

**HYPOGLYCEMIC AND HYPOLIPIDIMIC ACTIVITY OF  
ALCOHOLIC EXTRACT OF CITRUS AURANTIUM IN  
NORMAL AND ALLOXAN-INDUCED DIABETIC RATS.**

**Manoj Sharma<sup>1</sup>, J. Fernandes<sup>1</sup>, Dheeraj Ahirwar<sup>2</sup>, Ritesh Jain<sup>2\*</sup>**

1. NGSM Institute of Pharmaceutical Sciences, Mangalore, 575003, Kranataka, India.

2. School of Pharmacy, Choksey Engg. Collage, Bilaspur, 495007, Chhattisgarh, India.

**Summary**

The alcoholic extract of *Citrus aurantium* fruit peel was evaluated for its hypoglycemic and hypolipidimic activity in normal and alloxan-induced diabetic rats. The extract produced significant reduction ( $p < 0.001$ ) in blood glucose and also had beneficial effects on the lipid profile in normal as well as alloxan-induced diabetic rats at the end of the treatment period (21<sup>st</sup> day). The hypoglycemic and hypolipidimic activity of *Citrus aurantium* was compared with tolbutamide (100 mg/kg b.w.).

**Key words:** *Citrus aurantium*, diabetes mellitus, arteriosclerosis, tolbutamide.

**\* Address of the author for correspondence**

Ritesh Jain

Lecturer,

School of pharmacy, chuoksey Engg. Collage,  
Bilaspur-495007, India.

E-mail: [cec.ritesh@yahoo.com](mailto:cec.ritesh@yahoo.com)

FaX No.: +91-7752-212112

## Introduction

Diabetes mellitus is heterogeneous primary disorder of carbohydrate metabolism with multiple etiological factors; it generally involves absolute or insulin resistance, or both. Whatever the cause, diabetes ultimately leads to hyperglycemia, which is the landmark of this disease syndrome<sup>1</sup> and it has also been associated with an increased risk for premature arteriosclerosis due to increased in triglycerides and low density lipoprotein levels. Diabetes mellitus is an independent predictor of high risk for Coronary Heart Disease (CHD). CHD morbidity is two to four time higher in patients with diabetes than nondiabetics, and the mortality from CHD is up to 100% higher in diabetic patients than in nondiabetics over a 6-year period. About 70-80% of deaths in diabetic patients are due to vascular disease. Glucose control is essential, but this provides only minimal benefit with respect to CHD prevention.<sup>2</sup> An ideal treatment for diabetes would be a drug that not only controls the glycemic levels but also prevents the development of arteriosclerosis and other complications of diabetes.<sup>3</sup>

*Citrus aurantium* family rutaceae, commonly known in India as kumla nembu traditionally it is used in maintenance of diabetes.<sup>4</sup> The peel of *Citrus aurantium* contains pectin, pectin showed a significant hypoglycemic action in normal and diabetic rats.<sup>5</sup> *Citrus aurantium* is reported as having a weight reduction effect when combined with strict diet.<sup>6</sup> In addition, *Citrus aurantium* has been shown to be radioprotective because it is rich in flavanoids with anti-oxidative activity<sup>7</sup> and it is also reported cardiovascular effect<sup>8</sup> and antimutagenic activity<sup>9</sup>.

The objective of the present study was to evaluate the hypoglycemic and hypolipidemic activity of an alcoholic extract of the fruit peel of *Citrus aurantium* in normal and alloxan-induced diabetic rats.

## Material and Methods

### *Plant material and preparation of extract*

Fresh, unripe, green fruit of *Citrus aurantium* were collected in month of April from Ambah, District; Morena, M.P., India and were identified and authenticated by botanist Dr. R.A.S. Chauhan, department of botany, Ambah PG collage, Ambah. The fruit peel was shade dried at room temperature for 10 days and powdered with the help of a hand-grinding mill and the powder was passed through sieve no. 40.

