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Antidiabetic activity of ethanolic extract of *Euphorbia hirta* Linn

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ABSTRACT

Diabetes mellitus (DM) is a chronic disease caused by inherited and acquired deficiency in production of insulin by the pancreas, or by ineffectiveness of insulin produced, such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's systems in particular the blood vessels and nerves. Euphorbia hirta Linn (Euphorbiaceae) is commonly used in traditional medicine for the treatment of various diseases. The present study was carried out to evaluate the anti-diabetic effect of ethanolic extract of Euphorbia hirta Linn using various animal screening models. The ethanolic extract of Euphorbia hirta showed a significant hypoglycemic effect in the alloxan-induced diabetic rats. This laid the foundation to study the active compounds of such anti-diabetic plants that are responsible for the hypoglycemic activities.

Keywords: Hypoglycemic activity, *Euphorbia hirta*, Alloxan-induced diabetes, Diabetes mellitus.

INTRODUCTION

A study of ancient literature indicates that diabetes (madhumeha) was fairly well known as an entity in India. 'Madhumeha' is a disease in which a patient passes high sugar in urine and exhibits sweetness all over the body, i.e. in sweat, mucus, breath, blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20% [1]. Diabetes mellitus has now become an epidemic, with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS [2]. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades [3, 4].

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α–glucosidase inhibitors, glinides which are used as monotherapy or in combination to achieve better glycemic regulation, but many of these oral antidiabetic agents have a number of serious adverse effects [5]. Due to its high prevalence and potential deleterious effects on a patient's physical and psychological state, diabetes mellitus, which can result in a morbid condition, is a major medical concern several species of plants have been described as having antidiabetic property. The ethno botanical information reports about 800 plants that may possess antidiabetic potential [6]. Management of diabetes without any side effects is still a challenge to the medical system. Herbal Drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. The large number of plants described (176 species belonging to 84 families) clearly demonstrated the importance of herbal plants in the treatment of diabetes. The plant families, including the species, most studied for their confirmed hypoglycemic effects include: Leguminoseae (11 sp), Lamiaceae (7 sp), Liliaceae (8 sp), Cucurbitaceae (7 sp), Asteraceae (6 sp), Moraceae (6 sp), Rosaceae (6 sp), Euphorbiaceae (5 sp) and Araliaceae (5 sp) [6].

Present study deals with the plant *Euphorbia hirta* Linn is popularly known as *Euphorbia pilulifera*. Lyophilized aqueous extract of the plant has also been shown to exhibit sedative effects in mice [7]. The Swahilis and Sukumas in East Africa use *Euphorbia hirta* as a diuretic agent [8]. In traditional Ayurvedic medicines the whole aerial parts

is used in gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis), bronchial and respiratory diseases (asthma, bronchitis, hay fever, amoebiasis) [9].

The plant is used by many of triable Peoples of Nashik, Dhule & Nandurbar district of Maharashtra, but survey reveal that although the plant is used as folk medicine but there is no scientific investigation for its antidiabetic activity, therefore to verify the ethanoclaim of this plant we have decided to investigate the antidiabetic activity of *Euphorbia hirta* Linn by determining the effects on blood glucose level and lipid profile by using normal, alloxan-induced diabetic rats and fructose induce insulin resistance.

MATERIALS AND METHODS

Collection of plant material and authentication:
Euphorbia hirta Linn was collected from local area of Shirpur and authenticated by Dr. D. A. Patil Reader Department of Botany S.S.V.P.S College of Arts, Commerce and Science, Dhule (M.S.).

Preparation of Extract:
The plant was air-dried, and pulverized into coarse powder. The crude extract was obtained by soaking plant material in ethanol (2 liters) by cold maceration. Ethanolic extract was concentrated in rotary evaporator under vacuum.

Experimental Animals
Wistar rats weighing 150-200 gm were procured and were housed in polypropylene cages and maintained under standard conditions (12 hrs light and dark cycles, at 25 ± 30C and 35-60 % humidity). Standard palletized feed and tap water were provided *ad libitum*. Animals were acclimatized to laboratory conditions at least 24 hours before conducting the experiments (CPCSEA, India Registration No.651/02/C/CPCSEA).

