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Original article

Hypoglycemic Effect of Alcohol Extract of *Eugenia Jambolana* Seed Against Dexamethasone Induced Diabetes In Rats

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ABSTRACT

Objectives: The present study was planned to evaluate the hypoglycemic effect of alcohol extract of *Eugenia jambolana (E.J)* seed against Dexamethasone induced diabetes in Wistar rats. **Materials and Methods:** Rats were divided in to 9 groups. Group-I served as a control, Group-II: Dexamethasone (4mg/kg/i.p/6 days), Group-III: Metformin (500mg/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days), Group-IV: Metformin (1g/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days), Group-V: Rosiglitazone (8mg/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days), Group-VI: Rosiglitazone (16mg/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days), Group- VII: Alcohol extract of *E.J* (3gm/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days), Group-VIII: Alcohol extract of *E.J* (6gm/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days) Group-IX: Alcohol extract of *E.J* (12gm/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days). Standard drug and plant extract were administered their respective groups 1 to 6 days, from 7 to 12 days it was administered along with Dexamethasone (4mg/kg). On 12th day, after overnight fasting, a retro-orbital puncture was performed for obtaining blood samples to estimate the fasting blood glucose and insulin, and the same procedure was followed 30min, 60min and 120min after a glucose load of 2.5g/kg i.p for estimation of post glucose load blood glucose and insulin level. **Results:** Dexamethaosne group showed increased fasting and post glucose load the glucose and insulin levels when compared to normal control. Whereas *E.J* extract 12gm/kg group showed decreased glucose and insulin level compared to Dexamethasone group. Anti-hyperglycemic effect of *E.J* seed extract like as standard drugs. **Conclusion:** Prior administration of plant extract significantly prevents the Dexamethsone induced hyperglycemia in Wistar Albino rats.

KEYWORDS: Dexamethasone, Diabetes, *Eugenia jambolana*, Glucose, Insulin Resistance, Insulin

INTRODUCTION

Diabetes is a metabolic disorder. It occurs when the pancreas does not synthesis required insulin, or when the body cannot utilize the insulin. These leads to increased blood glucose level [1]. According to World Health Organization (WHO), the prevalence of diabetes is likely to increase by 35% be the year 2025 currently there are over 150 million diabetes worldwide and this likely to increase to 300 million or more [2]. Clinically diabetes is classified as Type-1 (Insulin Dependent Diabetes Mellitus), Type-2 (Non Insulin Dependent Diabetes Mellitus). Type-1 is due to deficiency of insulin secretion and type-2 is due to insulin resistance [3]. Insulin resistance is the one of the major cause in the development of type-2 diabetes mellitus.

It is a common pathological condition which body cells cannot respond to normal circulatory insulin level resulting in dysregulation of carbohydrate, protein and fat metabolism [4]. Hormones such as catecholamine, glycogen, cortisol and thyroxin act directly or indirectly or influence the release or action of other hormones their by causing insulin resistance. Long term or high dose administration of dexamethasone decreases glucose utilization, inhibit insulin secretion, stimulate glycogen secretion, protein catabolism,

increased hepatic glucose synthesis, increased blood free fatty acid level, muscle wasting, fatty liver, decreased body weight and micro and macro vascular complications [5]. Prolonged hyperglycemia during diabetes causes glycation of body proteins leads to diabetic retinopathy, nephropathy, nuropathy and atherosclerosis. High dose dexamethsone administration can induce all the diabetic symptoms in the rats [6]. Biguanides, Sulphonylureas, Thiozolidineones different classes of drugs available to treat diabetes patients. But administration of these drugs has undesired effects [7]. Alternative medicines like plant products are available in the market to treat diabetes. Selection of herbal medication depends mainly on effect of herb, safety profile, cost of the preparation and availability. Several medicinal plant products are available as a single or combined preparation for the treatment of diabetes mellitus.

E.J commonly known as Jamun or Indian Blackberry. Fruits of jamun have been indicated in Ayurveda, an ancient system of Indian Medicine for the treatment of diabetes mellitus [8]. In accordance to its claimed anti-diabetic effect in traditional medicine, EJ has been reported to have hypoglycemic effects both in experimental models and clinical studies [9]. *E.J* used as anti-inflammatory agent [10], psychotic disorders [11], antimicrobial agent [12], anti-viral drug [13] and anti-diarrheal agent [14]. The primary objective of the study was to examine the hypoglycemic potential of oral administration of alcohol extract of *Eugenia jambolana* seed powder against dexamethaosne 4mg/kg induced diabetes in rats.

