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Pharmacological Study

Therapeutic potency of saponin rich aqueous extract of *Scoparia dulcis* L. in alloxan induced diabetes in rats

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Abstract

Background: Diabetes mellitus is major metabolic disorders of carbohydrate metabolism. This leads to alter the multiple organ system. **Aims:** To investigate the antidiabetic and antioxidant effects of the saponin rich aqueous extract of *Scoparia dulcis* (SRE-SD) using alloxan-induced hyperglycemic rat model. **Material and Methods:** The single dose of alloxan was injected for the induction of diabetes in rats. The SRE-SD and glibenclamide were administered for 15 consecutive days from the 3rd day of alloxan administration. Quantity of food and water intake was measured at day 0, and 18. Further, body weight was recorded and blood samples were collected at different time intervals that is, day 0, 3, 8, 13, and 18. The oxidative biomarkers (i.e. thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and nitrite (NO^{2-}) levels were also estimated in the serum sample. **Results:** The SRE-SD showed a remarkable dose and time-dependent changes in alloxan-induced rise in the level of food consumption and water intake, serum glucose level, TBARS, NO^{2-} and fall in the level of GSH. Further, significant attenuation was observed at 20 and 30 mg/kg of SRE-SD treated group. **Conclusions:** These findings demonstrate that SRE-SD has both antidiabetic and antioxidant effects on the experimental model of diabetes in rat.

Key words: Alloxan, diabetes mellitus, oxidative stress, saponin, *Scoparia dulcis*

Introduction

Diabetes mellitus is one of the most common long lasting metabolic disorders.[1] The World Health Organization (WHO) reported that 171 million people worldwide suffer from diabetes in year 2000.[2] The expected total number of people with diabetes will be about 300 million by year 2025; this number would be double by the year 2030.[2,3] The use of Indian and Chinese ethno-botanicals has a long folkloric history for the treatment of diabetes mellitus.[4,5] The WHO has estimated that 80% of the world population use herbal medicine for their primary healthcare needs and also they are totally dependent on traditional medicine.[4,6] The use of complementary and alternative medicine, especially the consumption of botanicals have been increasing rapidly worldwide because of the less side-effects when compared with modern medicine.[7]

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Scoparia dulcis L. commonly known as "Sweet Broomweed" is widely used in Indian folk and Ayurvedic medicine for the treatment of diabetes mellitus.[8] Often this plant is considered as one of the source plant of *Pashanabheda* (*Bergenia ligulata*) of Ayurveda. It is a perennial herb widely distributed in tropical and sub-tropical regions. In these regions, fresh or dried *S. dulcis* plants have been traditionally used as remedies for various ailments such as stomach problems that is, peptic ulcer,[9] hypertension,[10] hyperlipidemia,[11] hepatic injury,[12] algesia, and inflammation.[13,14] It has various biologically active secondary metabolites such as carbohydrates, coumarins, phenols, saponins, glycosides, tannins, amino acids, flavonoids, terpenoids, catecholamine, noradrenaline, and adrenaline.[15,16] Moreover, it has also possess the various active chemical constituents that is, scoparic acid A, scoparic acid B, scoparic acid C, scopadulcic acid A and B, scopadiol, scopadulciol, dulcinol, scopadulin etc.[16,17] It has been documented to possess the various pharmacological actions such as antitumor, anticancer, antibacterial, antiviral, antifungal, antileukemia and antiaging.[16,18] Moreover, experimental reports suggest that it has potential antidiabetic action.[19,20]

Various antidiabetic agents from natural products including saponins, flavonoids, alkaloids, anthraquinones, terpenes, coumarins, phenolics, polysaccharides have been promised to possess the antidiabetic action.[21] Saponins are a class of

chemical compounds, it is one of major secondary metabolites has found in natural sources and it has found particular abundance in various plant species. Further, saponins have been documented that, it can be serving as major antihyperglycemic components.[22] *S. dulcis* is reported to possess the antidiabetic potential,[19,20] whereas there is no report for the responsible phytoconstituents for this activity. Therefore, our works is focused to investigate the therapeutic potency of saponin rich extract of *Scoparia dulcis* (SRE-SD) in alloxan-induced diabetes in rats.