Procurement of diagnostic kits and chemicals:
The diagnostic kits for the estimation of glucose, cholesterol, triglycerides, HDL-cholesterol, total protein, creatinine were purchased from RFCL Ltd. The Alloxan (Sigma chemicals, USA), Fructose (Qualigens fine chemical, Mumbai) respectively.

Acute toxicity study [10, 11]
Albino female mice weighing 25-30 gm were used for acute toxicity study. Acute toxicity study was carried out as per "Up & Down" method. The test drug was found to be safe up to the dose 5000 mg/kg body weight; hence $1/10^{th}$ of dose was taken as an effective dose (500mg/kg Body weight).

EXPERIMENTAL DESIGN
Albino Wistar rats were selected for the experimental model. The rats were fed with the standard diet and water *ad libitum* before the experiment. Room temperature was maintained between 20-30^{o}C along with the humidity 40–60%. The weights of selected rats were the 150-200 gm each. Diabetes was induced in rats that had been fasted for 12 hrs by intraperitoneal injection of 100 mg/kg body weight.

After one hour of Alloxan monohydrate freshly dissolved in sterile normal saline administration the animals were given feed *ad libitum*. The animals were allowed to drink 5 % glucose solution over night to overcome the drug induced hypoglycemia. The diabetic rats (glucose level > 250 mg/100ml) were divided into 7 groups of 6 rats each. Groups divide in a such way that –

Group I : Negative control
Group II : Diabetic control
Group III : Ethanolic extract of *Euphorbia hirta* of 100 mg/kg
Group IV : Ethanolic extract of *Euphorbia hirta* of 200mg/kg
Group V : Ethanolic extract of *Euphorbia hirta* of 400 mg/kg
Group VI : Ethanolic extract of *Euphorbia hirta* of 800 mg/kg
Group VII : Glibenclamide 5mg/kg.

Administration was continued for 21 days, once daily. Blood samples were collected from the retro-orbital plexus on day 1, 15, 21 of extract administration. The body weight of the animal was calculated.

Effect on Blood Glucose Level

A dose-dependent hypoglycemia was observed in animals treated with *Euphorbia hirta* Linn. Administration of the crude extracts, orally 30 min prior to glucose load showed improved glucose tolerance in normal rats. In alloxan-treated rats, the rise in blood glucose level reached its peak value on the 5th day and then remained stable throughout the study period. Treatment with all the four doses of *Euphorbia hirta* Linn (100, 200,400 and 800 mg/kg) produced significant reduction in the blood glucose level with maximum reduction being achieved with the dose 800 mg/kg ($P<0.01$). Giving ethanolic extract of *Euphorbia hirta* Linn (100,200,400 and 800 mg/kg) along with fructose feeding for 21 days significantly reduce the said parameters like glucose, cholesterol, triglyceride and creatinine value when compared to fructose alone fed group**.**

