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Isolation, Identification and Anti-Diabetic Activity of Quercetin-3-O-Rutinoside-7-O-Rhamnoside Extracted from *Cyperus Rotundus*

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ABSTRACT

Objective: To isolate a flavonoid glycoside from flowers of *Cyperus rotundus* using chromatography separation techniques, spectral characterization and its anti-diabetic activity.

Method: The structure of the isolated compound was purified, analyzed and characterized by chemical tests, HPLC and spectroscopic methods such as UV, IR, 1H-NMR and 13C-NMR. The blood sugar level was measured by digital display glucometer.

Results: HPLC analysis showed the presence of a flavonol glycoside and it's identified peak with the retention time of 24.8 min and exhibited two major peaks at 360 nm (band-I) and 261 nm (band-II).

Conclusion: The flowers of *Cyperus rotundus* have been found to contain Quercetin-3-O-rutinoside-7-O-rhamnoside. The highest activity of *Cyperus rotundus* 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 *Cyperus rotundus* flower extract.

KEYWORDS Anti-diabetic activity, *Cyperus rotundus*, HPLC, Quercetin-3-O-rutinoside-7-O-rhamnoside

INTRODUCTION

The plants are remarkable source of medicine throughout the world and still continue to occupy an important place in traditional as well as modern system of medicine. It is used frequently in tribal areas as well as in rural areas for the treatment of various diseases since a very longtime[1]. Nowadays, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase[2]. Phytochemicals isolated from plant sources are used for the prevention and treatment of cancer, heart disease, diabetes mellitus and high blood pressure[3].

Plant materials are used throughout developed and developing countries as home remedies, over-the-counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market. *Cyperus rotundus* (Family Cyperaceae), commonly known as 'Nagarmotha' is found throughout India[4]. It is a pestiferous perennial weed with dark green glabrous culms, arising from underground tubers[5]. *Cyperaceae* are the largest family in the monocotyledons consisting of 109 genera and approximately 5,500 species[6]. In Asian countries, the rhizomes of *C. rotundus*, which are used as traditional folk medicines for the treatment of stomach and bowel disorders and inflammatory diseases, have been widely, investigated[7]. *C. rotundus* is a traditional herbal medicine used widely as analgesic, sedative, antispasmodic, antimalarial, stomach disorders and to relieve diarrhoea[8]. Hence, taking into consideration the traditional evaluation and reported activities, the present study was planned to investigate the effect of Alloxan induced anti-diabetic activity in albino rats as animal model, as no work is done in this direction and to check possible role of plant in anti-diabetic activity.

Diabetes is a chronic disorder in metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance[9]. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes[10]. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism[11].

In the current scenario most of modern drugs have been isolated from natural sources such as medicinal plants containing a wide range of chemical compounds that serves as a leads for development of novel anti-diabetic agents. A wide range of plants have been reported in the literature to prevent and treat diabetes. This work mainly focuses on the role of the biomolecules from Indian traditional medicinal plants with anti-diabetic potential with diverse chemical structures. The present study was undertaken to test the plant *Cyperus rotundus* for its anti-diabetic activities of the extract on normal rats and the effect of the carbohydrate metabolizing enzymes and lipid.

MATERIALS AND METHODS

Collection of plant material

The fresh flowers of *Cyperus rotundus* were collected in the month of October - November from the land areas, Kattumannar koil, Cuddalore DT, Tamilnadu, India. These Plants were identified and authenticated by Dr. S. Dharmarajan, Assistant Professor & Head, Department of Botany, Thiru.Vi.Ka. Government Arts College, Bharathidasan University, Trichirappalli, Tamilnadu, India. The voucher specimen (TGACBOT-091) 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 air tight container for further use.

Isolation and Identification

The important stage in the experimental work includes first the isolation of chemical substances from the chosen plant and secondly, the characterization of those isolated compounds. The flowers of *Cyperus rotundus* (2.5 Kg) were extracted with 90% methanol (MeOH) (4 X 500ml) under reflux.

