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**Research Article**

# ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITIES OF *EUPHORBIA HIRTA* STEM EXTRACT

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## ABSTRACT

In the present study, antihyperglycemic and hypolipidemic effects of stem extract of *Euphorbia hirta* were studied in albino mice by administering graded oral doses (250 and 500 mg/kg body weight) of the extract for 21 days. The ethanolic and petroleum ether extracts of stems of the *E. hirta* were orally tested for 21 days in alloxan induced diabetic mice and blood glucose level was measured with glucometer. Administration of extract resulted in significant reduction in serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase levels but HDL levels and total proteins were found to be increased after treatments. The antioxidant activity of extracts were also evaluated by various antioxidant assays, including DPPH free radical scavenging activity, superoxide anion radical scavenging, nitric oxide scavenging and reducing power assay. The various antioxidant activities were compared to standard antioxidants such as butylated hydroxyl anisole and ascorbic acid. All the extracts showed antioxidant activity in all the tested methods.

**KEYWORDS**: *Euphorbia hirta*; antihyperglycemic; Hypolipidemia; Mice; Alloxan

## INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders having hyperglycemia, which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response[1]. Synthetic antidiabetic agents can produce serious side effects and they are not suitable for use during pregnancy[2]. Treatment of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity and less side effects. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycaemic agents from medicinal plants have become more important[3].

Euphorbia hirta (Euphorbiaceae), commonly known as "Dudhi" is an annual hairy plant. It is abundant in waste places along the roadsides and open grasslands. It is native to India and Australia[4]. The methanolic stem extract of plant had shown antidiabetic activity in streptozotocin induced diabetic mice[5]. The present study has been planned to investigate how the ethanolic and petroleum ether stem extracts of *E. hirta* influences lipid parameters in alloxan induced diabetic mice.

## MATERIAL AND METHODS

### Plant material

Stems of *Euphorbia hirta* 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, Kurukshetra, India. 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).

**Preparation of the plant extract**

The stems were washed with water and shade-dried. The dried stems were powdered by using dry grinder and passed through sieve. This powder was packed into soxhlet apparatus and extracted successively with petroleum ether (60-80$^0$C) and ethanol (yield 68.54 and 52.3% respectively). All the extracts were dried at 45$^0$C in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10 $^0$C.

**Animals**

Albino mice of either sex, weighing about 30-35g were used in the study. Animals were maintained under standard environmental conditions i.e. ambient temperature of 22 ± 2 $^0$C and at 45–55% relative humidity for 12 h, 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.

**Induction of diabetes**

Mice were made diabetic by a single intraperitoneal injection of alloxan (150 mg/kg i.p.) in sterile saline. Twelve days after alloxan injection, mice with blood glucose level >140 mg/dl were separated and used for the study. Blood glucose levels were measured using blood glucose test strips with elegance glucometer (Frankenberg, Germany) at weekly intervals till the end of study (i.e. 3 weeks). Blood glucose estimation and body weight measurement were done on 0, 7, 14 and 21 day after administration of extract orally[6].

**Experimental design**

All the diabetic animals were randomly divided into seven groups with six animals each and treated orally daily as follows:

Group I. Normal healthy control: given only vehicle (Tween 80, 5% v/v)
Group II. Diabetic control: given only vehicle (Tween 80, 5% v/v)
Group III. Diabetic mice given petroleum ether extract (250 mg/kg)
Group IV. Diabetic mice given petroleum ether extract (500 mg/kg)
Group V. Diabetic mice given ethanolic extract (250 mg/kg).
Group VI. Diabetic mice given ethanolic extract (500 mg/kg).
Group VII. Diabetic mice given Glibenclamide (10 mg/kg).

**Lipid profile**

After blood glucose estimation on day 21, whole blood was collected by cardiac puncture under mild ether anesthesia from mice. Serum cholesterol, triglycerides, HDL, VLDL and LDL were also evaluated in normal and alloxan induces diabetic mice. The total cholesterol is measured using diagnostic kits, Boehringer Mannheim, Germany. Total cholesterol, triglycerides and HDL cholesterol were determined[7-8]. The VLDL cholesterol was calculated using the formula (TG/5) mg/dl. The serum LDL cholesterol was also estimated[9].