MATERIALS AND METHODS

Animals

Male albino rats (Wistar strain) weighing 220-240g were used for the present study. The animals were housed in polypropylene cages at controlled temperature (26 ± 2^0C), relative humidity and light condition (12-12 hour's dark-light cycle). The rats were fed with standard laboratory diet and drinking water was given through a drinking bottle, throughout the experiment [15]. Experimental animals was kept institutional central animal house (Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamil Nadu). The animals were maintained as per CPCSEA regulations and the study was approved by Institutional Animal Ethics Committee, Nitte University, Mangalore, Karnataka and Institutional Animal Ethics Committee, Sree Mookambika Institute of Medical Sciences, Kulasekhram, Kanyakumari, Tamil Nadu.

Collection of Eugenia jambolana seeds

E.J fruits were collected from local areas in Nagercoil, Tamil Nadu. The fruits were washed with water and pulp was separated manually from the seeds. Seeds were washed thoroughly with tap water to remove all the traces of pulp from the seeds. Washed seeds were dried at room temperature. Dried seeds were grounded in an electric grinder to obtain coarse seed powder. The powder stored and used for extraction [16].

Preparation of alcohol extract

The seed powder (4kg) was extracted with alcohol in a Soxhlet apparatus. The resultant extract was filtered through Whatman no.1 filter paper. The extract was concentrated by keeping in water bath at 40^0C till all the solvent had completely evaporated from mixture. The yield of 10% concentrated extract was stored and used for study [17].

Experimental design

The rats were divided into 9 group's I-IX, each group consisting of six rats.

Group-I: Control (Normal Saline)

Group-II: Dexamethasone (4mg/kg/i.p/6 days)

Group-III: Metformin (500mg/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days)

Group-IV: Metformin (1g/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days)

Group-V:Rosiglitazone(8mg/kg/orally/1-12 days)+Dexamethasone (4mg/kg/i.p/7-12 days)

Group-VI:Rosiglitazone(16mg/kg/orally/1-12 days)+Dexamethasone(4mg/kg/i.p/7-12 days)

Group- VII: Alcohol extract of *E.J* (3gm/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days)

Group-VIII: Alcohol extract of *E.J* (6gm/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days)

Group-IX: Alcohol extract of *E.J* (12gm/kg/orally/1-12 days) + Dexamethasone (4mg/kg/i.p/7-12 days)

Procedure

Since alcohol extract of *E.J* seed powder and metformin was poorly soluble in water, a suspension was prepared in 2% gum acacia. Standard drugs and *E.J* seed extract doses prepared 10ml/kg basis. Animals in group-I received normal saline, group-II received Dexamethasone 4mg/kg/i.p for 6 days. Group-III and IV received metformin (500mg/kg and 1g/kg), group-V and VI received rosiglitazone (8mg/kg and 16mg/kg) and group-VII, VIII and IX (Alcohol extract of *E.J* seed 3gm/kg, 6gm/kg and 12gm/kg). Standard and *E.J* extract was administered orally 1-12 days and dexamsthasone (4mg/kg/i.p) from 7-12 days to their respective groups. All the group animals kept for overnight fasting. On 12^{th} day fasting glucose and insulin level were estimated. Intraperitonial Glucose Tolerance Test (IPGTT) was performed by administration of 2gm/kg glucose. At 30min, 60min and 120 min blood samples were collected and serum was used for the estimation of glucose (Oxidase peroxidase method by using fully automated auto analyses) and insulin (ELISA method by using ELISA reader). [18, 19]

Statistical analysis

The data expressed mean and standard error of mean. SPSS (20.0) version software used statistical analysis. ANOVA (Post hoc test) followed by Dunnet t test used to find statistical significant between the groups. P value less than 0.05 considered statistical significant at 95% confidence interval. [20]

RESULTS

Dexamethasone administered group showed increase in the blood glucose level compared to control group. Metformin, Rosiglitazone and plant extract administered groups showed

decrease in the plasma glucose level compared to group-II on 12th day (Table-1). The IPGTT results showed increase in serum glucose levels at 30, 60 and 120 min in the group-II compared to other groups. The serum glucose levels of group-II were compared with control, standard and test drugs at three (30, 60 and 120 min) different time periods. Group-II showed significant increase in the glucose levels at three time periods compared to other groups. Prior administration of standard (Metfromin, Rosiglitazone) and test drugs *(Eugenia jambolana* seed) showed significant decrease in the glucose levels compared to Dexamethasone group (Table-2).