STATISTICAL ANALYSIS

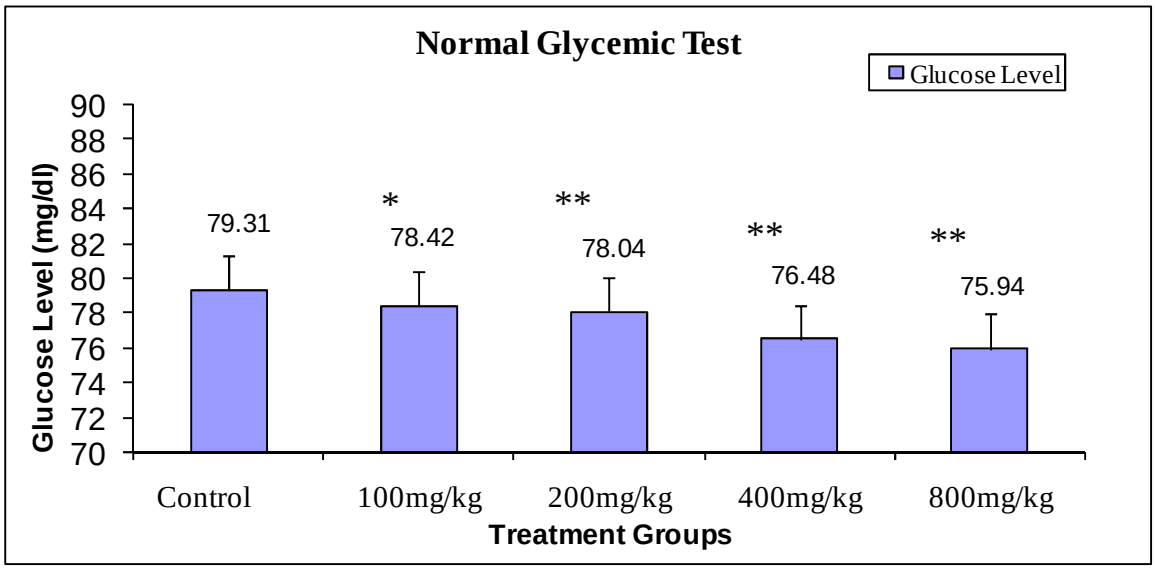
The data was statistically analyzed by one-way ANOVA followed by Dunnett multiple comparison test with equal sample size. The difference was considered significant when $p<0.01$. All the values were expressed as mean ± standard deviation (S.D.).

RESULTS

The Ethanolic Extract of *Euphorbia hirta* Linn showed positive test for flavonoids, saponins, glycosides, tannins, phenolics, steroids, proteins and carbohydrate. The various doses of ethanolic extract of *Euphorbia hirta* Linn were shows no mortality in mice. No toxic symptoms were observed even at the dose of 5000 mg/kg. The LD_{50} value of ethanolic extract of *Euphorbia hirta* Linn was calculated by using experimental data. A computer guided statistical program - AOT425statPgm was used for the determination of the LD_{50} value. The LD_{50} value was calculated more than 5000 mg/kg.

A dose-dependent hypoglycemia was observed in animals treated with *Euphorbia hirta* Linn **(Fig No 1)**. To determine significance of difference in hypoglycemia achieved by the four doses (100, 200, 400 and 800 mg/kg, p.o.) at 120 min, Student's *t*-test was applied and compared with the control group. A highly significant reduction ($P<0.01$) in blood glucose was observed.

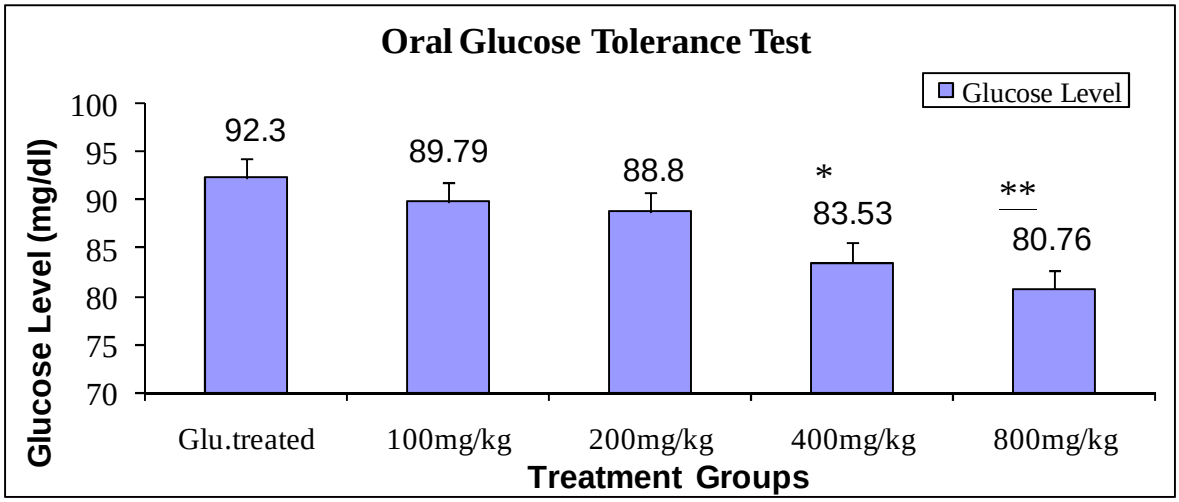
Fig No 1: Effect of Ethanolic Extract of *Euphorbia hirta* Linn on Glucose level in normal glycemic rats



