic extract of leaves of IS and its fractions on histopathological analysis of liver and kidney

The liver of the diabetic mice showed hypertrophy of hepatocytes, hepatocellular necrosis and vacuolization with vanishing of nuclei. Diabetic mice treated with glibenclamide (10 mg/kg) and the extract of leaves of IS and its fractions (200 mg/kg) showed the almost normal cellular architecture with normal nucleus and cytoplasm with less or no hepatocellular necrosis (Fig. 1).

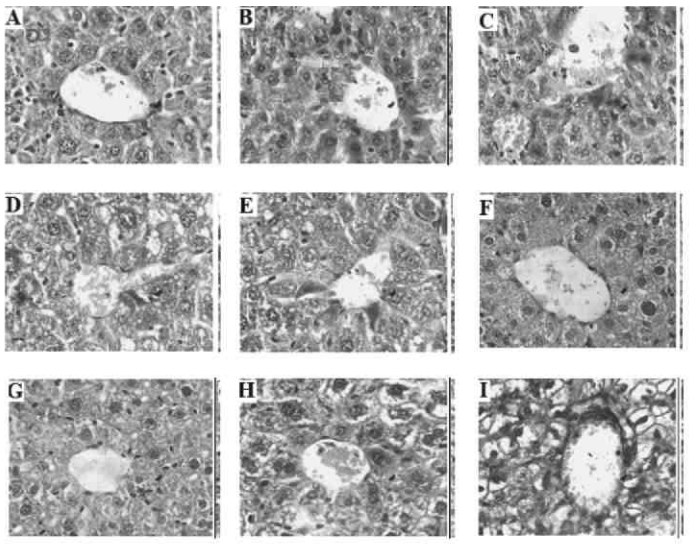

Fig. 1. Histology of liver in experimental mice after 28 days of treatment. (A) Normal control: normal liver showing normal hepatic cells and architecture. (B) Diabetic control: liver showing hypertrophy of hepatocytes, hepatocellular necrosis and vacuolization with vanishing of nuclei in STZ induced mice. (C) Diabetic+Glinbenclamide (5 mg/kg): attenuated STZ-induced hepatocellular necrosis in mice liver. (D) Diabetic+IS extract (200 mg/kg): section of liver showing normal hepatocellular architecture with normal and cytoplasm nucleus. (E) Diabetic+IS extract (100 mg/kg): section of liver showing hepatocellular architecture with normal nucleus and cytoplasm. (F) Diabetic+Ethyl acetate fraction of IS extract (200 mg/kg): section of liver showing normal hepatocellular architecture with normal nucleus and cytoplasm. (G) Diabetic+Ethyl acetate fraction of IS extract (100 mg/kg): section of liver showing normal hepatocellular architecture with normal nucleus, cytoplasm. (H) Diabetic+n-bulanol fraction of IS extract (200 mg/kg): section of liver showing nearly normal hepatocellular architecture. (I) Diabetic+ n-butanol fraction of IS extract (100 mg/kg): section of liver showing slightly cellular necrosis.

The kidney of normal control mice in STZ induced diabetic model showed the intact tubules and glomeruli whereas STZ treated group was found to cause degenerating tubules with desquamated epithelial cells in the lumen and glomerular congestion. Diabetic mice treated with glibenclamide (10 mg/kg) and the extract of leaves of IS and its fractions (200 mg/kg) showed the almost normal cellular architecture with intact tubules and glomeruli (Figure: 2).

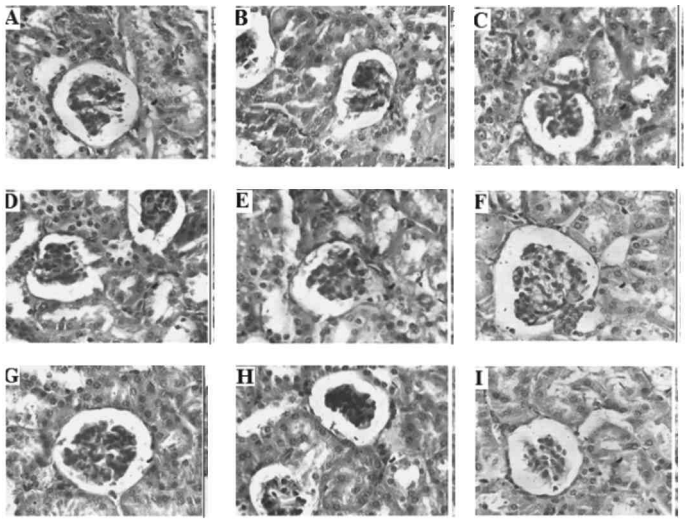

Fig. 2. Histology of kidney in experimental mice after 28 days of treatment. (A) Normal control: normal kidney showing intact tubules and glomeruli. (B) Diabetic control: kidney showing degenerating tubules with desquamated epithelial cells in the lumen and glomerular congestion in STZ induced mice. (C) Diabetic+Glinbenclamide (5 mg/kg): reduced STZ-induced degenerating tubules with desquamated epithelial cells in the lumen and glomerular congestion mice kidney. (D) Diabetic+IS extract (200 mg/kg): section of kidney showing normal cellular architecture with less glomerular congestion and degenerating tubules. (E) Diabetic+IS extract (100 mg/kg):

section of kidney showing normal cellular architecture with less glomerular congestion and degenerating tubules. (F) Diabetic+Ethyl acetate fraction of IS extract (200 mg/kg): section of kidney showing normal cellular architecture with less tubular damage and glomerular congestion. (G) Diabetic+Ethyl acetate fraction of IS extract (100 mg/kg): section of kidney showing normal cellular architecture with less tubular damage and glomerular congestion. (H) Diabetic+n-bulanol fraction of IS extract (200 mg/kg): section of kidney slight glomerular congestion with almost showing normal cellular architecture. (I) Diabetic+n-butanol fraction of IS extract (100 mg/kg): section of kidney slight glomerular congestion with almost showing normal cellular architecture.

