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# Evaluation of antidiabetic activity of *Euphorbia hirta* Linn. in streptozotocin induced diabetic mice

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*Euphorbia hirta* Linn. (Family-Euphorbiaceae) is widely used in traditional system of medicine to treat diabetes in India. The ethanolic extracts (250 and 500 mg/kg) of leaf, flower and stem were taken upundertaken to evaluate the antidiabetic activity against normal and streptozotocin (STZ) induced diabetic mice. Oral administration of the extracts for 21 days resulted in a significant reduction in blood glucose level. Chronic effects of the extracts on serum biochemistry were also studied and it was found that serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase levels were decreased significantly by all the extracts and Glibenclamide but HDL levels and total proteins were found to be increased after treatments. Thus, this study shows that *E. hirta* has antidiabetic action and the extracts should further be subjected to bioactivity guided drug discovery to isolate a lead compound responsible for this activity.

**Keywords:** *Euphorbia hirta*, Euphorbiaceae, *Dudhi*, Diabetes, Streptozotocin, Mice, Traditional medicine.

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## Introduction

Diabetes mellitus is the most common endocrine disorder. More than 150 million people are suffering from it worldwide[1] and it is likely to increase to 300 million by the year 2025. More than one-fifth of them are Indians and the International Diabetes Federation, declared India "Diabetic Capital of the World". Synthetic antidiabetic agents can produce serious side effects and they are not suitable for use during pregnancy. In view of the adverse effects associated with the synthetic drugs and considering natural medicine safer, cheaper and effective, traditional antidiabetic plants can be explored[2]. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important[3].

*Euphorbia hirta* Linn. (Family-Euphorbiaceae), commonly known as *Dudhi* is a slender, annual hairy plant, abundant in waste places and open grasslands (Plate 1). It is native to India and Australia. It has been reported to contain alkaloids, saponins, flavonoids and tannins. Traditionally, it is used in the treatment of gastrointestinal disorders, bronchial and respiratory diseases, kidney stones, diabetes and in conjunctivitis. It also exhibits anxiolytic, analgesic, antipyretic and anti-inflammatory activities. The literature survey revealed that there is no experimental evidence of antidiabetic effect of the plant. Therefore, the present study was carried out to investigate antidiabetic effect of ethanolic extracts of its leaf, flower and stem in streptozotocin induced diabetic mice[4-7].

Plate 1: *Euphorbia hirta* plant

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## Materials and Methods

### Chemicals

Streptozotocin was purchased from Hi Media, India. Total cholesterol (TC), serum high-density lipoprotein (HDL), serum creatinine (SC), serum urea (SU), serum alkaline phosphate (SAP) and triglyceride (TG) were assayed using standard kits from Erba Diagnostics Mannheim Gambh, Germany and Blood glucose level was measured using Elegance glucose meter (CT-X10) of Convergent Technologies, Germany.

### Plant material

The leaves, flowers and stems were collected in the month of September-October 2008 from campus of Kurukshetra University, Kurukshetra, India and was identified by Dr. B.D. Vashishta, Department of Botany, Kurukshetra University. A voucher specimen of the plant is preserved in the herbarium of the Faculty of Pharmaceutical Sciences, Kurukshetra University (No. IPS/KUK/E-1/2009). The materials were cleaned thoroughly with distilled water to remove any type of contamination. Washed leaves, flowers and stems were air dried in shade.

### Preparation of the plant extract

The plant parts were separately powdered in a grinder and passed through sieve. 500g of each powder was filled in the Soxhlet apparatus for extraction. The whole assembly of the Soxhlet apparatus was set up and first defatted by petroleum ether (60-80ºC) for 72h. After complete defatting, the drug powders were dried at room temperatures and extracted with ethanol for 48h. The alcoholic extracts of the different parts were dried at 45ºC in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10ºC.

### Phytochemical screening

The freshly prepared crude extracts were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using the following reagents and chemicals: Alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable foam and steroids with Libermann-Burchard reagent. These were identified by characteristic colour changes using standard procedures[8].

### Animals

Albino mice of either sex, weighing about 30-35g were used in the experiments. Animals were maintained under standard environmental conditions i.e. ambient temperature of 22 ± 2ºC and at 45-55% relative humidity for 12h, each of dark and light cycle and fed with a standard pellet mice diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*. All the studies were conducted in accordance with the Animal Ethical Committee of the University.

### Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method)[9]. Albino mice (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after that the extracts were administered orally at the dose level of 5mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300 and 2000 mg/kg body weight.

### Streptozotocin induced diabetes

Mice were made diabetic by a single intraperitoneal injection of streptozotocin (150 mg/kg i.p.) in sterile saline. Twelve days after streptozotocin injection, mice with blood glucose level >140mg/dl were separated and used for the study.

