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Evaluation of Antidiabetic Activity of *Oxalis corniculata* Linn. Whole Plant.

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ABSTRACT

Oxalis corniculata Linn. belongs to family Oxalidaceae is commonly known as creeping wood sorrel. It is somewhat delicate-appearing, low growing, herbaceous plant. The plant collected from Durgapur forest region in Dhenkanal district, Odisha, in the month of October – November was extracted successively with petroleum ether (60-80^0 C) and methanol. Preliminary phytochemical screening of extracts indicated the presence of flavonoids, carbohydrates, glycosides, saponins, phenolic compounds/tannins, proteins and amino acids in methanol extract. The methanol extract of *O. corniculata* was fractionated by column chromatography using a glass column packed with silica gel (100-200 mesh) and developed by gradient elution with chloroform and combination of chloroform : methanol in the increasing order of polarity (10%, 20% methanol in chloroform) which resulted in the fractions OCMF-1, OCMF-2 and OCMF-3, respectively. The antihyperglycaemic activity was evaluated in normal, glucose-loaded and Streptozotocin-induced hyperglycaemic rats (single and multi dose treatment). In normoglycaemic rats, the test extracts showed progressive fall of blood glucose level till the end of 8 h. In glucose loaded animals (OGTT), reduction in blood glucose level was observed after 60 minutes of administration of the test substances. The maximum reduction was observed at 4 h with methanol extract exhibiting maximum improvement in glucose tolerance. The extracts produced significant decrease in the blood glucose level in streptozotocin-induced hyperglycaemic rats when compared with the diabetic control group in the single dose treatment study at the tested dose level of 400 mg/kg of body weight. In multi-dose treated hyperglycaemic rats, both the extracts and fractions showed various degree of blood glucose reduction, among which OCMF-3 exhibited highest percentage of reduction in blood glucose level. Continuous administration of extracts and fractions for 14 days leads to significant decrease in serum total cholesterol, triglycerides, LDL and VLDL levels, while increase in total protein and HDL levels was recorded. The *in vitro* study showed an increased utilization of the glucose by α-amylase inhibition assay in presence of methanol extract which suggests that the test extract may inhibit the digestion and absorption of glucose through intestine. These findings suggest that the plant may be a potential source for the development of new oral antihyperglycaemic agent.

Keywords: *Oxalis corniculata* L., Extract, Fraction, Antidiabetic, Streptozotocin, α- Amylase

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INTRODUCTION

Diabetes is a complex and multifarious group of disorders characterized by hyperglycaemia that has reached epidemic proportions in the present century. Several classes of drugs such as biguanides and sulfonylureas are presently available to reduce blood sugar level in *Diabetes mellitus*. These drugs have side effects and thus searching for a new class of compounds is essential to overcome this problems. [1] Management of diabetes without any side effects using conventional medication is still a challenge to the medical community. There is continuous search for alternative drugs, because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries. Therefore, it is prudent to look for options in herbal medicine for diabetes as well. Approximately 140 million people worldwide suffer from diabetes. [2] The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable. [3] Traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing countries. India is well known for its herbal wealth. Medicinal plants like *Trigonella foenum-graecum*, *Allium sativum*, *Gymnema slyvestre* and *Syzigium cumini* have been studied for their usefulness in the treatment of *Diabetes mellitus*. [4] However, detailed studies on the efficacy, mechanism of action and safety of plant extracts are need to be properly explored.

Oxalis corniculata L. belongs to family Oxalidaceae is commonly known as creeping wood sorrel. It is one of the most demandable plant species in India having several gray areas which are the focus to the future researchers. It is a sub-tropical plant and originated from India. [5] *Oxalis* is the most diverse genus and consists of about 900 species. [6] It is somewhat delicate-appearing, low growing, herbaceous plant. It is distributed as a weed in damp shady places, roadsides, plantations, lawns, nearly all regions throughout the warmer parts of India and Ceylon, in the Himalayas up to 8,000 ft - cosmopolitan. [5-8] Traditionally, preparations from various parts of the plant, such as leaves, stem and root are used as remedies for various illnesses. [9] The plant is used as stimulant and tonic; beneficial in chest pain, convulsions, cramps and inflammatory tumour. [10] Fresh juice of plant is given in dyspepsia, piles, insomnia, anaemia and tympanitis. [11] Ground leaves are eaten as chutney that acts as blood purifier. [5] It is also used for giddiness, diarrhoea and dysentery; juice of leaves applied to open wound, relieves pain, paste of ground leaves and raw onions are applied to forehead for curing intense headache. [12] The plant is well known for its medicinal value as a good appetizer and as a remover of cough and piles. [13] The plant is reported to have anti-implantation and abortifacient, [14] wound healing, [15] anti-diarrhoeal, [16] hypoglycaemic effect [17] and anti-fertility activity. [18] Phytochemical investigation of *O. corniculata* revealed the presence of tannins, palmitic acid, a mixture of oleic, linoleic and stearic acids. [19] Methanol and ethanolic extracts of this plant show the presence of carbohydrate, glycosides, phytosterols, phenolic compounds, flavanoids, proteins (12.5%), amino acids and volatile oil. [13] The plant is reported to have several medicinal uses, but it is yet to be studied for its antidiabetic activity. The present investigation deals with the antidiabetic evaluation of extracts and fractions of *O. corniculata* whole plant by various experimental models.

