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# Pharmacological evaluation of leaves of *Jatropha curcas* L. for anti-diabetic activity in alloxan induced diabetic rats

JOSH KUMAR[1], SATYA PAL SINGH[2] and GOVIND KUMAR CHOUDHARY[3]

*Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145 India*

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ABSTRACT

Antidiabetic activity of hydroethanolic extract of *Jatropha curcas* leaves was evaluated using 25 rats equally divided into five groups of five rats each. Group 1 served as healthy control and group 2 as negative control. The study was conducted for the antidiabetic property evaluation after single injection of alloxan drug @ 100 mg/kg b.wt., i.p followed after 48 h by the treatment with the metformin (300 mg/kg) in group 3, hydroethanolic extract of *Jatropha curcas* leaves (JCE) (200 mg/kg) in group 4 and JCE (400 mg/kg) in group 5. Significant decrease was observed in haematological values of group 2. These values were significantly increased in groups 3 and 5. Total serum protein and albumin values were significantly reduced in group 2 as compared to group 1. But, significant increase was observed in group 3 and 5 in comparison to group 2. A significant increase in serum creatinine, urea, cholesterol, enzymes in group 2 and it significantly reduced in group 3 and group 5. Blood glucose level was significantly increased in group 2 as compared to group 1. Metformin and JCE treated groups had significantly reduced blood glucose level in comparison to group 2. In group 2, LPO level was significantly increased in RBC, liver and kidney but GSH levels were decreased significantly in RBCs, kidney and liver in comparison to group 1. LPO was significantly decreased in kidney and RBCs of group 3 and 5, but there was significant increase in GSH level in liver and RBCs of the group 3 in comparison to group 2.

**Key words** : Alloxan, Antidiabetic, *Jatropha curcas,* Metformin

Diabetes mellitus is a group of metabolic disorders that results from defects in insulin secretion, insulin action (sensitivity), or both with common clinical manifestation, hyperglycemia (WHO 1980). Diabetes is associated with oxidative stress leading to an increased production of superoxide radical, hydrogen peroxide and hydroxyl radical or a reduction in the antioxidant defense system (Dahecha *et al*. 2011). Different parts of the *Jatropha curcas* plant (family Euphorbiaceae) were traditionally used for the treatment of several disorders as anticancer, as an abortifacient, antiseptic, diuretic, purgative and haemostatic (Sharma *et al. 2*012). Phytochemical analyses have shown that different parts of *Jatropha curcas* plant contain phenolic, flavonoid, saponin and alkaloid compounds (Oskoueian *et al.* 2011). The hydroalcoholic and chloroform extract of leaves of *Jatropha curcus* demonstrated antidiabetic property in alloxan induced diabetic rats (Patil *et al*. 2011). The 50% ethanolic extract of leaves of *Jatropha curcas* L. produced antidiabetic effect in diabetic rats (Mishra *et al*. 2010). The *Jatropha curcas* has extreme commercial and medicinal properties.

Present address: [1]Veterinary Officer (drjosh34@gmail.com), Department of Animal Husbandry, [2]Professor and Head (sppharma@rediffmail.com), Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences. [3]Ph.D Scholar (drgovindvet@yahoo.co.in).

## MATERIALS AND METHODS

*Plant materials:* The *Jatropha curcas* leaves were collected from Medicinal Plant Research and Developmental Centre (MRDC)**,** G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand. Leaves were collected in clean stainless steel trays. Bulk amount of leaves were washed under running tap water and were wiped with dry muslin cloth**.** Leaves were chopped with clean sharp knife into small pieces. The drying of chopped material was done in a dryer under hot circulating air at 40ºC for 72 to 96 h. To get powder, grinding was done in a grinding mill having stainless steel blades. Ground powder was filled in air tight containers (plastic/glass).

