# Antidiabetic Activity of Leaves of *Jatropha Curcus* L. (Euphorbiaceae) in Alloxan Induced Diabetes Rats

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## Summary

The leaves of *Jatropha curcus* L. (Euphorbiaceae) has been traditionally used in the treatment of diabetes. Based on its traditional medicinal use, laves of *JC* was extracted with water: 95% ethanol (4:6 v/v). Diabetes to the rats was induced by administration of alloxan monohydrate. The antidiabetic property of hydro alcoholic extract (JCH) of *JC* was evaluated at a dose of 250 and 500 mg/kg in alloxan induced diabetic rats. The parameters tested were serum glucose and lipid profile (total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol). The JCH was found to be antidiabetic, significantly ($P<0.001$) decreases the serum glucose, total cholesterol, triglycerides and HDL and increases LDL level.The resulting JCH showed promising anti-diabetic potential.

**Key words:** *Jatropha curcus,* diabetes mellitus, alloxan, serum glucose, lipid profile

## Introduction

Diabetes mellitus (DM) is a common endocrine disorder caused by an absolute or relative lack of insulin and/or reduced insulin activity that results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism. The severity of diabetes is increasing day by day; the main cause of this problem is aging, urbanization and increasing privilege to obesity and physical inactivity. Now and in the future, it is important to quantify the prevalence of diabetes and to plan the allocation resources towards treatment and prevention of this disease.[1,2] Management of diabetes without any side effects is still a challenge for medical system.

A plenty of traditional herbal medicinal practices have been adopted for the diagnosis, prevention and treatment of diabetes. *Jatropha curcus* L. (*JC*) (Euphorbiaceae) is a drought resistant shrub or tree belonging to the family Euphorbiaceae, which commonly cultivated in Central and South America, South-East Asia, India and Africa.[3] The ethno medical uses of the *JC* leaves and roots include use a remedy for cancer, abortifacient, antiseptic, diuretic, purgative and haemostatic.[4] *JC* have traditionally been used for treatment of diabetes.[5] *JC* also possesses antidiabetic,[6] woundhealing[7], antitumor,[8] antiinflammatory[9] and antifungal[10] properties.

In view of this, the present was undertaken to perform acute toxicity and antidiabetic property of hydro alcoholic extract of leaves of *JC*.

## Materials and methods

### *Plant material*

The leaves of *JC* family Euphorbiaceae wase collected from Pune district, Maharashtra, India. The leaves was identified and authenticated by Head of the Botany Department, Sharadabai Pawar College, Malegaon, Baramati taluka, Pune district, India. Voucher specimens were deposited in a Herbarium of same institute for future reference. Leaves was dried in a shade and stored in the dark until use.

### *Chemicals and drugs*

Alloxan monohydrate, tolbutamide was purchased from Sigma Chemicals (St. Louis. MO USA). Chemical kits for estimation of blood glucose, total cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) were purchased from Erba Diagnostics (Mannheim, Germany). All other chemicals used were also of analytical grade.

### *Preparation of the extract*

Dried coarse powder of *JC* leaves extracted with 4:6 (v/v) mixture of water and 95% ethanol by Soxhlet extraction at $70^0$ C. The solvent was evaporated by a rotary evaporator and dried over a water bath at $45^0$ C to yield hydro alcoholic extract (JCH). Dried extract JCH weighed in an analytical balance and stored at $5^0$ C until use.

### *Animals*

The male albino rats (Wistar strain weighing 150-180 g) and albino mice (20–30 g) were procured from Shivnagar Vidya Prasarak Mandal's College of Pharmacy, Malegaon, Baramati taluka, Pune district, India and were housed in vivarium. The rasty were kept at $27^0 \pm 3^0$ C with relative humidity of 65 ± 10%) and a 12 h light/dark cycle. All the animals were fed a rodent pellet diet (Gold Mohr, Lipton India Ltd.) and water was allowed *ad-libitum* under strict hygienic conditions. The Institutional Animal Ethics Committee (IAEC) approved all the protocols of study (Reg. No. 1214/ac/08/CPCSEA).

