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Isolation, Identification and Anti-Diabetic Activity of Isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-Rhamnoside from *Solanum torvum*

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Abstract: ***To isolate a flavonoid glycoside fromflowers of Solanum torvum using chromatography separation techniques, spectralcharacterization and its anti-diabetic activity.The structure of the isolated compound was purified, analyzed and characterized by chemical tests, HPLC and spectroscopic methods such as UV, IR, ^{1}H-NMR and ^{13}C-NMR.HPLC analysis exhibited the presence of a flavonol glycoside and it's identified peak with the retention time of 26.8 min and showed two major peaks at 340 nm (band-I) and 260 nm (band-II). The flowers of Solanum torvum have been found to contain isorhamnetin-3-O-α-L-(3′′-E-p-coumaroyl)-rhamnoside.The blood sugar level was measured by digital display glucometer. The highest activity of Solanum torvum flower extract in this experiment was observed at the dose of 200 mg / kg of isolated compound while the reference drug glibenclamide (10 mg/kg) had a greater activity when associated with Solanum torvum flower extract.***

Keywords: Anti-diabetic activity, isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside, *Solanum torvum*, HPLC

1. Introduction

Plant materials are used all over developed and developing kingdoms as home remedies, over-the-counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the worldwide drug market. Natural products from plants provide a source for bioactive compounds and have the possible for developing some novel therapeutic agents. There has been an ever growing interest of drugs originating from plants which have been found to form asignificant class for disease control[1].Nowadays, the use of phytochemicals for pharmaceutical resolution has gradually increased in many countries. Phytochemicals isolated from plant sources are used for the anticipation and treatment of cancer, heart disease, diabetes mellitus and high blood pressure[2].

Flavonoids are one of secondary metabolites created by plants[3]. They play many dynamic roles such as pigmentation, pathogen resistance, UV light protection, growth and development in plants[4]. From a human point of view, flavonoids can give protection against many diseases because of their anti-oxidant activities. Flavonoids and phenolic acids have protective part in carcinogenesis, inflammation, atherosclerosis, thrombosis and have high anti-oxidant capacity. Furthermore, flavonoids have been reported as aldose reductase inhibitors blocking the sorbitol pathway that is linked to many problems associated with diabetes[5].

Diabetes Mellitus is a common and very prevalent disease affecting the citizens of both developed and developing nations. Diabetes is a chronic disorder in metabolism of carbohydrates, proteins, and fat due to complete or comparative deficiency of insulin secretion with/without varying degree of insulin resistance[6]. Effective blood glucose control is the key for preventing diabetic problems and improving quality of life in patients with diabetes[7]. Insulin is a key performer in the control of glucose homeostasis. Deficiency of insulin affects carbohydrate, fat and protein metabolism[8]. Therefore, searching for effective, low cost and less side effected hypoglycemic agents is significant.Based on this aspect, we have selected the plant*Solanum torvum* for treatment of diabetes mellitus.

The plant *Solanum torvum* belongs to the family Solanaceae. *Solanum torvum* commonly known sundaikkai in Tamil, is cultivated for its fruits which are used as an essential ingredient in South Indian population's diet. The genus *Solanum* is a hyper-diverse taxon of this family. There are about 2000 species of *Solanum* in the world that are mostly distributed in the tropical and sub-tropical areas, with a small number in the moderate areas[9]. Among the major chemical constituents of *Solanum torvum* are steroids, steroid saponins, steroid alkaloids, and phenols. A decoction of fruits is used in treatment of cough ailments and is also considered useful in cases of liver and spleen enlargement[10]. Pharmacological studies indicate that the stem and root of *Solanum torvum* have anti- tumour, anti-bacterial, anti-viral, anti-inflammatory and other medicinally important effects[11].

In the current scenario most of modern drugs have been isolated from natural sources such as medicinal plants comprising a wide range of chemical compounds that serves as a leads for enlargement of novel anti-diabetic agents. This work mainly efforts on the role of the biomolecules from Indian traditional medicinal plants with anti-diabetic potential with various chemical structures. The present study was undertaken to screen the phytochemical constituents of *Solanum torvum* flowers for its anti-diabetic activities of the extract on normal rats and the effect on blood glucose level.