*Preparation of 50% hydroethanolic extract from Jatropha curcas leaves:* The *Jatropha curcas* (10 g) was weighed and kept in 250 ml flask. Then 50 ml of water and 50 ml of ethanol was added, mixed gently after closing the mouth with parafilm. This solution was stirred with the help of magnetic stirrer for 1 h and kept in incubator shaker at 37ºC with gentle swirling at 120 rpm for overnight. The solution was then filtered with muslin cloth and doubly filtered with whatman filter paper No. 1. The filtrate thus

obtained was centrifuged at 5000 rpm at 37ºC for 10 min. The supernatant was collected and ethanol was evaporated with the help of Rota Vapour. The thick solution remaining at the bottom was collected and lyophilized in a vacuum lyophilizer. The yields per gram of sample were calculated.

*Animals:* Thirty Sprague Dawley albino rats of 2 to 2.5 month old age, weighing between 150 to 170 g were procured from Laboratory Animal Resource Centre, IVRI. These animals were kept in plastic cages and acclimatized for two weeks in the experimental laboratory animal shed of the College of Veterinary and Animal Sciences under standard managemental conditions. Standard rat feed and water was provided *ad libitum* throughout the experimental period.

*Experimental design:* Thirty rats of 2 to 2.5 months of age were divided randomly and equally into 5 groups i.e. 1 as control, 2 as diabetic, 3 as standard or positive control, 4 and 5 as ethanolic extract treatment group. All the rats were fasted for overnight, then body weight and blood glucose level was recorded. Alloxan drug mixed freshly with water was injected to all the rats except control group intraperitonially at the dose rate of 100 mg/kg body weight. Food and drinking water was given after two hours. Group 1 was kept as control. Group 2 was kept as diabetic control in which diabetes was induced with alloxan drug at the dose rate of 100 mg/kg body weight. Group 3 was kept as standard control in which diabetes induced was treated with metformin drug at the dose rate of 300 mg/kg body weight. Groups 4 and group 5 were kept as treatment group in which alloxan induced diabetic rats were treated with 50% hydroethanolic *Jatropha curcus* leaves extract (JCE) @ 200 mg/kg body weight and 400 mg/kg body weight respectively. Blood glucose levels were observed at weekly intervals.

Blood (0.5 ml) was collected in heparinized tubes for estimation of hematological parameters such as TEC and TLC, Packed cell volume and Haemoglobin (Jain 1986) and in non-heparinized tubes for the estimation of biochemical parameters such as total proteins (Koller 1984), albumin (Gendler 1984), globulin, total cholesterol (Warnick *et al*. 1985), glucose (Kaplan 1984), creatinine (Murray 1984) and urea by using standard diagnostic kits and aspartate aminotransferase and alanine aminotransferase (Moss and Henderson 1994) and alkaline phosphatase.

Estimation of different antioxidative parameters such as Lipid peroxidation product Malondialdehyde (LPO) (Rehman 1984) and Reduced Glutathione (GSH) (Sedlak and Lindsay 1968) in liver, kidney and brain and reduced Glutathione in erythrocytes (Prins and Loos 1969).

*Statistical analysis:* Statistical analysis of data was done by using graph pad software one way ANOVA technique. Comparisons were made with help of Tukey's Multiple Comparison Test. Statistically significant difference was considered at 5% level (Snedecor and Cochran 1989).

## RESULTS AND DISCUSSION

The blood glucose level was significantly ($P<0.05$) increased in alloxan induced diabetic group as compared to healthy control. Metformin and JC extract treated groups significantly ($P<0.05$) reduced blood glucose level in comparison to alloxan induced diabetic group (Table 1). A significant decrease in blood glucose levels was observed on 14th day in metformin treated group 3, low and high dose extract treated groups as compared to their 0 day blood glucose levels indicating the hypoglycemic action of metformin and hydroethanolic extract of *Jatropha curcas* leaves. An increase in the blood glucose level by alloxan might have occurred due to the destruction of pancreatic beta cells causing degranulation and reduction of insulin secretion. Anti-hyperglycemic activity of hydroethanolic extract of *Jatropha curcas* leaves could have been due to an improvement in glucose metabolism, its uptake and utilization in the body tissues (Olayiwola *et al.* 2004)**.** The improved insulin secretion might be responsible for anti-hyperglycemic activity of hydroethanolic extract of *Jatropha curcas* leaves. This finding also corroborated with findings of Mishra *et al*. (2010), Olayiwola *et al.* (2004) and Farag *et al.* (2014).