The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was fractionated with peroxide free ether (3 x 250 ml) and ethyl acetate (4 x 250 ml) (Sigma Aldrich Co., India).

The ether fraction was concentrated *in vacuo* and left in an ice-chest for a week. A yellow solid that separated was filtered and studied. On crystallization from MeOH, pale yellow needles were obtained [melting point: 314-315˚C]. It was readily soluble in organic solvents and sparingly in hot water. It reduced ammoniacal $AgNO_3$ in the cold and Fehling's solution on heating. It answered the Horhammer-Hansal, Wilson's boric acid and Gibb's tests. It chromatographic behavior and UV spectral data were all similar to those for the free aglycone flavonoid. The R_f values are indicated in Table 1. It had λ_{max}^{MeOH} nm 255, 325, 370; (+ NaOMe) 251, 275, 419; (+$AlCl_3$ with and without HCl) 255, 269, 342, 430; (+NaOAc) 270, 390; (NaOAc/H_3BO_3) 261, 295 sh, 366. It was identified as quercetin and the identity was confirmed by paper chromatography (p.c) and melting point (m.p) with an authentic sample of quercetin from *Physalis minima*[12, 13].

The ethyl acetate fraction was concentrated *in vacuo* and left in an ice-chest for a few days. A yellow solid [m.p. 319-320˚C] that separated was filtered and studied. It came out as pale yellow crystals on recrystallization from MeOH. It reduced ammoniacal $AgNO_3$ solution but not Fehling's solution. It appeared deep purple under UV that turned yellowish green on exposure to NH_3. It responded to Wilson's boric acid, Molisch and Gibb's tests, but did not answer the Horhammer-Hansal tests. It had λ_{max}^{MeOH} nm 261, 270, 360; (+NaOMe) 410, 370, 296, 270; (+$AlCl_3$) 261, 282, 366; ($AlCl_3$ +HCl) 284, 294, 370; (+NaOAc) 410, 370, 296, 270; (+NaOAc/ H_3BO_3) 274, 256.

Supporting evidence for the structure of the flavonol glycoside is provided by the HPLC (Shimadzu, Columbia), UV (Perkin Elmer Spectrophotometer), IR (Perkin - Elmer spectrometer) and NMR (400 MHz, DMSO-d_6 and TMS) spectral data were recorded on a Bruker AMX 400 NMR spectrometer. Chemical shifts were reference to the respective residual solvent peaks and the values were recorded in δ.

Quercetin-3-O-rutinoside-7-O-rhamnoside

Yellow solid; m.p. 319-320˚C; RT 24.8 min; UV λ_{max}^{MeOH} (log ε) 261, 360 nm; IR (KBr): νm_{ax} 3277, 2954, 2845, 1640, 1548, 1450, 1411, 1356, 1237, 1097 and 1014 cm^{-1}; ^{1}H-NMR spectrum δ (400 MHz, DMSO-d_6, TMS): δ ppm 6.363 (H-6), 6.404 (H-8), 7.384 (H-2′), 7.243 (H-5′), 7.240 (H-6′), 5.165 (H-1″), 4.043 (H-1‴), 1.143 (Rha- CH_3), 12.5 (5-OH), 3.03 ~ 3.98 (Rest of sugar protons); ^{13}C- NMR(400 MHz, DMSO-d_6 and TMS): δ ppm 177.63 (C-4); 164.22 (C-7); 161.31 (C-5); 160.03 (C-9); 156.48 (C-2); 148.54 (C-3′); 145.65 (C-4′); 133.29 (C-3); 125.53 (C-1′); 120.99 (C-6′); 116.32 (C-5′); 115.20 (C-2′); 114.88 (C-1‴); 104.11 (C-10); 101.8 (C-1⁗); 98.79 (C-6, 8); 93.76 (C-1″); 77.54 (C-3″, 5″, 4⁗); 76.59 (C-2″, C-4‴); 76.51 (C-2‴); 75.84 (C-3‴); 74.46 (C-4″); 73.42 (C-2⁗); 73.2 (C-3⁗); 71.31 (C-5‴); 69.99 (C-5⁗); 67.33 (C-6″); 19.53 (C-6‴); 16.4 (C-6⁗).