2. Materials and Methods

Collection of plant material

The fresh flowers of *Solanum torvum*were collected in the month of November - January from the area of Cauvery river basin, Thanjavur, Tamilnadu, India. These Plants were identified and authenticated by Dr. S. Sambathkumar, Assistant Professor, Department of Botany, Government Arts College, Thiruvannamalai, Tamilnadu, India. The voucher specimen (GACBOT-189)was maintained in our research laboratory for future reference. The collected fresh flower materials were washed properly and dried in shade. Dried plant material was subjected to reduction to coarse powdered and stored in airtight container for further use.

Isolation and Identification

The important stage in the experimental work includes first the isolation of chemical substances from the selected plant and secondly, the characterization of those isolated compounds. The flowers *Solanum torvum* (2.5 Kg) were extracted with 90% methanol (MeOH) (6 X 500ml) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was fractionated with peroxide free ether (5 x 250 ml) and ethyl acetate (8 x 250 ml) (Sigma Aldrich Co., India).

The residue from ethyl acetate fragment was taken up in acetone and left in an ice-chest for two days when a yellow solid separated. It prominent a greenish brown colour with alc. Fe^{3+}, formed yellow precipitate with basic lead acetate solution and reduced ammoniacal $AgNO_3$ but not Fehling's solution. It contributed yellow colour with aqueous NaOH intense yellow with Con.H_2SO_4 and magenta colour with Mg/HCl. It appeared deep purple under UV which turned yellow and disclosure to NH_3. It retorted to Wilson's Boric Acid, Gibbs and Molisch's tests but not answer to Horhammer- Hansel tests.

Supporting evidence for the structure of the flavone glycoside is provided by the HPLC (Shimadzu, Columbia), UV (Perkin Elmer Spectrophotometer), IR (Perkin - Elmer spectrometer) and NMR (125 MHz and 500 MHz, $CDCl_3$) spectral data were recorded on a Bruker AMX NMR spectrometer. Chemical shifts were reference to the equivalent residual solvent peaks and the values were recorded in δ.

Isorhamnetin-3-O-α-L-(3″-E-*p*-coumaroyl)-rhamnoside

Yellow amorphous solid; m.p. 198-200°C; RT 26.8 min; UV λ_{max}^{MeOH} (log ε) 260 - 340 nm; IR (KBr): ν_{max} 3974, 3421, 2929, 2106, 1640, 1415, 1244, 1059, 923, 862, 818, 778 and 715 cm^{-1}; ^1H (500 MHz, $CDCl_3$)δ ppm: 7.94 (H-6), 7.83 (H-8), 7.82 (H-2′), 7.79 (H- 6′), 7.81 (H-5′), 5.85 (H-1″), 5.84 (H-2″), 5.487 (H-3″), 5.484 (H-4″), 4.02 (H-5″), 0.76 (H-6″), 7.64 (H-2‴), 7.628 (H-3‴), 7.622 (H-5‴), 7.64 (H-6‴), 7.620 (H-7‴), 7.631 (H-8‴), 11.52 (5-OH), 8.016 (3-OH), 8.015 (7-OH), 7.99 (4′-OH);^{13}C-NMR (125 MHz, $CDCl_3$)δ ppm: 146.9 (C-2), 132.5 (C-3), 180.0 (C-4), 150.3 (C-5), 116.4 (C-6), 164.3 (C-7), 89.7 (C-8), 148.6 (C-9), 121.7 (C-10), 121.8 (C-1′), 124.6 (C-2′), 124.4 (C-3′), 124.9 (C-4′), 127.7 (C-5′), 128.2 (C-6′), 116.4 (C-1″), 76.8 (C-2″), 76.6 (C-3″), 77.3 (C-4″), 69.0 (C-5″), 19.7 (C-6″), 128.3 (C-1‴), 128.7 (C-2‴), 128.8 (C-3‴), 145.9 (C-4‴), 130.2 (C-5‴), 131.4 (C-6‴), 131.9 (C-7‴), 143.4 (C-8‴), 146.8 (C-9‴).

Hydrolysis of the glycoside

The glycoside liquefied in hot aqueous methanol was hydrolyzed with H_2SO_4 (5%) at 100°C for around 2 hrs. The excess of alcohol was distilled off *in vacuo* and the consequent aqueous solution was extracted with ether. The residue from ether fraction was isolated as nominated below. The glycoside was exposed to partial hydrolysis by treatment with 10% formic acid in cyclohexane and the resultant solution extracted with ethyl acetate.

Phytochemical screening of plant extract

A small amount of the dry extract was used for the phytochemical tests[12]for compounds which contain flavonoids, carbohydrates, glycosides, proteins and tannins.

