Effect of *Eugenia Jambolana* Leaves Extracts on Blood Glucose Levels of Experimental Diabetic Rabbits

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Summary

The objective of our study was to examine the antidiabetic potential of oral administration of *Eugenia jambolana* leaves extracts. 100mg/kg b.w. of each extract was given to streptozotocin-induced diabetic rabbits as well as to nicotinamide-streptozotocin-induced diabetic rabbits. Fasting blood glucose levels were measured before the treatment and 10th, 20th & 30th day of the treatment. Our results showed that aqueous as well as alcoholic extract of *Eugenia jambolana* leaves have no effect on fasting blood glucose levels of streptozotocin-induced diabetic rabbits and streptozotocin induces Type I diabetes mellitus. Aqueous extract of *Eugenia jambolana* leaves also have no effect on fasting blood glucose levels of nicotinamide-streptozotocin-induced diabetic rabbits. However, alcoholic extract of *Eugenia jambolana* leaves decrease the blood glucose level 64% (P<0.01) of nicotinamide-streptozotocin-induced diabetic rabbits. The study also showed that nicotinamide-streptozotocin induces Type II diabetes mellitus.

Keywords: *Eugenia jambolana*; Hypoglycaemic activity; Leaf extract.

Introduction

Diabetes mellitus is characterised by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism.[1] During the last two decades, traditional systems of medicine and medicinal plant research have become topic of global interest and importance. In many developing nations of the world, large numbers of people still rely heavily on traditional healers and medicinal plants to meet their daily primary healthcare needs. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are prescribed widely even when their biologically active compounds are unknown.[2] *Eugenia jambolana;* different parts of the plant, such as seeds, bark, fruit, and leaves have been used to treat diabetes traditionally.[3,4]

The objective of our study was to examine the antidiabetic potential of oral administration of *Eugenia jambolana* leaves extracts in experimentally induced diabetes in rabbits. The results of this preclinical study could prove useful for phase 2 clinical trials in which the morbidity and mortality of diabetes mellitus complicated by the side effects of drug-induced hypoglycemia may be reduced by the practice of integrated medicine.

Materials and Methods

Collection of plant material

The leaves of *Eugenia jambolana* were collected from District Multan of Pakistan. The material was identified in the Department of Biochemistry, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad. A voucher specimen has been kept for future reference.

Preparation of Aqueous Extract

Six-hundred grams of powdered *Eugenia jambolana* leaves were boiled in 5 litters of distilled water for 15 min. The decoction was taken and allowed to cool for 30 min at room temperature ($24 \pm 2°$ C). The decoction was filtered twice and the filtrate was dried in an oven (56°C) for 3 days. The resulting material yielded 80 g (13.33 % w/w).

Preparation of Alcoholic Extract

Six-hundred grams of the leaves powder were soaked with 2 litters of ethanol for 3 days. The filtrate was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40°C. The extract yielded was 132 g (22 % w/w).

Experimental Animals

Adult albino rabbits of either sex weighing 1.5 - 2 kg were used in the study. All the rabbits were kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained in a well-ventilated animal house with 12 h light and dark cycle. They were fasted for 18 h prior to the experiment, allowing access to water only. The experimental protocols were approved by the Institutional Animal Ethics Committee.

Experimental Design

A total of 90 Rabbits were divided into three groups:

Group A: Normal Rabbits (Normal control) receiving 1.5 ml of physiological NaCl-solution ($n = 10$)

Group B: Streptozotocin-induced diabetic rabbits; they were further divided into four groups ($n = 10$ each)

Group B-I: Diabetic control receiving 1.5 ml of physiological NaCl-solution as vehicle.

Group B-II: Diabetic rabbits treated with glibenclamide (3 mg/kg b.w./day) in the same vehicle.

Group B-III: Diabetic rabbits treated with Aqueous Extract of *Eugenia jambolana* (100 mg/kg b.w./day) in the same vehicle. Group B-IV: Diabetic rabbits treated with Alcoholic Extract of *Eugenia jambolana* (100 mg/kg b.w./day) in the same vehicle.

