

Anti-Diabetic Activity of Inorganic Metals of *Eugenia Jambolana* Lam. (Myrtaceae) Flowers

Tehzeeb-ul-Nisa, M. Imran Qadir* and Salman Akbar Malik¹

College of Pharmacy, GC University, Faisalabad, Pakistan
¹Department of Biochemistry, Faculty of Biological Sciences,
Quaid-i-Azam University, Islamabad

*Correspondence:

M Imran Qadir, College of Pharmacy, GC University, Faisalabad, Pakistan
mrimranqadir@hotmail.com

Summary

Medicinal plants have been the subject of intense research due to their potential as sources of commercial drugs or as lead compounds in drug development. The aim of this study was to evaluate the hypoglycemic activity of *Eugenia jambolana* Lam. (Myrtaceae) flower extracts and the ash obtained from flower. 100 mg/kg of body weight of the extracts were orally administered to streptozotocin-induced diabetic rabbits and to nicotinamide-streptozotocin-induced diabetic rabbits. 4.8 mg/kg of body weight of ash was also given to nicotinamide-streptozotocin-induced diabetic rabbits to detect whether the hypoglycemic activity of the extracts is due to organic constituents or inorganic metals. Our results showed that aqueous as well as alcoholic extract of *Eugenia jambolana* flowers have no effect on fasting blood glucose levels of streptozotocin-induced diabetic rabbits. Aqueous extract of *Eugenia jambolana* flower also have no effect on fasting blood glucose levels of nicotinamide-streptozotocin-induced diabetic rabbits. However, alcoholic extract of *Eugenia jambolana* flower decrease the blood glucose level ($P < 0.01$) in nicotinamide-streptozotocin-induced diabetic rabbits. There was highly significant reduction ($p < 0.01$) in fasting blood glucose levels of ash-treated animals. Alcoholic extract of *Eugenia jambolana* flowers has hypoglycaemic activity on nicotinamide-streptozotocin-induced diabetic rabbits due to its inorganic metals constituents.

Key Words: Hypoglycemic activity, Extracts, Ash

Introduction

Medicinal plants have been the subject of intense research due to their potential as sources of commercial drugs or as lead compounds in drug development.¹ Diabetes is one of the most commonly occurring problems round the globe. Hyperglycaemia is a major characteristic of diabetes.² Even from early civilization, herbs have been considered to be a powerful tool in treating illnesses. In places where physicians cannot reach, natives have made-up their own creation of herbs and plants to deal with the common afflictions of daily life. Sometimes, these herbal treatments are far more superior and effective than its chemical counterparts, not to mention even safe and inexpensive. Nowadays, because of the expensive treatment for diabetes as well as the contraindications that these medications give, a lot of people are trying to discover the wonders of herbal treatments for diabetes mellitus. *Eugenia jambolana* Lam. Family Myrtaceae; different parts of the plant, such as seeds and fruit have been used for hyperglycemia.^{3,4}

The aim of this study was to evaluate the hypoglycemic property of orally given *Eugenia jambolana* flower extracts in experimentally induced diabetes in rabbits.

Materials and Methods

The hypoglycemic activity of *Eugenia jambolana* flowers was determined according to the protocol described by Qadir *et al.*, (2009).⁵

Collection of plant material

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

Preparation of Aqueous Extract

600 g of powdered *Eugenia jambolana* flower were boiled in 5 liters of distilled water for 15 min. The decoction was allowed to cool for 30 minutes at room temperature (25° C) and was filtered twice. The filtrate was dried in an oven (56°C) for 3 days. The resulting material yielded 80.00 g (13.33 % w/w) of semi-solid extract.

Preparation of Alcoholic Extract

600 grams of the flower powder were soaked in 2 liters of ethanol for 3 days and filtered. The filtrate was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40°C. The extract yield was 73.20 g (12.20 % w/w) in the form of semi-solid material.

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 free 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 saline per day orally ($n = 10$).

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 saline per day orally.

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

Group B-III: Diabetic rabbits treated with Aqueous Extract of *Eugenia jambolana* (100 mg/kg b.w./day orally) in physiological saline.

Group B-IV: Diabetic rabbits treated with Alcoholic Extract of *Eugenia jambolana* (100 mg/kg b.w./day orally) in physiological saline.

Group C: Nicotinamide-streptozotocin-induced diabetic rabbits; they were also further divided into four (C-I to C-IV) groups as described above ($n = 10$ each).

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 used in 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 used in 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 before 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 until analyzed. Fasting blood glucose levels were measured by using commercially available kit manufactured by Randox, Germany.