Animals

Male Wistar rats weighing 200 to 250 g were used for study and were kept in animal house at 24 ± 2°C with relative humidity 44 - 56 % along with light and dark cycles of 12 h respectively. Animals were provided with standard diet and water ad libitum. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of IAEC and experimental protocol was approved by Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No. BDU/IAEC/2011/31/29.03.2011).

Experimental Design

The animals were divided into six groups each containing six animals.

Group-I: Served as normal control. Control rats received only normal saline.

Group-II: The Second group of rats with diabetes was induced by intraperitoneal injection of alloxan for 2 days.

Group-III: Alloxan treated rats were administered the Glibenclamide (10 mg / kg) and served as standard.

Group-IV: Alloxan treated rats were administered the Isorhamnetinglycosidedrug from extract of *Solanum torvum*(100 mg / kg)

Group-V: Alloxan treated rats were administered the Isorhamnetinglycosidedrug from extract of *Solanum torvum*(200 mg / kg)

Group-VI: Alloxan treated rats were administered the methanolic extract of *Solanum torvum*(300 mg / kg)

After the treatment period all the groups of rats were euthanized by anesthesia using chloroform vapor and the rats were sacrificed by decapitation. Then the blood was collected in a tube for analysis.

Acute Toxicity Studies

Acute toxicity studies were conceded out according to the literature[13]. Animals of either sex were fasted for eighteen hours and used. A dose of 200 mg/kg of Isorhamnetinglycosidefrom extract of *Solanum torvum* were administrated orally to 12 mice, additionally three mice were kept as control. The control group received distilled water.

Then they were absorbed for 72 hours. Since no mortality was absorbed and the behavioral pattern was unaffected. No depth was absorbed at the end of the study.

Evaluation of Anti-Diabetic Activity

Before starting the experiment, animals were separated according to their body weight. The animals were injected intraperitoneally with freshly prepared alloxan monohydrate (150 mg / kg) in normal saline solution. Alloxan administration resulted in significant elevation of glucose level and reduction in body weight. Diabetes was confirmed by the elevated blood glucose levels determined at 72 h. The blood sugar level was measured by digital display glucometer (One touch - Johnson & Johnson Ltd.). Initial blood sample were taken before the oral administration of the standard drug glibenclamide, isolated compound at doses100, 200 mg/kg and*Solanum torvum*methanol extracts. The blood glucose level test was done on the normal, diabetic and treated diabetic rats were measured at 0, 4, 8 and 12 days after oral administration of glibenclamide and different concentration of isolated compound isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside.

Biochemical Analysis

After blood glucose estimation on day 12, whole blood samples were drawn from the tail vein during the course of the experiment. At the end of the experimental period (12 days), the rats were anesthetized with chloroform following a 12-hour fast. Blood samples were drawn by cardiac puncture into plain tubes. The blood samples were centrifuged at 3500 rpm for 20 minutes using a refrigerated centrifuge at 4°C (Remi Laboratory Instruments, Mumbai, India). The serum collected was stored at −20°C until needed. Serum albumin was determined using the bromcresol green method with an Autopak kit. The total protein present in serum was estimated by the Biuret method[14]using an Autopak kit. Globulin levels were calculated from total protein and albumin measurements. Serum was separated and analyzed for serum cholesterol[15], serum triglycerides by enzymatic DHBS colorimetric method[16], serum HDL[17], serum LDL[18], serum creatinine[19], serum urea[20] and levels of hemoglobin using the ion exchange resin method[21]with kits purchased from Diotek India Ltd, Mumbai, India. To the animals, standard drug glibenclamide tablets (10 mg / kg orally) and the test isolated compound (100 and 200 mg / kg orally) were administered by dissolving in 2% Twen-80/water and normal saline respectively.

Statistical analysis

The experimental results has expressed as statistical comparisons of Mean ± SEM carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test. P values less than 0.05 has considered as statistically significant.

