Research Article

Hypoglycemic and Hypolipidemic Activity of Scopoletin (Coumarin Derivative) in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Scopoletin, a derivative of coumarin, is a benzopyrone in nature, and present in the various plants. In the present study, scopoletin at a dose of 1mg/kg p.o. once a day and scopoletin at a dose of 1mg/kg p.o. at two times in a day was evaluated for the hypoglycemic and hypolipidemic activity in Wistar albino rats in streptozotocin induced diabetic rats. Glimepiride (0.11mg/kg) was used as reference standard for the activity comparison. Fasting blood glucose level and plasma lipid profiles were measured in diabetic and non diabetic rats at the end of the experiment. Scopoletin showed significant reduction in blood glucose level and lipid level in streptozotocin induced diabetic rats that is comparable to Glimepiride, with more promising decrease in glucose concentration observed in scopoletin two times a day than the scopoletin given once a day. The finding of the present investigation showed that the scopoletin at 1mg/kg dose is very helpful in diabetes and its complication. It showed the significant anti hyperglycemic and anti hyperlipidemic activity in Streptozotocin induced diabetic rats.

Keywords: Hypoglycemic, Hypolipidemic, Scopoletin, Streptozotocin.

INTRODUCTION

Diabetes, as we all know, is a very old disease prevailing among mankind. Traces of Indian diabetes mellitus can be found from the age of Charak (400 B.C. *CharakSamhita)* and Shushruta (200 B.C. *ShushmtaSamhita),* where diabetes is called as "*Madhu-Meha".*[1] Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels, which result from defects in insulin secretion, or action, or both.[2] Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide. The countries with the largest number of diabetes people in the year 2025 will be India, China, and United States. There are more than 30 million people with diabetes mellitus in India and the incidence is increasing.[3] As a matter of fact there are different types of Diabetes Mellitus. Out of all the types, Type-2 is most common and the data says it accounts for 85% of all incidences worldwide. It is the major cause of end stage renal disease, non-traumatic lower limb amputation, adult blindness and profound cardiovascular morbidity and mortality.[4] Traditional systems of medicines always played important role in meeting the global health care needs.[5] It's a proven historical fact that, mankind has been using herbs for the treatment of diabetes as they possess less cost, strength and effectiveness, better tolerance, more safety, less side- effects, ready availability, and ecofriendly in nature.[6] Now scientists are working to discover a more effective and safe hypoglycemic agent using the plants constituents.[7] Therefore the present work have been planned to evaluate the hypoglycemic activity of scopoletin.

Scopoletin, a derivative of coumarin, is a benzopyrone in nature, and found in the root of plants in the genus *Scopolia* like *Scopoliacarniolica* or *Scopolia japonica*, in chicory, in *Artemisia scoparia*, in the passion flower, in *Brunfelsia*, in *Viburnum prunifolium* or *Kleinhovia hospita,* Noni, manaca, stevia, Agle marmelos etc. Chemical name is 7-Hydroxy-6-methoxycoumarin.[8,9] Scopoletin possesses antioxidant property, scavenged superoxide anion in the xanthine/xanthine oxidase reaction system in a concentration-dependent manner.[10] Scopoletin obtaind from fruits of Tetrapleura tetraptera (Mimosaceae),has hypotensive effect.[11] It also shows antidepressant activity, angiogenic activity and antifungal activity.[12-14] Scopoletin is coumarin derivative and coumarin is reported for hypoglycemic activity.[15]

Considering the above facts, the present study was undertaken to evaluate the anti hyperglycemic and anti hyperlipidemic activity of scopoletin in streptozotocin induced diabetic rats.

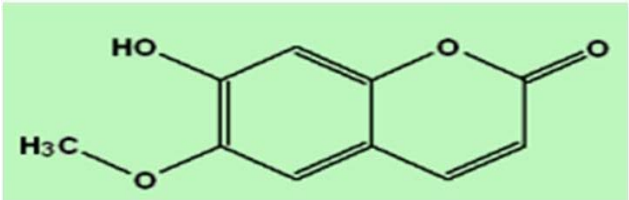


Figure 1: Structure of Scopoletin

MATERIALS AND METHODS

Chemicals

STZ and nicotinic acid (Himedia laboratories Pvt. Ltd. Mumbai)**,** Glimepiride (Alkem Pvt., Mumbai), Scopoletin S2500 (Sigma Aldrich USA), EDTA **(**LR, Qualigens), D-glucose Anhydrous and Citric acid anhydrous (Nice chemicals Pvt. Ltd. Cochin), Sodium citrate 2-hydrate **(**Merk Specialities Pvt. Ltd. Mumbai).

