ANTIHYPERGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF METHANOLIC EXTRACT OF *EUPHORBIA PROSTRATA* ON ALLOXAN INDUCED INDUCED DIABETIC RABBITS

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Abstract

In past numerous medicinal plants had been used for the control of diabetes because of their antihyperglycemic effect but there was no scientific proof of it. Aim of current study was to evaluate antihyperglycemic and hypolipidemic effect of whole plant of *Euphorbia prostrata* (Family: Euphorbiaceae) in order to validate its traditional use in diabetes, by native people of Cholistan desert, Pakistan. Whole plant (methanolic extract) of *Euphorbia prostrata* (EP) (250 and 500 mg/Kg/day, per oral for 14 days) was evaluated in alloxan induced diabetic rabbits (150 mg/Kg., i/p.) by serum biochemical parameters. Glibenclamide (5 mg/Kg/day, p.o. for 14 days) was used as standard antidiabetic drug. Alloxan induced diabetic groups had elevated levels of fasting blood glucose, cholesterol and triglyceride levels when compared with control group. EP extract (both doses of 250 and 500 mg/Kg) exhibited antidiabetic effect by significant reduction of fasting blood glucose levels. 500 mg/Kg dose of EP produced more significant ($P< 0.05$) results as compared to 250 mg/Kg dose. Serum cholesterol and triglyceride levels were also lowered doen at the end of study. Therefore, outcome of the present study validate the traditional claims on antihyperglycemic effects of *Euphorbia prostrata* (whole plant).

Keywords: Antihyperglycemic; Euphorbia prostrata; Hypolipidemic; Medicinal plants

Introduction

Diabetes is one of the major health issues in current scenario and is affecting many people from different walks of life in almost every country of the world (Modak *et al*., 2007). Diabetes is a chronic disorder and is associated with abnormal metabolism of carbohydrate, fat and protein. Elevated levels of both fasting and post-paradinal blood sugar is the specific characteristic of diabetes. Auto-immune and non-auto-immune responses cause destruction of pancreatic β-cells resulting in type 1 diabetes (Kanatsuka *et al*., 2006). There are circulating immune markers against pancreatic islets known as anti-islet cell antibodies or β-cell antigens (Buchanan and Xiang, 2005). That is the reason for patient's dependency on external supply of insulin but those suffering from Type II diabetes known as non-insulin dependent, can not properly respond to insulin. Type II diabetics can be therefore treated by changes in diet intake, exercise and by use of medicines. Type II diabetes is more prevalent form among two and constitutes about 90% of the whole. Symptoms can be same for both types such as: (i) raised levels of blood glucose; (ii) increased need to drink water; (iii) repeated urine output; (iv) increased food intake and weight loss; (v) blurred vision; (vi) nausea and vomiting; (vii) fatigue and weakness; (viii) restlessness and changes of mood etc.

Adverse side effects are reported by the use of synthetic oral antidiabetic drugs commonly used for the diabetes treatment (Akhtar and Iqbal, 1991) and (Holman and Turner,

1991). On the other hand, theses drugs are also not safe during pregnancy (Larner, 1985). In recent years, use of herbal origin medicines had grown and these medicines are becoming popular in developed as well as developing countries. Natural origin is one of the reasons of increased popularity along with low cost and fewer side effects (Modak *et al*., 2007).

Medicinal plants are in vogue from many centuries for the management of diabetes but very less scientific research had been conducted on these drugs to rule out potentially active constituent and their mechanism of action. *Euphorbia prostrata* commonly known as Hazardani is an annual herb, which belongs to family Euphobeaceae and is abundantly found in India, Pakistan and Africa (Singla and Pathak, 1990). The dried leaves and seeds are both slightly aromatic, and are considered as stimulant, astringent, anthelmintic and laxative. The juice from the fresh plant is used to treat ringworm. Among some tribes of India (Nag pur) the pounded whole plant, mixed with sugar and water, is used to stop diarrhea. An essential oil distilled from the plant is used in medicinal soaps and to treat erysipelas and as a mosquito and fly repellent (Parrotta, 2001).

Several flavonoids like apigenin, quercetin, luteolin (and their glucosides), phenolic acids like gallic acid, ellagic acid and tannins are reported to be present in *Euphorbia prostrata*. These active constituents affect the inflammatory process by means of its action on the enzymes involved such as cyclooxygenase, protein kinase C, hyaluronidase, 5 – lipoxigenase, etc, (Wallis, Text Book of Pharmacognosy). The hypoglycemic activity of *Euphorbia prostrata* was reported earlier by Singla and Pathak (1990) but systematic and scientific investigations had not been carried out on *Euphorbia prostrata*.