MATERIALS AND METHODS

Chemicals and instruments

Streptozotocin (STZ) was procured from Sigma life science (Mumbai), α- Amylase was procured from Hi-media laboratories and Glibenclamide tablet (Daonil; Emcure- Sanofi India Ltd.) was purchased from local market. Total cholesterol, HDL cholesterol, LDL cholesterol, VLDL, total protein, triglycerides, total bilirubin and creatinine were assayed by using the kits procured from Span Diagnostics Ltd. (India). Blood glucose level was measured by using Dr. Morpen Gluco meter (Model No-BG-03)

Plant materials and preparation of extracts

Oxalis *corniculata* L. was collected from Durgapur forest region in Dhenkanal district, Odisha, in the month of October – November and authenticated by Dr. K. B. Satapathy, P.G. Department of Botany, Utkal University, Bhubaneswar, Odisha, India. After authentification, the whole plant was collected in bulk quantity, washed under running tap water to remove the adhering dirt and shade dried at room temperature. The dried

plant material was crushed to course powder by using mechanical grinder. The powdered plant material was extracted successively with petroleum ether (60-80^0 C) and methanol. The extracts were concentrated by evaporating the solvent under reduced pressure using Rotary evaporator (IKA Rv 10 V digital). The yield of petroleum ether and methanol extracts were found to be 4.99 and 19.24 % w/w respectively.

Preliminary phytochemical screening of extracts

The preliminary phytochemical screening of petroleum ether and methanol extracts of *O. corniculata* was performed according to the method described by Gupta *et al*. [20] The tests were based on the visual observation of colour change or formation of a precipitate after the addition of specific reagents.

Fractionation of the methanol extract of *O. corniculata*

The methanol extract of *O. corniculata* was further fractionated by column chromatography since it exhibited promising blood glucose lowering effect. The dried methanol extract (5 g) was reconstituted in a minimum amount of chloroform and heated to dissolve. Then it was adsorbed onto small amount of silica gel (100-200 mesh; in dry form) and heated in a water bath by continuous stirring for evaporation of the solvent. Then it was air dried. A glass column (60 cm height and 2.5 cm diameter) was packed with silica gel (100-200 mesh, 250 g) by suspending it in chloroform and allowed to settle. The dried silica gel impregnated with extract was loaded into the column. The column was developed by gradient elution with chloroform and combination of chloroform : methanol in the increasing order of polarity (10%, 20% methanol in chloroform) which resulted in the fractions OCMF-1, OCMF-2 & OCMF-3, respectively. 30 eluates of 50 ml each were collected with each solvent system (i.e. a total of 90 eluates). The fractions of each solvent system were concentrated separately by evaporating the solvent under reduced pressure and used for further studies.

Animals and approval status

Albino rats of either sex weighing between 150-200 g were used for the experiment. Animals were housed in a group of six in polypropylene cages at controlled room temperature 25 ± 2 ^{0}C, relative humidity 55% and 12 h light : dark cycle. They were fed with standard chow diet and water *ad libitum* during the experiment. The experimental protocol of the animal experiments was approved by the IAEC of P.G. Department of Zoology and Biotechnology, Utkal University, Bhubaneswar (Regd. No: 192/ CPCSEA, 22-05-2000).

Preparation of test extracts and fractions for animal experiments

The suspension of petroleum ether and methanol extracts and fractions of the methanol extract was prepared by using distilled water and Tween-40 which was used for animal experiments.

Acute oral toxicity study of extracts and fractions

Swiss albino mice of either sex were fasted overnight prior to the experiment. The study was carried out as per OECD guidelines, 2000. The different doses of the extracts and fractions were administered to mice by oral route in different dose levels of 1000, 2000, 3000 and 4000 mg/kg of body weight. The food was withheld for further 3-4 h after drug administration to avoid any complications relating to absorption of test extracts and fractions arising from food. Animals were critically observed individually at least once during the first 30 minutes of dosing followed by occasional observation for first 24 h and continued for 72 h for the recording of mortality, if any. The LD_{50} was calculated according to Miller and Tainter. [21] One-tenth (1/10th) of the lethal dose was taken as a screening dose. [22] The rats were observed continuously and the following profiles were observed.

- Behavioral profile: Alertness, restlessness, irritability, and fearfulness
- Neurological profile: Spontaneous activities, reactivity, touch response, pain response, and gait
- Autonomic profile: Defecation and urination.

