

# ANTIHYPERGLYCEMIC ACTIVITY OF FLAVONOIDS FROM METHANOLIC EXTRACT OF AERIAL PARTS OF *SCOPARIA DULCIS* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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**ABSTRACT:** The objective of the present study was to study the antihyperglycemic effects of flavonoids from methanolic extract of aerial parts of *Scoparia dulcis* leaves in normal, glucose loaded and streptozotocin induced diabetic rats. The methanolic extract of *S. dulcis* at a dose of 100, 200, 400 mg/kg of body weight (p.o) and standard drug glibenclamide 500 µg/kg, in all three animal models. The extract exhibited significant hypoglycemic activity in all three animal models when compared with a standard antidiabetic agent Glibenclamide. The hypoglycemia produced by the extract may be due to increased uptake of glucose at tissue level and or increase in pancreatic β-cell function or due to inhibition of intestinal glucose absorption of glucose. The findings of the present study suggest that the methanolic extract of *Scoparia dulcis* produced significant antihyperglycemic activity in STZ induced diabetic rat which is comparable to Glibenclamide (a standard oral hypoglycaemic agent).

**Key words:** *Scoparia dulcis*, Flavonoids, Diabetes, Streptozotocin.

## INTRODUCTION

Diabetes word is derived from Greek, and it means a siphon. Aretus the Cappadocian, a Greek physician during the second century A.D., named the condition diabainein. He described patients who were passing too much water (polyuria) - like a siphon. The word became "diabetes" from the English adoption of the Medieval Latin diabetes<sup>1</sup>.

Flavonoids are the compounds that are widely found in fruits and vegetables. They have a broad range of biological activities<sup>2</sup>. They function as powerful antioxidants, as phytoestrogens, and can alter the activities of important cell-signalling enzymes, such as tyrosine kinases, phosphodiesterases, and phosphoinositide kinases<sup>3</sup>. Some may also have

antidiabetic activity. Studies of the *in vivo* and *in vitro* effects of various flavonoids on glucose metabolism have shown opposite and often controversial results. This is probably because of the different structural characteristics of the molecules and the different experimental designs used<sup>4</sup>.

*Scoparia dulcis* is a shrub belonging to family, Scrophulariaceae synonym sweet broom weed. Every part of the plant is used as a cure for toothache<sup>5</sup>. A decoction of the root is given in blennorrhagia and in excessive menstruation<sup>6</sup>. The root is considered astringent, mucilaginous, and emollient. An infusion of the bitter leaves is prescribed in febrile conditions<sup>7</sup>.

The aim of the present study was to demonstrate the antihyperglycemic effects of flavonoids from methanolic extract of aerial parts of *Scoparia Dulcis* in glucose tolerance test, normal and streptozotocin induced diabetic rats.

## MATERIAL AND METHODS

### Chemicals and instruments used

The following chemicals were used in the study: Streptozotocin (SISCO Research Laboratories Pvt Ltd. India.), Glibenclamide (Cadilla Pharmaceuticals), Sodium CMC.

### Plant material

Dry plants of *Scoparia dulcis* were collected from, Zarkhand near Sammet shikharji in the month of December and its authentication was confirmed by Taxonomist, Prof. and Head of Botany Dept., Sardar Patel University, Vallabh Vidyanagar and deposited this plant to Dept. of pharmacognosy, A R College of pharmacy, Vallabh Vidhyanagar(ARGH). VJS/SD-35

### Preparation of plant extracts

Dried plant material (5 kg) was ground in a waring blender and sifted through a wire screen (mesh size 2 mm × 2 mm). The powdered material (500 gm) was exhaustively extracted with methanol. The extracts were filtered and concentrated on a rotary evaporator.

### Isolation of Flavonoids

The methanolic extract was subjected to column chromatography. Column was prepared with silica gel and methanol. Methanolic extract (6 g) was dissolved in minimum volume of chloroform: methanol which was used as eluting agent. The fraction obtained. The fraction (39-167) and fraction (184-295) gave single spot in chloroform: ethyl acetate: methanol (6:2:3) solvent system with spraying reagent Boric acid (3%) and Oxalic acid (10%). Both compounds gave positive test for flavonoids i.e shinoda test. The column was repeated thrice to get sufficient amount of flavonoids for hypoglycemic activity.

### Animals

Wistar rats of both sexes (150-200 g) were maintained under standard animal house conditions, fed standard pellet diet (Hindustan Lever Ltd. Bombay) and allowed water *ad libitum*. Fasted animals were deprived of food for at least 16 hr, but were allowed free access to water. The study was approved by the Institutional Animal Ethical Committee of A.R.College of pharmacy, Vallabh vidhyanagar.