There was significant ($P<0.05$) decrease in Hb, PCV, TEC and TLC values in alloxan induced diabetic group in comparison to healthy control and significantly ($P<0.05$) increased in metformin treated group and high dose extract treated group. These findings corroborated with findings of Mansi and Lahham, 2008. The haemoglobin was lowered significantly in groups 2, 3 and 5 in comparison to control with significantly higher value in group 3 and 4 as compared to group 2. This might have been due to the occurred hypoglycemia converting haemoglobin to glycosylated

Table 1. Effect of hydroethanolic extract (1:1) of *Jatropha curcas* leaves following its daily oral administration for 14 days on blood glucose (mg/dl) (Mean ± SE, n=5) in alloxan induced diabetic rats

| Treatments (dose, mg/kg body weight) | Days | | |
|---|---|---|---|
| | 0 days | 7 days | 14 days |
| Control | 90±2.00 | 88±1.82 | 89±1.82 |
| Alloxan (100) | 238.4±16.85$^{p}$ | 237.8±14.00$^{p}$ | 227.8±16.60$^{p}$ |
| Alloxan (100) + Metformin (300) | 246.4±10.17$^{p}$ | 194±9.01$^{pq}$ | 131±9.18$^{abpq}$ |
| Alloxan (100) + JCE (200) | 237.8±17.36$^{p}$ | 204.8±9.79$^{p}$ | 169.2±8.51$^{abpq}$ |
| Alloxan (100) + JCE (400) | 249.4±14.77$^{p}$ | 205.8±12.32$^{p}$ | 154±6.16$^{apq}$ |

a, Significant difference ($P<0.05$) as compared to day 0 within same column; b, significant difference ($P<0.05$) as compared to day 7 within same column; p, significant difference ($P<0.05$) as compared to control group within same row; q, significant difference ($P<0.05$) as compared to alloxan (100 mg/kg) as group within same row. JCE, hydroethanolic extract of *Jatropha curcas* leaves.

haemoglobin resulting in fall in total haemoglobin in alloxan induced diabetic rats (Sheela and Augusti 1992).

Total serum protein and albumin values were significantly (P<0.05) reduced in negative control group as compared to healthy control. But, significant (P<0.05) increase was observed in metformin treated and high dose extract treated group in comparison to negative control. An improvement in protein profile was observed in this investigation which might have occurred due to hepatoprotective efficacy of the treatment with metformin and *Jatropha curcas* leaves extract. These findings are in agreement with the finding of Shaikh *et al.* 2010.

A significant (P<0.05) increase in serum creatinine, serum urea, cholesterol, AST, ALT and ALP was observed in alloxan induced diabetic group as compared to the healthy control and significantly (P<0.05) reduced in metformin treated group and high dose extract group. Enzyme activities of AST, ALT and ALP were significantly (P<0.05) reduced in the treatment groups in comparison to the alloxan induced diabetic group (Table 2). There was significant increase in serum urea, creatinine and total cholesterol in negative control group as compared to healthy control and significant decline was observed in JCE treated group in comparison to alloxan induced diabetic group. This could be due to antioxidant property of *Jatropha curcas* hydroethanolic extract (Huang *et al.* 2014).

An increase in the serum levels of urea and creatinine is an indication of probable kidney damage and they also increase due to hepatic urea production from amino acid metabolism. Similar results were reported by Awasthy *et al.* 2010 in rats.

Enhanced cholesterol level is also an indicator of liver dysfunction which is indicated by enhanced AST and ALT activity in this study (Mishra *et al.* 2010) and JCE have hepatoprotective effect. This is in agreement with finding of Shaikh *et al.* 2010.

