ANTIDIABETIC EFFECT OF *EUPHORBIA HIRTA* LEAVES IN ALLOXAN INDUCED DIABETIC MICE

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Summary

The present study was aimed to investigate the antidiabetic effect of *Euphorbia hirta* leaves extract in normal and alloxan-induced diabetic mice. The ethanolic and petroleum ether extracts of leaves of the *E. hirta* were orally tested at doses of 250 and 500 mg/kg body weight for 21 days in normal and alloxan induces diabetic mice and blood glucose level was measured with glucometer. Serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase, High Density Lipoprotein (HDL) and total proteins levels were also evaluated in normal and alloxan induces diabetic mice. Oral administrations of the both extracts significantly decreased 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 both the extracts and glibenclamide but HDL levels and total proteins were found to be increased after treatments.

Keywords: *Euphorbia hirta*; Alloxan; Diabetic; Mice

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Introduction

Diabetes is a metabolic disorder characterized by deficiency in production of insulin by pancreas, or by ineffectiveness of the insulin produced (1). 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 as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored (2). Since times immemorial, various plants have been used in the treatment of diabetes. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important (3).

Euphorbia hirta (Euphorbiaceae), commonly known as 'Dudhi' is abundant in waste places along the roadsides and open grasslands. It is native to India and Australia (4). The *E. hirta* have been reported to contain alkaloids, saponins, flavonoids, tannins phenolic acids and amino acids (5). Traditionally, *E. hirta* is used in treatment of gastrointestinal disorders, bronchial and respiratory diseases, kidney stones, diabetes and in conjunctivitis. It also exhibits anxiolytic, analgesic, antipyretic and anti-inflammatory activities (6, 7). Our 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 and petroleum ether leaf extracts of *E. hirta* in alloxan induced hyperglycemia in mice.

Materials and Methods

Chemicals

Alloxan was purchased from Loba chemie Pvt. Ltd. Mumbai, India . Total cholesterol (TC), serum high-density lipoprotein (HDL), serum Creatinine (SC), serum urea (SU), serum alkaline phosphate (SAP) and triglyceride (TG) standard kits were obtained from Erba diagnostics Mannheim Gambh, Germany and Blood glucose level was measured using Elegance glucose meter (CT-X10) of Convergent Technologies, Germany .

Plant material

E. hirta leaves (Euphorbiaceae) 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).

Extraction

The leaves were washed with water and shade-dried. The dried leaves 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 67.4 and 52.4% respectively). All the extracts were dried below 45 ^{0}C in rotary evaporator and stored in airtight containers in refrigerator below 10^{0}C.

Animals

Albino mice of either sex, weighing about 30-35g were used for the studies. Animals were allowed to acclimize before the test and were also conditioned at an ambient temperature of 22 ± 2 ^{0}C and at 45–55% relative humidity for 12 h, each of dark and light cycle. Animals were fed on standard pellet mice diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*. The study was approved by the Animal Ethical Committee of the University.

Induction of diabetes

Mice were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Loba Chemie, Bombay; 150 mg/kg i.p.) in sterile saline (8). Twelve days after Alloxan injection, mice with blood glucose level of >140mg/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 (8).

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 alcoholic extract (500 mg/kg).
Group VII. Diabetic mice given Glibenclamide (10 mg/kg).

Biochemical assays

After blood glucose estimation on day 21, 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 alloxan induces diabetic mice. Total cholesterol and triglyceride were determined by the method of Rifai *et al*. (9). Serum urea and creatinine were assayed by the method of Tomas (10, 11). Total proteins (12) and alkaline phosphatase were assayed by the method of Wilkinson et al. (13) and HDL by the method of Burstein *et al* ., (14).

Statistical analysis

All the data were expressed as mean ± S.E.M. Statistical analysis was carried using Student's t-test to analyze the significance between the groups. A value of $p < 0.05$ was considered to be significant.

Results

In the present study, the antidiabetic potential of ethanolic and petroleum ether leaves extracts of *E. hirta* is measured in alloxan induced mice. Daily administration of both the extracts for three weeks led to a dose dependent fall in blood glucose levels. Maximum effect seems to reach after 14 days of treatment and remains constant in third week. The antidiabetic effect of the extracts on the blood glucose levels of diabetic mice is shown in Table 1.

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

Groups	Blood glucose level (mg/dl)			
	Initial day	Day 7	Day 14	Day 21
Normal control	72.25 ± 0.89	73.5 ± 1.0	73.75 ± 0.86	75.5 ± 1.58
Diabetic control	185 ± 2.88	186.2 ± 1.7	189.25 ± 1.25	192.5 ± 1.73
Petroleum ether extract (250mg/kg)	181.75 ± 7.5	154.75± 8.0**	136.3 ± 1.0**	98.3 ± 0.6**
Petroleum ether extract	196 ± 4.54	155 ± 5.75**	145.3 ± 5.3**	110.3±2.72**

(500 mg/kg)				
Ethanolic extract (250 mg/kg)	182.25±4.69	141.2± 3.81**	110 ± 2.23**	82.0 ± 1.41**
Ethanolic extract (500 mg/kg)	188 ± 3.39	138.75± 4.8**	109.5 ± 6.3**	79.75 ± 0.5**
Glibenclamide (10 mg/kg)	156.5 ± 5.95	116.5± 5.24**	83.75 ± 4.5**	194.75±2.84*

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

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 2).