After a period of 72 h the rats were observed for any lethality or death. Since no mortality was observed up to the dose level of 4000 mg/kg body weight (b.w.), so the dose of 400 mg/kg was fixed for screening of anti-diabetic activity.

Evaluation of antidiabetic activity of extracts

The antidiabetic activity of the extracts (petroleum ether and methanol) of *O. corniculata* whole plant was assessed in normoglycaemic, glucose-loaded, streptozotocin-induced single dose and multi dose treated hyperglycaemic animals, including the *in-vitro* antidiabetic potentials using α- amylase assay method.

Evaluation of activity on normoglycaemic animals [23]

Healthy Wistar albino rats of either sex weighing 150-200 g deprived of food for 12 h before the experiment were divided into five groups of six rats each. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of tail and the fasting blood glucose level was estimated. Group-I animals served as control received only solvent (distilled water + Tween 40, 2 ml/kg, b.w.), group-II standard group received Glibenclamide (10 mg/kg b.w.) and group-III and IV served as test group animals were treated with the suspensions of petroleum ether and methanol extracts of *O. corniculata* (400 mg/kg, b.w.). All the treatments were made by oral route. The blood glucose level was determined at 0, 1, 2, 4, 6 and 8 h after administration of test extracts and standard. The blood samples were collected from tail vein of the animals and blood glucose level was measured by using glucose oxidase-peroxidase reactive strips and glucometer.

Evaluation of activity on glucose-loaded animals (OGTT)

The oral glucose tolerance test was performed as per the method of Shirwaikar. [24] In this method, rats were fasted for 16 h before and during the experiment. Rats were divided into four groups of six rats each. Group-I solvent treated group received (distilled water + Tween 40, 2 ml/kg); Group-II standard group was treated with Glibenclamide (10 mg/kg, b.w.); and test groups Group-III and IV received suspensions of petroleum ether and methanol extracts of *O. corniculata* (400 mg/kg, b.w.) respectively. Glucose (3 g/kg) was fed 30 minutes after the administration of vehicle, standard and test extracts. Blood was withdrawn from tail vein of the animal at 0, 1, 2, and 4 h of glucose administration. The blood glucose level was estimated by using glucose oxidase-peroxidase reactive strips and glucometer.

Evaluation of activity on streptozotocin-induced diabetic animals (Single dose) [23]

The effect of extracts on blood glucose level was studied in STZ-induced diabetic rats. The rats were divided into five groups of six rats each and fasted for 12 h with free access of water. Six normal rats were treated only with solvent and served as solvent control. The treatments were made orally as: Group-I Solvent control (distilled water + Tween 40, 2 ml/kg, b.w.); Group-II Diabetic control (distilled water + Tween 40, 2 ml/kg, b. w.); Group-III Glibenclamide (10 mg/kg); Group-IV Petroleum ether extract (400 mg/kg); Group-V Methanol extract (400 mg/kg). The blood glucose level was estimated at 0, 1, 2, 4, 8, and 10 h following the treatment.

Evaluation of antihyperglycaemic activity of extracts and fractions on STZ-induced diabetic animals (Multi-dose)

The Wistar albino rats of either sex of body weight of 150-200 g were divided into eight groups, six animal each (n=6) and kept fasting for 24 h. Diabetes was induced by intra-peritoneal injection of STZ freshly dissolved in citrate buffer (pH 4.5) immediately before use at a dose of 65 mg/kg body weight. [25] Six normal rats were treated only with solvent and served as solvent control. In order to avoid STZ induced hypoglycaemic mortality, 5% glucose solution was given for 24 h to STZ treated rats. [26] After 72 h of STZ administration, the blood glucose levels were measured and the rats showing blood glucose level greater than 220 mg/dl were considered to be diabetic and were used for the present study. Group-I served as solvent control received only solvent (distilled water + Tween 40, 2 ml/kg b.w.); Group-II Diabetic control (distilled water + Tween 40, 2 ml/kg b.w.); group-III served as standard group received standard drug, Glibanclamide (10 mg/kg b.w.) by oral route once daily for 14 days. Test group animals received various extracts and fractions. Group-IV & V received petroleum ether and methanol extracts and Group-VI to VIII received the fractions, OCMF-1, OCMF-2 & OCMF-

3 at the dose of 400 mg/kg body weight, respectively. The blood samples were collected from tail vein and blood glucose level was measured. The blood glucose levels were determined on 1st, 2nd, 4th, 7th and 14th day after administration of solvent, standard drug and test extracts and fractions. [27]

Serum lipid profile [28]

The lipid profile was done on 14th day after induction of diabetes. The serum lipid parameters such as total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and very low density lipoproteins, total bilirubin and creatinine were estimated using commercial kits (Span diagnostics, Mumbai).