Lack of clinical data and scientific research is the main blocking stone in the use of herbal origin medicine for the treatment of various illnesses these days. There is continuous need of scientific research for proving safety and efficacy of herbal medicines from simple biological assays to drug standardization along with toxicity and safety studies. Present study was carried out to investigate the antihyperglycemic and hypolipidemic effects of the whole plant of *E. prostrata* in diabetic rabbits.

Material and method

Chemicals

All chemicals and drugs of analytical grade were obtained commercially. Alloxan monohydrate (98 %) was used to induce diabetes (Acros Organics, USA).Glibenclamide used to control diabetes. Commercial kits for the estimation of serum glucose, cholesterol and trigycerides were purchased from Human Weisbaden,, Germany.

Plant material and extraction procedure

Fresh plants of *Euphorbia prostrata* were collected locally from nearby areas f Cholistan desert and were authenticated from Cholistan Institue of Desert Studies, the Islamia University of Bahawalpur, Pakistan. A voucher specimen (3402/CIDS/IUB) was deposited in the herbarium.

Preparation of Extract

Plant materials were dried under shade and crushed to get coarse powder. The coarse powder (1430 g) was macerated with 70% methanol for 15 days with frequent shaking. After 15 days, filteration was carried out through muslin cloth initially and then through Whatman filter paper. The filtrate was subjected to evaporation at low temperature (30 to 40 °C) and under reduced pressure on rotary evaporator (Heidolph Laborota 4000 efficient, Germany) until semisolid residue was obtained. The final extract obtained was in the form of thick viscous paste with dark brownish color and approximate yield was 61 g. For convenient

administration, the dry extract powder was encapsulated after weighing. Glibenclamid was used as a reference antidiabetic agent.

Experimental animals

Healthy rabbits of either sex (local breed), weighing from 1.5-2 Kg were purchased from the local market. They were kept in animal house of Faculty of Pharmacy & Alternative Medicine, The Islamia University of Bahawalpur. Standard housing conditions were maintained for rabbits and provided standard pellet diet and water ad labitum. All procedures were approved prior to performance by Institutional Animals Ethics committee.

Induction of Diabetes

Diabetes mellitus was induced by intraperitoneal administration of alloxan monohydrate (98%) at a dose of 150 mg/kg body weight, dissolvd in normal saline to the overnight fasted rabbits. Fasting blood glucose (FBG) level of rabbits was checked up regularly for one week to get stable hyperglycemia. Blood glucose level (BGL) was checked by Merck microlab 300 (Merck, Germany). Rabbits showing fasting blood glucose level ≥ 200 mg/dl were destined for screening antidiabetic effects of the crude extract.

Experimental design

The experimental period was 22 days. The first 8 days were for the induction of diabetes in rabbits and the following 14 days were the investigational period with crude methanolic extract of *Euphorbia prostrata*.

There were five groups of six rabbits each.

Group-1: Normal saline treated rabbits (Normal control-NC)

Group-2: Normal saline treated diabetic rabbits (Diabetic Control-DC)

Group-3: Glibenclamide (5 mg/kg body weight) treated diabetic rabbits (Standard Control-SC)

Group-4: *Euphorbia prostrata* extract (250 mg/kg/day)) treated diabetic rabbits (*Euphorbia prostrata* crude extract-Ep.Ce 250 mg/kg/day)

Group-5: *Euphorbia prostrata* extract (500 mg/kg/day)) treated diabetic rabbits (*Euphorbia prostrata* crude extract-Ep.Ce 500 mg/kg/day)

Blood sampling and biochemical analysis

Feeding was stopped 12 hrs before blood sampling. Blood samples were drawn at the1st, 3rd, 7th and 14th day of study in vacuum tubes without the anticoagulant. Blood was centrifuged at 3000 rpm for 10 minutes to separate the serum after which it was tested for glucose. At the end of the experimental period (day 14) the blood samples collected were also tested for serum cholesterol and triglycerides. Effect of extract was studied up to 6 hours on BGL.

Statistical analysis

Results were presented as the mean standard deviation (SD). A one-way analysis variance was performed using SPSS-17 and Graphpad Prism 5 statistical software. Tukey's test was used for comparing the groups. The values were considered significantly different when the pvalue was lower than 0.05.