**Other Biochemical parameters**

Serum creatinine, urea, alkaline phosphatase and total proteins levels were also evaluated in normal and alloxan induces diabetic mice. Serum urea and creatinine were assayed by the method of L.Tomas[10-11]. Total proteins and alkaline phosphatase were also assayed[12-13].

**Antioxidant assay**

Antioxidant activity of *E. hirta* (alcoholic stem extract) was evaluated by following methods: DPPH free radical scavenging activity[14], Superoxide radical scavenging assay[15-16], Nitric oxide scavenging activity[17]and Reducing power assay[18].

**Statistical analysis**

All the values of body weight, blood glucose and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and comparison between the groups was made by student t- test. A valve of $p < 0.001$ was considered significant.

## RESULTS

### Effect on blood glucose level

Daily treatment of *stem extract* for three weeks led to a dose dependent fall in blood glucose levels. Maximum effect seems to reach after 15 days of treatment and remains constant in third week. Antihyperglycemic effect of the extracts on the blood glucose levels of diabetic mice is shown in Table 1. Administration of Alloxan led to elevation of blood glucose levels, which was maintained over a period of three weeks. As shown both extracts induced significant ($p < 0.001$) antihyperglycemic effects in dose-dependent fashion when compared to the control group (**Table 1**).

### Effect on lipid profile

Serum cholesterol and triglyceride levels were decreased significantly ( $p<0.01$) by both extracts and Glibenclamide as compared to diabetic control but HDL level was found to be increased after treatments (**Table 2**).

### Effect on other biochemical parameters

Serum creatinine, urea and alkaline phosphatase levels were found to be decreased whereas total proteins were found to be increased after treatments (**Table 3**).

### Effect on Body weight

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 was diminished by the extract treatments after 14 days of treatment (**Table 4**).

### Antioxidant Effect

Both the extracts have significant antioxidant activity compared to other well characterized, standard antioxidant systems. Free radical scavenging potential was assessed against DPPH. The scavenging effect of alcoholic extract on the DPPH radical was 59.95%, at a concentration of 250 µg/ml. The reductive capabilities of extract were compared with ascorbic acid and BHA. The extract showed dose dependent reducing power. In the Nitric oxide scavenging study, crude extract was checked for its inhibitory effect on nitric oxide production. It also showed scavenging of superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The percentage inhibition of superoxide generation by 250 µg/ml concentration of extract was measured as 59.67%. The antioxidant activity of extract and standard compounds were compared by using specific in vitro methods (**Table 5**).

## DISCUSSION

Alloxan is cytotoxic to the pancreatic β cells thus it is an effective diabetes-induction agent. It has been widely used to induce diabetes mellitus in experimental animal models allowing investigation of hypoglycemic agents in the treatment of diabetes[19-20]. Alloxan injection consistently produced symptoms characteristic of diabetes mellitus including hyperglycemia, decreased insulin levels, polyuria and weight loss. Significant reduction of blood glucose levels is observed in alloxan induced diabetic mice treated with *E. hirta stem exract* ($p < 0.001$).

The repeated administration of *Euphorbia hirta* extract for a period of 21 days resulted in a significant decrease in lipid parameter levels of various tissues when compared to the diabetic control. It is not known whether *E. hirta* has a direct effect on lipids or the present hypolipidemia is achieved due to controlled hyperglycemia.