### Experimental design

Animals were fasted overnight and were divided in to nine groups of six each. Group I served as normal healthy control. Group II served as untreated diabetic control. Group III and IV received leaf extract 250mg/kg and 500mg/kg per body weight (b.w.), respectively. Group V and VI received flower extract (250mg/kg and 500mg/kg b.w., respectively). Group VII and VIII received stem extract (250mg/kg and 500mg/kg b.w., respectively). Group IX received Glibenclamide (10mg/kg b.w.). Blood samples were collected on 0, 5, 10 and 15 day after oral administration and blood glucose levels were measured using blood glucose test strips with elegance glucometer (Frankenberg, Germany).

***Biochemical assays***

After blood glucose estimation on day 15, whole blood was collected by cardiac puncture under mild ether anesthesia from mice. Serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase, HDL and total proteins levels were also evaluated in normal and streptozotocin induced diabetic mice. Total cholesterol and triglyceride were determined[10]. Serum urea and creatinine were assayed by the method of Tomas[11]. Total proteins[12] and alkaline phosphatase[13] were assayed by the method of Wilkinson *et al* and HDL cholesterol level was also measured[14].

**Statistical analysis**

All values were expressed as mean ± standard error of mean (S.E.M.) and comparison between the groups were made by Analysis of variance (ANOVA), followed by student t- test. A value of $P<0.001$ was considered significant.

## Results and Discussion

**Phytochemical screening**

Phytochemical analyses of the crude extract revealed the presence of flavonoids, steroid, alkaloids, tannins and saponins.

**Acute toxicity studies**

This study showed no mortality up to the dose of 2,000mg/kg body weight. So, the extracts are safe for long term administration.

**Antidiabetic activity**

The effects of the treatment with all extracts and Glibenclamide on blood glucose concentration in normal fasted and diabetic mice after treatment are shown in Table 1. At the end of experiment (15$^{th}$day) blood glucose level was 80.6 ± 1.8 and 77.6 ± 1.4 mg/dl in the diabetic mice treated with 250 and 500mg/kg b.w. of the leaves extract, respectively. Whereas flower and stem extracts at dose of 500mg/kg b.w. also showed significant reduction ($P<0.001$) in blood glucose level on diabetic mice at 15$^{th}$day of the study.

**Body weight**

Furthermore, daily treatment of all extracts for two weeks led to a dose dependent fall in blood glucose levels. Maximum effect seems to reach after 14 days of treatment and remains constant thereafter. Normal healthy control was found to be stable in their body weight but diabetic mice showed reduction in body weight. In this study, the decrease of body weights

Table 1—Long-term effects of *E. hirta* extracts on the blood glucose levels in normal and diabetic mice

| S.No. | Groups and doses (mg/kg, b.w.) | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|---|
| | | Initial day | Day 5 | Day 10 | Day 15 |
| 1 | Normal control | 72.25 ± 0.89 | 73.5 ± 1.0 | 73.75 ± 0.86 | 75.5 ± 1.58 |
| 2. | Diabetic control | 185 ± 2.88 | 186.2 ± 1.7 | 189.25 ± 1.25 | 192.5 ± 1.73 |
| 3 | Leaf extract (250) | 192 ± 2.6 | 151 ± 1.5 | 110.6 ± 3.0* | 80.6 ± 1.8** |
| 4. | Leaf extract (500) | 190.3 ± 4.98 | 148 ± 1.1** | 108 ± 3.4* | 77.6 ± 1.4** |
| 5. | Flower extract (250) | 197.3 ± 4.3 | 157.6 ± 5.2** | 129.6 ± 3.2* | 89.3 ± 2.5** |
| 6. | Flower extract (500) | 194 ± 2.8 | 148.3 ± 3.1 | 116.6 ± 5.3* | 84.6 ± 1.8** |
| 7. | Stem extract (250) | 185.3 ± 4.2 | 154 ± 1.1 | 134.6 ± 2.4 | 88.6 ± 1.2** |
| 8. | Stem extract (500) | 186.3 ± 4.3 | 146.3 ± 5.3* | 121.3 ± 1.4 | 80.3 ± 0.9** |
| 9. | Glibenclamide (10) | 194.75 ± 2.84 | 156.5 ± 5.95 | 110.5±5.24 | 75.73 ± 4.5** |

Data represent means ± S.E.M., n=6 in each group, *$P<0.05$, ** $P<0.001$, No. of sample tested=7