Materials and Methods

Drugs and chemicals

Alloxan monohydrate was purchased from Explicit Chemical Pvt. Limited, Pune, India. 5,5'-dithio, bis (2-nitrobenzoic acid), bovine serum albumin, reduced glutathione (GSH) were purchased from Sisco Research Laboratories, Mumbai. Thiobarbituric acid was purchased from Loba Chemie, Mumbai. Glibenclamide was procured from Sun Pharmaceuticals, India. The glibenclamide (GLUCOVANCE®) has been used as a positive control in this study.

Plant material

Scoparia dulcis plants were collected from the paddy fields of Kalkurichy, Kanyakumari District. The plant was identified and authenticated. The specimen was deposited in the same department for future reference (No. SD/158/06/2013).

Preparation of aqueous extracts

Whole plants of *S. dulcis* materials were washed individually with clean sterile water and oven dried for 1 h at 16°C. Three hundred grams of *S. dulcis* material was made into a fine powder and soaked in 150 ml of distilled water (aqueous extract) for 24 h. The slurry was placed in a clean, sterile glass container and shaken vigorously to allow for proper extraction. The slurry was filtered using a Whatman filter paper no 42, the extract was air dried and stored at 4°C for further preparation. The yield of aqueous extract of *S. dulcis* was obtained 11.36% w/w, dry weight basis.

Preparation of saponin rich extracts *Scoparia dulcis*

Saponins rich extract was prepared from aqueous extract of *S. dulcis* as described method in previous study.[23] with some modification. Briefly, the aqueous extract of *S. dulcis* (300 g) was refluxed with n-butanol for 2 h and n-butanol soluble constituents were separated by filtration. The n-butanol layer was sequentially washed with distilled water, alkali (2% KOH) and distilled water again. The n-butanol layer was evaporated and dried under vacuum to obtain a clear powder of crude saponin. The yield of crude saponin of *S. dulcis* was obtained 25.3% w/w, dry weight basis.

Determination of saponin contents by high-performance liquid chromatography analysis

The saponin content of SRE-SD was determined by using high-performance liquid chromatography (HPLC).[24] The extracts were filtered through a 0.45 μm membrane filter (Millipore, Bedford, USA) before injection into the column and analyzed by HPLC. Determination of saponin was carried out using a C_{18} column (4.6 × 150 mm, ID; particle size 5 μm). The mobile phase consisted of methanol, water, and acetic acid in the ratio of 60:34:6 (v/v/v) at a flow rate of 1.5 ml/min. The mobile phase was filtered and degassed prior to use. The injection volume was 20 μl and the detection wavelength was set at 254 nm and 25°C. A total of 5 mg of ursolic acid (Sigma-Aldrich) was weighed into a 100 ml volumetric flask and diluted to volume with triple deionized water for a final concentration of 0.05 mg/ml for using as a saponin standard. The compounds appearing in chromatograms were identified on retention times and spectral data by comparison with standards. All analyses were performed on triplicate of the extracts. The values were expressed as mean ± standard deviation (SD).

Animals

Male Wistar rats (200–220 g, purchased from Animal Husbandary, Kanyakumari) were used in the present investigation. The rats were allowed free access to feed and tap water under strictly controlled pathogen free conditions with room temperature 25°C ± 2°C, relative humidity (30–70%) on 12 h light and dark cycle, lighted between time intervals of 6:00 AM and 6:00 PM. The rats were fed on standard rodent pellet chow and acclimatization to their environment for 2 weeks before the commencement of the experiment. The study protocol was duly approved by Institutional Animal Ethical Committee (193/98/CPCSEA; dated April 15, 2005) of the department and care of the animals was carried out as per the guidelines of the committee for the purpose of Committee for the Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

Induction of diabetes

Diabetes was induced by a single intra-venous administration of freshly prepared alloxan monohydrate (40 mg/kg; dissolved in 1 ml normal saline) on overnight fasted rats (i.e. day 0) as described method of Ahmed.[25] After 48 h, blood samples were collected from the rat tail vein puncture method and rats fasting glucose levels were checked by commercial diagnostic device method in alloxan treated animals. The fasting glucose above 210 mg/dl along with showing clear signs of diabetes that is, polyuria, polyphagia and polydipsia were considered and selected for further study. Animals with fasting serum glucose <200 mg/dl were rejected.