Hydrolysis of the glycoside

The glycoside dissolved in hot aqueous methanol was hydrolyzed with H_2SO_4 (5%) at 100˚C for about 2 hrs. The excess of alcohol was distilled off *in vacuo* and the resulting aqueous solution was extracted with ether. The residue from ether fraction was studied as described below. The glycoside was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexane and the resulting solution extracted with ethyl acetate.

Phytochemical screening of plant extract

A small amount of the dry extract was used for the phytochemical tests[14] for compounds which include alkaloids, flavonoids, tannins, saponins, glycosides, phenol and terpenoids while steroids, coumarin and cardiac glycosides are absent in all the crude extracts.

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 Quercetin glycoside drug from extract of *Cyperus rotundus* (100 mg / kg)

Group-V: Alloxan treated rats were administered the Quercetin glycoside drug from extract of *Cyperus rotundus* (200 mg / kg)

Group-VI: Alloxan treated rats were administered the methanolic extract of *Cyperus rotundus* (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 carried out according to the literature[15]. Animals of either sex were fasted for eighteen hours and used. A dose of 200 mg/kg of Quercetin glycoside from extract of *Cyperus rotundus* 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 *Cyperus rotundus* 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 Quercetin-3-O-rutinoside-7-O-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[16] using an Autopak kit. Globulin levels were calculated from total protein and albumin measurements. Serum was separated and analyzed for serum cholesterol[17], serum triglycerides by enzymatic DHBS colorimetric method[18], serum HDL[19], serum LDL[20], serum creatinine[21], serum urea[22] and levels of hemoglobin using the ion exchange resin method[23] 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.

RESULTS AND DISCUSSION

Chemical Constituents

The flowers of *Cyperus rotundus* have been found to contain Quercetin-3-O-rutinoside-7-O-rhamnoside. The UV spectrum of the aglycone exhibited two major peaks at 360 nm (band-I) and 261 nm (band-II), to reveal a flavonoid skeleton. The band I absorption of the glycoside is reminiscent of a flavonol skeleton. A comparison of band I absorption of glycoside and that of the aglycone revealed that may be 3-glycosylation in the flavonol. The presence of a free –OH at C-5 in the glycoside and the aglycone is evident from its positive response to Wilson's boric acid test. A shift of +58 nm on the addition of $AlCl_3$-HCl showed the presence of a free 5-OH in the A-ring. A shift of +30 nm was observed in the case of $AlCl_3$ without acid, which also revealed that O-dihydroxyl group. A bathochromic shift of 15 nm in the aglycone in NaOMe spectrum is suggestive of the presence of a free –OH at C-4′ in both aglycone and glycoside. This is also supported by the absence of any characteristic shift (band I) in NaOAc-H_3BO_3 spectra of both the glycoside and the aglycone. Addition of NaOAc did not result in any bathochromic shift in band II of the methanol spectrum of the glycoside, but under similar treatment of the aglycone there was a bathochromic shift of 18 nm suggesting another site of glycosylation at C-7 in the glycoside.