Values are expressed as mean ±SEM n= 6
Data was analyzed by one way ANOVA followed by Dunnet test
*P<0.01= Highly significant **, P<0.05= Significant**

The ethanolic extract of *Euphorbia hirta* L ($P<0.01$) have shown significant increase in glucose tolerance. The blood glucose levels were reduced considerably within 90 minutes of the drug administration. Administration of ethanolic extract of *Euphorbia hirta* Linn (800 mg/kg) to glucose-fed rats induced time dependent hypoglycemic effect **(Fig No 2)**.

Fig No 2: Effect of Ethanolic Extract of *Euphorbia hirta* Linn on Glucose level in orally Glucose treated rats



Values are expressed as mean ±SEM n= 6
Data was analyzed by one way ANOVA followed by Dunnet test
*P<0.01= Highly significant **, P<0.05= Significant**

In alloxan-treated rats, all the four doses of *Euphorbia hirta* Linn (100, 200,400 and 800 mg/kg) produced significant reduction in the blood glucose level with maximum reduction being achieved with the dose 800 mg/kg (P<0.01) **(Table 1)**. The peak reduction in blood glucose level with all the four doses was observed at the end of the 21st day of treatment. Similar effects were also observed in the lipid profile **(Fig No 3)**.

All the four doses of *Euphorbia hirta* Linn also produced significant increase in the HDL level with the maximum elevation being produced with the dose of 800 mg/kg (P<0.01). Vehicle control animals were found to be stable in their body weight while diabetic rats showed significant reduction in body weight during 21 days **(Table 1)**.

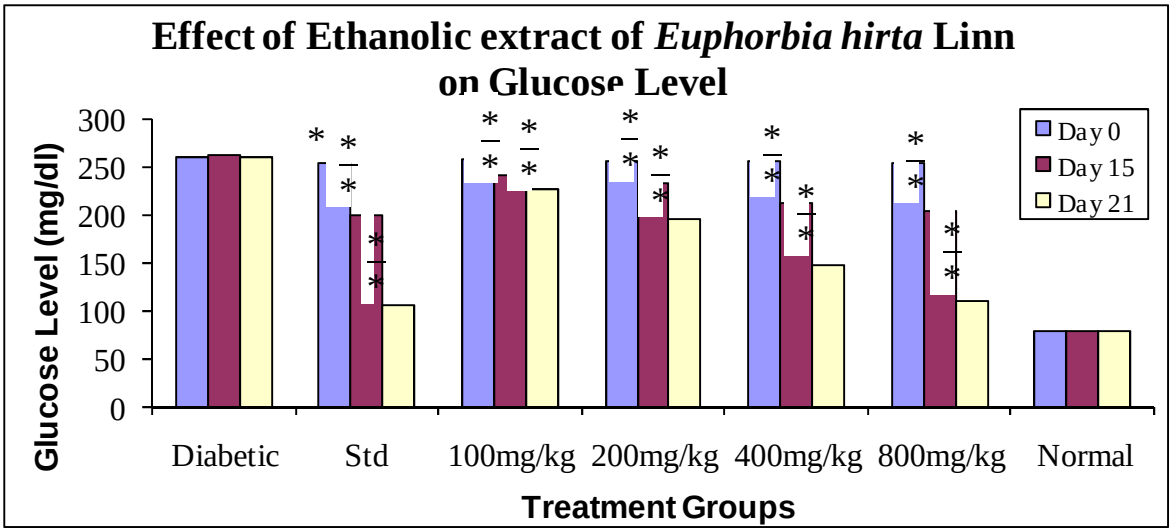
Alloxan caused weight reduction, which was reversed by ethanolic extracts of *Euphorbia hirta* Linn after 21 days of treatment. HDL levels were increased by glibenclamide (p<0.01), ethanolic extract (p<0.01) compared with diabetic control **(Table 2)**.