Experimental protocol

This study consists of seven groups, each group comprised of six Wistar rats (n = 6). After the development of hyperglycaemic (>210 mg/dl) rats were randomly allotted in Group 2–7.

Group 1 (Normal control): Rats were subjected without administration of the vehicle and drugs in this study.

Group 2 (Alloxan control).

Group 3 (Vehicle control): Diabetic rats were treated orally with 5 ml/kg of distilled water for 18 consecutive days from the 3rd day of alloxan administration.

Group 4–6 (SRE-SD-10, 20 and 30 mg/kg respectively): Diabetic rats were treated orally with SRE-SD at the dose of 10,

20, 30 mg/kg of body weight/day for 18 consecutive days from the 3rd day of alloxan administration.

Group 7 (Glibenclamide 1.25 mg/kg): Diabetic rats were treated orally with glibenclamide at the dose of 1.25 mg/kg of body weight/day for 18 consecutive days from the 3rd day of alloxan administration.[25] The graphical study design was expressed in Figure 1.

The dose was suspended in 1% w/v of carboxy methyl cellulose and it was administered through a gastric gavage by using oral cannula. Further, measurement of body weight, food and water consumption as well as blood sample analysis were performed different time intervals that is, day 0, 2, 8, 14, and 20. In serum glucose level was determined using one touch electronic glucometer (Life Scan, Johnson and Johnson Limited, Mumbai, India) and blood biomarkers that is, thiobarbituric acid reactive substances (TBARS),[26] reduced GSH,[27] nitrite (NO^{2-})[28] and total protein[29] levels were also estimated by spectrophotometric method.

Statistical analysis

The data were analyzed by two-way analysis of variance followed by Bonferonni's *post-hoc* test by using Graph-Pad prism software, California, USA. Values were expressed as mean standard deviation (mean ± SD). $P < 0.05$ was considered to be statistically significant.

Results

Total saponin content level in aqueous extract of *Scoparia dulcis*

The aqueous extract of *S. dulcis* contained 38.64% of total saponins when compared to ursolic acid (100%). The triplicate value of total saponin value has shown to possess the 0.350 ± 0.011 mg of ursolic acid equivalent per ml. The chromatogram of SRE-SD and standard saponin that is, ursolic acid (real time value is 19.93 [Figure 2]).

Effect of saponin rich aqueous extract of *Scoparia dulcis* on serum glucose level

Administration of alloxan resulted in significant rise serum glucose level when compared to normal control group. The administration of SRE-SD have shown to possess the attenuating effect on alloxan-induced diabetes in dose-dependent manner. Moreover, the significant ameliorative effect has been observed in 20 and 30 mg/kg of SRE-SD treated group. The vehicle treated group did not produce the any significant changes when compared to alloxan treated group. Further, the administration of glibenclamide has also been produced the significant ($P < 0.05$) ameliorative effect when compared to alloxan treated groups [Figure 3].

Effect of saponin rich aqueous extract of *Scoparia dulcis* on bodyweight, food and water consumption levels

Administration of alloxan resulted in significant rise in food and water consumption and decrease in bodyweight levels when compared to normal control group. The administration of SRE-SD shown to possess the attenuating effect on alloxan-induced above changes in a dose-dependent manner. Moreover, the significant ameliorative effect has been observed in 20 and 30 mg/kg of SRE-SD treated group. The vehicle treated group did not produce the any significant changes when compared to alloxan treated group. Further, the administration of glibenclamide has also been produced the significant ($P < 0.05$) ameliorative effect on bodyweight, fluid intake, and food consumption levels when compared to alloxan treated groups [Table 1].