Detection of activity due to inorganic metals

To detect whether the hypoglycemic activity of the extracts is due to organic constituents or inorganic metals, the flower powder showing positive results were reduced to ash and given to the experimental animals with dose of 4.8 mg/kg b.w. (calculated that 137 grams of flower powder yield 100 mg of alcoholic extract or 4.8 mg/kg of ash) by dissolving in 1 ml of water.

Statistical analysis

Data was expressed as mean blood glucose levels \pm 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* flowers 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* flowers on fasting blood glucose levels, when compared with the diabetic control.

Effect of aqueous and alcoholic extracts of *Eugenia jambolana* flowers 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* flowers on fasting blood glucose levels through out the 30 days, when compared with the diabetic control. However, there was highly significant reduction 38.83% ($p < 0.01$) in fasting blood glucose levels of alcoholic extract-treated animals, when compared with the diabetic control.

Effect of ash of *Eugenia jambolana* flowers on fasting blood glucose levels (mg/dl) of nicotinamide-streptozotocin-induced diabetic rabbits is given in Table 3. There was highly significant reduction ($p < 0.01$) in fasting blood glucose levels of ash-treated animals, when compared with the diabetic control.

Table 1: Effect of aqueous and alcoholic extracts of *Eugenia jambolana* flower on fasting blood glucose levels (mg/dl) of streptozotocin-induced diabetic rabbits (mean \pm 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 \pm 1.7	271.8 \pm 5.9	287.2 \pm 3.7	292.8 \pm 2.1
Glibenclamide 3 mg/kg b.w. (positive control)	272.7 \pm 2.2	209.6 \pm 6.9*	145.6 \pm 2.3*	88.8 \pm 1.9*
Aqueous extract 100 mg/kg b.w.	272.3 \pm 6.1	268.1 \pm 5.2	281.8 \pm 2.5	279.4 \pm 3.3
Ethanol extract 100 mg/kg b.w.	296.1 \pm 5.4	284.8 \pm 4.7	277.7 \pm 5.7	288.7 \pm 5.5

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

Table 2: Effect of aqueous and alcoholic extracts of *Eugenia jambolana* flower on fasting blood glucose levels (mg/dl) of nicotinamide-streptozotocin-induced diabetic rabbits (mean \pm 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 \pm 2.5	279.9 \pm 2.9	291.4 \pm 4.1	297.1 \pm 4.2
Glibenclamide 3 mg/kg b.w. (positive control)	288.4 \pm 3.2	178.1 \pm 3.2*	100.6 \pm 4.4*	92.8 \pm 5.3*
Aqueous extract 100 mg/kg b.w.	282.2 \pm 5.2	293.8 \pm 3.7	280.4 \pm 4.0	272.6 \pm 6.2
Ethanol extract 100 mg/kg b.w.	279.6 \pm 4.1	192.9 \pm 2.9*	113.4 \pm 4.5*	78.2 \pm 5.2*

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

Table 3: Effect of ash of *Eugenia jambolana* flower on fasting blood glucose levels (mg/dl) of nicotinamide-streptozotocin-induced diabetic rabbits (mean \pm SEM).

Treatment groups	Blood glucose at different days after treatment (mg/dl)			
	Day 0	Day 10	Day 20	Day 30
Diabetic control (negative control)	285.2 \pm 2.3	278.8 \pm 2.2	292.3 \pm 4.3	298.1 \pm 4.3
Glibenclamide 3 mg/kg b.w. (positive control)	289.9 \pm 4.2	177.1 \pm 2.2*	103.5 \pm 3.4*	91.7 \pm 4.3*
Ash in water 4.8 mg/kg b.w.	285.5 \pm 3.1	199.4 \pm 3.2*	107.3 \pm 3.5*	83.5 \pm 4.8*

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

Discussion

Eugenia jambolana is being widely used to treat diabetes by the traditional practitioners for over many centuries.^{6,7} The bark of this plant is astringent, antihelmenthic, antipyretic, antidysentric and useful in certain urinary disorders, excessive thirst, hemorrhages, ulcer and vaginal discharges. The juice is helpful in treating inflammation and swelling on the liver and spleen.⁸ Seeds of *Eugenia jambolana* have the hypoglycemic activity and lowers blood glucose levels by improving oral glucose tolerance.^{9,10} Leaves of *Eugenia jambolana* have also been searched for antidiabetic activity. But the literature related to evaluation of hypoglycemic activity of *Eugenia jambolana* flowers is limited.