It is accepted that oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism's antioxidant potential[21]. Various studies have shown that diabetes is associated with increased formation of free radicals and decrease in antioxidant potential. Glucose itself and hyperglycemia-related increased protein glycosylation are important sources of free radicals[22]. Elevated glucose causes slow but significant non-enzymatic glycosylation of proteins in diabetes[23]. Glucose auto-oxidise in the presence of transition metal ions generating oxygen free radicals, which make the membrane vulnerable to oxidative damage. Other possible sources include elevated plasma lipids leading to increased lipid oxidation and decreased levels of antioxidant defense systems[24]. Due to these events, the balance normally present in cells between radical formation and protection against them is

disturbed. This leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids. So, there are evidences to show the role of free radicals in diabetes and studies indicate that tissue injury in diabetes may be due to free radicals[25]. In both insulin dependent (type 1) and non-insulin-dependent diabetes (type 2) there is increased oxidative stress[26]. Ethanolic stem extract showed free radicals scavenging effect on DPPH, nitric oxide and superoxide radicals.

The exact mechanism is still unclear but it may be due antioxidant and free radical scavenging effect of the plant and presence of flavanoids, tannins and other phenolic compounds in the extracts.

## CONCLUSION

From this study, we can conclude that *Euphorbia hirta* stem extracts has significant antihyperglycemic effects. These extracts also showed improvement in parameters like lipid profile, body weight and other biochemical parameters. The extract has protective effect against free radicals. Further studies are required to identify the active constituents.

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

| S.No. | Groups | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|---|
| | | Initial day | Day 7 | Day 14 | Day 21 |
| 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. | Petroleum ether extract (250 mg/kg) | 192.75 ± 3.9* | 177.50 ± 4.12 | 165 ± 3.13** | 118.75 ± 2.1** |
| 4. | Petroleum ether extract (500mg/kg) | 177.5 ± 3.22* | 160.50 ± 3.71 | 145.6 ± 1.32** | 110 ± 3.58** |
| 5. | Alcoholic extract (250 mg/kg) | 195 ± 4.08 | 148.5 ± 2.85** | 123 ± 4.74** | 85.75 ± 2.32** |
| 6. | Alcoholic extract (500 mg/kg) | 180.25 ± 2.0 | 129.25 ± 3.9** | 102 ± 2.7** | 78.25 ± 0.86** |
| 7. | Glibenclamide (10 mg/kg) | 194.75 ± 2.84 | 156.5 ± 5.95** | 116.5 ± 5.24** | 83.75 ± 4.5* |

Data represent means ± S.E.M. *$p$<0.05, **$p$ <0.001

**Table 2: Effect of chronic exposure to *E. hirta* extracts on lipid profile (mg/dl)**

| Groups | Cholesterol | Triglycerides | HDL cholesterol | VLDL Cholesterol | LDL Cholesterol |
|---|---|---|---|---|---|
| Normal control | 159.58 ± 5.8 | 82.42 ± 5.1 | 35 ± 1.9 | 16.48 ± 1.5 | 97.52 ± 2.1 |
| Diabetic control | 257.83 ± 14.6 | 250.62 ± 12.7 | 30 ± 1.2 | 50.12 ± 1.4 | 177.71 ± 7.4* |
| Pet. ether extract (250 mg/kg) | 191.41 ± 13.8 | 192 ± 3.62 | 37.28 ± 1.4 | 38.4 ± 2.3 | 115.73 ± 3.5 |
| Pet. ether extract (500 mg/kg) | 186.56 ± 1.2* | 182 ±7.41 | 31.39 ± 1.9* | 36.4 ± 2.7 | 188.77 ± 2.7* |
| Ethanolic extract (250 mg/kg) | 159.72 ± 6.3 | 130 ± 1.4 | 46.27 ± 1.8 | 26 ± 3.1* | 87.45 ± 3.7* |
| Ethanolic extract (500 mg/kg) | 153.41 ± 6.1 | 141 ± 11.2 | 48.82 ± 1.7 | 28.2 ± 1.7* | 76.39 ± 3.8 |
| Glibenclamide (10 mg/kg) | 120.16 ± 5.7* | 102 ± 6.5* | 64.52 ± 1.9 | 20.4 ± 2.8* | 35.24 ± 5.4* |