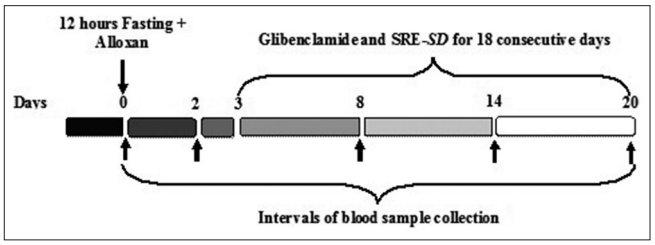

Figure 1: The study design of alloxan induced diabetes mellitus. Glibenclamide has been used as a reference drug. Saponin rich aqueous extract of *Scoparia dulcis* (20 and 30 mg/kg, p.o.) and glibenclamide (1.25 mg/kg, p.o.) were administered once a day for 18 days and blood samples were collected at different time intervals (i.e., day 0, 2, 3, 8, 14 and day 20)

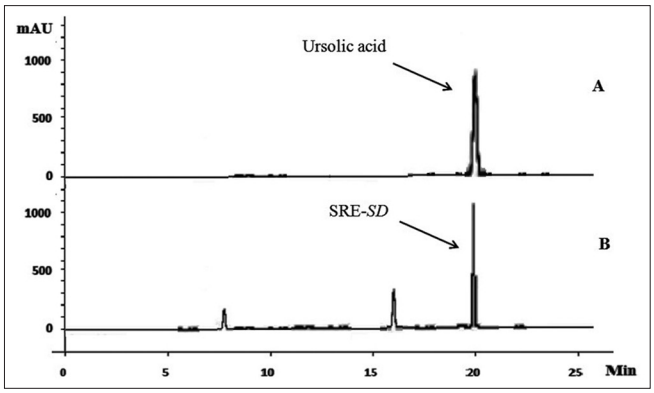

Figure 2: High-performance liquid chromatography (HPLC) chromatogram of saponin rich aqueous extract of *Scoparia dulcis* (SRE-SD) and standard compounds HPLC peak in a and b indicates saponin contents of SRE-SD and reference samples, that is, ursolic acid respectively (real time value is 19.93). The peaks in HPLC chromatogram represent saponin concentrations which were expressed as ursolic acid equivalent

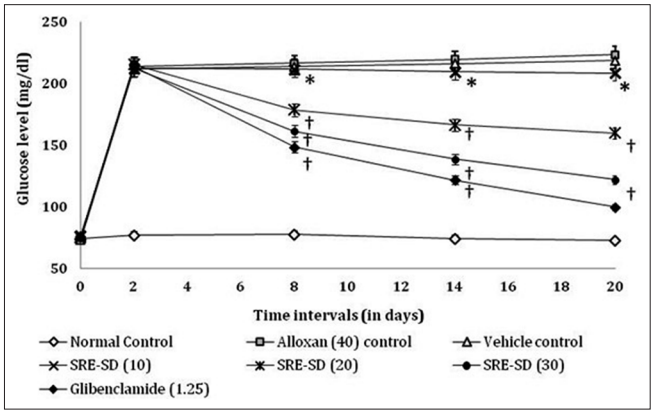

Figure 3: Effect of saponin rich aqueous extract of *Scoparia dulcis* on alloxan-induced serum glucose levels $F(6, 41) = 4331.053$; $P < 0.001$. Digits in parentheses indicate dose in mg/kg. (*$P < 0.05$ vs. normal control group, †$P < 0.05$ vs. alloxan control group)

Table 1: Effect of SRE-SD on body weight, fluid intake and food consumption changes in alloxan-induced diabetic rats