Streptozotocin induces type I diabetes^{11,12} and nicotinamide-streptozotocin results in type II diabetes.¹³⁻¹⁷ The present study indicates that the both aqueous and alcoholic extracts of *Eugenia jambolana* flowers have no effect on fasting blood glucose levels of streptozotocin-induced diabetes. Aqueous extract of *Eugenia jambolana* flowers also have no hypoglycemic activity in nicotinamide-streptozotocin-induced diabetes. But alcoholic extract of *Eugenia jambolana* flowers decreases the fasting blood glucose levels in nicotinamide-streptozotocin-induced diabetic rabbits. The ash of *Eugenia jambolana* flowers also decreases the fasting blood glucose levels in nicotinamide-streptozotocin-induced diabetic rabbits. This indicates that some inorganic metals present in alcohol soluble form compounds in *Eugenia jambolana* flowers have the anti-diabetic activity. anti-diabetic activity due to inorganic metals in *Eugenia jambolana* seeds already has been proved by Ravi et al., (2004).¹⁸

Conclusion

By this study it is concluded that alcoholic extract of *Eugenia jambolana* flowers has hypoglycaemic activity on nicotinamide-streptozotocin-induced diabetic rabbits. And inorganic metals present in alcohol soluble form compounds in *Eugenia jambolana* flowers have the anti-diabetic activity.

References

1. Cordell GA, Beecher CWW, Pezzuto JM. (1991). Can ethnopharmacology contribute to the development of new anticancer drugs? *J Ethnopharmacol*, 32, 117–133.
2. Arky RA. (1982). Clinical correlates of metabolic derangements of diabetes mellitus in: Complications of Diabetes mellitus. Kozak GP. (ed.), Saunders WB. Philadelphia, 16-20.
3. Bramachari HD, Augusti KT. (1961). Hypoglycaemic agents from Indian indigenous plants. *J Pharmacy and Pharmacol*, 13, 381–382.
4. Rahman AU, Zaman K. (2001). Medicinal plants with hypoglycemic activity. *J Ethnopharmacol*, 26, 1–56.
5. Qadir MI, Nisa TU, Malik SA. (2009). Effect of *Eugenia Jambolana* Leaves Extracts on Blood Glucose Levels of Experimental Diabetic Rabbits. *Pharmacologyonline*, 3, 829-835.
6. Bansal R, Ahmad N, Kidwi JR. (1981). Effects of oral administration of *Eugenia jambolana* seeds and chlorpropamide on blood glucose level and pancreatic cathepsin B in rats. *Indian J Biochem Biophys*, 18, 377.
7. Kolhi RK, Singh RH. (1985). *Eugenia jambolana* a plant drug with potential antidiabetic: A review. *J Sci Res Plant Med*, 1, 21–8.
8. Deshpande DJ. (2008). *A hand book of herbal remedies*. Jodhpur India: Agrobios.
9. Pandey M, Khan A. (2002). Hypoglycaemic effect of defatted seeds and water soluble fibre from the seeds of *Syzygium cumini* (L.) Skeels (Linn.) skeels in alloxan diabetic rats. *Indian J Exp Biol*, 40, 1178–82.
10. Ravi K, Sathish Sekar DS, Subramanian S. (2004). Hypoglycemic activity of inorganic constituents in *Eugenia jambolana* seed on streptozotocin-induced diabetes in rats. *Biol Trace Elem Res*, 99, 145–55.
11. Pechhold K. (2001). Low dose streptozotocin-induced diabetes in rat insulin promoter-mCD80-transgenic mice is T cell autoantigen-specific and CD28 dependent. *J Immunol*, 166(4), 2531–2539.
12. Rolandsson O, Haney M F, Hägg E, Biber B, Lernmark Å. (2002). Streptozotocin Induced Diabetes in Minipig: A Case Report of a Possible Model for Type 1 Diabetes? *Autoimmunity*, 35, 261 – 264.
13. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillarire-Buys D. (1998). Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes*, 47, 224-9.
14. Novelli M, Fabregat ME, Fernandez-Alvarez J, Gomis R, Masiello P. (2001). Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes. *Mol Cell Endocrinol*, 175, 57-66.
15. Rebel U, Larsen MO, Rolin B, Carr RD, Wilken M, Sturis J. (2002). NN221: A long-acting glucagons-like peptide-1 derivative with antidiabetic in glucose tolerant pigs. *Eur J Pharmacol*, 451, 217-25.
16. Larsen MO, Rolin B, Wilken M, Carr RD, Gottfredsen DF. (2003). Measurements of insulin secretory capacity and glucose intolerance to predict pancreatic β cell mass in vivo in the nicotinamide/streptozotocin Göttingen mini pig, a model of moderate insulin deficiency and diabetes. *Diabetes*, 52, 118-23.
17. Sridhar SB, Sheetal UD, Pai MRSM, Shastri MS. (2005). Preclinical evaluation of the antidiabetic effect of *Eugenia jambolana* seed powder in streptozotocin-diabetic rats. *Braz J Med Biol Res*, 38(3), 463-468.
18. Iyengar BS, Dorr RT, Remers WA. (2004). Chemical basis for the biological activity of Imexon and related Cyanaziridines. *J Med Chem*, 47, 218-23.