Table 1: Effect of Ethanolic extract of *Euphorbia hirta* Linn, Glibenclamide and Alloxan on Glucose, triglyceride, cholesterol, and creatinine levels

Groups	Days	Glucose Level (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)
Negative control (Normal)	0	79.41±2.09	80.01±1.38	72.32±0.65	0.65±0.01
	15	78.48±1.85	80.39±0.85	73.13±0.49	0.64±0.01
	21	79.25±1.49	79.25±1.22	73.37±0.63	0.64±0.01
Diabetic control	0	260.7±2.33	199.9±0.60	142.3±0.99	1.83±0.05
	15	261.6±2.21	199.8±0.55	141.2±0.57	1.89±0.02
	21	259.8±2.09	199.4±0.54	141.6±0.77	1.90±0.01
Standard (glibenclamide)	0	254.2±1.68*	193.5±0.61**	129.8±0.92**	1.44±0.01**
	15	199.1±1.34**	131.6±1.69**	104.3±0.69**	1.10±0.01**
	21	106.7±1.69**	94.5±0.79**	85.8±1.32**	0.78±0.01**
Ethanolic Extract 100mg/kg	0	257.7±1.16	199.0±0.41	139.8±0.77	1.82±0.022
	15	240.8±0.78**	186.3±1.76**	136.5±0.80*	1.63±0.03**
	21	226.6±3.52**	170.7±2.47**	125.5±1.85**	1.54±0.02**
Ethanolic Extract 200mg/kg	0	255.9±1.08	197.8±0.49	138.5±0.43*	1.75±0.024
	15	233.4±1.61**	167.3±2.26**	125.9±1.85**	1.54±0.01**
	21	195.8±2.08**	151.8±2.06**	109.7±1.40**	1.37±0.01**
Ethanolic Extract 400mg/kg	0	256.3±1.27	196.3±0.44**	132.1±0.66**	1.66±0.01**
	15	212.5±1.91**	155.5±1.98**	120.5±1.35**	1.30±0.02**
	21	148.8±209**	136.3±2.02**	100.8±0.59**	1.03±0.01**
Ethanolic Extract 800mg/kg	0	255.1±0.93	195.0±0.64**	131.9±1.02**	1.51±0.02**
	15	203.5±1.67**	136.5±2.27**	110.9±1.48**	1.16±0.01**
	21	111.0±2.21**	95.84±1.62**	90.2±0.84**	0.80±0.02**

Values are expressed as mean ±SEM n= 6
Data was analyzed by one way ANOVA followed by Dunnet test
*P<0.01= Highly significant **, P<0.05= Significant**

Fig no 3: Effect of Ethanolic extracts of *Euphorbia hirta* Linn on blood glucose level



Fructose feeding (dose of 30 % orally) significantly increase serum glucose, cholesterol, triglyceride, and creatinine when compared with normal. However, there was significant decrease HDL and Protein Levels. Giving ethanolic extract of *Euphorbia hirta* Linn (100,200,400 and 800mg/kg) along with fructose feeding for 21 days significantly reduce the said parameters like glucose, cholesterol, triglyceride and creatinine value when compared to fructose alone fed group **(Table 3)**.

Table 2: Effect of Ethanolic extract of *Euphorbia hirta* Linn, Glibenclamide and Alloxan on Body weight, Total Protein, and Serum HDL levels

Groups	Days	Body weight (gm)	Total protein (gm/dl)	Serum HDL (mg %)
Negative control (Normal)	0	220.3±6.25	6.755±0.074	45.69±0.707
	15	222.0±5.18	6.683±0.069	46.16±1.198
	21	220.3±6.54	6.657±0.0545	47.19±0.714
Diabetic control	0	209.8±5.40	4.255±0.155	32.51±0.356
	15	192.0±3.06**	4.227±0.149	32.43±0.351
	21	176.3±2.96**	4.295±0.121	32.66±0.278
Standard (glibenclamide)	0	205.3±4.78	4.838±0.069**	37.92±0.698**
	15	201.5±4.68**	5.665±0.129**	39.47±0.721**
	21	198.2±4.66**	6.250±0.046**	46.90±0.645**
Ethanolic Extract 100mg/kg	0	204.3±4.44	4.488±0.123	33.77±0.370
	15	194.5±1.84**	4.778±0.178	34.63±0.368
	21	181.8±3.12**	5.008±0.183*	36.82±0.268**
Ethanolic Extract 200mg/kg	0	208.2±6.87	4.503±0.142	34.78±0.413*
	15	195.5±4.62**	4.858±0.203	37.03±0.481**
	21	185.2±4.49**	5.190±0.243*	39.71±0.409**
Ethanolic Extract 400mg/kg	0	204.7±5.75	4.710±0.090	36.88±0.521**
	15	199.5±4.09**	5.012±0.266*	38.72±0.496**
	21	191.0±4.12**	5.500±0.191**	40.77±0.418**
Ethanolic Extract 800mg/kg	0	202.7±3.27	4.750±0.068*	37.26±0.665**
	15	199.7±3.07**	5.313±0.149**	39.97±0.521**
	21	193.0±2.48**	5.978±0.097**	45.77±0.709**