Results

Antihyperglycemic effect

The administration of methanolic extract showed a significant reduction in serum glucose level in alloxanized rabbits. One week after alloxan administration, serum glucose

values were almost 3-folds higher in all diabetic groups as compared to NC group. No statistical difference was observed among the diabetic groups before the treatment. Serum glucose levels showed a significant reduction in all treatment groups in comparison to the non diabetic rats during the experimental period which was more pronounced from the 7th to the 14th day. After 14 days the blood glucose in the diabetic control still recorded an elevated value (table 1).

Reduction in serum glucose values in diabetic control group was however, non-significant. When compared to the serum glucose level on day 1 after diabetes induction, methanolic extract treatments (250 and 500mg/Kg body weight) showed a significant reduction in serum glucose levels over the experimental period comparable with the standard drug (Glibenclamide). However, maximal reduction in blood glucose was seen in 500mg/Kg body weight over the experimental period (fig. 1). Although the methanolic extract treatments (250 and 500mg/Kg body weight) showed a significant (P< 0.05) reduction in serum glucose levels as compared to standard drug (table 1).

Table 1: Effect of *Euphorbia prostrata* (Ep.Ce) on Fasting Blood Glucose levels (mg/dl) of alloxan induced diabetic rabbits

Effect of *Euphorbia prostrata* (Ep.Ce) on Fasting Blood Glucose levels (mg/dl) of alloxan induced diabetic rabbits					
Sr. #	**Treatment Groups**	**1st Day**	**3rd Day**	**7th Day**	**14th Day**
1	**Normal Control** (N/S 1 ml/Kg)	70.90 ± 3.17	71.01 ± 2.75	70.33 ± 2.52	71.18 ± 2.59
2	**Diabetic Control** (N/S 1 ml/Kg)	262.43 ± 7.41	251.91 ± 6.08	248.13 ± 6.11	253.38 ± 6.09
3	**Standard Control** (Glibenclamide 5 mg/Kg)	273.15 ± 6.90	135.95 ± 6.08	131.25 ± 3.92	124.16 ± 4.42
4	**Ep.Ce 250 mg/Kg**	262.85 ± 2.90	183.26 ± 1.19	180.63 ± 0.93	178.21 ± 0.81
5	**Ep.Ce 500 mg/Kg**	269.38 ± 5.16	166.81 ± 2.87	164.45 ± 2.65	161.80 ± 2.55

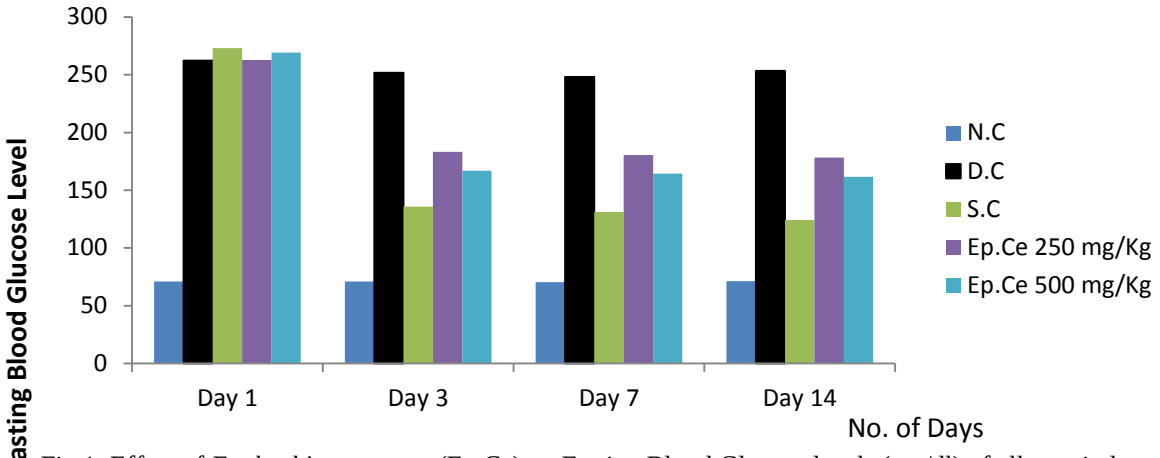

Fig 1: Effect of *Euphorbia prostrata* (Ep.Ce) on Fasting Blood Glucose levels (mg/dl) of alloxan induced diabetic rabbits

(N.C = Normal Control, D.C = Diabetic Control, S.C = Standard Control (Glibenclamide 5 mg/Kg)

Values are expressed as Mean ± SEM and n = 6. Tuckey test was used to compare means. P values were considered as P < 0.05 as significant (*). Both Extract treated groups were compared to positice control, negative control and standard control.