Table 2—Effect of *E. hirta* extracts on the body weight in normal and diabetic mice

| S. No. | Groups and doses (mg/kg, b.w.) | Change in body weight | | | |
|---|---|---|---|---|---|
| | | Initial day | Day 5 | Day 10 | Day 15 |
| 1. | Normal control | 27.3 ±1.9 | 27.9 ± 1.5 | 31.1 ± 3.8 | 28.5 ± 1.1 |
| 2. | Diabetic control | 30.3 ± 1.2 | 28.5 ± 3.2 | 27.9 ± 3.2 | 26.2 ± 2.4 |
| 3. | Leaf extract (250) | 31.1 ± 1.9 | 29.2 ± 0.6 | 28.3 ± 2.7 | 30.7 ± 1.7* |
| 4. | Leaf extract (500) | 29.8 ± 2.1 | 27.1 ± 2.4 | 26.1 ± 3.0 | 28.3 ± 1.4* |
| 5. | Flower extract (250) | 28.6 ± 1.7 | 26.7 ± 2.0 | 25.4 ± 2.4 | 28.5 ± 2.4* |
| 6. | Flower extract (500) | 29.4 ± 1.9 | 27.9 ± 1.2 | 26.0 ± 2.6 | 28.3 ± 1.2* |
| 7. | Stem extract (250) | 31.7 ± 2.5 | 30.4 ± 1.7 | 28.7 ± 1.9 | 30.6 ± 2.1* |
| 8. | Stem extract (500) | 32.6 ± 1.4 | 30.8 ± 1.9 | 28.6 ± 1.4 | 30.7 ± 0.9* |
| 9. | Glibenclamide (10) | 26.27±1.80 | 27.7±2.06 | 29.89±2.25* | 30.46±1.91* |

Data represent means ± S.E.M., N=6 in each group, *$P<0.01$, No. of sample tested=7

Table 3—Effect of chronic exposure to *E. hirta* extracts on plasma lipid and biochemistry

| S. No. | Groups and doses (mg/kg, b.w.) | Cholesterol (mg/dl) | Triglycerides (mg/dl) | Creatinine (mg/dl) | Urea (mg/dl) | Alkaline phosphatase | H.D.L cholesterol | Total Proteins (g/dl) |
|---|---|---|---|---|---|---|---|---|
| 1. | Normal control | 148 ± 4.9 | 84.3 ± 4.6 | 0.6 ± 0.4 | 21.0 ± 0.4 | 116.2 ± 3.9 | 34.1 ± 1.8 | 8.3 ± 1.7 |
| 2. | Diabetic control | 259 ± 13.7 | 256.1 ± 11.4 | 1.8 ± 0.1 | 79.4 ± 2.7 | 364.2 ± 5.7 | 28.1 ± 1.4 | 3.9 ± 5.1 |
| 3. | Leaf extract (250) | 154.7 ± 5.9 | 138.4 ± 2.1 | 0.67 ± 0.1 | 37.2 ± 1.7 | 156.3 ± 10.1* | 47.2 ± 1.7 | 4.7 ± 0.2 |
| 4. | Leaf extract (500) | 148.4 ± 11* | 142.1 ± 1.9 | 0.64 ± 0.3* | 39.5 ± 2.2* | 151 ± 4.3 | 51.9 ± 2.2 | 4.9 ± 1.6* |
| 5. | Flower extract (250) | 154.2 ± 6.3 | 132.3 ± 1.7* | 0.64 ± 0.4 | 38.5 ± 1.7 | 162.5 ± 9.1* | 52.3 ± 1.7* | 5 ± 2 |
| 6. | Flower extract (500) | 150.7 ± 4.7* | 130.5 ± 1.7* | 0.52 ± 0.4 | 32.7 ± 1.4 | 151.6 ± 8.7* | 57.9 ± 1.5* | 5.4 ± 1.7 * |
| 7. | Stem extract (250) | 160.4 ± 4.6 | 134.4 ± 1.4* | 0.59 ± 0.1 | 33.6 ± 2.1 | 154.4 ± 3.7 | 54.2 ± 1.9 | 5.7 ± 0.9 |
| 8. | Stem extract(500) | 146.1 ± 9.5 | 146.8 ± 2.4 | 0.67 ± 0.1 | 36.7 ± 1.9 | 147.8 ± 5.2* | 58.6 ± 1.1* | 4.6 ± 1.1 |
| 9. | Glibenclamide (10) | 120.1 ± 5.7 | 102.6 ± 6.5* | 0.42 ± 0.0 | 30 ± 3.2* | 110 ± 3.9 | 64.5 ± 1.9 | 8.4 ± 1.4 |

Data represent means ± S.E.M., N=6 in each group, * $P<0.01$, No. of sample tested=7

were significantly diminished ($P<0.01$) by the extracts treatments after 14 days of treatment (Table 2).

**Biochemical estimation**

Serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase levels were decreased significantly by all extracts of *E. hirta* and Glibenclamide but HDL levels and total proteins were found to be increased after treatments (Table 3). The extract exhibited anti-diabetic property in streptozotocin induced diabetic mice, as evident from blood glucose levels. The activity may be due to presence of tannins and flavonoids[15].

## Conclusion

The results revealed that *E. hirta* extracts possess significant antihyperglycemic activity in streptozotocin-induced diabetic mice. Further studies are necessary to elucidate in detail the mechanism of action of the medicinal plant at the cellular and molecular levels. These extracts also showed improvement in parameters like body weight, lipid profile and other biochemical parameters.

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