4. Discussion

Streptozotocin (STZ) has been extensively used to induce type-I diabetes mellitus in experimental animal models. STZ, a 2-deoxy-D-glucose with an N-nitrosomethylurea moiety at second carbon atom was responsible to produce diabetes in laboratory animals. The presence of 2-deoxy-D-glucose facilitates uptake of streptozotocin into the pancreatic β cells through GLUT 2 [28] and the N-nitrosomethylurea moiety triggers DNA fragmentation in pancreatic β cells through formation of alkylating free radicals which results β-cell necrosis.[29] The action of STZ on mitochondria generates SOD anions, which leads to diabetic complications.[30-32] Based on the above perspectives, the current study was carried out to determine the hypoglycaemic activity of ethanolic extracts IS and its fractions glucose-loaded hyperglycemic and streptozotocin-induced diabetic mice. 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 STZ induced hyperglycemia, oral administration of ethanolic extract of IS and its fractions for 28 days showed a significant decrease in the levels of blood glucose. The possible mechanism by which the extract and its fractions reduced the blood glucose level in diabetic mice might be due to stimulation of surviving β cells leading to increase in insulin secretion.

STZ-induced diabetes is characterized by a severe loss in body weight. The decrease in body weight is due to the increased muscle destruction or degradation of structural proteins cause muscle wasting.[33] Diabetic mice treated with ethanolic extract of IS and its fractions showed an improvement in body weight as compared to the diabetic control mice which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis.

Diabetes is associated with profound alterations in the plasma lipid, triglycerides and lipoprotein profile and with an increased risk of coronary heart disease.[34] High level of total cholesterol and triglycerides is one of the major factors for coronary heart disease and arthrosclerosis which are the secondary complications of diabetes.[35] Diabetic mice were treated with ethanolic extract of IS and its fractions showed reduction in the serum lipid level. From this study, we can conclusively state that the ethanolic extract of IS and its fractions could modulate blood lipid abnormalities.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. SGOT, SGPT and ALP are reliable markers of liver function. [36] Marked elevations in SGOT, SGPT and ALP in diabetic control animals indicated the hepatocellular damage. [37] In STZ-induced diabetic the liver cells are necrotized and the integrity of plasma membrane decrease causes an increase in the activities of SGOT, SGPT and ALP in plasma. Treatment with ethanolic extract of IS and its fractions reduced 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.

Reduction in plasma total protein and elevation in serum urea, creatinine levels observed in diabetic control animals. 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. [38] 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 mice.

Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen due to decrease in insulin secretion. Insulin promotes deposition of glycogen in the liver by stimulating glycogen synthase and inhibiting the glycogen phosphorylation.[39, 40] The increased hepatic glycogen level in the diabetic mice treated with ethanolic extract of IS and its fractions was observed, which may be due to increase in insulin secretion.

Oxidative stress plays a major role in the causation of diabetes. Free radicals are generated in disproportionate manner in diabetes mellitus cause lipid peroxidation.[41] In the present study, significant increase in the levels of lipid peroxidation observed in liver and kidney of diabetic control mice might be due to reduction in antioxidant defense or due to the increase in free radicals generation. Treatment with ethanolic extract of IS and its fractions significantly reduced levels of lipid peroxidation in liver and kidney.

SOD, CAT and GPx are enzymatic antioxidants prevent cells from being exposed to oxidative damage by direct elimination of reactive oxygen species (ROS).[42] In diabetes mellitus, antioxidant enzymes SOD, CAT and GPx are inactivated due to high concentration of glucose thus increase the availability of superoxide anion ($O2.^{-}$) and hydrogen peroxide in the biological systems, which in turn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation.[43, 44] The ethanolic extract of IS and its fractions treatment increased the SOD, CAT and GPx activities indicating the efficacy of the extract in attenuating the oxidative stress in diabetic liver and kidney.

Histological studies of liver of the diabetic mice revealed that the ethanolic extract of IS and its fractions significantly reduced hypertrophy of hepatocytes and hepatocellular necrosis. The kidney histological exposed extensively improved architecture with intact tubules and glomeruli in the diabetic mice treated with ethanolic extract of IS its fractions.

Our previous study has shown that *Ipomoea staphylina* leaves are rich in phenolic and flavonoid content.[45] Phenolic compounds and flavonoids are known for hypoglycaemic and antioxidant properties.[29, 46] Thus, the significant antidiabetic effect of *Ipomoea staphylina* could be due to the presence of more than one active principle and their synergistic properties.

5. Conclusion

This study shows that the ethanolic extract of IS leaves and its fractions has beneficial effects on blood glucose level. The extract and its fractions restored the altered total cholesterol, triglycerides, serum enzymes (SGOT, SGPT and ALP), total protein and liver glycogen levels to near normal and also enhanced the activities of endogenous antioxidant enzymes SOD, CAT and GPx. 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.

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Conflicts of Interest

The authors report no conflict of interest.

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