Values are expressed as mean ±SEM n= 6
Data was analyzed by one way ANOVA followed by Dunnet test
*P<0.01= Highly significant **, P<0.05= Significant**

Table 3: Effects of Ethanolic extract of *Euphorbia hirta* Linn, Glibenclamide and Fructose on Glucose, triglyceride, cholesterol, and creatinine levels

Groups	Days	Glucose Level (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)
Negative control (Normal)	0	79.88±0.94	81.15±1.89	70.44±1.60	0.673±0.008
	15	79.08±1.03	80.25±0.78	76.73±1.56	0.688±0.007
	21	80.14±1.00	79.94±0.56	81.02±1.08	0.678±0.029
Diabetic control	0	227.0±1.64	189.1±1.93	148.1±1.32	1.863±0.029
	15	224.5±1.85	189.8±1.32	147.5±0.45	1.835±0.015
	21	223.6±1.31	189.6±1.18	147.2±0.59	1.853±0.016
Standard (glibenclamide)	0	209.2±0.97**	182.0±1.04**	136.8±0.72**	1.458±0.030**
	15	146.5±1.99**	138.5±1.79**	111.5±1.64**	1.218±0.017**
	21	102.0±2.16**	86.43±1.38**	84.82±1.08**	0.780±0.023**
Ethanolic Extract 100mg/kg	0	224.9±1.44	187.6±0.91	145.6±0.88	1.810±0.028
	15	213.4±1.87**	186.7±0.92	140.1±0.38**	1.760±0.016*
	21	199.5±1.74**	177.9±0.87**	120.5±1.02**	1.537±0.019**
Ethanolic Extract 200mg/kg	0	223.9±1.09	185.0±0.77	144.1±0.67*	1.763±0.021*
	15	196.4±2.48**	179.0±0.72**	137.9±0.54**	1.652±0.008**
	21	170.2±3.35**	154.5±0.70**	117.7±0.81**	1.410±0.016**
Ethanolic Extract 400mg/kg	0	221.6±1.02*	185.1±0.53	140.3±0.62**	1.673±0.018**
	15	185.8±1.65**	156.6±1.22**	131.6±0.53**	1.500±0.020**
	21	132.7±2.43**	126.1±1.17**	104.6±1.01**	1.262±0.018**
Ethanolic Extract 800mg/kg	0	218.5±1.23**	183.8±0.55*	138.8±0.58**	1.563±0.013**
	15	162.3±2.25**	151.5±2.16**	118.4±0.93**	1.325±0.022**
	21	108.7±2.32**	93.55±1.49**	90.06±1.65**	0.878±0.020**

Values are expressed as mean ±SEM n= 6
Data was analyzed by one way ANOVA followed by Dunnet test
*P<0.01= Highly significant **, P<0.05= Significant**

DISCUSSION

Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas [12, 13]. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis [14, 15]. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells [16]. Experimental studies reveals that the ethanolic extracts from *Euphorbia hirta* Linn produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats. The mechanism was found to be an insulin-independent mechanism. The mechanism by which plant extracts has been proposed to inhibit hepatic glucose production, to inhibit intestinal glucose absorption or to correct insulin resistance. It also proves the traditional claim with regard to *Euphorbia hirta* Linn for its anti-diabetic activity.

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