Group C: Nicotinamide-streptozotocin-induced diabetic rabbits; they were further divided into four groups ($n = 10$ each)

Group C-I: Diabetic control receiving 1.5 ml of physiological NaCl-solution as vehicle.

Group C-II: Diabetic rabbits treated with glibenclamide (3 mg/kg b.w./day) in the same vehicle.

Group C-III: Diabetic rabbits treated with Aqueous Extract of *Eugenia jambolana* (100 mg/kg b.w./day) in the same vehicle. Group C-IV: Diabetic rabbits treated with Alcoholic Extract of *Eugenia jambolana* (100 mg/kg b.w./day) in the same vehicle.

Induction of Experimental Diabetes

Streptozotocin-induced diabetes: A freshly prepared solution of streptozotocin (60 mg/kg) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally. Hyperglycemia was confirmed by elevated blood glucose levels determined at 72 h and then on day 7 after injection. The rabbits with fasting blood glucose 200-300 mg/dl were taken for the experiment.

Nicotinamide-streptozotocin-induced diabetes: In group C, diabetes was induced by a single intraperitoneal injection of 60 mg/kg streptozotocin 15 min after the intraperitoneal administration of 120 mg kg^{-1} nicotinamide. Hyperglycemia was confirmed by elevated blood glucose levels determined at 72 h and then on day 7 after injection. The rabbits with fasting blood glucose 200-300 mg/dl were taken for the experiment.

Collection of blood and analytical procedure

Blood samples (approx. 0.3 ml) were collected by puncturing the marginal ear vein of each rabbit of a group before the treatment and 10th, 20th& 30th day of the treatment. The samples were collected into vials containing EDTA as anti-coagulant. They were stored at 4°C in a refrigerator before the analysis. Fasting blood glucose levels were measured by using commercially available kit manufactured by Randox, Germany.

Statistical analysis

Data was expressed as mean blood glucose levels ± SEM (standard error of mean). Statistical analysis was made by using Student's *t*-test. P values of 0.05 and less were taken to imply statistical significance between the means.

Results

Effect of aqueous and alcoholic extracts of *Eugenia jambolana* leaves on fasting blood glucose levels (mg/dl) of streptozotocin-induced diabetic rabbits is given in Table 1. There was no significant effect of aqueous or alcoholic extract of *Eugenia jambolana* leaves on fasting blood glucose levels, when compared with the diabetic control. Moreover animals treated with glibenclamide (3 mg/kg) also did not showed significant effect on streptozotocin-induced diabetes throughout the 30 days.

Table 1: Effect of aqueous and alcoholic extracts of *Eugenia jambolana* leaves on fasting blood glucose levels (mg/dl) of streptozotocin-induced diabetic rabbits (mean ± SEM).

Treatment groups	Blood glucose at different days after treatment (mg/dl)			
	Day 0	Day 10	Day 20	Day 30
Diabetic control (negative control)	268.5 ± 1.7	271.8 ± 5.9	287.2 ± 3.7	292.8 ± 2.1
Glibenclamide 3 mg/kg b.w. (positive control)	272.7 ± 2.2	269.6 ± 6.9	268.6 ± 2.3	272.8 ± 1.9
Aqueous extract 100 mg/kg b.w.	270.6 ± 7.2	285.4 ± 2.8	279.2 ± 4.2	294.8 ± 3.1
Ethanol extract 100 mg/kg b.w.	279.7 ± 2.7	283.8 ± 4.8	298.1 ± 5.3	298.7 ± 6.5

Effect of aqueous and alcoholic extracts of *Eugenia jambolana* leaves on fasting blood glucose levels (mg/dl) of nicotinamide-streptozotocin-induced diabetic rabbits is given in Table 2. It is worthy to mentioned that animals treated with glibenclamide (3 mg/kg) showed highly significant reduction in blood glucose level (p<0.01). There was no significant effect of aqueous extract of *Eugenia jambolana* leaves on fasting blood glucose levels through out the 30 days, when compared with the diabetic control. However, there was highly significant reduction 64% (p<0.01) in fasting blood glucose levels of alcoholic extract-treated animals, when compared with the diabetic control.