Experimental animals

Selected 30 numbers of adult male Wistar albino rats were housed in animal house (Regd. No. 926/ab/06/CPSCSEA, 22-02-2006) of Roland Institute of Pharmaceutical Sciences, Berhampur. The animals were kept in polystyrene cages under standard laboratory conditions i.e. at temperature of 25±1°C, relative humidity of 60±2% and were exposed to a 12h photoperiod and fed on normal chaw pellet (Rayan's biotechnologies Pvt. Ltd, Hyderabad, A.P.

Induction of diabetes and method of blood collection

Type II diabetes mellitus was induced in overnight fasted rats by a single intraperitonial injection of 120 mg/kg of NA dissolved in normal saline followed by freshly prepared STZ 50 mg/kg in 0.1M citrate buffer (pH 4.5) intravenously 15 min afterwards.[16,17] After 3 days, blood was collected from sublingual vein of overnight fasted rats. Fasting blood glucose level was measured in the autoanalyser (3000 Evolution, BSI Italy) using commercially available biochemical kits. The threshold value of the fasting plasma glucose to diagnose diabetes was taken as >200 mg/dl. Only those rats that were found to have plasma glucose level >200 mg/dl were used in the study.

Experimental design

In the experiment, a total of 30 rats were used in the overall experiment. 6 rats were kept in a group and 5 such groups were made. Total time of the experiment, after inducing the STZ diabetes was 6 weeks. Group I: non-diabetic rats treated with normal saline. Group II: diabetic control (STZ+ NA) treated with normal saline. Group III and IV: were diabetic rats treated with Scopoletin (1 mg/kg)[18] once a day and Scopoletin (1 mg/kg) two times a day. Group V: diabetic rats treated with 0.11mg/kg of Glimepiride.[19] At the end of experiment, pancreas, liver and kidney were dissected out for histopathological studies.

Collection of blood and determination of plasma glucose and other biological parameters

Animals were fasted overnight. Blood (0.5 ml) was withdrawn from the sublingual vein under ether anesthesia and was collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ ml of blood). The micro tubes were centrifuged at 4000 rpm at 4°C for 20 min to obtain clear plasma. The plasma was then analyzed for glucose, triglyceride, and total cholesterol in the autoanalyser (3000 Evolution, BSI Italy) using commercially available biochemical kits.

Oral glucose tolerance test

The rats were kept on fasting overnight for performing the OGTT. The rats were however allowed to drink water and the treatment groups were administered the respective drugs at the regular time. Glucose (2 g/kg) was fed to each animal 10 min after collecting blood at 0 min. 0.1 ml blood was withdrawn from the sublingual vein under ether anesthesia at 0min, 30min, 60 min and 120 min and was collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ ml of blood). The micro tubes were centrifuged at 4000 rpm at 4°C for 20 min to obtain clear plasma. The plasma was then analyzed for glucose through the above mentioned method.[20]

Histopathological Study of rat's pancreas

At last, all the rats of the different groups, which survived, were sacrificed by cervical dislocation method. After that pancreas of the rats of different groups were removed and treated with the ice-cool saline immediately. The pancreases were fixed in 10% of neutral formalin. The sections of 3-5 mm thickness were stained with hematoxylin and eosin for histopathological studies.[21]

Statistical analysis

All values were expressed mean ± standard error mean. Statistical analysis of *in vivo* results were performed by one-way analysis of variance (ANOVA) followed by Dunnet t-test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of scopoletin on body weight

The pharmacological investigation on the basis of body weight was performed on streptozotocin-induced diabetic rats and non-diabetic rats. The body weight was slightly increased in normal control rats compared to initial body weight whereas streptozotocin-induced diabetic rats showed loss of body weight (135 ± 2.88g) after 6 weeks as compared with initially weight of diabetic rats (177.5 ± 2.13g). However, body weight of diabetic rats was restored by treating with Glimepiride (0.11 mg/kg) and scopoletin (at a dose of 1 mg/kg OD and 1 mg/kg BD, p.o.) shown in table 1.

Table 1: Body weights of streptozotocin-induced diabetic rats after treatment with Scopoletin

	Initial body wt	Final body wt
Normal control	180.33 ± 0.75	190 ±1.82
Diabetic control	177.5 ± 2.13	135 ± 2.88
Scopoletin (OD)	181.67 ± 2.1	175.83 ± 2.38**
Scopoletin (BD)	186.67 ± 3.33	182.5 ± 2.13**
Glimepiride	192.5 ± 2.13	187.5 ± 2.81**

Values are expressed as mean ± SEM, n=6 Statistical analyses were carried out by Dunnet*t*-test. **P< 0.01 as compared to diabetic control group.

