

Antidiabetic and Hypolipidemic Activity of *Citrus medica* Linn. Seed Extract in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Introduction: The objective of the present investigation was to evaluate the antidiabetic and hypolipidemic activity of petroleum ether extract of *Citrus medica* Linn. seeds in streptozotocin (STZ) induced diabetic model in rats. **Methods:** The study was carried out using albino rats of either sex weighing 150-200 gm. One group was selected as control group (buffer alone) and four groups of STZ induced diabetic rats ($n = 5$ in each group) were administered vehicle (1% tween 80), seed extract (200 and 400 mg/kg, p.o.) of *C. medica* Linn. and standard drug glibenclamide (5 mg/kg) for 15 days after 10 days of single dose of STZ (60 mg/kg) intraperitoneal administration. Blood samples were collected by retro-orbital puncture and were analyzed for blood glucose, serum cholesterol, triglycerides, HDL, LDL and VLDL on days 0, 3, 10 and 25 by using diagnostic kit. **Results:** The petroleum ether extract of *C. medica* Linn. seeds (200 and 400 mg/kg, p.o.) induced significant reduction ($p < 0.05$) of fasting blood glucose, serum cholesterol, serum triglycerides, LDL and VLDL in dose dependent manner after 15 days of drug administration. Though 200 mg/kg/day seed extract for 15 days was not showing any change in HDL level, while 400 mg/kg/day dose significantly increased HDL level in diabetic rats. **Conclusion:** So it is concluded that *C. medica* Linn. seeds have significant antidiabetic, hypocholesterolemic and hypolipidemic activity.

Key words: Antidiabetic, hypolipidemic, *Citrus medica* Linn., blood glucose.

INTRODUCTION

Diabetes mellitus is a chronic disease that has a significant impact on health, quality of life as well as on the health care system. It is a disease characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism and by complications like retinopathy, microangiopathy and nephropathy.^[1] These metabolic abnormalities result, in part, from a deficiency of the blood sugar lowering hormone insulin; this deficiency in insulin results in type 1 diabetes or insulin dependent diabetes mellitus (IDDM). Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) is a result of hyperglycemia caused by overproduction of glucose at the hepatic level

or because of abnormal β cell function or insulin resistance at target cells.^[2]

In diabetic rats, the impaired utilization of carbohydrate leads to accelerated lipolysis, resulting in hyperlipidemia.^[3] NIDDM has also been associated with an increased risk of premature arteriosclerosis due to increase in triglycerides and low density lipoprotein levels. About 70-80% deaths in diabetic patients are due to vascular diseases. Thus an ideal treatment for diabetes would be a drug that not only controls the blood sugar level but also prevents the development of arteriosclerosis and other complications of diabetes.^[4] Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma, hepatorenal disturbances and are unsafe during pregnancy.^[5-7]

There has been an increasing demand for the use of natural products with antidiabetic and antihyperlipidemic activity. This is largely because insulin cannot be used orally and insulin injections are associated with the risk of hypoglycemia and impairment of hepatic and other body functions. The undesirable side effects of synthetic hypoglycemic drugs,

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and the fact that they are not suitable for use during pregnancy, have made researchers look towards hypoglycemic drugs of plant origin.^[8-10]

Several members of the genus *Citrus* (Rutaceae) are being used traditionally in a wide variety of ethnomedical remedies. One among them is *Citrus medica* Linn. It is commonly known as matulunga in Sanskrit, citron in English and bijapura or bara nimbu in Hindi.^[13] Ripe fruits of *C. medica* Linn. are potent anti-scorbutic, tonic, stomachic, used in vomiting and expellant of poison. Seeds are used in piles, biliousness, inflammations and as vermifuge, stimulant and cardiac tonic.^[14] The objective of the present study was to evaluate the hypoglycemic and hypolipidemic activity of petroleum ether extract of seeds of *C. medica* Linn. in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant material

The seeds of *C. medica* Linn. were collected locally. The plant was identified as *C. medica* Linn. by an authorized taxonomist at National Botanical Research Institute, Lucknow. Voucher specimen number 97840 has been deposited in the same institute.