The IR spectrum indicated the presence of ν_{max} 3277, 2954, 2845, 1640, 1548, 1450, 1411, 1356, 1237, 1097 and 1014 cm^{-1}. HPLC analysis showed the presence of a flavonol glycoside and it's identified peak with the retention time of 24.8 min. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, TMS), the A-ring protons at C-6 and C-8 appear separately as doublet at δ 6.363 ppm and δ 6.404 ppm. The hydroxyl group at C-5 appears distinct singlet at δ 12.5 ppm. The H-1″ signal of the glucose moiety appears at δ 5.165 ppm and is located down field from the bulk of the sugar protons. The β-linkage of the glucose moiety 3-OH is evident from the large coupling constant of H-1″ had it been a 7-rhamnoside. H-1″ would have given rise to a complex multiplet instead of sharp doublet. The H-1‴ of rhamnose resonates at δ 4.043 ppm. The methyl proton of the rhamnose resonates at δ1.143 ppm, the remaining sugar protons appear in the region of δ 3.03 to 3.98 ppm.

The ^{1}H and ^{13}C NMR spectra showed the expected signals in the aromatic region for the quercetin moiety in the flavonoids. In the ^{1}H - NMR spectrum, A-ring protons at C-6 and C-8 appear separately at δ 6.363 ppm and δ 6.404 ppm respectively. Due to glycosylation at C-3 position, C-2 and C-4 carbon absorb at δ 156.48 ppm and 177.63 ppm respectively. The C-6 and C-8 carbons resonate at δ 98.79 ppm. In ^{13}C NMR signals corresponding to the anomeric carbon of glucose were found at 93.76 ppm and those corresponding to rhamnose were seen at 114.88 ppm. The attachment of the rhamnose to C-6″ of the glycosyl moiety was evidenced by the downfield shift of the glycosyl C-6″ carbon resonance to δ 67.33 ppm and accompanying up field shift of the resonances of the adjacent carbons C-5″ to 77.54 ppm. The down field shift of the signal of the two anomeric carbon indicates their involvement in a glycosylation. The C-6″ and C-6‴ appear at δ 67.33 ppm and δ 19.53 ppm respectively. The ^{13}C-NMR signal resonates at 164.22 ppm has attributed to C-7, those corresponding to rhamnose were seen at 101.8 ppm (C-1‴) and C-6‴ appear at 16.4 ppm.

By comparing their HPLC, UV, IR, ^{1}H-NMR and ^{13}C-NMR data it was proved to be Quercetin-3-O-rutinoside-7-O-rhamnoside (Fig. 1) with those reported for similar compound[24, 25].

Anti-diabetic activity

The treatment of diabetes with pharmaceutical proved much safer than synthetic drugs throughout the world and gained importance in recent years[26]. The anti-diabetic effect of the test compounds on the blood glucose levels of diabetic rats is shown in Table 2. Twelve days of daily treatment of different test compounds of *Cyperus rotundus* led to a dose-dependent fall in blood sugar levels by 36-65%. Effect seems to reach maximum after 12th day of treatment and remains constant their after. From the results it is revealed that the test isolated compound Quercetin-3-O-rutinoside-7-O-rhamnoside from *Cyperus rotundus* at a dose level 100 and 200 mg / kg, showed significant reduction in blood sugar level from 1 to 8th day in progressive manner comparable to standard glibenclamide. There is no significant 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 96.67±3.79, 95.78±3.79 and 94.33±2.52 respectively at the 12th day hour while the reference drug glibenclamide (10 mg / kg) decreased the blood glucose levels by 59.36%. The highest activity of *Cyperus rotundus* 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 *Cyperus rotundus* flower extract.

In diabetes mellitus, the loss of body weight is caused by increase in muscle wasting and catabolism of fat and proteins. A decrease in body weight was registered in case of diabetic control group rats while in tested groups the weight loss was reversed. Table 3 showed the body weight changes in the normal and experimental animals in each group. The mean body weight of the diabetic rats decreased compared to diabetic treated rats. There was a significant reduction in body weight of the diabetic rats compared with normal and diabetic treated rats. The administration of *Cyperus rotundus* restored these levels significantly (P <0.001) towards normal. Diabetic rats treated with the methanol extract showed an increase in body weight compared to diabetic control. This may also be due to the protective effect of the extract in controlling muscle wasting i.e. reversal 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 149.87 ± 5.58, 153.33 ± 3.51, 153.67 ± 3.79 and 145.00 ± 6.25 mg/kg respectively.