### Acute toxicity and selection of doses

The acute toxicity studies were carried out in adult female albino rats weighing about 150-200 g, by

up and down method as per OECD 425 guidelines<sup>8</sup>. Over night fasted animals received test drug at a dose of 2000 mg/kg body weight orally. Then the animals were observed continuously once in half an hour for next 4 hrs and then after 24 hrs for general behavioral, neurological, autonomic profiles and to find out mortality. The extract was found safe up to a dose of 2000 mg/kg body weight.

### Oral glucose tolerance test<sup>9</sup>

The oral glucose tolerance test was performed in overnight fasted normal animals. Rats divided into four groups (n=6) were administered 2% gum acacia solution, flavonoids 100 mg/kg, 200 mg/kg & 400 mg/kg and glibenclamide (500 µg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of flavonoids. Blood was withdrawn from the retro-orbital sinus and blood glucose levels were estimated at 0, 30, 60, 90, and 120 min of flavonoids administration.

### Normoglycemic study

For, normoglycemic study rats were divided into five groups (n=6) and were administered 2% gum acacia solution, flavonoids 100 mg/kg, 200 mg/kg and 400 mg/kg and glibenclamide (500 µg/kg) respectively. Blood glucose levels were estimated on days 0, 4, 8 and 12.

### Induction of experimental diabetes<sup>10</sup>

Diabetes was induced in overnight fasted rats by single intraperitoneal injection of 50 mg/kg of body weight of Streptozotocin (SISCO Research Laboratories Pvt Ltd. India.) in Citrate buffer PH 4.5, Hyperglycemia was confirmed by the elevated blood glucose level determined at 48 hr after the dose. Animal that exhibited glycosuria after 48h was tested by urine test strips (Uristix, Bayer diagnostics Ltd, India) were considered as diabetic.

### Experimental and design

The animals were divided into five groups of six animals in each group:

- Group I** : - Normal healthy control;
- Group II** : - Diabetic control (Streptozotocin 50 mg/kg i.p)
- Group III** : - Diabetic + Extract (100 mg/kg body weight, orally)
- Group IV** : - Diabetic + Extract (200 mg/kg body weight, orally)
- Group V** : - Diabetic + Extract (400 mg/kg body weight, orally)
- Group VI** : - Diabetic + Glibenclamide (500 µg/kg body weight, orally)

## Sample collection

### Blood sampling

At the end of day 12, blood samples were collected from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hemocrit Capillaries, Mucaps). Blood was collected in to fresh vials containing anticoagulant antiserum was separated in a centrifuge at 3000 rpm for 5 minutes at room temperature and was directly used for estimating serum glucose levels using span diagnostic kits.

### Statistical analysis

The results were expressed as mean  $\pm$  SD. Statistical analysis were carried out using paired t-test and one-way ANOVA followed by Bonferroni's test. Differences below  $P < 0.05$  implied statistically significance.

## RESULT AND DISCUSSION

Diabetes mellitus is a major endocrine disorder in which the homeostasis of carbohydrates, protein and lipid metabolism is improperly regulated by the insulin, resulting in elevation of fasting and post prandial blood glucose levels<sup>11</sup>.

Preliminary phytochemical screening of the methanolic extract of *Scoparia dulcis* reveals the presence of flavonoids, saponins, phenolic compounds and terpenoids. Different doses of flavonoid extract were screened for their oral toxicity. No mortality was recorded till 4000 mg/kg with flavonoid extract, hence the extracts were found to be safe up to the dose levels of 4000 mg/kg.

Blood glucose levels of all groups at different doses after glucose tolerance test are shown in table I. The level of blood glucose was significantly decreased in flavonoid extract treated rats (Group II, III & IV) as compared to control animals (Group I). However, the level of blood glucose was returned to near normal

concentrations in diabetic rats treated with flavonoid extract (400 mg/kg) and glibenclamide (group V). Flavonoid extract at dose of 400 mg/kg showed comparable effect to that of glibenclamide.

Blood glucose levels of flavonoid extract treated groups at different doses after normoglycemic study are shown in table II. A significant hypoglycemic activity was found at 12<sup>th</sup> day with 100, 200, 400 mg/kg doses. Flavonoids significantly reduced blood glucose level at 12<sup>th</sup> day as compared to zero day for each treatment. In normoglycemic study, flavonoids from methanolic extract of *S.Dulcis* showed significant reduction of serum glucose levels and these effects were dose dependent.

Blood glucose levels of flavonoid extract treated groups at different doses in streptozotocin induced diabetic rats are shown in table IV. After oral administration of 100, 200 & 400 mg/kg of the flavonoids extract of *S.Dulcis* a significant reduction was observed in blood glucose level after day 8 and this hypoglycemic effect became more pronounced after day 12. The effect of flavonoid extract at a dose of 400 mg/kg was comparable to the glibenclamide, a standard antidiabetic agent.

The glibenclamide effects on glucose can be attributed to the enhanced activity of the  $\beta$  cells of the pancreas, resulting in secretion of the large amount of insulin<sup>12</sup>. Fasting blood glucose level in diabetic rats is an important basal parameter for monitoring diabetes<sup>13</sup> and it has shown that the *S.Dulcis* causes the antihyperglycemic effect by reducing the fasting blood glucose level.