The powdered material was successively extracted with petroleum ether and alcohol in soxhlet's apparatus. Aqueous extract was prepared by maceration process with water for 72 hours. The drug was extracted with each solvent till complete extraction is effected. The solvents were removed under reduced temperature from the concentrated extracts. Each extract was concentrated by distilling off the solvent to obtain the crude extractives. The percentage yield of ether extract, alcoholic and aqueous extract were 2.3 %, 28.5 %, 16.3 %, respectively.

#### ***Phytochemical Screening***

Preliminary phytochemical screening of the extracts was carried out by the standard procedure<sup>10</sup>.

#### ***Animals and Housing condition***

Laboratory bred adult albino rats of 16-18 weeks age and of either sex, weighing 180-220 g was selected. The animals were maintained under standard laboratory condition at  $25 \pm 2^{\circ}$  C, relative humidity  $50 \pm 15$  % and normal photo period (12 h dark / 12 h light) used for experiment. Commercial pellet diet (Pranav Agro Industries Ltd, Bangalore, India) and water provided *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by regulatory body of the government.

#### ***Determination of LD<sub>50</sub>***

The acute oral toxicity (AOT) of alcoholic and aqueous extract of fruit peel of *Citrus aurantium* were determined by using nulliparous, non pregnant female albino rats (Wistar strains) weighing between 180-220 g those maintained under standard husbandry conditions. The animals were fasted 12 hrs prior to the dosing. Animals were administered (orally) with single dose of extracts dissolved in 2% w/v acacia and observed for its mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OECD guideline 425. All the animals were also observed for long term toxicity (14 Days). The LD<sub>50</sub> of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA<sup>11</sup>

#### ***Induction of experimental diabetes***

Animals were allowed to fast 24 h and were injected with alloxan monohydrate (Loba Chemie Pvt. Ltd. Mumbai, India) dissolved in sterile normal saline at a

dose of 150 mg/kg body weight intraperitoneally. After one week rats with mild diabetes and hyperglycemic (200-300 mg/dl) were used for the experiment.

### ***Experimental protocol***

**Normal Rats:** Normal rats were divided into four groups, each group having six animals. Group I served as control and received 1% w/v suspension of CMC at a dose of 10ml/kg b.w. Group II and Group III received alcoholic extract of *Citrus aurantium* in 1% CMC at a dose of 300 mg/kg and 500 mg/kg b.w. respectively. Group IV received the standard drug tolbutamide at a dose 100 mg/kg b.w. Serum glucose and lipid profile were estimated before starting the treatment and weekly (0, 7, 14, and 21 days) thereafter up to the end of the treatment period.

**Diabetic rats:** diabetic rats were also divided in to four groups as described above. Serum glucose and lipid profile were determine at the day 0, 7, 14, 21.

### ***Collection of blood and Estimation of blood glucose and lipid profile.***

The rats were anesthetized with anesthetic ether and blood sample were drawn from retroorbital method. Blood subjected to centrifugation to obtained serum.

Serum was analyzed for serum glucose, serum triglyceride, serum total cholesterol, HDL- cholesterol by using the Span diagnostic kit. LDL-cholesterol and VLDL-cholesterol was calculated as described by Friedewald et al.<sup>12</sup>

### ***Statistical analysis***

The results have been expressed as mean  $\pm$  standard error of mean (S.E.M). Difference in means were compared using one way analysis of variance (ANOVA) followed by Tukey Kramer's post hoc test.  $P < 0.05$  were considered statistically significant.

## **Results**

Preliminary phytochemical studies of our study revealed that presence of glycoside, carbohydrates, pectin, flavanoids, phenolics compounds and tannins in alcoholic extract. Different doses of alcoholic extract were screened for their oral toxicity. No mortality was recorded till 5000 mg/kg with alcoholic extract, hence the extracts were found to be safe upto the dose levels of 5000 mg/kg.