### *Acute toxicity studies*

The acute toxicity of JCH was determined in experimentally maintained female albino mice (20–30 g). The animals were fasted for a period of overnight prior to the experimentation. The CPCSEA fixed dose (OECD Guideline No. 420) method was followed for acute toxicity studies (Mrs. Prema Veeraraghavan. Expert consultant, CPCSEA, OECD guide line No. 420; Oct 2000)

### *Induction of diabetes in rats*

The rats were intraperitoneally injected with alloxan monohydrate dissolved in normal saline at dose of 150 mg/kg. After 2 weeks rats with serum blood glucose levels higher that 260 mg/dL were selected for experiment.[11]

### *Experimental design for antidiabetic activity*

The rats were divided into 5 groups (n=5). Group I (the control group) received 0.5 mL saline, Group II was untreated diabetic rats and group III were diabetic rats treated with tolbutamide 80 mg/kg. Groups IV was diabetic rats treated with JCH 250 mg/kg and group V were diabetic rats treated with JCH 250 mg/kg. Tolbutamide was used as the standard antidiabetic treatment throughout the experiment.

The animals were carefully monitored on each day. Animals described as fasted were deprived of food for at least 12 h but were allowed free access to drinking water. Fasting blood glucose measurements were performed on days 0, 7 and 14 of the study. Blood samples were collected using the retro orbital method at weekly intervals until the end of study and were processed for estimation of serum glucose by the glucose oxidase-peroxidise (GOD-POD) method.[12,13,14]

***Estimation of lipid profile***

Serum samples from all the experimental rats were collected for the estimation of lipid parameters, total cholesterol by the oxidase-*p*-aminophenazone (CHOD-PAP) method, triglycerides by the glycerol phosphate oxidase (GPO) Triender method, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol.[15]

***Statistics***

Data were expressed as the mean ± SEM for all experiments. Significant differences between groups were calculated according to one-way analysis (ANOVA) followed by Tukey's multiple comparison tests. Values corresponding to $P<0.001$ were considered statistically significant.

## Results

***Extract preparation***

The sample (100 g leaves powder) extracted with water and 95% ethanol showed the best recovery rate. The yield of extraction was 15.13 % w/w.

***Acute toxicity***

The oral acute toxicity ($LD_{50}$) was determined by following OECD guidelines no 420 of extract JCH. The LD50 of extract JCH was found to be 2500 mg/kg.

***Antidiabetic activity***

The rats were treated with extract JCH at a dose of 250 and 500 mg/kg. Blood glucose measurements were performed on days 0, 7 and 14. It was observed that, JCH demonstrated antidiabetic property at both dose, significantly ($P<0.001$) decreases serum glucose level relative to untreated diabetic rats (Table 1).

***Lipid profile***

The extract JCH was found to be antilipidemic, significantly ($P<0.001$) decreases the serum total cholesterol, triglycerides and high density lipopropetin (HDL) and increases serum low density lipoprotein (LDL) level relative to untreated diabetic rats (Table 2).

**Table 1: Effect of JCH at a dose 250 and 500 mg/kg for 2 weeks on serum glucose level in diabetic rats.**

| Group | Treatment | Average Serum Glucose (mg/dl) | | |
|---|---|---|---|---|
| | | Day 0 | Day 7 | Day 14 |
| I | Normal | 85.25 ± 6.23 | 90.35 ± 5.42 | 96.48 ± 7.59 |
| II | Diabetic Control | 336.21 ± 3.26 | 358.57 ± 4.36 | 362.32 ± 8.26 |
| III | Diabetic + Tolbutamide (80 mg/kg ) | 297.26 ± 4.56*** | 186.60 ± 5.26*** | 105.37 ± 4.87*** |
| IV | Diabetic + JCH (250 mg/kg ) | 319.68 ± 4.56* | 298.23 ± 3.25** | 263.55 ± 3.78*** |
| V | Diabetic + JCH (500 mg/kg ) | 310.57 ± 3.42* | 289.31 ± 4.62*** | 233.47 ± 5.18*** |

All values are Mean ± SEM (n=5).
Statistical comparisons between each treatment and untreated diabetic were carried out by one way ANOVA followed by Tukey's multiple comparison tests.
*P< 0.05, **P< 0.01, *** P < 0.001 when compare to untreated diabetic group.