Lipid profile

Serum lipid levels were measured at the start and end of the experimental time period (figs. 2 and 3). Separate tables provided with figs. (2 and 3). The total cholesterol (TC)

concentrations of diabetic control rabbits showed a significant increase compared with those of the normal control rats. However, rabbits receiving an oral administration of the methanolic extracts (250 and 500mg/Kg body weight) and glibenclamide had significantly ($p<0.05$) lower concentrations of TC comparable to diabetic control group on day 14th at the end of experimental period. The administration of the methanolic extracts and the standard drug was able to restore and further decrease the total cholesterol which was statistically more pronounced ($p<0.05$) in the groups receiving the methanolic extracts. High dose of methanolic extract (500mg/Kg body weight) was significantly better than the low dose (250mg/Kg body weight) in restoring the total cholesterol and triglycerides. Thus both doses of extract significantly corrected the hypercholesterolemia and hypertriglyceridemia coupled with hyperglycemia.

Table 2: Effect of *Euphorbia prostrata* (Ep.Ce) on Serum Cholesterol levels (mg/dl) of alloxan induced diabetic rabbits

Effect of *Euphorbia prostrata* (Ep.Ce) on Serum Cholesterol levels (mg/dl) of alloxan induced diabetic rabbits			
Sr. #	**Treatment Groups**	**0 Day**	**14th Day**
1	**Normal Control** (N/S 1 ml/Kg)	80.70 ± 2.89	81.85 ± 1.79
2	**Diabetic Control** (N/S 1 ml/Kg)	145.25 ± 5.54	151.20 ± 3.35
3	**Standard Control** (Glibenclamide 5 mg/Kg)	157.63 ± 4.33	100.53 ± 3.19
4	**Ep.Ce 250 mg/Kg**	158.73 ± 6.47	134.46 ± 7.94
5	**Ep.Ce 500 mg/Kg**	158.28 ± 8.61	126.54 ± 6.95

Fig 2: Effect of *Euphorbia prostrata* (Ep.Ce) on Serum Cholesterol levels (mg/dl) of alloxan induced diabetic rabbits

Values are expressed as Mean ± SEM and n = 6. Tuckey test was used to compare means. P values were considered as $P < 0.05$ as significant (*). Both Extract treated groups were compared to positice control, negative control and standard control.

Table 3: Effect of *Euphorbia prostrata* (Ep.Ce) on Serum Triglyceride levels (mg/dl) of alloxan induced diabetic rabbits

Effect of different doses of *Euphorbia prostrata* (Ep.Ce) on Serum Triglyceride levels (mg/dl) of alloxan induced diabetic rabbits			
Sr. #	**Treatment Groups**	**0 Day**	**14th Day**
1	**Normal Control** (N/S 1 ml/Kg)	105.70 ± 2.83	108.95 ± 3.20
2	**Diabetic Control** (N/S 1 ml/Kg)	167.74 ± 3.56	171.41 ± 2.98
3	**Standard Control** (Glibenclamide 5 mg/Kg)	168.80 ± 3.13	101.19 ± 2.17
4	**Ep.Ce 250 mg/Kg**	156.14 ± 3.24	129.32 ± 2.59
5	**Ep.Ce 500 mg/Kg**	156.36 ± 5.61	123.80 ± 4.32

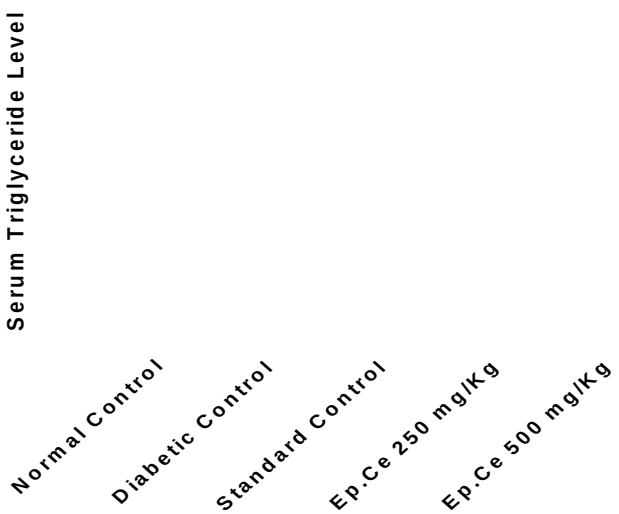

Fig 3: Effect of *Euphorbia prostrata* (Ep.Ce) on Serum Triglyceride levels (mg/dl) of alloxan induced diabetic rabbits

Values are expressed as Mean ± SEM and n = 6. Tuckey test was used to compare means. P values were considered as $P < 0.05$ as significant (*). Both Extract treated groups were compared to positice control, negative control and standard control.