Table-1: Effect of *Eugenia jambolana* seed extract on fasting serum glucose levels (mg/dl) in Dexamethsone injected rats on 12th day

Groups	Initial	On 12th day
G-I	94.87± 1.07	98.13±0.66
G-II	96.61± 0.54	195.69±0.49*
G-III	88.92±0.23	122.89± 0.56*,†
G-IV	82.18± 0.18	113.78±0.12*,†,‡
G-V	86.12±0.34	132.12±0.34*,†, §
G-VI	82.19±0.34	117.78±0.28*,†‡, ‖
G-VII	86.45±0.56	134.34±0.45*,†,‡,§, ¶
G-VIII	86.78± 0.23	106.67±0.34*,†,‡,‖,**
G-IX	87.89± 0.13	95.67±0.97†,‡,§,‖,¶,**,††

[(*P<0.01 compared to normal control, †P<0.01 compared to diabetic control, ‡P<0.01 compared to Metfromin (500mg/kg), §P<0.01 comapred to Metfromin (1g/kg), ‖P<0.01 compared to Rosiglitazone (8mg/kg),¶P<0.05 compared to Rosiglitazone (16mg/kg), **P<0.03 compared to *E.J* extract (3gm/kg), ††P<0.01 compared to *E.J* extract (6gm/kg)]

Table-2: Effect of *Eugenia jambolana* seed extract on serum glucose levels of glucose loaded hyperglycemic rats (IPGTT) on 12th day

Groups	0 min	30 min	60 min	120 min
G-I	98.13±0.66	125.07± 2.59	149.34±1.76	105.26±2.46
G-II	195.69±0.49*	249.38±0.73*	338.51±0.71*	220.11± 0.54*
G-III	122.89± 0.56*,†	156.95± 0.17*,†	174.23± 0.12*,†	110.98± 0.87*,†
G-IV	113.78±0.12*,†,‡	124.34± 0.13*,†,‡	159.93± 0.87*,†,‡	103.34± 0.18*,†,‡
G-V	132.12±0.34*,†, §	154.89± 0.23*,†, §	179.78± 0.45*,†, §	126.34± 0.34*,†, §
G-VI	117.78±0.28*,†,‡,‖	136.98± 0.76*,†,‡,‖	168.23± 0.23*,†,‡,‖	117.78± 0.45*,†,‡,‖
G-VII	134.34±0.45*,†,‡,§,¶	159.34±0.57*,†,‡,§,¶	226.15±0.89*,†,‡,§,¶	141.56±0.45*,†,‡,§,¶
G-VIII	106.67±0.34*,†,‡,‖,**	137.94±0.56*,†,‡,‖,**	185.89±0.45*,†,‡,‖,**	138.56±0.34*,†,‡,‖,**
G-IX	95.67±0.97†,‡,§,‖,‡,**, ††	104.89±0.67†,‡,§,‖,‡,**, ††	139.56±0.96†,‡,§,‖,‡,**, ††	119.05±1.67†,‡,§,‖,‡,**, ††

[(*P<0.01 compared to normal control, †P<0.01 compared to diabetic control, ‡P<0.01 compared to Metfromin (500mg/kg), §P<0.01 comapred to Metfromin (1g/kg),‖P<0.01 compared to Rosiglitazone (8mg/kg),¶P<0.05 compared to Rosiglitazone (16mg/kg), **P<0.03 compared to *E.J* extract (3gm/kg), ††P<0.01 compared to *E.J* extract (6gm/kg)]

The serum insulin level was estimated on 12th day and during IPGTT at three different time periods. Significant increase in the serum insulin level was observed in the Dexamethsone administered group compared to control; standard and plant extract groups at the end of experiment (Table-3). The serum insulin levels was estimated at three different time periods during IPGTT and compared. Dexamethsone group showed increased insulin levels compared to control and other groups at three 30, 60 and 120 min. Metfrmin, Rosiglitazone and *E.J* seed extract administered groups showed significant decrease in the serum insulin levels at all the three time levels (Table-4).