***Effect of alcoholic extract of Citrus aurantium on Normal rats:***

A significant reduction ( $P < 0.001$ ) in blood glucose levels was observed at the end of the second week (14 th day) of treatment with ethanolic extract (300 mg/kg and 500 mg/kg b.w.) of *Citrus aurantium* in the normal rats; this was further lowered after 21 days of treatment. The maximum reduction in blood glucose level was seen at a dose of 500 mg/kg b.w of *Citrus aurantium* extract administration. However the effect of ethanolic extract of *Citrus aurantium* was less than that of tolbutamide.[Figure - 1].

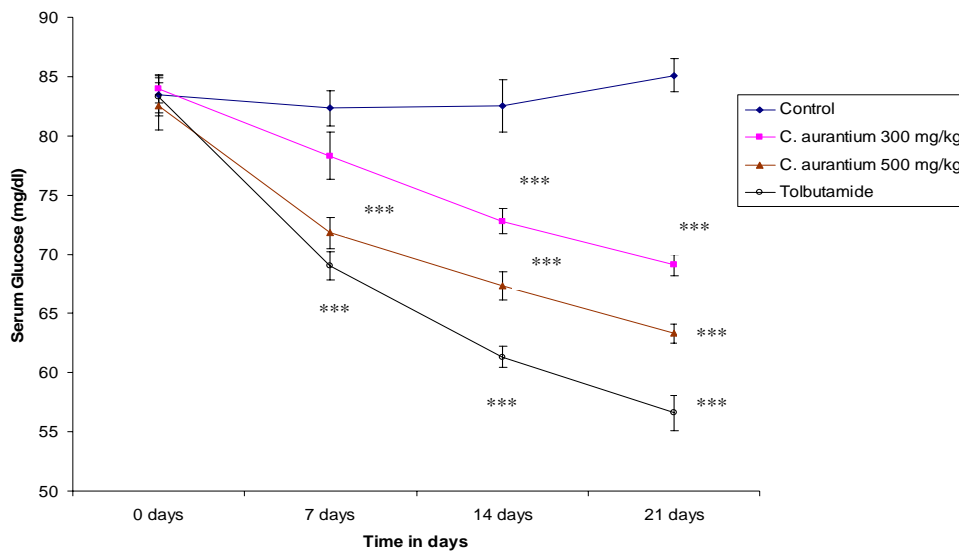
As the blood glucose-lowering effect of 500 mg/kg b.w. of the ethanolic extract was more, only the effect of this dose on the lipid profile of normal as well diabetic albino rats is shown in [Table – 1 & 2]. Administration of the ethanolic extract led to a significant fall in the level of triglycerides, total cholesterol, LDL, and VLDL, and improved the HDL levels, in normal rats. Tolbutamide also showed reduction in the levels of triglycerides, total cholesterol, LDL, and VLDL, and improved the HDL, after 21 days [Table - 1].