Biochemical estimation

The biochemical parameters (Table 4) such as serum cholesterol, serum triglycerides, serum LDL, serum creatinine and serum urea levels were decreased significantly by glibenclamide and HDL levels were increased by glibenclamide, test compounds and methanol extracts. Administration of isolated compound Quercetin-3-O-rutinoside-7-O-rhamnoside at dose of 200 mg / kg from *Cyperus rotundus* 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.

The results also showed the levels of hemoglobin in control and experimental groups of rats. A significant decrease in the level of hemoglobin was observed in diabetic rats when compared to control rats. Administration of isolated compound at dose of 200 mg / kg from *Cyperus rotundus* flower to diabet-

ic rats resulted in the restoration of hemoglobin level to near normal. There was a significant increase in total hemoglobin in the diabetic control rats (13.59 ± 0.28). After treatment with the methanol extract and the isolated compound at a dose of 100 mg / kg, the animals showed a decrease in hemoglobin levels to 12.36 ± 0.27 and 12.51 ± 0.36 respectively. The diabetic rats also treated with glibenclamide showed 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 significant 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 significant markers of renal dysfunction.[27] On administering isolated compounds at doses 100, 200 mg / kg and methanol extracts for 12 days, serum urea and creatinine steadily returned to near normal. Administering 200 mg / kg test isolated compound orally to diabetic rats decreased serum urea and creatinine, more effectively and could be explained by the regenerative ability of the renal function. The observed increase in creatinine level in diabetic rats is mainly due to renal dysfunction and is altered to near normal by oral administration of isolated test compound from *Cyperus rotundus* flowers for 12 days. The present investigation 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 significant increase in the levels of total cholesterol and triglycerides. The administration of 200 mg / kg test isolated compound and the glibenclamide reverse the level of lipids significantly ($p < 0.05$ and $p < 0.01$).

CONCLUSION

Our study clearly shows that *Cyperus rotundus* caused marked hypoglycemic activity in alloxan induced diabetic rat model which indicates anti-diabetic potentials of the methanol extract and test isolated compounds. The compound Quercetin-3-O-rutinoside-7-O-rhamnoside isolated from *Cyperus rotundus* flowers tested for anti-diabetic activity as it significantly lowered the serum glucose levels and increased the body weight of diabetic rats. In this experiment, the dose of 200 mg / kg produced the highest anti-diabetic effect and this may suggest that this dose may be the effective anti-diabetic drug even though there might have some limitation due to the crude nature of the extract.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

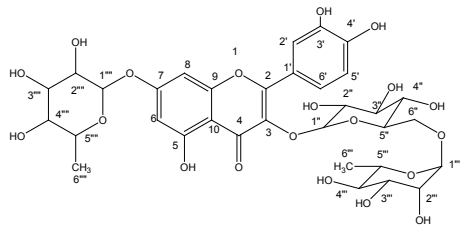

Fig 1: Quercetin-3-O-rutinoside-7-O-rhamnoside

Table 1: R_f (X100) values of the constituents of the flowers of *Cyperus rotundus*
(Whatman No.1, Ascending, 30 ± 2°C)

Compounds	*Developing Solvents								
	a	b	c	d	e	f	g	h	i
[1]Glycoside	39	45	75	68	80	24	37	87	46
Glycoside (authentic)	40	44	75	67	80	24	37	86	46
[2]Aglycone	-	-	5	18	49	93	96	62	86
Quercetin (authentic)	-	-	5	19	50	93	97	62	87

Compounds

[1]Glycoside - Quercetin-3-O-rutinoside-7-O-rhamnoside

[2]Aglycone - Quercetin

***Solvent key**

a → H_2O

b → 5% aq.HOAc

c → 15% aq.HOAc

d → 30% aq.HOAc

e → 60% aq.HOAc

f → BAW (n-BuOH: HOAc: H_2O=4:1:5, upper phase)

g → phenol saturated with water

h → forestol (HOAc: conc. HCl)

i → TBA (t-butanol-acetic acid-water, 3:1:1)