***In-vitro* antidiabetic activity**

Inhibition assay for α-amylase activity

α-Amylase was premixed with the methanol extract at various concentrations (25-250 µg/ml) and starch (0.5% solution) was added as a substrate to start the reaction. This was carried out at 37 ^{0}C for 5 min and terminated by addition of 2 ml of DNS (3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100 ^{0}C and diluted with 10 ml of distilled water in an ice bath. α-Amylase activity was determined by measuring the spectrum at 540 nm using colorimeter. [29] The IC_{50} value is defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions.

$$\% \text{ inhibition} = \frac{\text{Abs 540 control - Abs 540 test}}{\text{Abs 540 control}} \times 100$$

Statistical analysis

All the results are expressed as mean ± SEM. Comparison was made between the test groups and diabetic control. The data were statistically analysed by one way analysis of variance (ANOVA) followed by Dunnett's t-test and p value less than 0.05 was considered significant.

RESULTS

Preliminary phytochemical screening

The study indicates the presence of flavonoids, carbohydrates, glycosides, saponins, phenolic compounds/tannins, proteins and amino acids in methanol extract (Table 1).

Table 1: Preliminary phytochemical screening of different extracts of *O. corniculata* L. whole plant

Test	Petroleum ether extract	Methanol extract
Alkaloids	-	-
Flavonoids	-	+
Carbohydrates	-	+
Glycosides	-	+
Phytosterols	-	-
Fixed Oils & fats	-	-
Saponins	-	+
Phenolic compounds / Tannins	-	+
Proteins & Amino acids	-	+

(+) denotes present; (-) denotes absent

Acute oral toxicity study

The gross observational results revealed that the extracts and fractions did not show any sign of toxicity and mortality up to 72 h of the study at the dose level of 4000 mg/kg b.w. Hence, 400 mg/kg b.w. was fixed as the screening dose during antidiabetic evaluation.

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Effect of extracts on normoglycaemic rats

The effect of extracts on blood glucose level of normal rats is presented in Table 2. The test extracts at 400 mg/kg body weight showed a significant fall of blood glucose level when compared with solvent control group at the end of 8 h ($p<0.01$). Methanol extract exhibited the highest reduction of blood glucose level with the percentage reduction of 7.50 followed by petroleum ether extract (5.53%).

Table 2: Effect of extracts of *O. corniculata* L. on blood glucose levels in normoglycaemic animals

Groups and treatment	Blood glucose level (mg/dl)						% decrease at the end of 8 hrs
	0h	1h	2h	4h	6h	8h	
Solvent control (2 ml/kg)	98.16 ± 0.60	97.66 ± 0.61	97.50 ± 0.76	96.83 ± 0.60	85.83 ± 0.60	93.33 ± 0.49	--
Glibenclamide (10 mg/kg)	92.83 ± 0.60	85.66 ± 0.66*	79.66 ± 0.88**	67.83 ± 0.79**	63.50 ± 0.76**	52.50 ± 0.84***	43.74
Petroleum ether extract (400 mg/kg)	95.50 ± 0.61	94.50 ± 0.84*	93.66 ± 0.71*	92.66 ± 0.88*	90.50 ± 0.76**	88.16 ± 0.60**	5.53
Methanol extract (400 mg/kg)	93.66 ± 1.63	92.83 ± 0.54*	91.66 ± 0.80**	90.50 ± 0. 92**	89.66 ± 0.71**	86.33 ± 1.14**	7.50

Values expressed as mean ± SEM (n=6). The data were statistically analysed by one-way ANOVA, followed by Dunnet's t-test. p values less than 0.05 were considered significant. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Effect of extracts on glucose loaded hyperglycaemic rats

As per the results depicted in Table 3, petroleum ether and methanol extracts showed significant fall of blood glucose level with $p<0.01$ & $p<0.001$, respectively at 4 h following the administration of test substances. Methanol extract exhibited maximum reduction of blood glucose and better glucose tolerability (32.30%) as compared to petroleum ether extract (22.92%).

Table 3: Effect of extracts of *O. corniculata* L. on blood glucose levels in glucose loaded animals

Groups and treatment	Blood glucose level (mg/dl)				
	Pre-treatment	Post-treatment			
	0 hr	1hr	2 hr	4 hr	% decrease at the end of 4 hrs
Solvent control (2 ml/kg)	85.83 ± 1.30	140.50± 0.76	133.00± 0.36	124.33± 0.49	--
Glibenclamide (10 mg/kg)	79.66 ± 0.55	126.17± 1.44*	99.83 ± 0.54**	76.66 ± 0.76***	38.34
Petroleum ether extract (400 mg/kg)	90.16 ± 0.70	135.67±0.88*	127.83±1.72*	95.83±1.53**	22.92
Methanol extract (400 mg/kg)	81.16±0.60	131.17±0.94*	120.67±1.17**	84.16±0.60***	32.30

Values expressed as mean ± SEM (n=6). The data were statistically analysed by one-way ANOVA, followed by Dunnet's *t*-test. p values less than 0.05 were considered significant. Rats of all groups were loaded with glucose (2 g/kg p.o.) 30 min after extracts, Glibenclamide and water * $p < 0.05$; ** $p < 0.01$; ***: $p<0.001$.