Effect of scopoletin on plasma glucose, TC and TG level

The biochemical parameters were measured for plasma blood glucose level, total cholesterol and total triglyceride level. Streptozotocin treated rats resulted in elevation of fasting blood glucose, triglycerides and total cholesterol levels as compared to the normal control rats as noted at end of the study. When diabetic rats treated with scopoletin at a dose of 1mg/kg OD and 1 mg/kg BD, p.o for 6 weeks showed significant (P <0.01) reduction in

fasting blood sugar levels (208.5 ± 2.03 mg/dl and 166.5 ± 1.25 mg/dl respectively). However, the standard drug glimepiride (0.11mg/kg, p.o) exhibited potent anti-diabetic activity with maximum reduction of fasting blood sugar level (142.83 ± 1.4 mg/dl) on 6 weeks as compared to the diabetic control. There was a significant (*P* <0.01) reduction in triglycerides and total cholesterol levels of diabetic rats treated with scopoletin (1mg/kg OD and 1mg/kg BD, p.o) as compared to diabetic controland shown in table 2.

Table 2: Effect of Scopoletin on FBS, TC and TG in normal and diabetic rats for 6 weeks

BBP	Days	Group 1	Group 2	Group 3	Group 4	Group 5
FBS (mg/dl)	0 week	88.6±2.77	256.0±1.75	240.5±3.22	234.3±2.84	249.5±2.01
	6 week	95.17±2.24	296.5±2.6##	208.5±2.03**	166.5±1.25 **	142.83±1.4**
TC (mg/dl)	0 week	103.5±0.4	169.9±0.7	161.89±1.51	159.1±2.86	153.33±1.58
	6 week	100.5±0.88	175.3±0.75##	118.3±0.98**	105.0±2.86**	98.2±1.58**
TG (mg/dl)	0 week	87.5±0.99	145.6±0.8	141.3±0.98	143.5±0.75	138.5±0.84
	6 week	98.7±1.55	188.7±2.0##	130.4±1.5**	114.0±1.46**	102.3±0.59**

Values are expressed as mean ± SEM. n=6/group. Group-I served as normal control. Group-II served as diabetic control. Group III received 1mg/kg p.o scopoletin (OD). Group IV received 1mg/kg p.o scopoletin (BD). Group V received 0.11mg/kg p.o glimepiride. BBP-Biochemical parameters, FBS-fasting blood sugar, TC-total cholesterol, TG-triglycerides. ##P <0.01, non-diabetic group vs. diabetic group. **P <0.01, treated diabetic groups vs. diabetic control group.

Table 3: Effect of Scopoletin in oral glucose tolerance test

	0min	30 min	60min	120 min
Group I	85.83±0.7	143.5±0.62	134.16±0.74	120±0.57
Group II	286.5±0.76##	345.17±1.07##	323.5±1.02##	317.83±0.7 ##
Group III	199.5±3.96 **	287±5.08 **	266±3.71**	224±4.16 **
Group IV	167.33±0.84 **	253.66±0.88 **	235.5±0.56**	190.16±0.94 **
Group V	142.33±3.7 **	197.3±5.9 **	177.5±6.25**	155±6.36 **

Values are expressed as mean ± SEM, n=6 Statistical analysis was carried out by Dunnet *t*-test. **P< 0.01 as compared to diabetic control group. ## p<0.01 as compared with non-diabetic group.