3. Results and Discussion

Chemical Constituents

Compound was obtained as a yellow amorphous solid (m.p: 198-200^{o}C), which also gave positive color reactions for a hydroxyl flavone with several reagents[22]. IR spectrum showed the presence of hydroxyls (3421 cm^{-1}), carbonyl (1640 cm^{-1}), the aromatic ring (1415 cm^{-1}) and meanwhile strong absorption at 260 and 340 nm was observed in UV spectrum[23]. HPLC analysis exhibited the presence of a flavone glycoside. The identified peak with the retention time of 26.8 min (Figure 1).The ^{1}HNMR spectrum exhibited five aromatic protons signals appearing at (δ 7.947, H-6; δ7.839, H-8; δ 7.822, H-2'; δ 7.811, H-5'; δ 7.794, H-6') are typical of an AX system in B ring and their parallel carbon signals seem at δ116.4, 89.7, 124.6, 127.7, 128.2 respectively. Also methoxy protons signal was existing at δ 3.957 ppm (3H, s) which showed with the carbon resonance at δ 124.4 (C-3'). These statistics clearly definite the characteristic pattern of isorhamnetin as aglycone[24]. In addition, an anomeric rhamnose proton has predictable in this spectrum as a doublet at δ 5.85 ppm and also respect to the question of α- or β-linkage of the sugar moieties, it has been found the coupling constant *J* =2.0 Hz corresponded to the anomeric proton of α - linked rhamnose[25]. The methyl protons of the sugar rhamnose look at δ 0.763 ppm which is therefore allocated to a 6-deoxy sugar (rhamnose) and rest of the sugar protons seem in the range δ 4.022 - 5.846 ppm.The ^{1}H-NMR spectrum of the compoundalso presented signals attributed to sugar moieties and a *p*-coumaroyl residue. The arrangements of the sugar units were allocated after hydrolysis of compoundcompared to those of reliable sugar samples. The lower field shifts of H-6''' (δ 7.642) of one glycosyl unit optional the substitution site of the *p*-coumaroyl unit. Also the signals at 7.620 and 7.631 ppmconsigned to transolefinic protons suggested the presence of p-coumaric acid as the acyl moiety. In the ^{13}CNMR spectrum, the signal on 180.0 ppm (C=O) sustained this proposal.The ^{13}C NMR spectrum contained 31 carbon signals, 15 of them gave to the flavonol aglycone and one was methoxy carbon signal shown the isorhamnetin and remaining 15 signals has ascribed to sugar rhamnose with addition of *p*-coumaroyl unit. The sugar moiety has proved to be acylated at C-3 of the aglycone as deduced from the anomeric proton at δ_H 5.85 and δ_C 132.5 ppm, which were in close arrangement to achieved the isolated compound was isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside(Figure 2).

Anti-diabetic activity

The anti-diabetic effect of isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside on the blood glucose levels of diabetic rats is shown in Table 1. Twelve days of daily treatment of different test compounds of *Solanum torvum* led to a dose-dependent fall in blood sugar levels by 36-65%. Effect seems to reach maximum after 12th day of treatment and leftovers constant their after. From the results it is exposed that the test isolated compound isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside from *Solanum torvum* at a dose level 100 and 200 mg / kg, exhibited significant reduction in blood sugar level from 1 to 8th day in progressive manner comparable to standard glibenclamide. There is no substantial change in the blood glucose levels of rats in group I that received Tween 80 solutions (negative control). This decreased the blood glucose levels by 97.87±3.72, 96.98±3.74and 95.63±2.63respectively at the 12th day. The highest activity of *Solanum torvum* flower extract in this experiment was observed at the dose of 200

mg / kg of isolated compound while the reference drug glibenclamide (10 mg/kg) had a superior activity when compared with *Solanum torvum* flower extract.

Table 2 displayed the body weight changes in the normal and experimental animals in each group. The mean body weight of the diabetic rats decreased related to diabetic treated rats. There was a substantial reduction in body weight of the diabetic rats compared with normal and diabetic treated rats. The administration of *Solanum torvum* restored these levels significantly towards normal. Diabetic rats treated with the methanol extract indicated an increase in body weight compared to diabetic control. This may also be due to the shielding effect of the extract in controlling muscle wasting of gluconeogenesis. The mean body weight of the animals after 12 days of treatment with methanol extract, isolated compound (100 and 200 mg / kg) and glibenclamide was 140.87±5.58, 136.00±0.58, 144.33±3.51 and 146.67±3.79 mg/kg respectively.