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Table 4: Effect of extracts and of *O. corniculata* L. on blood glucose levels in single dose treated streptozotocin induced diabetic animals

Groups and treatment	Blood glucose level (mg/dl)						
	0h	1h	2h	4h	8h	10 hr	% decrease at the end of 10 hrs
Solvent control (2 ml/kg)	87.66 ± 0.88	88.50 ± 0.99	89.83 ± 1.04	92.50 ± 1.08	89.33 ± 0.95	87.66 ± 1.02	--
Diabetic control	279.17 ± 0.70 [b]	277.67 ± 0.76 [a]	278.17 ± 0.70 [b]	285.67 ± 0.88 [a]	276.17 ± 0.60 [c]	275.83 ± 0.74 [a]	--
Glibenclamide (10 mg/kg)	266.83 ± 0.60**	218.67 ± 0.88*	190.33 ± 0.88**	147.17 ± 0.60**	121.33 ± 0.71*	106.33 ± 1.14***	61.45
Petroleum ether extract (400 mg/kg)	263.83 ± 0.60*	254.17 ± 0.94**	237.50 ± 1.05*	221.33 ± 0.66*	184.17 ± 1.01*	160.67 ± 0.88**	41.75
Methanol extract (400 mg/kg)	261.83 ± 1.44**	256.17 ± 0.79**	219.67 ± 1.05*	176.83 ± 0.94*	139.83 ± 0.60*	117.50 ± 0.76***	57.40

Values expressed as mean ± SEM (n=6). The data were statistically analysed by one-way ANOVA, followed by Dunnet's *t*-test. p values less than 0.05 were considered significant. Solvent control vs Diabetic control [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. Diabetic control vs all other groups * $p < 0.05$; ** $p < 0.01$; ***: $p < 0.001$.

Effect of extracts on STZ-induced diabetic animals (Single dose)

The results revealed that methanol extract exhibited highest reduction of blood glucose level with the percentage reduction of 57.40 followed by petroleum ether extract (41.75%) at 10 h after administration of test substances when compared with the diabetic control group (Table 4).

Effect of extracts and fractions on STZ-induced diabetic animals (Multi-dose)

The effect of extracts and fractions on STZ-induced diabetic rats in multi dose treatment is presented in Table 5. The results revealed that the fraction, OCMF-3 exhibited highest reduction of blood glucose level with the percentage reduction of 64.90 followed by the fractions, OCMF-1 (62.43%) and OCMF-2 (62.33%). However, methanol and petroleum ether extracts respectively showed 58.93 and 51.63% reduction of blood glucose level at the end of 14th day when compared with the diabetic control group.

Table 5: Effect of extracts and fractions of *O. corniculata* L. on blood glucose levels in multi dose treated streptozotocin induced diabetic animals

Groups and treatment	Blood glucose level (mg/dl)					
	day 1	day 2	day 4	day 7	day 14	% decrease at the end of 14th Day
Solvent control (2 ml/kg)	91.16 ± 0.60	93.33 ± 0.95	90.83 ± 0.60	93.66 ± 0.66	89.33 ± 0.95	--
Diabetic control	280.17 ± 0.47 [b]	282.50 ± 0.76 [a]	302.50 ± 0.76 [c]	317.33 ± 0.88 [b]	337.67 ± 0.88 [b]	--
Glibenclamide (10 mg/kg)	267.67 ± 0.66*	253.33 ± 0.88*	236.33 ± 0.88**	172.33 ± 0.66**	106.50 ± 0.76***	68.46
Petroleum ether Extract (400 mg/kg)	263.83 ± 0.60	266.17 ± 0.60*	262.67 ± 0.84*	212.33 ± 0.71*	163.33 ± 0.88*	51.63

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Methanol extract (400 mg/kg)	264.83 ± 0.60	257.67 ± 0.66	247.17 ± 0.94	186.67 ± 0.88	138.67 ± 0.55	58.93
OCMF-1 (400 mg/kg)	269.33 ± 0.76*	270.33 ± 0.49*	249.17 ± 0.94**	176.33 ± 0.71*	126.83 ± 0.60**	62.43
OCMF-2 (400 mg/kg)	266.83 ± 0.60*	265.83 ± 0.90**	251.67 ± 0.88*	170.50 ± 0.76**	127.17 ± 1.19*	62.33
OCMF-3 (400 mg/kg)	273.67 ± 0.86*	272.50 ± 0.76**	257.67 ± 0.88*	181.17 ± 0.79***	118.50 ± 0.76***	64.90

Values expressed as mean ± SEM (n=6). The data were statistically analysed by one-way ANOVA, followed by Dunnet's *t*-test. p values less than 0.05 were considered significant. Solvent control vs Diabetic control [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. Diabetic control vs all other groups * $p < 0.05$; ** $p < 0.01$; ***: $p < 0.001$.