Groups	Day 0	Day 2	Day 8	Day 14	Day 20
Body weight (g)					
Normal control	210.7±1.8	211.3±2.3	212.6±1.9	212.7±1.4	213.4±2.1
Alloxan (40) control	211.9±1.6	211.3±2.2	176.8±2.7*	159.3±1.6*	149.4±2.7*
Vehicle control	211.4±1.5	211.6±1.8	179.4±2.1	162.8±2.3	152.7±2.1
SRE-SD (10)	210.6±1.7	209.9±1.7	186.2±1.9	171.6±2.1	159.5±2.3
SRE-SD (20)	211.6±1.5	210.8±1.6	200.6±1.6†	195.2±2.5†	191.1±1.7†
SRE-SD (30)	212.3±1.4	211.7±1.5	204.3±1.9†	200.5±1.8†	197.3±1.9†
Glibenclamide (1.25)	211.5±1.9	210.9±2.2	208.8±2.1†	206.1±1.7†	203.9±1.6†
Fluid intake (ml/100 g of rat/day)					
Normal control	35.4±1.4	36.4±1.3	35.1±1.4	34.9±1.7	36.3±1.3
Alloxan (40) control	34.2±1.2	51.2±1.8	67.3±1.7*	69.2±1.2*	71.6±1.4*
Vehicle control	37.5±2.1	50.4±1.2	68.2±1.2	69.1±1.3	70.3±1.1
SRE-SD (10)	38.3±3.3	49.6±2.9	66.1±1.4	67.4±1.9	69.2±2.4
SRE-SD (20)	36.2±2.6	51.1±2.7	50.2±2.1†	48.2±1.4†	45.2±1.8†
SRE-SD (30)	35.4±2.4	52.4±2.3	45.8±2.7†	44.1±1.9†	43.1±1.7†
Glibenclamide (1.25)	35.4±1.9	50.6±2.5	42.7±2.3†	39.4±2.3†	38.5±2.2†
Food consumption (g/100 g of rat/day)					
Normal control	6.9±0.3	6.4±0.9	6.7±0.2	6.5±0.3	6.4±0.7
Alloxan (40) control	6.7±0.4	5.9±0.3	9.2±0.8*	12.4±0.4*	14.3±1.1*
Vehicle control	6.2±0.8	6.5±0.4	9.7±0.4	11.2±0.3	13.4±0.5
SRE-SD (10)	6.3±0.3	6.1±0.8	10.2±0.3	11.1±0.6	12.3±0.7
SRE-SD (20)	5.8±0.9	5.8±0.2	8.9±0.6†	8.3±0.2†	7.8±0.2†
SRE-SD (30)	6.6±0.6	6.6±0.7	8.4±0.8†	7.8±0.4†	7.4±0.4†
Glibenclamide (1.25)	6.5±0.4	6.3±0.5	8.1±0.3†	7.9±0.4†	7.5±0.8†

F (6, 41)=29.891; *P*<0.001; for fluid intake, *F* (6, 41)=735.926; *P*<0.001; for food consumption, *F* (6, 41)=783.614; *P*<0.001. Digits in parentheses indicate dose in mg/kg. **P*<0.05 versus normal control group, †*P*<0.05 versus alloxan control group. SRE-SD: Saponin rich extracts-*Scoparia dulcis*

Effect of saponin rich aqueous extract of *Scoparia dulcis* on oxidative stress marker levels

Administration of alloxan resulted in significant rise in TBARS, NO^{2-} and decrease in GSH levels when compared to normal control group. The administration SRE-SD shown to possess the attenuating effect on alloxan-induced oxidative stress marker changes in a dose-dependent manner. Moreover, the significant ameliorative effect has been observed in 20 and 30 mg/kg of SRE-SD treated group. The vehicle treated group did not produce the any significant changes when compared to alloxan treated group. Further, the administration of glibenclamide has also been produced the significant ($P < 0.05$) ameliorative effect on TBARS, GSH, and NO^{2-} levels when compared to alloxan treated groups [Table 2].