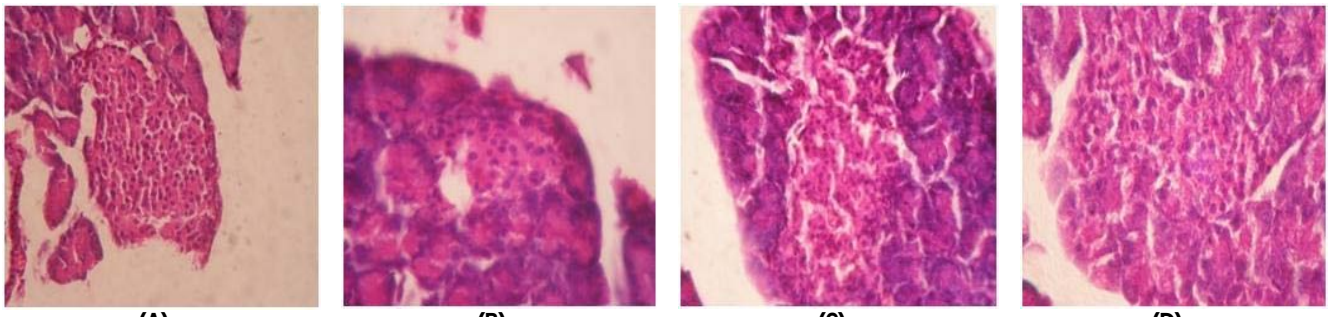
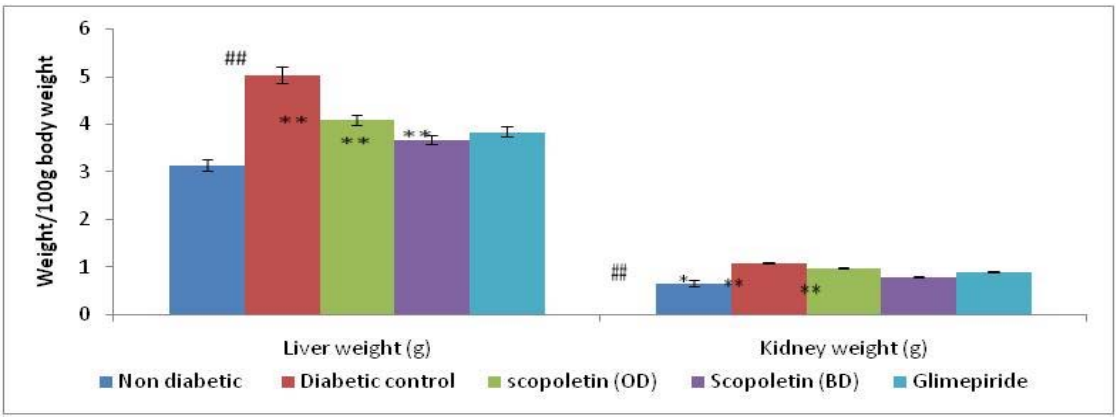


Figure 2: Photomicrographs rat pancreas stained by haematoxylin and eosin (magnification 400x) of untreated diabetic rats(A), STZ induced diabetic rats (B) , Scopoletin (BD) treated rats (C) and Glimepiride treated rats (D).



Values are expressed as mean ± SEM, n=6 Statistical analysis was carried out by Dunnett-test. *P< 0.05 as compared to diabetic control group, **P< 0.01 as compared to diabetic control group. ## p<0.01 as compared with non-diabetic group.

Figure 3: Effect of scopoletin on rat's liver and kidney

Effect of scopoletin on oral glucose tolerance test (OGTT)

In oral glucose tolerance test a significant reduction in blood glucose level (compared with initial level) was also observed with scopoletin 1 mg/kg OD and 1 mg/kg BD with the maximum fall of (190.16 ± 0.94mg/dl) reduction in blood glucose concentration at the end of 120min with scopoletin 1mg/kg BD . On the other hand, scopoletin 1mg/kg OD showed (224 ± 4.16mg/dl) reduction in blood glucose concentration at 120 min after glucose administration. The standard drug Glimepiride showed maximum lowering glucose concentration of (155 ± 6.36mg/dl) at the end of 120 min. Shown in table 3.

Histopathology of Pancreas

In histopathological study the non- diabetic rats were characterized by normal acini, and normal cellular population in the islets of langerhans in pancreas. Finally, a comparison was made between diabetic group and the non- diabetic group and it was found that islets tissue of diabetic rats were characterized by disrupted boundaries and abnormal distribution of cytoplasm, it also shows impaired structure and reduction in number of cells. Scopoletin (BD) showed slight regeneration of β-cells was seen when compared with diabetic control. Glimepiride showed restoration of normal cellular population of islets and shown in figure 2.

Effect of Scopoletin on organ weight in streptozotocin induced diabetic rats

Figure 3 shows the liver and kidney weight in STZ induced diabetic rats. It was observed that the weight of liver and kidney in proportion to the body weight was more in diabetic rats as compared to the non-diabetic rats. Enlargement of liver might be due to increased accumulation of triglyceride. Renal hypertrophy might be due to an increase protein synthesis or decrease in the degradation of renal extracellular matrix components.

Various experimental papers suggest that scopoletin possesses an ability to reduce the blood glucose level on various diabetic models but no one chooses the streptozotocin induced diabetic model. The present study was carried out to explore the effect of scopoletin on blood glucose and plasma lipid profile on streptozotocin-induced diabetic rats. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues.[22] In the present study, an increase in blood sugar levels in diabetic rats was observed after the induction of diabetes by streptozotocin. This was prevented by treating diabetic rats with scopoletin (at a dose of 1 mg/kg OD and 1 mg/kg BD, p.o) for 6 weeks. The standard drug Glimepiride has been used to treat diabetes, which stimulate insulin secretion from pancreatic beta cells, it may be suggested that the mechanism of action of scopoletin is similar to Glimepiride.

CONCLUSION

The results provided a scientific validation of the scopoletin and suggested that the scopoletin has promising therapeutic activity for the maintenance of diabetes mellitus.

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