Table 2: Effect of Quercetin-3-O-rutinoside-7-O-rhamnoside from *Cyperus rotundus* 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	97.67±10.69	96.67±3.79	95.78±3.79	94.33±2.52
II	Diabetic Control	388.67±6.11	401.67±3.51	405.67±2.08	411.33±2.52
III	Standard (Alloxan + glibenclamide 10 mg/kg)	374.33±4.04	284.33±2.52	243.33±4.04	152.00±4.58
IV	Alloxan + Quercetin Drug (100 mg/kg)	390.33±2.08	279.67±8.62	188.00±7.00	152.00±3.61
V	Alloxan + Quercetin Drug (200 mg/kg)	386.33±4.73	247.33±5.03	168.33±5.51	134.67±8.33
VI	Alloxan + Methanolic Extract (300 mg/kg)	387.46±4.27	290.81±2.53	192.43±4.08	154.63±4.51

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 of Quercetin-3-O-rutinoside-7-O-rhamnoside from *Cyperus rotundus* 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	162.67±1.53	157.33±6.43	152.67±2.08	155.67±3.21
II	Diabetic Control (Alloxan)	163.00±1.00	143.67±2.52	137.67±2.52	131.33±3.06
III	Standard (Alloxan + glibenclamide 10 mg/kg)	162.33±2.89	147.67±4.16	146.33±3.21	145.00±6.25
IV	Alloxan + Quercetin Drug (100 mg/kg)	163.67±0.58	154.00±2.65	142.33±4.04	153.33±3.51
V	Alloxan + Quercetin Drug (200 mg/kg)	163.67±3.79	152.33±1.53	155.33±2.52	153.67±3.79
VI	Alloxan + Methanolic Extract (300 mg/kg)	162.98±2.53	149.61±2.54	144.48±3.02	149.87±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 4: Effect of Quercetin-3-O-rutinoside-7-O-rhamnoside from *Cyperus rotundus* 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)	14.33±0.42	15.46±0.27	12.21±0.28	14.64±0.39	12.51±0.36	13.59±0.45	12.36±0.27
2	Albumin (g/dl)	3.85±0.05	3.82±0.07	3.16±0.05	3.91±0.07	3.03±0.07	3.86±0.06	3.58±0.05
3	Globulin (g/dl)	2.66±0.06	2.61±0.04	1.80±0.04	2.64±0.06	2.42±0.04	2.90±0.02	2.60±0.06
4	Serum Urea (mg/dl)	26.61±0.68	26.20±0.26	38.60±0.53	22.23±0.49	38.41±0.37	47.90±0.79	40.17±0.68
5	Serum Creatinine (mg/dl)	0.84±0.053	0.83±0.025	1.32±0.053	0.93±0.042	0.83±0.044	1.40±0.020	0.98±0.054
6	Serum Chloesterol (mg/dl)	169.67±2.08	166.33±1.53	97.00±2.65	141.67±2.08	112.67±2.52	141.33±1.53	138.38±2.68
7	Serum triglycerides (mg/dl)	61.97±1.00	61.13±0.23	41.67±1.53	62.83±1.04	51.50±0.50	58.33±0.58	53.60±1.00
8	Serum Protein (g/dl)	6.83±0.40	6.32±0.10	5.15±0.13	4.63±0.04	7.40±0.08	7.88±0.07	6.40±0.10
9	HDL (mg/dl)	33.67±1.53	34.04±0.07	27.67±1.53	34.80±0.72	28.00±1.00	41.17±1.04	36.49±1.52
10	LDL (mg/dl)	64.00±1.73	64.50±0.50	51.67±0.58	57.93±0.70	55.33±0.58	62.70±0.61	54.29±1.76

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.

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