Discussion

In the present investigation, administration of alloxan-induced a significant development of diabetic changes that is, fasting blood glucose, bodyweight, food as well as water consumption levels and oxidative stress marker changes that is, TBARS, reduced GSH, as well as NO^{2-} levels in rats. Moreover, treatment of SRE-SD has shown to produce significant attenuating effect on alloxan-induced diabetic and antioxidant changes. These observations are in line with others research reports, that is suggested, crude extract of *S. dulcis* has possess the ameliorative effect in streptozotocin-induced of diabetic changes.[20,30,31]

Alloxan monohydrate is a pyrimidine derivative has been commonly used as a pharmacological tool for induction of diabetes mellitus in experimental animals. The pathogenesis of alloxan-induced diabetes is mainly through the ability of destroying the insulin-producing beta cells of the pancreas.[32,33] *In vitro* studies have also shown that alloxan is selectively toxic to pancreatic beta cells, causing cell necrosis.[34] The cytotoxic action of alloxan is mediated through rise in oxidative stress by free radical (reactive oxygen species and reactive nitrogen species) generations along with a massive increase of cytosolic calcium concentration, leading to a rapid destruction of beta cells.[35] Oxidative stress has been reported to be the most common and important mechanism in diabetes mellitus and their complications.[36,37]

Many natural products, especially plants-derived medicines have been promised to treat and management of diabetes mellitus in experimental animal and human trials via antioxidative mechanism.[21,38,39] The research report from literature and this study has been revealed that, the *S. dulcis* has antidiabetic and antioxidant potential.[20,31] Numerous studies has been reported that herbal constituents such as saponins, tannins, flavonoids, phenolic compounds, terpenoids, glycosides alkaloids etc., shown to possess the free radical scavenging role *in vitro* as well as in biological systems and also ameliorated the oxidative injury and disease progress.[40] However, among the all-natural compounds, saponins and flavonoids are reported to possess the potential antidiabetic action.[41,42]

Table 2: Effect of SRE-SD on oxidative stress marker levels in alloxan-induced diabetic rats

Groups	Day 0	Day 2	Day 8	Day 14	Day 20
TBARS level (nmol/ml)					
Normal control	0.98±0.02	1.00±0.01	0.99±0.04	0.97±0.03	0.99±0.03
Alloxan (40) control	0.99±0.01	1.08±0.03	1.11±0.06*	1.19±0.04*	1.26±0.09*
Vehicle control	0.97±0.01	1.07±0.01	1.10±0.04	1.18±0.03	1.25±0.01
SRE-SD (10)	0.99±0.02	1.02±0.01	1.12±0.02	1.17±0.03	1.22±0.04
SRE-SD (20)	0.99±0.02	1.01±0.02	1.05±0.04†	1.09±0.03†	1.15±0.07†
SRE-SD (30)	0.99±0.02	1.03±0.01	1.01±0.02†	1.06±0.02†	1.13±0.06†
Glibenclamide (1.25)	0.99±0.02	1.02±0.01	0.97±0.01†	0.98±0.04†	0.99±0.02†
GSH level (mg/dl)					
Normal control	39.73±1.71	38.92±1.57	40.28±1.42	39.36±1.51	38.17±1.39
Alloxan (40) control	40.48±1.93	31.05±1.69	24.93±1.07*	18.19±1.42*	13.16±1.27*
Vehicle control	38.64±1.32	30.93±1.92	25.74±1.71	19.26±1.27	12.73±1.95
SRE-SD (10)	37.37±1.58	31.28±1.41	25.02±1.34	20.07±1.04	16.92±1.31
SRE-SD (20)	38.42±1.30	32.27±1.59	30.73±1.93†	27.53±1.62†	24.47±1.63†
SRE-SD (30)	39.26±1.27	30.09±1.09	34.37±1.62†	33.71±1.51†	31.92±0.94†
Glibenclamide (1.25)	39.18±1.83	32.76±1.47	36.91±1.27†	36.26±1.67†	35.89±1.23†
Nitrite level (µmol/l)					
Normal control	5.21±0.64	5.32±0.41	5.19±0.32	5.23±0.61	5.34±0.57
Alloxan (40) control	5.33±0.33	8.48±0.42	12.62±0.45*	16.71±0.47*	17.26±0.74*
Vehicle control	5.34±0.89	7.29±0.47	11.97±0.74	17.03±0.28	17.38±0.41
SRE-SD (10)	5.46±0.52	8.32±0.25	11.69±0.62	14.46±0.38	15.16±0.54
SRE-SD (20)	5.19±0.49	8.19±0.39	8.41±0.71†	9.37±0.86†	10.01±0.63†
SRE-SD (30)	5.34±0.61	8.21±0.43	7.14±0.45†	8.08±1.06†	8.76±0.45†
Glibenclamide (1.25)	5.39±0.32	8.42±0.52	6.38±0.53†	6.92±0.91†	7.83±0.52†