Preparation of the extract

The collected seeds were washed thoroughly under running tap water, dried under sun and coarsely powdered. The petroleum ether extract of course powder was prepared by Soxhlet extraction at 40 °C for 3-4 h, and then the solvent was recovered at 40 °C under reduced pressure. The extract was oily, pale yellow coloured liquid, stored at room temperature till used in the experiment.

Animals

Healthy adult ~~male~~ Wistar rats between 2-3 months of age and weighing 150-175 g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12-h light and 12-h dark cycle; 25 ± 5 °C; 35-60% humidity), the animals were fed with standard rat pellet diet and provided water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee and the CPCSEA.

Experimental design and induction of diabetes

The animals irrespective of sex were distributed into five groups (with six animals in each group) as follows: (I) control group, (II) diabetic control group, (III) diabetic group treated with 200 mg/kg/day of *Citrus medica* Linn. extract, (IV) diabetic group treated with 400 mg/kg/day of *Citrus medica* Linn. extract, and (V) diabetic group treated with Glibenclamide 5 mg/kg/day.

Animals of groups II, III, IV and V were rendered diabetic by a single intraperitoneal (i.p.) injection of 60 mg/kg of streptozotocin (STZ) freshly prepared in 0.1 M of citrate buffer (pH 4.5). Animals of group I were injected with buffer alone. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined after 72 h and after 10 days of STZ injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >126 mg/dl.^[13] Only those rats that were found to have permanent NIDDM were used for the study.

Ten days after the STZ injection, animals of group III received 200 mg/kg/day, group IV received 400 mg/kg/day of *Citrus medica* Linn. extract and group V received Glibenclamide 5 mg/kg/day for 15 days. The *Citrus medica* Linn. extract and Glibenclamide were administered orally as a suspension in 1% v/v tween 80. While animals of group II were received only 1% v/v of tween 80.

Collection of blood and estimation of biochemical parameters

Blood was withdrawn from the retroorbital sinus under ether inhalation anesthesia. The fasting blood glucose levels and lipid profile were determined on day 0 and after day 3, 10, and 25. Fasting blood glucose was determined by Modified Roeschlau's Method.^[14] The blood sugar level was measured by using Agappe diagnostics kit (Ernakulan, Kerala). The blood sugar, serum triglyceride and total cholesterol levels were measured by spectrophotometric methods using Agappe diagnostic kits (Ernakulan, Kerala). HDL (high density lipoproteins)-cholesterol was determined using the diagnostic kit of Piramal Healthcare, Mumbai. LDL (low density lipoproteins)-cholesterol and VLDL (very low density lipoproteins)-cholesterol were calculated by the equations (Friendewald's Formula):^[15]

$$\text{LDL (mg/dl)} = \frac{\text{Total Cholesterol} - \text{HDL Cholesterol} - \text{Triglyceride}}{5}$$

$$\text{VLDL (mg/dl)} = \text{Total cholesterol} - \text{HDL} - \text{LDL}$$

Statistical analysis

The data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Values were considered to be significant at $P \leq 0.05$.

RESULTS

A single dose of STZ at a dose of 60 mg/kg body weight causes a significant diabetogenic response in rats. The increase in glucose level in diabetic groups (II-V) was found to be highly significant ($p < 0.001$) when compared to normal control group I. The diabetes was maintained even after ten days of STZ administration. Changes in blood

glucose of various groups are presented in Table 1. *C. medica* Linn. seed extract caused dose dependent significant reduction of fasting blood glucose in groups III and IV as compared to the diabetic control group. The antihyperglycemic effect of 400 mg/kg/day of extract for 15 days, being comparable to that of glibenclamide. Glibenclamide showed a 28.1% decrease in glucose level as compared to the diabetic control group. Administration of vehicle to STZ induced diabetic rats resulted in an increase in the level of triglycerides, total cholesterol, LDL, and VLDL, and decreased HDL, even after 25 days; same effect in rats of group III to V till tenth day of STZ administration. Continuous administrations of the petroleum ether extract (200 and 400 mg/kg/day for 15 days) of *C. medica* Linn. seeds lead to significant decrease ($p < 0.05$) in the level of triglycerides, total cholesterol, LDL, and VLDL in the diabetic rats, while it increased the level of HDL (Table 2).