Table-3: Effect of *Eugenia jambolana* seed extract on fasting serum insulin levels (ng/ml) in Dexamethsone injected rats on 12th day

Groups	Initial	On 12th day
G-I	2.53±0.16	3.05±0.15
G-II	3.28±0.16	8.30±0.12*
G-III	1.65±0.23	2.83±0.78*,†
G-IV	1.89±0.19	2.16±0.34*,†,‡
G-V	1.55±0.98	2.87±0.56*,†, §
G-VI	1.65±0.45	2.43±0.45*,†,‡,‖
G-VII	2.68±0.23	5.23±0.12*,†,‡,§,¶
G-VIII	1.54±0.65	2.78±0.23*,†,‡,‖,**
G-IX	1.65±0.83	2.17±0.45†,‡,§,‖,‡,**, ††

[(*P<0.01 compared to normal control, †P<0.01 compared to diabetic control, ‡P<0.01 compared to Metfromin (500mg/kg), §P<0.01 comapred to Metfromin (1g/kg),‖P<0.01 compared to Rosiglitazone (8mg/kg),¶P<0.05 compared to Rosiglitazone (16mg/kg), **P<0.03 compared to *E.J* extract (3gm/kg), ††P<0.01 compared to *E.J* extract (6gm/kg)]

Table-4: Effect of *Eugenia jambolana* seed extract on serum insulin levels (ng/ml) of glucose loaded hyperglycemic rats (IPGTT) on 12th day

Groups	0 min	30 min	60 min	120 min
G-I	3.05±0.15	3.78±0.17	5.30±0.29	4.35±0.36
G-II	8.30±0.12*	9.43±0.14*	13.53±0.14*	10.61±0.13*
G-III	2.83±0.78*,†	3.28±0.67*,†	4.06±0.45*,†	2.56±0.23*,†
G-IV	2.16±0.34*,†,‡	2.15±0.45*,†,‡	3.10±0.87*,†,‡	2.01±0.12*,†,‡
G-V	2.87±0.56*,†, §	3.01±0.89*,†, §	3.98±0.67*,†, §	2.14±0.45*,†, §
G-VI	2.43±0.45*,†,‡,‖	2.98±0.13*,†,‡,‖	3.17±0.23*,†,‡,‖	2.02±0.34*,†,‡,‖
G-VII	5.23±0.12*,†,‡,§,¶	5.67±0.89*,†,‡,§,¶	6.12±0.89*,†,‡,§,¶	3.96±0.45*,†,‡,§,¶
G-VIII	2.78±0.23*,†,‡,‖,**	3.04±0.23*,†,‡,‖,**	3.04±0.23*,†,‡,‖,**	2.45±0.67*,†,‡,‖,**
G-IX	2.17±0.45†,‡,§,‖,‡,**, ††	2.08±0.34†,‡,§,‖,‡,**, ††	2.95±0.24†,‡,§,‖,‡,**, ††	1.96±0.23†,‡,§,‖,‡,**, ††

[(*P<0.01 compared to normal control, †P<0.01 compared to diabetic control, ‡P<0.01 compared to Metfromin (500mg/kg), §P<0.01 comapred to Metfromin (1g/kg),‖P<0.01 compared to Rosiglitazone (8mg/kg),¶P<0.05 compared to Rosiglitazone (16mg/kg), **P<0.03 compared to *E.J* extract (3gm/kg), ††P<0.01 compared to *E.J* extract (6gm/kg)]

DISCUSSION

High dose or long term administration of dexamethsone induces insulin resistance in rats. Glucocortiocides induce gene expression in rats and causes changes in carbohydrate, fat and protein metabolism resulting in hyperglycemia, hyperlipidemia, increased free amino acids. These changes increases oxidative stress, it has been accused to cause insulin resistance. The present study administration of dexamthsone (4mg/kg/i.p) for 6 days showed hyperglycemia and hyperinsulinemia compared to the control group. The increase in blood glucose levels in Dexamethasone administered group due to catabolism of glycogen or synthesis of glucose [21].

The anti-diabetic activity of *E.J* seed extract may be due to increased used of glucose by cells, inhibition of gluconeogenisis and stimulation glycogen synthesis. Studies conducted by using active principal isolated from *E.J* seed. The study results explained administration of LH-II isolated from *E.J* seed decreased plasma glucose levels [22]. Rosiglitazone, Metformin and plant extract treatment inhibited dexametahsone induced diabetes. The anti-diabetic activity of plant may be attributed to the increase in the sensitivity to insulin and the subsequent increase in the glucose uptake.

CONCLUSION

In conclusion alcohol extract of *Eugenia jambolana* seed has hypoglycemic activity against dexamethaosne induced diabetic in rats. This investigations reaffirms the *E.J* seed extract as oral agent as add on therapy to oral hypoglycemic agents to treat diabetes mellitus. Further studies to isolate and screen the active phytochemical compound for hypoglycemic activity.

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