Biochemical estimation

The biochemical parameters (Table 3) such as serum cholesterol, serum triglycerides, serum LDL, serum creatinine and serum urea levels were reduced significantly by glibenclamide and HDL levels were improved by glibenclamide, test compounds and methanol extracts. Administration of isolated compound isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside at dose of 200 mg / kg from *Solanum torvum*flower to diabetic rats for 12 days resulted in the restoration of total protein, urea and creatinine levels towards near normal as in glibenclamide treated diabetic rats. Administration of isolated compound at dose of 200 mg / kg from *Solanum torvum*flower to diabetic rats resulted in the restoration of hemoglobin level to near normal. There was a substantial increase in total hemoglobin in the diabetic control rats (12.37±0.05). After treatment with the methanol extract and the isolated compound at a dose of 100 mg / kg, the animals indicated a decrease in hemoglobin levels to 12.44±0.02 and 12.91±0.35respectively. The diabetic rats also treated with glibenclamide indicated a restoration in hemoglobin levels. In rats treated with isolated compound at a dose of 200 mg / kg, hemoglobin levels were found to be in the near normal range. There was a substantial increase in serum albumin, globulin, and total protein content in the treated diabetic rats compared with the diabetic controls.

Diabetic hyperglycemia produces elevation of urea and creatinine which are considered to be important markers of renal dysfunction[26]. On administering isolated compounds at doses 100, 200 mg / kg and methanol extracts for 12 days, serum urea and creatinine steadily resumed to near normal. Administering 200 mg / kg test isolated compound orally to diabetic rats decreased serum urea and creatinine, more efficiently and could be explained by the recreating ability of the renal function. The observed increase in creatinine level in diabetic rats is mainly due to renal dysfunction and is changed to near normal by oral administration of isolated test compound from *Solanum torvum*flowers for 12 days. The present research showed the level of lipids in normal and tested animals, there is an increase in serum lipoproteins (total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein). There was a significant decrease in the level of HDL-cholesterol and a substantial increase in the levels of total cholesterol and triglycerides.

4. Conclusion

The presence of isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside in ethyl acetate extracts of *Solanum torvum*flowers has reported for the first time. Our research clearly shows that *Solanum torvum*caused noticeable hypoglycemic activity in alloxan induced diabetic rat model which indicates anti-diabetic potentials of the methanol extract and test isolated compounds. The compound isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside isolated from *Solanum torvum*flowers tested for anti-diabetic activity as it suggestively lowered the serum glucose levels and increased the body weight of diabetic rats. In this experiment, the dose of 200 mg / kg produced the maximum anti-diabetic effect and this may suggest that this dose may be the real anti-diabetic drug even though there strength have some limitation due to the crude nature of the extract.

5. Conflict of Interest

The authors declare no conflict of interest.

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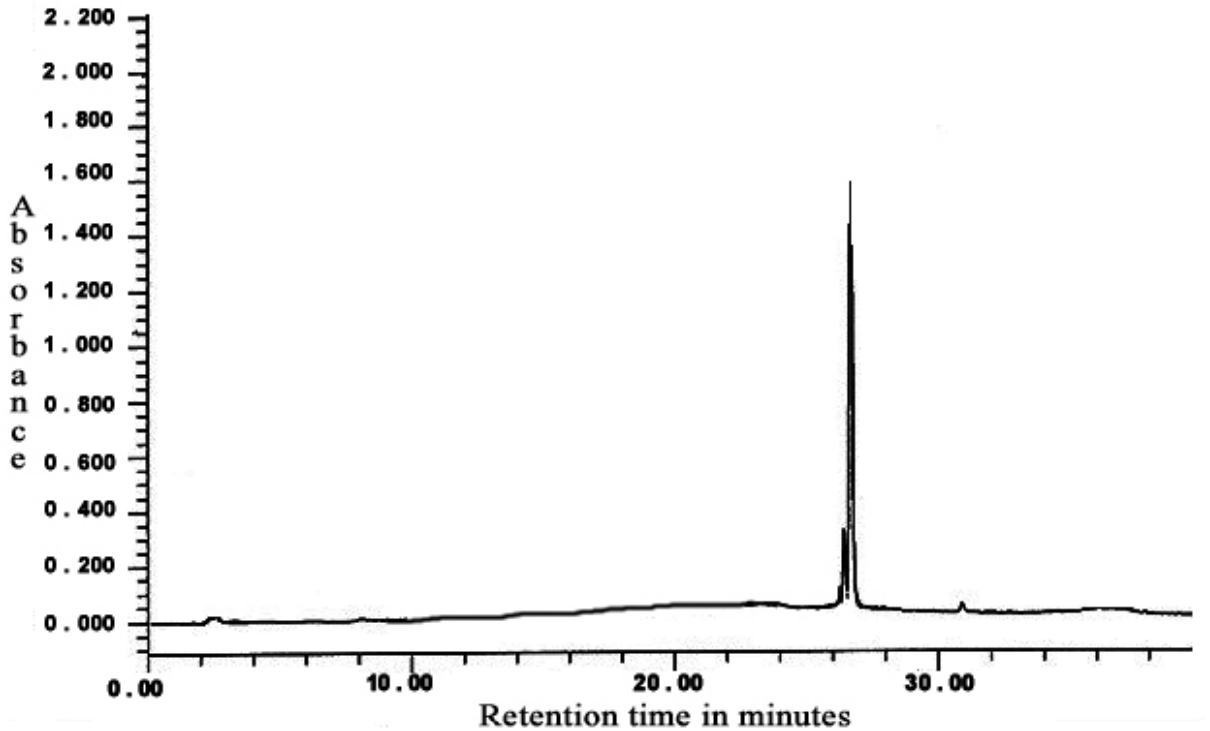