Biochemical parameters of STZ-induced diabetic animals after 14th day

The results of extracts and fractions on biochemical parameters of STZ-induced diabetic animals after 14th day treatment are depicted in Table 6. Overall, the fractions of methanol extract showed significant activity as compared to the crude methanol and petroleum ether extracts. The fractions at a dose of 400 mg/kg b.w. was found to exhibit highest degree of action in reducing the triglyceride, total cholesterol, total bilirubin, creatinine, LDL and VLDL levels followed by methanol and petroleum ether extracts. Furthermore, the fraction, OCMF-3 and methanol extract significantly increase the total protein and HDL levels after 14th day treatment.

Table 6: Effect of extracts and fractions of *O. corniculata* L. on Lipid profile and other biochemical parameters in multi dose treated streptozotocin induced diabetic rats

Groups and treatment	Lipid profile and other biochemical parameters							
	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TP (g/dl)	TB (mg/dl)	Creatinine (mg/dl)
Solvent control (2 ml/kg)	123.17 ± 1.19	87.16 ± 0.70	61.33 ± 0.88	73.16 ± 1.01	17.66 ± 0.88	07.66 ± 0.66	0.58 ± 0.11	0.66 ± 0.08
Diabetic control (STZ-65 mg/kg)	245.83 ± 0.94	132.83 ± 0.94	33.66 ± 0.66	135.67 ± 0.55	38.66 ± 0.66	3.83 ± 0.70	2.33 ± 0.49	2.21 ± 0.41
Glibenclamide (10 mg/kg)	119.83 ± 1.01	67.33 ± 1.05	57.33 ± 1.14	83.83 ± 0.94	18.16 ± 0.60	6.50 ± 0.76	0.88 ± 0.23	0.66 ± 0.15
Petroleum ether extract (400 mg/kg)	138.67 ± 0.88	93.33 ± 0.88	38.16 ± 1.13	105.67 ± 1.05	25.33 ± 0.71	5.50 ± 0.42	1.00 ± 0.01	0.97 ± 0.03
Methanol Extract (400 mg/kg)	130.17 ± 1.07	83.33 ± 1.14	41.33 ± 0.86	99.83 ± 0.94	22.66 ± 0.88	6.16 ± 0.79	1.02 ± 0.05	0.90 ± 0.10
OCMF-1 (400 mg/kg)	134.67 ± 0.88	80.83 ± 0.94	42.33 ± 1.33	95.95 ± 0.76	20.83 ± 0.94	5.83 ± 0.83	1.00 ± 0.03	0.95 ± 0.04
OCMF-2 (400 mg/kg)	133.17 ± 0.94	85.66 ± 0.88	40.83 ± 0.87	91.50 ± 0.76	23.66 ± 0.88	6.16 ± 1.07	0.97 ± 0.07	0.94 ± 0.02
OCMF-3 (400 mg/kg)	127.17 ± 0.70	78.33 ± 0.71	45.66 ± 0.38	106.17 ± 0.70	19.66 ± 0.88	6.33 ± 0.80	0.94 ± 0.01	0.92 ± 0.02

Values expressed as mean ± SEM (n=6). The data were statistically analysed by one-way ANOVA, followed by Dunnet's *t*-test. p values less than 0.05 were considered significant. TC-Total cholesterol, HDL - High density lipoprotein, LDL - Low density lipoprotein, VLDL- Very low density lipoprotein, TP-Total protein, TG-Triglycerides, TB-Total bilirubin.

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Table 7: α--amylase inhibition assay of methanol extract of *O. corniculata* L

Sample	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)
Acarbose (standard)	10	39.53±0.17	21.30
	20	45.58±0.71	
	30	60.61±0.80	
	40	69.30±0.55	
	50	74.10±0.94	
	60	76.39±0.66	
***O. corniculata* methanol extract**	25	40.64±0.89	64.84
	50	47.58±0.51	
	100	57.87±0.72	
	150	68.87±0.75	
	200	76.83±0.64	
	250	82.15±0.84	

All determinations were carried out in triplicate manner and values are expressed as mean ± SEM. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

***In-vitro* α-amylase inhibition assay**

Acarbose is a standard drug for α-amylase inhibition. Acarbose at a concentration of (10-60µg/ml) showed α-amylase inhibitory activity from 39.53 ± 0.17 to 76.39 ± 0.66% with an IC_{50} value 21.30 µg/ml (Table 7). Methanol extract (25-250 µg/ml) of *O. corniculata* exhibited potent α-amylase inhibitory activity in a dose dependent manner. Methanol extract showed highest inhibitory activity from 40.64 ± 0.89 to 88.15 ± 0.84 with an IC_{50} value of 64.84µg/ml.