***Effect of alcoholic extract of Citrus aurantium on alloxan-induced diabetic rats:***

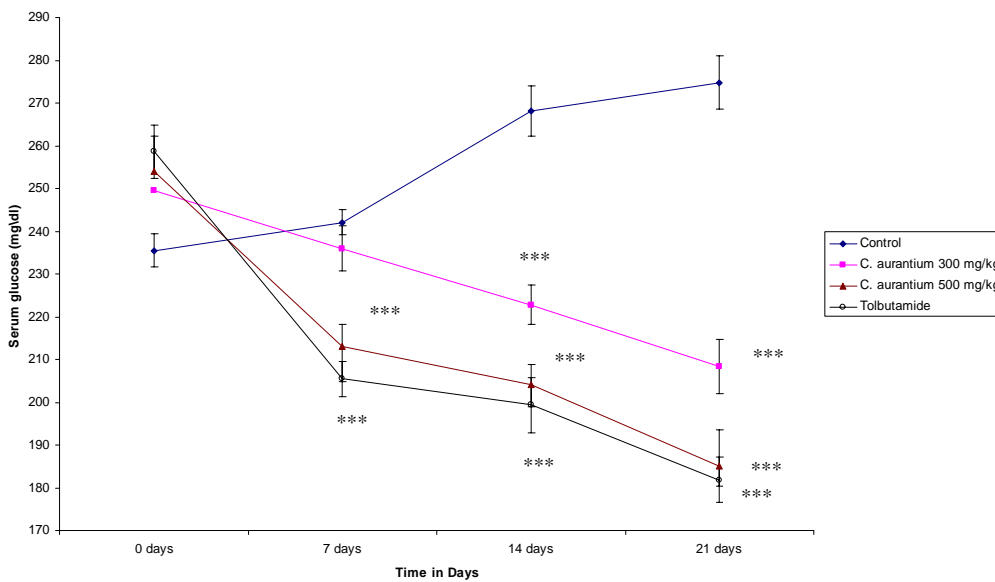
On repeated administration of ethanolic extract at doses of 300 mg and 500 mg/kg b.w.for 21 days, a significant ( $P < 0.001$ ) dose-dependent decrease in blood glucose of the diabetic rats was seen as compared to the vehicle-treated group. [Figure - 2].

Administration of vehicle to alloxan-induced diabetic rats resulted in an increase in the level of triglycerides, total cholesterol, LDL, and VLDL, and decreased HDL, after 21 days. Continuous administration of the ethanolic extract (500 mg/kg b.w.) of *Citrus aurantium* led to significant decrease in the level of triglycerides, total cholesterol, LDL, and VLDL in the diabetic rats, while it increased the level of HDL, effect of ethanolic extract of *Citrus aurantium* on lipid profile was more than that of tolbutamide [Table - 2].

**Figure 1: Effect of *Citrus aurantium* and Tolbutamide on blood glucose level in normal rats.**



**Figure 2: Effect of *Citrus aurantium* and Tolbutamide on blood glucose level in diabetic rats.**



**Table: 1** Effect of administration with *Citrus aurantium* and tolbutamide on serum lipid parameter level in Normal rats (on 21 day).

S. No.	Treatment	Serum Lipid Parameter (mg/dl)					
		TG	TCH	HDL-C	LDL-C	VLDL	
1.	Normal Control	66.7 ±3.4	102.16 ±2.69	50.83 ±2.32	38.08 ±4.27	13.25 ±0.711	
2.	ALCA 500 mg/kg	52.1* ±3.45	88.06** ±2.01	53.83 ±2.91	23.71* ±1.63	10.51* ±0.68	
3.	Tolbutamide 100mg/kg	51.8* ±2.3	96.7 ±1.86	53.5 ±2.74	33.26 ±3.74	9.98** ±0.46	
<b>One way-ANOVA</b>		df	17	17	17	17	
		f	7.28	10.24	0.37	4.59	7.77

Values are expressed as mean ± S.E.M. n=6

\*  $P < 0.05$ , \*\* $P < 0.01$  vs. control (Normal), using one-way ANOVA followed by Tukey Kramer's post hoc test.

**Table: 2** Effect of administration with *Citrus aurantium* and tolbutamide on serum lipid parameter level in Diabetic rats (on 21 day).

S. No.	Treatment	Serum Lipid Parameter (mg/dl)					
		TG	TCH	HDL-C	LDL-C	VLDL	
1.	Diabetic Control	205.08 ±14.96	137.65 ±9.95	25.66 ±2.6	70.83 ±8.8	41.03 ±2.98	
2.	ALCA 500 mg/kg	117.5*** ±11.41	97.03** ±4.89	42.36** ±3.2	35.1** ±2.15	18.29*** ±1.53	
3.	Tolbutamide 100mg/kg	147.57** ±6.4	111.16* ±5.05	34.2 ±1.89	47.43* ±4.4	29.5** ±1.28	
<b>One way-ANOVA</b>		df	17	17	17	17	
		f	15.02	8.51	10.19	9.58	30.2

Values are expressed as mean ± S.E.M. n=6

\*  $P < 0.05$ , \*\* $P < 0.01$  \*\*\*  $P < 0.001$  vs. control (Diabetic), using one-way ANOVA followed by Tukey Kramer's post hoc test.