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

Groups	Change in body weight			
	Initial day	Day 7	Day 14	Day 21
Normal control	27.3±1.93	27.96±1.58	31.11±3.85	28.59±1.11
Diabetic control	30.37±1.25	26.53±3.22	28.95±3.21	27.2±2.43
Petroleum ether extract (250 mg/kg)	32.44±0.4	30.93±0.70	27.52±0.81*	29.91±0.91
Petroleum ether extract (500 mg/kg)	30.34±2.32	28.76±2.38	25.71±2.12	27.75±2.02
Ethanolic extract (250 mg/kg)	28.67±2.4	26.94±2.41	25.13±2.32*	27.09±2.41
Ethanolic extract (500 mg/kg)	27.41±2.29	25.90±2.32	23.35±2.51	25.46±2.51
Glibenclamide (10 mg/kg)	26.27±1.80	27.7±2.06	29.89±2.25	30.46±1.91*

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

Serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase levels were decreased significantly by both the extracts and glibenclamide but HDL levels and total proteins were found to be increased after treatments Table 3).
The results of present study indicated that the petroleum ether and ethanolic extracts of *Euphorbia hirta* possesses significant hypoglycemic activity.

Discussion

Various studies have shown that *Diabetes mellitus* is also associated with increased formation of free radicals and decrease in antioxidant potential. It is accepted that oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism's antioxidant potential (15) Traditional plant medicines are used throughout the world for treatment of various diseases and disorders. Worldwide, over 1200 species of plants have been recorded as traditional medicine for diabetes
Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (16). Therefore, investigation on such agents from traditional medicinal plants has become more important. *Euphorbia hirta* had showed *in vitro* antioxidant potential (17). So, free radical scavenging and antioxidant effect may be responsible for its antidiabetic effect. It is possible that extract exert its effect by causing hypoglycemia.

Euphorbia prostrata, the plant of same genus has been reported to have hypoglycemic effect (18). Keeping in view of this and traditional uses, the ethanolic and petroleum ether extracts of *E. hirta* were investigated for its antidiabetic activity and long-term effects on the body weights. The diabetic state was induced by intraperitoneal injection of alloxan. In this study alloxan effectively induced diabetes in all animals studied. The animals having blood glucose levels above 140 mg/dl were selected for the experiment. It was observed that there was also significant weight gain in *E. hirta* treated diabetic mice compared with untreated diabetic mice.
This study observed decrease in serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase levels by both extracts of *E. hirta* and glibenclamide but HDL levels and total proteins were found to be increased after treatments. The mechanism of action of reduction of blood glucose levels after administration (p.o.) of the extracts is not clear. The extracts should further be subjected to bioactivity guided drug discovery to isolate a lead compound responsible for this activity.

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Alkaline phosphatase	H.D.L cholesterol	Total Proteins (g/dl)
I	149.58±5.8	82.42±5.1	0.61±0.1	24.24±1.8	119.46±3.7	35.00±1.9	7.1±2.3
II	257.83±14.6	250.62±12.7	1.53±0.1	72.00±2.4	351.40±6.2	30.00±1.2	4.3±4.62
III	172.33±4.7	181.21±6.0	0.90±0.1	42.19±2.7	219.83±6.4*	35.42±1.4	4.7±3.7
IV	164.00±6.8*	189.46±5.8*	0.92±0.2	48.20±1.0	250.66±5.7	32.19±1.5	5.1±6.4
V	152.26±6.1	139.91±2.1*	0.63±0.1*	36.83±3.9*	166.78±17.2	45.67±2.1*	5.2±4.9
VI	149.18±5.7	148.64±1.98	0.58±0.1	34.00±2.4*	182.46±8.9*	40.93±1.6	4.6±9.7*
VII	120.16±5.7*	102.00±6.5*	0.42±0.0*	30.00±3.2*	110.27±3.9*	64.52±1.9	8.4±1.4

Table 3. Effect of *E. hirta* extracts on various biochemical parameters

Data represent means ± S.E.M. *P<0.01

Conclusion

Ethanolic and petroleum ether extracts of *E. hirta* exhibited significant antidiabetic activity in alloxan- induced mice. The former extract showed better effects than the latter one. These extracts also showed improvement in parameters like body weight, lipid profile and other biochemical parameters. Further studies are required to identify the active fractions.

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