Figure 1:HPLC of Isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside

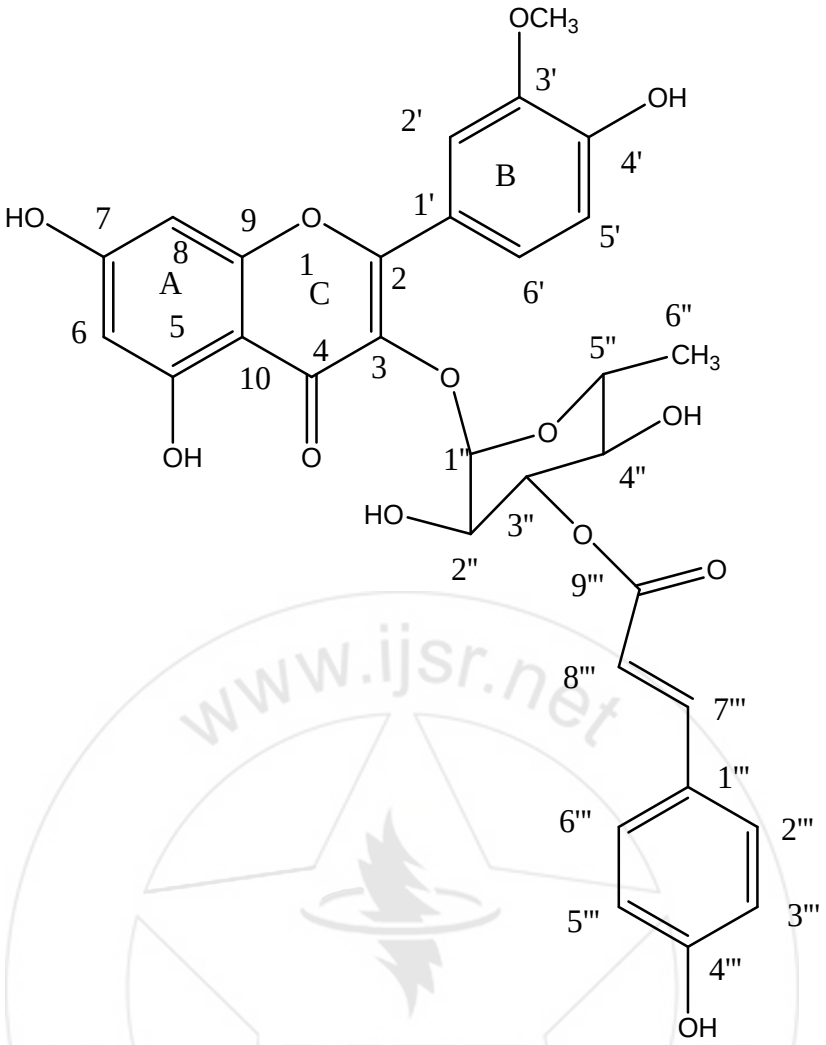

Figure 2: Structure of Isorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside

Table 1: Effect ofisorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside isolated from *Solanum torvum*on fasting blood glucose level in alloxan induced diabetic rats