ALCA=Alcoholic extract of *Citrus aurantium*, TG=Triglyseride, TCH= Total Cholesterol, HDL-C= HDL-cholesterol, LDL-C= LDL-cholesterol, VLDL= very low density lipoprotein.

### Discussion

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, which produces inadequate glucose control and leads to acute and chronic complications. Premature and extensive arteriosclerosis involving renal, peripheral, and cardiovascular vessels remain the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk for coronary heart disease. A reduction in serum lipids, particularly of the LDL and VLDL fraction and triglycerides, should be considered as being beneficial for the long-term prognosis of these patients.<sup>13</sup> Lowering of blood glucose and plasma lipid levels through dietary modification and drug therapy seems to be associated with a decrease in the risk of vascular disease.

In the present study, treatment with *Citrus aurantium* ethanolic extract (500 mg/kg b.w.) in normal rats produced significant decrease in blood glucose level. The hypoglycemic effect may be due to increased secretion of insulin from the b-cells of the pancreas, i.e., pancretotrophic action.<sup>14</sup> The results were comparable with that of tolbutamide, which acts by stimulation of insulin release,<sup>15</sup> thus further confirming that the extract lowers the blood glucose by a similar action.

Moreover, *Citrus aurantium* produced significant beneficial effects in the lipid profile in euglycemic rats, reducing triglycerides, total cholesterol, LDL, and VLDL, and increasing HDL, significantly. The ethanolic extract increased secretion of insulin from b-cells of pancreas; this increased secretion of insulin stimulates fatty acid biosynthesis and also the incorporation of fatty acids into triglycerides in the liver and adipose tissue.<sup>16</sup>

Alloxan, a beta cytotoxin, induces 'chemical diabetes' in a wide variety of animal species by damaging the insulin-secreting cells of the pancreas. Literature sources indicate that alloxan rats are hyperglycemic.<sup>17</sup> The use of lower doses of alloxan (150 mg/kg b.w./i.p.) produced a partial destruction of pancreatic b-cells even though the animals became permanently diabetic. Thus, these animals have surviving b-cells and regeneration is possible.<sup>18</sup> It is well known that the sulfonylureas (tolbutamide) act by directly stimulating the b-cells of the Islets of Langerhans More Details to release more insulin and these compounds are active in mild alloxan-induced diabetes where as they.<sup>17</sup> Since our results show that tolbutamide reduced the blood glucose levels in the diabetic animals, the state of diabetes is not severe.

Prolonged administration of an ethanolic extract of *Citrus aurantium* leads to significant reduction in blood glucose level, which is in agreement with other studies.<sup>18,20</sup> The hypoglycemic activity of the drug was due to the regeneration of



pancreatic cells that were partially destroyed by alloxan, and potentiation of insulin secretion from surviving b-cells of the islets of Langerhans.<sup>21</sup>

Diabetic rats were observed to have increased plasma lipids, which are responsible for several cardiovascular disorders.<sup>22</sup> The higher lipid levels seen in diabetic rats was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones.<sup>23,24</sup> The ethanolic extract leads to regeneration of the b-cells of the pancreas and potentiation of insulin secretion from surviving b-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. In this context, a number of other plants have also been reported to have antihyperglycemic, antihyperlipidemic, and insulin stimulatory effects.<sup>25,26,27</sup>

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 ethanolic extract, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about. Further studies are needed to identify the chemical constituents of the ethanolic extract of *Citrus aurantium* that may be responsible for the hypoglycemic and hypolipidemic activity.

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