Table 2. Effect of hydroethanolic extract (1:1) of *Jatropha curcas* leaves following its daily oral administration for 14 days on hematobiochemical parameters (Mean ± SE, n=5) in alloxan induced diabetic rats

| Parameters | (Control) | Alloxan (100) | Alloxan (100) + Metformin (300) | Alloxan (100) + JCE (200) | Alloxan (100) + JCE (400) |
|---|---|---|---|---|---|
| Hb (%) | 14.4±0.40 | 11.2±0.32$^{a}$ | 13.2±0.24$^{b}$ | 12.1±0.26$^{a}$ | 12.88±0.30$^{ab}$ |
| PCV (%) | 40±1.14 | 30±1.30$^{a}$ | 39±1.84$^{b}$ | 35.8±2.33 | 38.2±1.85$^{b}$ |
| TEC ($10^{6}$/µL) | 5.64±0.14 | 4.02±0.14$^{a}$ | 5.42±0.26$^{b}$ | 4.90±0.16$^{ab}$ | 5.2±0.11$^{b}$ |
| TLC ($10^{3}$/µL) | 5.5±0.11 | 4.78±0.16$^{a}$ | 4.5±0.17$^{a}$ | 4.8±0.10$^{a}$ | 4.6±0.15$^{a}$ |
| Total protein (g/dl) | 7.00 ±0.34 | 5.20±0.27$^{a}$ | 7.10±0.10$^{b}$ | 6.50±0.23$^{b}$ | 7.08±0.20$^{b}$ |
| Albumin (g/dl) | 4.2±0.22 | 2.62±0.29$^{a}$ | 3.9±0.23$^{b}$ | 3.42±0.34 | 3.72±0.17$^{b}$ |
| Globulin (g/dl) | 2.80±0.13 | 2.58±0.43 | 3.20±0.14 | 3.08±0.36 | 3.36±0.19 |
| BUN (mg/dl) | 30.20±1.43 | 43.80±1.59$^{a}$ | 34.00±1.41$^{b}$ | 38.00±1.30$^{a}$ | 36.00±1.14$^{b}$ |
| Creatinine (mg/dl) | 0.58±0.05 | 1.39±0.07$^{a}$ | 0.62±0.04$^{b}$ | 1.20±0.07$^{ac}$ | 0.91±0.06$^{abcd}$ |
| Total cholesterol | 75.60±2.98 | 138.80±5.63$^{a}$ | 93.40±4.87$^{b}$ | 120.40±6.05$^{ac}$ | 109.20±4.16$^{ab}$ |
| AST (U/L) | 39.80±1.50 | 97.47±0.70$^{a}$ | 54.19±6.20$^{b}$ | 73.48±6.00$^{ab}$ | 68.17±7.81$^{ab}$ |
| ALT (U/L) | 44.00±1.01 | 101.34±2.36$^{a}$ | 60.08±4.25$^{b}$ | 76.50±6.23$^{ab}$ | 71.56±5.95$^{ab}$ |
| ALP (U/L) | 160.01±4.12 | 219.87±8.90$^{a}$ | 170.36±4.38$^{b}$ | 194.78±3.87$^{abc}$ | 182.36±3.71$^{b}$ |

a, Significant difference (P<0.05) as compared to day 0 within same column; b, significant difference (P<0.05) as compared to day 7 within same column; p, significant difference (P<0.05) as compared to control group within same row; q, significant difference (P<0.05) as compared to alloxan (100 mg/kg) as group within same row. JCE, hydroethanolic extract of *Jatropha curcas* leaves.

Table 3. Effect of hydroethanolic extract (1:1) of *Jatropha curcas* leaves following its daily oral administration for 14 days on antioxidative parameters (Mean ± SE, n=5) in liver, kidney and erythrocytes of alloxan induced diabetic rats