DISCUSSION

Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in patients with diabetes. Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of microvascular complications.^[16] STZ is widely used to induce diabetes in experimental animals. As STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, NO has been proposed to contribute to STZ induced DNA damage.^[17,18] STZ was also found to generate reactive oxygen species which contribute to DNA fragmentation and evoke deleterious changes in the cells.^[19,20] The present investigation studied the hypoglycemic and hypolipidemic potential of *C. medica* Linn. seeds in STZ-induced diabetic rats. *C. medica* Linn. seeds extract has shown significant decrease of fasting blood

Table1: Effect of *C. medica* extract on fasting blood sugar level in diabetic rats

Group	Treatment	Fasting blood sugar level (mg/dl)			
		0 day	3 rd day	10 th day	25 th day
1.	Control	116.342 ± 2.512	110.419 ± 4.384	110.943 ± 3.703	124.688 ± 3.424
2.	Diabetic Control	107.912 ± 3.769	204.679 ± 4.893 ^{##}	177.9 ± 5.012 ^{##}	181.162 ± 3.577 ^{##}
3.	200 mg extract	108.02 ± 3.758	207.904 ± 4.212 ^{##}	200.512 ± 2.327 ^{##}	138.695 ± 2.224 [*]
4.	400 mg extract	118.747 ± 2.251	214.874 ± 4.666 ^{##}	205.864 ± 1.848 ^{##}	132.755 ± 1.425 ^{**}
5.	Glibenclamide	116.206 ± 3.664	188.296 ± 5.87 ^{##}	184.408 ± 6.087 ^{##}	130.116 ± 1.689 ^{**}

Value represents mean ± SEM (n = 6); [#]p < 0.05, ^{##}p < 0.001, significant when compared to normal control group (Group I); ^{*}p < 0.05, ^{**}p < 0.001, significant when compared to diabetic control group (Group II); One way ANOVA followed by Tukey's multiple comparison test.

Table2: Effect of *C. medica* extract on lipid profile in STZ-induced diabetic rats