Data represent means ± S.E.M.*$p$<0.01

**Table 3: Effect of chronic exposure to *E. hirta* extracts on other biochemical parameters**

| Groups | Creatinine (mg/dl) | Urea (mg/dl) | Alkaline phosphatase | Total proteins (g/dl) |
|---|---|---|---|---|
| Normal control | 0.61± 0.1 | 24.24 ±1.8 | 119.46 ± 3.7 | 7.1 ± 2.3 |
| Diabetic control | 1.53 ± 0.1 | 72.00 ± 2.4 | 351.40 ± 6.2 | 4.3 ± 4.62 |
| Pet. ether extract (250mg/kg) | 0.79 ± 0.6 | 50.46 ± 3.9 | 201.00 ± 6.8 | 4.9 ± 3.1* |
| Pet. ether extract (500 mg/kg) | 0.84 ± 0.1 | 42.21 ± 1.7* | 220.37 ± 7.4 | 5.4 ± 1.9 |
| Ethanolic extract (250 mg/kg) | 0.59 ± 0.5 | 38.61 ± 2.2 | 164.29 ±16.4 | 5.7±1.4 |
| Ethanolic extract (500 mg/kg) | 0.64 ± 0.1 | 35.74 ± 2.2* | 169.81±10.7 | 5.65 ± 1.8* |
| Glibenclamide (10 mg/kg) | 0.42 ± 0.1* | 30.00±3.2* | 110.27±3.9* | 8.4 ± 1.4* |

Data represent means ± S.E.M., *$p$<0.01

**Table 4: Effect of *E. hirta* extracts on the body weight in normal and diabetic mice**

Data represent means ± S.E.M., *$p$<0.01

| S.No. | Groups | Change in body weight | | | |
|---|---|---|---|---|---|
| | | Initial day | Day 7 | Day 14 | Day 21 |
| 1. | Normal control | 27.3 ± 1.93 | 27.96 ± 1.58 | 31.11 ± 3.85 | 28.59 ± 1.11 |
| 2. | Diabetic control | 30.37 ± 1.25 | 26.53 ± 3.22 | 28.95 ± 3.21 | 27.2 ± 2.43 |
| 3. | Petroleum ether extract (250 mg/kg) | 30.81 ± 2.1 | 28.28 ± 2.32 | 26.48 ± 1.95 | 28.49 ± 1.95* |
| 4. | Petroleum ether extract (500 mg/kg) | 28.32 ± 2.5 | 27.07 ± 1.95 | 23.72 ± 0.64 | 28.32 ± 2.41* |
| 5. | Alcoholic extract (250 mg/kg) | 24.58 ± 1.41 | 23.44 ± 2.5 | 21.29 ± 1.29 | 24.58 ± 1.70 |
| 6. | Alcoholic extract (500 mg/kg) | 27.42 ± 0.8 | 25.05 ± 1.29 | 23.02 ± 0.76 | 27.42 ± 1.70* |
| 7. | Glibenclamide (10 mg/kg) | 26.27 ± 1.80 | 27.7 ± 2.06* | 29.89 ± 2.25 | 30.46 ± 1.91* |

**Table 5: Antioxidant profile of *Euphorbia hirta* stem extract**

| Sample | Sample conc. (µg/ml) | DPPH radical scavenging activity (%inhibition) | Superoxide anion scavenging activity (%inhibition) | Percentage scavenging of nitric oxide | Reducing power activity[a] (absorbance) |
|---|---|---|---|---|---|
| Alcoholic extract | 250 | 59.95 ± 1.9* | 59.67 ± 0.9* | 28.54 ± 2.4* | 0.352 |
| Ascorbic acid | 250 | 95.44 ± 1.4* | 69.16 ± 1.6 | 39.86 ± 2.1 | 0.292 |
| BHA | 250 | 88.05 ± 1.0* | 67.74 ± 3.1* | 30.89 ±2.7* | 0.248 |

[a]Increased absorbance indicates increased reducing power All values represent Mean ± S.E.M., * $p$<0.01

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