Table 2: Effect of aqueous and alcoholic extracts of *Eugenia jambolana* leaves on fasting blood glucose levels (mg/dl) of nicotinamide-streptozotocin-induced diabetic rabbits (mean ± SEM).

Treatment groups	Blood glucose at different days after treatment (mg/dl)			
	Day 0	Day 10	Day 20	Day 30
Diabetic control (negative control)	286. 7 ± 2.5	279.9 ± 2.9	291.4 ± 4.1	297.1 ± 4.2
Glibenclamide 3 mg/kg b.w. (positive control)	288.4 ± 3.2	178.1 ± 3.2*	100.6 ± 4.4*	92.8 ± 5.3*
Aqueous extract 100 mg/kg b.w.	284.4 ± 4.5	277.6 ± 7.2	279.2 ± 4.2	294.8 ± 3.1
Ethanol extract 100 mg/kg b.w.	281.6 ± 5.1	121.9 ± 3.8*	97.7 ± 3.5*	101.3 ± 4.1*

* P<0.01 compared with the initial level of blood glucose of the rabbits (Day 0) in the respective group.

Discussion

Results obtained in the present study showed that aqueous as well as alcoholic extract of *Eugenia jambolana* leaves have no effect on fasting blood glucose levels of streptozotocin-induced diabetic rabbits. The study also showed that streptozotocin induces Type I diabetes mellitus, as the blood glucose levels were increased but glibenclamide fail to lower the levels. This is in the support of idea of Okamato, 1981 and Sridhar *et al.*, 2005.[5,6]

Aqueous extract of *Eugenia jambolana* leaves also have no effect on fasting blood glucose levels of nicotinamide-streptozotocin-induced diabetic rabbits. However, alcoholic extract of *Eugenia jambolana* leaves has the hypoglycemic activity on nicotinamide-streptozotocin-induced diabetic rabbits. The study also showed that nicotinamide-streptozotocin induces Type II diabetes mellitus, as the blood glucose levels were decreased by glibenclamide. This is in the support of idea of Masiello *et al.*, 1998, Novelli *et al.*, 2001, Rebel *et al.*, 2002 and Larsen *et al.*, 2003.[7-10]

Two different studies have been made recently to evaluate the hypoglycemic activity of *Eugenia jambolana* leaves extracts. But both of them failed due to their approach. Pepato *et al.,* (2001)[11] and Teixeira *et al.,* (2006)[12] failed to search the hypoglycemic activity of *Eugenia jambolana* leaves because they used water extracts (decoctions) only. Further more Pepato *et al.,* 2001 used streptozotocin-induced diabetic animals that are of Type I. [11]

The result of our study suggests that the substances responsible for the hypoglycaemic activity of *Eugenia jambolana* leaves are probably polar in nature and more soluble in ethanol than in water. Ethanol extracts of a plant are usually known to contain many chemical compounds each of which is capable of producing definite biological activities.[13] The constituents present in the alcoholic extract of *Eugenia jambolana* leaves may probably stimulate the pancreatic β cells to produce insulin or possess an extra-pancreatic hypoglycemic mechanism of action as they affect on non-insulin dependent diabetes (Type II). Like the plant extract, glibenclamide also produced a significant reduction in the blood glucose level of nicotinamide-streptozotocin-induced diabetic rabbits. The present findings appear to be in consonance with the earlier suggestion of Jackson and Bressler (1981) [14] that sulphonylureas such as glibenclamide have extra-pancreatic hypoglycaemic mechanism of action secondary to their causing insulin secretion and the attendant glucose uptake into and utilization by the tissues.

It may be concluded from this study that alcoholic extract of *Eugenia jambolana* leaves has hypoglycaemic activity on nicotinamide-streptozotocin-induced diabetic rabbits. This activity could be attributed to certain polar compounds of different nature present in *Eugenia jambolana* leaves. Further investigations are in progress to isolate these active principles and to determine their mechanism of action.

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