Parameters	Period	Experimental Groups					
		Normal control	Diabetic control	<i>C. medica</i> extract			Glibenclamide
				200 mg/kg	400 mg/kg	5 mg/kg	
TCH	0 day	100.38 ± 1.65	99.74 ± 1.82	111.4 ± 2.52	115.97 ± 1.98	107.76 ± 1.87	
	3 days	101.93 ± 1.87	176.45 ± 4.74 ^{##}	191.01 ± 1.94 ^{##}	189.73 ± 1.96 ^{##}	189.79 ± 1.26 ^{##}	
	10 days	101.20 ± 2.17	186.10 ± 1.52 ^{##}	190.60 ± 1.73 ^{##}	191.59 ± 2.35 ^{##}	192.13 ± 1.73 ^{##}	
	25 days	102.92 ± 1.99	187.95 ± 1.36 ^{##}	141.37 ± 2.72 [*]	136.34 ± 3.54 ^{**}	108.72 ± 1.08 ^{**}	
TG	0 day	81.79 ± 3.70	91.59 ± 2.77	93.07 ± 2.34	109.70 ± 6.52	98.46 ± 6.184	
	3 days	82.89 ± 2.52	224.50 ± 6.59 ^{##}	200.95 ± 5.16 ^{##}	213.44 ± 3.46 ^{##}	207.15 ± 4.47 ^{##}	
	10 days	86.20 ± 3.18	206.63 ± 4.72 ^{##}	204.25 ± 4.1 ^{##}	212.99 ± 9.10 ^{##}	210.23 ± 5.32 ^{##}	
	25 days	79.57 ± 2.7	213.27 ± 4.54 ^{##}	155.62 ± 3.41 [*]	147.05 ± 3.53 [*]	102.51 ± 2.18 ^{**}	
HDL	0 day	39.42 ± 1.30	35.13 ± 1.0	31.11 ± 1.45	35.86 ± 0.85	38.67 ± 1.21	
	3 days	38.77 ± 1.60	29.82 ± 1.07 [#]	27.65 ± 2.28 [#]	29.71 ± 1.10 [#]	30.79 ± 1.28 [#]	
	10 days	34.74 ± 1.30	30.27 ± 0.72 [#]	26.59 ± 0.80 [#]	28.31 ± 0.61 [#]	30.72 ± 1.32 [#]	
	25 days	36.82 ± 1.11	32.46 ± 0.88 [#]	27.04 ± 0.9	31.88 ± 1.11 [*]	33.35 ± 1.42 [*]	
LDL	0 day	44.60 ± 2.70	46.28 ± 2.46	61.38 ± 4.03	58.162 ± 2.932	49.56 ± 1.50	
	3 days	44.28 ± 2.6	101.73 ± 4.74 ^{##}	123.16 ± 1.59 ^{##}	117.33 ± 2.66 ^{##}	117.56 ± 2.49 ^{##}	
	10 days	49.19 ± 1.78	114.49 ± 1.52 ^{##}	123.15 ± 2.37 ^{##}	120.67 ± 3.38 ^{##}	119.37 ± 3.1 ^{##}	
	25 days	50.18 ± 3.14	113.84 ± 2.39 ^{##}	108.20 ± 2.56	94.45 ± 2.60 [*]	90.86 ± 1.59 [*]	
VLDL	0 day	16.25 ± 0.58	18.32 ± 0.55	18.61 ± 0.46	21.94 ± 1.30	19.53 ± 1.36	
	3 days	15.85 ± 0.76	44.9 ± 1.31 ^{##}	40.18 ± 1.03 ^{##}	42.68 ± 0.69 ^{##}	41.43 ± 0.89 ^{##}	
	10 days	17.27 ± 0.63	41.32 ± 0.95 ^{##}	40.85 ± 0.82 ^{##}	42.59 ± 1.82 ^{##}	42.032 ± 1.604 ^{##}	
	25 days	15.85 ± 0.54	42.65 ± 0.90 ^{##}	31.02 ± 0.76 [*]	29.01 ± 0.80 [*]	27.49 ± 0.43 [*]	

Value represents mean ± SEM (n = 6); [#]p < 0.05, ^{##}p < 0.001, significant when compared to normal control group (Group I); ^{*}p < 0.05, ^{**}p < 0.001, significant when compared to diabetic control group (Group II); One way ANOVA followed by Tukey's multiple comparison test.

sugar levels at doses of 200 and 400 mg/kg/day in diabetic rats as compared to sugar levels in untreated diabetic rats.

The level of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia^[21] and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities.^[22] In our study also, the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with plant extract significantly decreased both cholesterol and triglyceride levels, the extract also decreased LDL and VLDL levels and increased useful HDL level in diabetic rats. This implies that the petroleum ether extract of *C. medica* Linn. seeds may prevent or be helpful in reducing the complications of lipid profile seen in many diabetics in whom hyperglycemia and hypercholesterolemia coexist quite often.^[23]

Possibly the petroleum ether extract may lead to regeneration of the β -cells of the pancreas and potentiation of insulin secretion from surviving β -cells; the increase in insulin secretion and the consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. A number of other plants have also been reported to have antihyperglycemic, antihyperlipidemic, and insulin stimulatory effects.^[24,25]

It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The decrease of cholesterol and LDL levels achieved by administration of *C. medica* Linn. seeds extract, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about.

As the chemical constituents and medicinal uses of *C. medica* Linn. seeds have been reported, the observed effects could be attributed to the presence of various phytoconstituents including limonin, limonol and nomilinic acid present in the seeds.^[26]

CONCLUSION

From the results of the present study, it can be concluded that *C. medica* Linn. seeds are potent antihyperglycemic and hypolipidemic agent. The biological efficacy may be

even higher after the isolation and purification of the compounds. Further studies are needed to identify the chemical constituents that may be responsible for the above activities.

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