DISCUSSION

Oxalis corniculata L. is a medicinally important plant indigenous to tropical and subtropical regions of the world. The present study has been undertaken to evaluate the antihyperglycaemic activity of *O. corniculata* in normal, glucose-loaded and STZ-induced hyperglycaemic rats (single and multi dose treatment). Sulfonylureas like glibenclamide are commonly used as a standard antidiabetic drug in STZ-induced diabetes to compare the efficacy of a variety of antihyperglycaemic compounds. [30] In normoglycaemic rats, the test extracts showed progressive fall of blood glucose level till the end of 8 h. Among the extracts, methanol extract showed maximum activity. In glucose loaded animals (OGTT), reduction in blood glucose levels was observed after 60 minutes of administration of the test substances. The maximum reduction was observed at 4 h where both methanol and petroleum ether extracts showed a significant reduction in blood glucose level and methanol extract exhibited maximum improvement in glucose tolerance.

The extracts produced significant decrease in the blood glucose level in STZ-induced hyperglycaemic rats when compared with the diabetic control group in the single dose treatment study at the tested dose levels. In multi-dose treated hyperglycaemic rats, both the extracts and fractions of the methanol extract showed various degree of blood glucose reduction, among which OCMF-3 exhibited highest percentage of reduction in blood glucose level. This might suggest that the said effect is due to extra intestinal action of the test substances. [31] From the results of the biochemical parameter study, it was observed that there was an increase in total bilirubin and serum creatinine levels in streptozotocin induced diabetic rats. However, 14 days of administration of extracts and fractions lead to a significant fall in total bilirubin and serum creatinine levels when compared with the diabetic control group. It was also observed that there was an increase in serum total cholesterol, triglycerides, LDL and VLDL levels and decrease in total protein and HDL levels in STZ-induced hyperglycaemic rats. Continuous administration of petroleum ether and methanol extracts and fractions of methanol extract for 14 days leads to significant decrease in serum total cholesterol, triglycerides, LDL and VLDL levels, while increase in total protein and HDL levels was recorded. The results indicate that, the treatment of diabetic rats with *O. corniculata* prevents the alteration in serum biochemistry values and returns nearer to their normal values, which supports its anti-diabetic activity.

Medicinal plants that exhibit anti-diabetic activity usually possess active substances which are able to mimic the action of insulin or which exert similar effect on the β-cells of the pancreas, causing them to synthesize and secrete insulin. [32] The blood glucose lowering ability of the test substances showed encouraging results in our study among which methanol extract and fractions of methanol extract showed maximum potency. Lack of insulin affects the metabolism of carbohydrates, proteins, fat and causes significance disturbance of water and electrolyte homeostasis. [32] Recent advances in understanding the activity of intestinal enzymes have lead to the development of newer pharmacological agents. α-Amylase is important in carbohydrate digestion and glucose absorption. [33] The *in vitro* study showed an increased utilization of the glucose by α-amylase inhibition assay in presence of methanol extract which suggests that the test extract may inhibit the digestion and absorption of glucose through intestine. The possible mechanism might be the potentiation of pancreatic secretion of insulin from existing β-cells of islets, and inhibition of digestion and absorption of glucose through intestine as evidenced by the significant increase in glucose utilization by α-amylase inhibition assay.

CONCLUSION

The experimental results of the present investigation conclude that the extracts and fractions of *Oxalis corniculata* L. whole plant showed various degree of antihyperglycaemic effect, among which methanol extract and fractions of methanol extract especially OCMF-3 showed potent antihyperglycaemic activity in streptozotocin induced multi-dose treated diabetic animals. The fraction, OCMF-3 exhibited highest activity among the test substances which suggests the presence of higher concentration of active constituents in it. The methanol extract also showed persuasive inhibition of glucose digestion and absorption in α-amylase inhibition assay. The antihyperglycaemic activity of the test substances was comparable with the standard drug. These findings suggest that the plant may be a potential source for the development of new oral antihyperglycaemic agent.

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REFERENCES

[1] Rao K, Giri B, Kesavulu R, and Apparao M. Effect of oral administration of bark of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. J. Ethnopharmacol., 2001; 74: 69–74.
[2] WHO. (1999). *Diabetes mellitus*. Fact sheet number 138 and 236, Geneva.
[3] Djrolo F, Houngbe H, Avode G, Addra B, Kodjoh N, Avinadje M and Monterio B. Le diabète lié à la mal nutrition (Diabète tropical). Médecine Afrique Noire, 1998; 45 (8/9): 538-42.
[4] Grover JK, Yadav S and Vata V. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol., 2002; 81: 81– 100.
[5] Badwaik H, Singh MK, Thakur D, Kumar TG, Tripathi DK. The Botany, Chemistry, Pharmacological and Therapeutic application of *Oxalis corniculata* Linn. International Journal of Phytomedicine, 2009; 3: 14-19.
[6] Dreyer LL, Esler KJ, Zietsman J. Flowering phenology of South African *Oxalis* - Possible indicator of climate change, South African Journal of Botany 2006; 72: 150-156.
[7] Kathiriya AK, Das K, Joshipura M, Mandal N. *Oxalis corniculata* Linn. -The Plant of Indian subtropics. Herbal Tech Industry 2010; 1: 7-11.
[8] Madhava, KS, Sivaji K, Tulasi RK. Flowering plants of Chitoor district, Andhra Pradesh, India. Student Offset Printers, Tirupati; 2008, p. 54.
[9] Gupta G, Singh V, Singh M, Bisht R, Maurya H. Analgesic activity of ethanolic extract from *Oxalis corniculata* Linn (Oxalidaceae) in mice. Inventi Rapid Ethanopharmacology 2010; 2: 15-18.
[10] Chatterjee A, Prakashi SC. "The Treatise on Indian Medicinal Plants", New Delhi, CSIR, 1994; p. 118-119.
[11] Achola KJ, Mwangi JW. "Pharmacological activity of *Oxalis corniculata*". Int J Pharmacognosy 1995; 33(3): 247-249.