Group	Treatment	Fasting blood glucose level (mg/dl)			
		Initial	4th day	8th day	12th day
I	Normal Control	98.57±8.60	97.87±3.72	96.98±3.74	95.63±2.63
II	Diabetic Control	378.67±5.17	391.67±2.52	398.67±1.06	402.33±1.50
III	Standard (Alloxan + glibenclamide 10 mg/kg)	366.30±4.04	275.42±2.52	237.24±4.04	146.05±4.58
IV	Alloxan + Isorhamnetin Drug (100 mg/kg)	380.14±1.01	269.81±2.05	178.06±1.08	142.09±3.42
V	Alloxan + Isorhamnetin Drug (200 mg/kg)	375.23±1.07	237.05±2.02	156.07±3.05	124.02±1.07
VI	Alloxan + Methanolic Extract (300 mg/kg)	379.21±2.02	283.01±1.72	184.26±3.08	146.09±1.07

Values are expressed in Mean ± Standard Deviation (n=6)
Superscript letters represent $P<0.05$(Duncan test). Group II compared with Group III, IV and V. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 2: Effect ofisorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside isolated from *Solanum torvum*on body weight in alloxan induced diabetic rats

Group	Treatment	Body weight of the animal (g)			
		Initial	4th day	8th day	12th day
I	Normal Control	153.67±1.53	148.33±0.58	144.67±2.08	146.67±3.21
II	Diabetic Control (Alloxan)	155.00±1.00	136.67±3.21	126.67±0.58	123.33±1.53
III	Standard (Alloxan + glibenclamide 10 mg/kg)	156.33±1.53	138.67±1.00	137.33±3.21	136.00±0.58
IV	Alloxan +Isorhamnetin Drug(100 mg/kg)	154.67±0.58	145.00±2.65	133.33±1.00	144.33±3.51
V	Alloxan + IsorhamnetinDrug(200 mg/kg)	155.67±5.58	143.33±1.00	146.33±0.58	146.67±3.79
VI	Alloxan + Methanolic Extract (300 mg/kg)	153.98±1.53	138.61±2.54	135.48±3.02	140.87±5.58

Values are expressed in Mean ± Standard Deviation (n=6)

Superscript letters represent *P*<0.05(Duncan test). Group II compared with Group III, IV and V. **P*<0.05, ***P*<0.01, *** *P*<0.001.

Table 3: Effect ofisorhamnetin-3-O-α-L-(3′′-E-*p*-coumaroyl)-rhamnoside isolated from *Solanum torvum*on 12th day on biochemical parameters in alloxan induced diabetic rats (mg/dl)

S. No.	Biochemical parameter	First Day	12th day (M±SD)					
			Treatment					
			I	II	III	IV	V	VI
1	Haemoglobulin (g/dl)	14.83±0.02	15.06±0.10	12.37±0.05	14.28±0.39	12.91±0.35	13.90±0.40	12.44±0.02
2	Albumin (g/dl)	3.87±0.05	3.83±0.07	3.15±0.05	3.89±0.07	3.02±0.05	3.79±0.06	3.47±0.05
3	Globulin (g/dl)	2.68±0.02	2.63±0.04	1.84±0.05	2.68±0.06	2.49±0.04	2.96±0.02	2.68±0.04
4	Serum Urea (mg/dl)	26.69±0.68	26.26±0.07	38.79±0.53	21.96±0.49	39.02±0.02	48.03±0.05	40.94±0.04
5	Serum Creatinine (mg/dl)	0.88±0.04	0.84±0.02	1.34±0.05	0.96±0.04	0.87±0.05	1.36±0.04	1.04±0.02
6	Serum Chloesterol (mg/dl)	168.82±0.04	165.45±0.05	98.09±1.01	142.46±2.08	114.72±0.02	145.90±1.01	136.05±0.04
7	Serum triglycerides (mg/dl)	62.46±0.04	62.07±0.21	42.81±0.04	63.08±1.02	52.82±0.05	59.72±0.02	54.07±0.01
8	Serum Protein (g/dl)	6.91±0.20	6.45±0.01	5.29±0.10	4.78±0.04	7.59±0.02	7.91±0.04	6.54±0.15
9	HDL (mg/dl)	34.49±1.04	35.88±0.04	28.64±1.05	35.91±0.04	29.58±0.01	42.09±0.02	37.56±0.05
10	LDL (mg/dl)	64.09±1.05	62.71±0.50	53.58±0.01	58.04±0.02	56.08±0.50	63.74±0.60	55.38±0.04

Values are expressed in Mean ± Standard Deviation (n=6)
Superscript letters represent *P*<0.05(Duncan test). Group II compared with Group III, IV and V. **P*<0.05, ***P*<0.01, *** *P*<0.001.