In conclusion, the methanolic extract of *S.Dulcis* produced significant antihyperglycemic activity against STZ induced diabetic rats. Further studies are needed to identify the flavonoids of the methanolic extract of *S.Dulcis* that may be responsible for the antihyperglycemic activity.

**Table I. Effect of ethanolic extract on serum glucose level (mg/dL) in glucose tolerance test in glucose loaded rats.**

Group	Treatment	0 min	30 min	60 min	90 min	120 min
1	Control (Vehicle )	90.3 $\pm$ 1.3	111.4 $\pm$ 1.1	101.2 $\pm$ 2.1	96.4* $\pm$ 1.3	90.6* $\pm$ 1.6
2	Flavonoid extract (100mg/kg)	86.2 $\pm$ 2.0	98.6 $\pm$ 2.1	92.2 $\pm$ 1.6	87.4 $\pm$ 1.8	80.8 $\pm$ 1.4
3	Flavonoid extract (200mg/kg)	85.9 $\pm$ 1.6	97.2 $\pm$ 1.2	90.6 $\pm$ 1.5	84.2* $\pm$ 1.3	80.4* $\pm$ 2.3
4	Flavonoid extract (400mg/kg)	82.6 $\pm$ 2.1	86.3 $\pm$ 1.4	82.1 $\pm$ 1.2	78.4* $\pm$ 1.2	72.5 $\pm$ 1.7
5	Glibenclamide (500 $\mu$ g/kg)	83.7 $\pm$ 1.9	89.9 $\pm$ 1.6	84.3 $\pm$ 1.4	79.5* $\pm$ 1.1	71.4 $\pm$ 1.8

Values are expressed as mean  $\pm$  SEM; n=6 in duplicate for each treatment; \*statistically significant difference from the corresponding zero time value;  $p < 0.05$ ;

**Table II. Effect of different ethanolic extracts on serum glucose level (mg/dL) in normal fasted animals**

Groups	Dose	Day 0	Day 4	Day 8	Day 12
1	Control (Vehicle )	86.7 ± 1.3	83.1 ± 1.6	79.4 ± 5.2	78.5 ± 2.4
2	Flavonoid Extract (100 mg/kg)	90.1 ± 2.6	86.2 ± 1.3	83.8* ± 1.7	80.2* ± 1.2
3	Flavonoid extract (200 mg/kg)	84.2 ± 1.9	79.6 ± 2.1	75.3 ± 1.5	73.2* ± 1.1
4	Flavonoid extract (400 mg/kg)	83.1 ± 0.9	76.1 ± 1.3	74.6* ± 2.5	71.1 ± 1.6
5	Glibenclamide (500 µg/kg )	87.4 ± 1.6	79.3 ± 0.9	71.2* ± 1.2	66.2* ± 1.5

Values are expressed as mean ±SEM; n=6 in duplicate for each treatment; \*statistically significant difference from the corresponding zero time value; p<0.05

**Table III: Effect of different ethanolic extracts on serum glucose level (mg/dL) in alloxan diabetic rats**

Groups	Dose	Day 0	Day 4	Day 8	Day 12
1	Control (Vehicle )	297.2 ± 3.8	287.6 ± 3.9	268.4 ± 3.2	254.4 ± 4.2
2	Flavonoid extract (100 mg/kg)	288.3 ± 2.9	273.2 ± 3.7	257.3 ± 4.7	234.6 ± 3.6
3	Flavonoid extract (200 mg/kg)	291.6 ± 3.7	269.3* ± 4.1	253.3 ± 3.6	228.3 ± 4.3
4	Flavonoid extract (400 mg/kg)	283.2 ± 3.6	258.3* ± 3.2	230.6* ± 2.5	193.8* ± 3.6
5	Glibenclamide (500 µg/kg)	294.2 ± 4.2	259.2* ± 4.6	227.2 ± 4.1	184.2* ± 4.9

Values are expressed as mean ±SEM; n=6 in duplicate for each treatment; \*statistically significant difference from the corresponding zero time value; p<0.05

**Table IV: Effect of different flavonoid on body weight (gm) of rats**

Group s	Treatment	Initial (g)	Final (g)
1	Control (Vehicle )	171.6 ± 7.2	149.3 ± 5.3
2	Flavonoid extract (100 mg/kg)	168.4 ± 5.8	151.2 ± 9.7
3	Flavonoid extract (200 mg/kg)	162.1 ± 6.4	146.4 ± 7.9
4	Flavonoid extract (400 mg/kg)	169.2 ± 7.1	153.2 ± 5.6
5	Glibenclamide (500 µg/kg)	176.2 ± 8.2	164.5 ± 6.9

Values are expressed as mean ± SEM; n=6 in duplicate for each treatment

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