F (6, 41)=5239.4; *P*<0.001; for reduced glutathione, *F* (6, 41)=952.601; *P*<0.001; for nitrite, *F* (6, 41)=746.413; *P*<0.001. Digits in parentheses indicate dose in mg/kg. **P*<0.05 versus normal control group, †*P*<0.05 versus alloxan control group. TBARS: Thiobarbituric acid reactive substances, GSH: Glutathione, SRE-SD: Saponin rich extracts-*Scoparia dulcis*

Saponins has been documented to possess the ameliorate effects from disease progress by various number cellular mechanisms such as reduction in the lipid peroxidation, cytokines, chemokines, pro-apoptotic, and pro-inflammatory mediators, cellular calcium accumulation, and oxidation of 2'-deoxyribose; increased in the levels of reduced GSH, antiinflammatory and antiapoptotic mediators as well as physiological process that is, rise in the activity of myeloperoxidase, superoxide dismutase and GSH peroxidase; decreased in the levels creatine kinase, catalase levels.[43-45] Moreover, it has been reported that, saponins are possess the potential free radical scavenging action on hydroxyl and superoxide radicals along with suppression of inducible nitric oxide synthase, cyclooxygenase-2, and tumor necrosis factor-alpha expression.[44,46] Soponins have also been demonstrated that, it possesses the inhibitory action on opening of the potassium adenosine-triphosphate (K_{ATP}) channel.[44] Glibenclamide inhibits the opening of ATP-sensitive K_{ATP} channels, which represents a protective mechanism in type-II diabetes mellitus in experimental animal as well as human.[47-49] It also possesses the potential antioxidant and antiinflammatory actions.[48,50] Therefore, glibenclamide has been selected as a positive control in this investigation.

Conclusion

Saponin rich aqueous extract of *S. dulcis* has potent antidiabetic and antioxidant effects thus might be a potential therapeutic agent for the management of diabetes mellitus. However, the more elaborative and mechanistic studies are required to explore their possible therapeutic action.

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हिन्दी सारांश

सॅपोनिन से परिपूर्ण स्कोपारिया डलसिस के जलीय सत्व का एलोक्झान प्रेरित मधुमेही चूहों पर प्रभाव

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मधुमेह एक कार्बोहाड्रेड चयापचय का मुख्य विकार है। मधुमेह से अन्य शारिरीक प्रणालीओं मे विकार उत्पत्ती हो सकती है। यह अध्ययन सॅपोनिन से परिपूर्ण स्कोपारिया डलसिस के जलीय सत्व की एण्टीडायबेटीक (मधुमेह विरोधी) और एण्टीऑक्सिडण्ट गतीविधि जानने हेतु एलोक्झान प्रेरित मधुमेही चूहों मे देखा गया। एलोक्झान की एकल मात्रा से चूहों मे मधुमेह प्रेरित किया गया। मधुमेह प्रेरित करने के ३ दिन बाद परीक्ष्य औषधी का जलीय सत्व एवम् ग्लिबेनक्लेमाईड लगातार १५ दिन तक दिया गया। जल एवं अन्नग्रहण की मात्रा ० और १२ वे दिन पर नाँपी गयी। शरीर भार तथा रक्त के नमूने ०, ३, ८, १३ और १२ वे दिन मे लिए गए। औषधी जलीय सत्व से मात्रा एवम् कालानुसार अन्न और जल ग्रहण, रक्तगत शर्करा, टी.बी.ए.आर.एस, नायट्राइट की मात्रा में वृद्धी और जी.एस.एच. मे क्षय पाया गया। उपरोक्त निरीक्षणों से यह सिद्ध होता है कि स्कोपारिया डलसिस के जलीय सत्व मे एण्टीडायबेटीक तथा एण्टीऑक्सिडेण्ट की क्षमता है।