Discussion

Diabetes mellitus is metabolic disorder and its incidence is increasing rapidly worldwide. As diabetes is caused by wide variety of factors there is a continuous need to rule out different and appropriate ways of treatment to handle this challenging disease (Akah *et al*., 2011). In this regard to establish the scientific basis for utilization of *Euphorbia prostrata* in diabetes management, antihyperglycemic study of methanolic extract was carried out on diabetic rabbits.

Results of current study exhibited a defined role of methanolic extract of *Euphorbia prostrata* plant in reducing serum glucose level in alloxan induced diabetic rabbits. It was therefore considered worthwhile to perform antihyperglycemic activity of Ep.Ce. Result of current study showed that Ep.Ce decreased serum glucose level in diabetic rabbits. Methanolic extract in both doses produced significant ($P < 0.05$) hypoglycemic effect 6 hours after administration but maximum decrease in fasting blood glucose was observed at the dose of 500 mg/kg body weight. Alloxan causes tissue injury of pancreas by production of free radicals (Akah *et al*., 2011). That's why regeneration of islet beta cell after destruction by alloxan is suggested to be the primary mechanism of recovery of alloxan-injected rabbits after administration of drugs (Akah *et al*., 2011). It was also observed that variable dose of extract had begun hypoglycemic effect after 2 hours of extract administration. Maximum decrease in serum glucose levels was produced at 6^{th} hour after administration of Ep.Ce which showed that it takes about 6 hours or even more time for active ingredient(s) of extract or its metabolites to enter into circulation and target tissues to bring about glucose lowering effect. But the action of Ep.Ce is slower as compared to sulfonylureas and biguanides. Most effective dose (500 mg/kg) had closest effect as of synthetic drug glibenclamide.

It had been reported earlier that treatment with plant origin drugs should be continued for longer time duration to get maximum effect (Grover *et al*., 2000). To get maximum effect of Ep.Ce on diabetic rabbits, extract was orally administered once a day for 14 days, the period which produced a significant decrease in fasting serum glucose level of diabetic

rabbits. Such results confirmed the previous studies that effectiveness of drugs depends on the cumulative effect of active principles (Obatomi *et al*., 1994). Thus, Ep.Ce effectively controled serum glucose levels and maintained normal glucose level which can be helpful in preventing the microvascular complications related with diabetes.

In diabetes induced by alloxan there is also increase in serum cholesterol and triglyceride levels. Serum cholesterol and triglycerides levels were brought to near normal by the treatment with Ep.Ce in diabetic rabbits. Hypocholestremic and hypotriglyceridemic effects can be achieved by tight control over serum cholesterol level. This is in agreement with the facts that (1) control of serum glucose is the major determinant of total and very low density lipoprotein along with triglyceride levels in blood (Markku Laakso, 1995) and (2) better serum glucose control after sulfonyl urea treatment reduces serum VLDL and total triglycerides levels((Huupponen *et al*., 1984), (Taskinen *et al*., 1985), (Hughes *et a*l., 1985) and *(Taskinen et al.*, 1986). The methanolic extract of *E. prostrata* has hypolipidemic effect in addition to antihyperglycemic effect in diabetic rabbits.

Conclusion

A good control of serum glucose is the cornerstone in the management of diabetes. In the present study both doses of *E. prostrata* exhibited anti-hyperglycemic and hypolipidemic activity in alloxan-induced diabetic rabbits. The management of diabetes includes a combination of antihyperglycemic drug treatment with lipid-lowering effects. This plant could be used as potential therapeutic drugs for the management of diabetes type 2 and dyslipidemia associated with it. Further biochemical and pharmacological studies are under way to evaluate the mechanism of antihyperglycemic and hypolipidemic activity of *E. prostrata* plant. A long term study however, is imperative as plant products are slow in action than the synthetic drugs and at higher doses may also exhibit a plateau effect which would not help in diabetes management.

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