[12] Runyoro DKB, Ngassapa OD, Matee MIN, Joseph CC, Moshi MJ. Medicinal plants used by Tanzanian traditional healers in the management of Candida infections. Journal of Ethanopharmacology 2006; 106: 158-165.
[13] Raghvendra MP, Satish S, Raveesha KA. Phytochemical analysis and antibacterial activity of *Oxalis Corniculata*, a known medicinal plant. My Science; 2006; 1: 72-78.
[14] Sharagouda K, Saraswati BP. Antiimplantation and abortifacient activities of *Oxalis corniculata* in albino rats. Nig J Nat Prod Med 2007; 11: 58-60.
[15] Taranalli AD, Tipare SV, Kumar S, Torgal SS. Wound healing activity of *Oxalis corniculata* whole plant extract in rats. Ind J Pharm Sci 2004; 66: 444-6.
[16] Pierre W, Evelyne N, Telesphore BN, Sylvie LW, Albert K. Antidiarrhoeal activity of aqueous and methanol extracts of *Oxalis corniculata* in rats. J Exp Biol 2005; 1(1): 256-60.
[17] Krishnaveni A, *et al*. Hypoglycaemic effect of *Oxalis corniculata* in normal rabbits. Asian J Exp Sci 2006; 103(12): 710.
[18] Sharangouda K, Saraswati BP. Post-coital antifertility activity of whole plant of *Oxalis corniculata* (Oxalidaceae) on female albino rats. Pak J Pharm Sci 2006; 1: 63-67.
[19] Han ST. Medicinal plants in the South Pacific. WHO Regional Publications, Western specific series no. 19; 1998, p. 135.
[20] Gupta G. Sedative, antiepileptic and antipsychotic effects of *Viscum album* L. (Loranthaceae) in mice and rats. J. Ethnopharmacol. (2012) doi:10.1016/j.jep.2012.03.013
[21] Ghosh MN, *Fundamentals of Experimental Pharmacology*, 3rd ed, (Hilton & Company, Kolkata, India) 2005; 194.
[22] Paget G E & Barnes JM, in *Pharmacometrics*, (Academic press, New York) 1983, 115.
[23] Kar D, Maharana L, Pattnaik S & Dash G. Studies on hypoglycaemic activity of *Solanum xanthocarpum* Schrad. & Wendl. fruit extract in rats, J Ethnopharmacol, 2006; 108: 251–256.
[24] Shirwaikar A, Rajendran K, Barik R. Effect of aqueous bark extract of *Garuga pinnata* Roxb. in streptozotocin–nicotinamide induced type-II *Diabetes mellitus*, J Ethnopharmacol, 2006; 107 : 285–290.
[25] Andallu B & Varadacharyulu NC. Control of hyperglycemia and retardation of cataract by mulberry (*Morus indica* L.) leaves in streptozotocin diabetic rats, Indian J Exp Biol., 2002; 40: 791-795.
[26] Theodorou N A, Vrbova H, Tyhurst M & Howell SL. Management of intestinal amoebiasis by an indigenous drug Kantaki karanja (*Caesalpinia crista* L.), *Diabetol*, 1980; 18: 313-318.
[27] Kesari AN, Gupta RK, Singh SK, Diwakar S, Watal G. Hypoglycaemic and antihyperglycaemic activity of *Aegle marmelos* seed extract in normal and diabetic rats, J Ethnopharmacol, 2006; 107: 374–379.
[28] Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal Biochem, 72 (1976) 248–254. 29.
[29] Miller GL. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. Anal Chem, 1959; 31: 426-428.
[30] Day C, Cartwright T, Provost J, Bailey CJ. Hypoglycaemic effect of *Momordica charantia* extracts, Planta Med, 1990; 56(5):426-429.
[31] Keto M and Miura T. Planta Med. 1996; 62, 440-443.
[32] Frier BM, Fisher M. *Diabetes mellitus*. In: Boon NA, Colledge NR, Walker BR, Hunter JAA, (Ed.), Davidson's principle and practice of medicine, 20th ed. Churchill Livingstone, Elsevier: Ediburgh, 2006; 805-845.
[33] Liu F, Ooi VE, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Science, 1997; 60:763-771.