**Table 2: Effect of JCH at a doses 250 and 500 mg/kg for 2 weeks on serum total cholesterol, triglycerides, HDL and LDL (mg/dl) in diabetic rats**

| Group | Treatment | Average serum lipid profile (mg/dl) | | | |
|---|---|---|---|---|---|
| | | Total Cholesterol | Triglycerides | HDL Cholesterol | LDL Cholesterol |
| I | Normal | 64.28 ± 3.51 | 79.64 ± 3.12 | 48.60 ± 3.72 | 48.53 ± 4.36 |
| II | Diabetic Control | 95.28 ± 4.01 | 143.88 ± 3.52 | 26.14 ± 5.61 | 108.23 ± 7.59 |
| III | Diabetic + Tolbutamide | 58.78 ± 3.61*** | 81.10 ± 4.00*** | 41.86 ± 5.94** | 55.34 ± 5.46** |
| IV | Diabetic + JCH (250 mg/kg ) | 71.23± 3.11*** | 98.52 ± 3.33 *** | 32.89 ± 3.19** | 65.31 ± 3.71** |
| V | Diabetic + JCH (500 mg/kg ) | 67.92 ± 4.62*** | 91.41 ± 2.63*** | 39.16 ± 4.01*** | 58.43 ± 3.99*** |

All values are Mean ± SEM (n=5).
Statistical comparisons between each treatment and untreated diabetic were carried out by one way ANOVA followed by Tukey's multiple comparison tests.
**P< 0.01, *** P < 0.001 when compare to untreated diabetic group.

## Discussion

Diabetes is a chronic metabolic disorder affecting significant portion of the global population. A sustained reduction in hyperglycaemia will decrease the risk of developing microvascular diseases and reduce their complications.[16] The conventional therapies for diabetes have many shortcomings in terms of side effects and high rates of secondary failure. On the other hand, herbal extracts are expected to have similar efficacies without the side effects of conventional drugs.[17]

Alloxan first identified as a pyrimidine derivative in 1838, is one of the most prominent diabetogenic chemicals in diabetes research.[18,19] Alloxan induces diabetes in animals[20] as a result of specific necrosis of pancreatic β cells.[21,22] The resulting insulinopenia causes a state of experimental diabetes mellitus which is known as alloxan diabetes.[23,24] Alloxan is capable of generating reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid.[25,26,27]

The leaves of *JC* has a reach source of several biological molecules produced via secondary metabolism such as flavonols, alkaloids and coumarins.[12] In present study we extracted leaves of *JC* with hydro alcohol and observed that extract JCH was fond to be potent antidiabetic and antilipidemic. The antidiabetic and hypoglycemic property of JCH in alloxan induced diabetic rats may be due to(a) potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β cells of islets of Langerhans or its release from bound insulin, and/or (b) enhanced glucose utilization by peripheral tissues. In this context a number of other plants have been observed to have similar type of hypoglycaemic effect.

The administration of JCH significantly reduces the level of serum glucose, triglycerides, LDL cholesterol and total cholesterol in diabetic rats. A significant elevation of HDL cholesterol levels was also observed. These results, together with previous reports of flavonoids having antidiabetic properties, suggest that *JC* have the potential to be developed as antidiabetic compounds.

## Conclusion

It is evident from the present work that, extract JCH has a potential anti-diabetic as well as anti-hyperlipidemic effect, which is bioactivity of great relevance to diabetes mellitus complication therapy.

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