| | | (Control) | Alloxan (100) | Alloxan (100) + Metformin (300) | Alloxan (100) + JCE (200) | Alloxan (100) + JCE (400) |
|---|---|---|---|---|---|---|
| LPO (nM MND/g) | Liver | 14.71±1.63 | 32.55±3.81$^{a}$ | 19.25±1.37$^{b}$ | 23.29±1.46$^{b}$ | 20.36±1.39b |
| | Kidney | 25.07±1.51 | 40.31±3.46$^{a}$ | 28.07±2.47$^{b}$ | 34.81±2.60 | 28.44±3.26$^{b}$ |
| | Erythrocyte | 17.44±2.49 | 33.46±4.12$^{a}$ | 19.31±1.80$^{b}$ | 25.12±1.50 | 19.72±1.47$^{b}$ |
| GHS (nM/g) | Liver | 0.92±0.16 | 0.51±0.05$^{a}$ | 0.91±0.06$^{b}$ | 0.48±0.06$^{ac}$ | 0.58±0.01 |
| | Kidney | 0.59±0.06 | 0.45±0.05 | 0.60±0.08 | 0.48±0.06 | 0.58±0.01 |
| | Erythrocyte | 1.29±0.12 | 0.37±0.16$^{a}$ | 0.89±0.10$^{b}$ | 0.51±0.06$^{a}$ | 0.83±0.12 |

a, Significant difference (P<0.05) as compared to group 1 within same column; b, significant difference (P<0.05) as compared to group 2 within same column; JCE, hydroethanolic extract of *Jatropha curcas* leaves.

Significant decline in AST, ALT and ALP level was observed in JCE treated group as compared to control group and diabetic group. The rise in activity of serum AST, ALT and ALP enzymes has been attributed to the damaged structural integrity of the liver. Reduction in AST, ALT and activity in this study indicated the hepatoprotective effect of hydroethanolic extract *Jatropha curcas* leaves (Shaikh *et al.* 2010).

In alloxan induced diabetic rats, LPO level was significantly (P<0.05) increased in RBC, liver and kidney but GSH levels were decreased significantly (P<0.05) in RBCs and liver in comparison to the healthy control (Table 3). LPO was significantly (P<0.05) decreased in kidney and RBCs of metformin treated and high dose extract treated group but significant (P<0.05) increase in GSH level in liver and RBCs of the metformin treated group was observed in comparison to the alloxan induced diabetic group.

An increase in LPO levels in liver, kidney and erythrocytes as evidenced by the increased production of malondialdehyde (MDA) it was further associated with decrease in activity of antioxidant enzymes GSH in liver, kidney and erythrocytes in diabetic group as compared to the JCE treated group. The level of LPO is decreased in JCE treated group that indicate that Jatropha have antioxidant effect which is in agreement with finding of Huang *et al.* 2014.

Glutathione antioxidant system plays a pivotal role in cellular defence against reactive free radicals and other oxidant species (Meister and Anderson 1983). In this study, enhanced lipid peroxidation (LPO) in RBC, liver and kidney and diminished GSH in RBC, kidney and liver of group 2 suggested that alloxan produced oxidative stress in erythrocytes, liver and kidney. Glutathione is an endogenous protective agent that plays a critical role in intracellular antioxidant defence. GSH efficiently scavenges reactive oxygen species (ROS) and detoxifies various chemicals by forming conjugates with these harmful substances (Yang *et al. 1*997). It is concluded from this study that hydroethanolic extract of *Jatropha curcas* leaves could further be investigated to develop a strategy for herbal remedy for the prevention and treatment of diabetes in man and animals.

The study was conducted for the antidiabetic property evaluation after single injection of alloxan drug @ 100 mg /kg b.wt., i.p followed after 48 hours by the treatment with the metformin (300 mg/kg) in group 3, hydroethanolic extract of *Jatropha curcas* leaves (JCE) (200 mg/kg) in group 4 and JCE (400 mg/kg) in group 5. Group 1 served as healthy control and group 2 as negative control. Blood glucose level was significantly (P<0.05) increased in group 2 as compared to group 1. Metformin and JCE treated groups had significantly (P<0.05) reduced blood glucose level in comparison to group 2. Significant (P<0.05) decrease in haematological values group 2. These values were significantly (P<0.05) increased in group 3 and 5. In group 2, LPO level was significantly (P<0.05) increased in RBC, liver and kidney but GSH levels were decreased significantly (P<0.05) in RBCs, kidney and liver in comparison to the group 1. LPO was significantly (P<0.05) decreased in kidney and RBCs of group 3 and 5, but significant (P<0.05) increase in GSH level in liver and RBCs of the group 3 in comparison group 2.

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