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Preclinical evaluation of the antidiabetic effect of *Eugenia jambolana* seed powder in streptozotocin-diabetic rats

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Abstract

The world is facing an explosive increase in the incidence of diabetes mellitus and cost-effective complementary therapies are needed. The effects of *Eugenia jambolana*, a household remedy for diabetes, were studied. Streptozotocin diabetic female albino Wistar rats weighing 150-200 g (N = 6) were fed *E. jambolana* seed powder (250, 500 or 1000 mg/kg) for 15 days. Diabetic rats fed 500 and 1000 mg/kg seed powder showed an increase in body weight on day 20 in relation to day 5 (6 ± 4.7, 9 ± 7.8 *vs* diabetic control -16 ± 7.1 g, $P < 0.001$), a decrease in fasting blood glucose (75 ± 11.9, 123 ± 14.4 *vs* diabetic control -34 ± 12.1 mg/dl, $P < 0.001$), a difference in post-treatment fasting and peak blood glucose (38 ± 11.9, 36 ± 14.2 *vs* diabetic control 78 ± 11.9 mg/dl, $P < 0.001$), and a difference in liver glycogen (50 ± 6.8, 52 ± 7.5 *vs* normal control 90 ± 6.6 µg/g of liver tissue, $P < 0.001$). Tri-terpenoids, tannins, gallic acid, and oxalic acid were the chemical constituents detected in *E. jambolana* seed. The best results were obtained with an oral dose of 500 mg/kg. Subacute toxicity studies with a single administration of 2.5 and 5.0 g/kg seed powder showed no mortality or abnormality. These data on the antidiabetic effect of *E. jambolana* seed are adequate for approval of phase 2 clinical trials to evaluate this seed powder as complementary therapy in type 2 and type 1 diabetes.

Key words
- Diabetes
- *Eugenia jambolana* seed
- Antidiabetic effect
- Preclinical data
- Liver glycogen
- Toxicity studies

Introduction

There is growing awareness of the role and practice of integrated medicine in the field of metabolic disorders. This is based in part on a flood of scientific data reported about medicinal plants including those with antidiabetic potential (1) and partly on the support provided for its practice by governmental agencies as well as the WHO (2). At our center there has been a compelling need to conduct phase 2 clinical trials in a select group of type 2 diabetes mellitus patients, combining an established oral hypoglycemic agent with *Eugenia jambolana* synonym *Syzygium cumini*, *Syzygium jambolanum*,

family Myrtaceae, prescribed by Ayurveda, the ancient Indian form of medicine. Institutional Ethics Committee clearance for clinical trials requires as a prerequisite, data from preclinical animal studies and acute, subacute toxicity tests. We felt that the necessary preclinical data should be obtained from plants grown locally in view of contradictory reports about the use of a tea prepared from *E. jambolana* (3) and its lack of an antidiabetic effect on rats with streptozotocin (STZ)-induced diabetes (4). Furthermore, there is a lack of scientific data on *E. jambolana* grown in the Western Ghats of coastal India though the seed is a household remedy and is prescribed in these parts of India by Ayurveda medicine for diabetes mellitus. It is also well known that the chemical constituents isolated from the plant can vary according to humidity, soil, climate, and geography.

The primary objective of the study was to examine the antidiabetic potential of subchronic oral administration of *E. jambolana* seed powder in rats with STZ-induced diabetes. The results of the 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.

Material and Methods

Plant material and treatment preparation

The seed powder of *E. jambolana* was obtained from plants grown in the herbarium of an Ayurvedic establishment in the Dakshina Kannada district of Karnataka State located in the Western Ghats of coastal India. The plant was identified by Dr. Kakunje Gopalkrishna Bhat and authenticated material was deposited in the Herbarium of the Botanical Survey of India in Pune. The seeds were collected during the month of April; 500 g of seeds dried in sunlight yielded 450 g of *E. jambolana* seed powder.

Animals, treatment and chemical analysis

Female albino Wistar rats from the same colony weighing 150-200 g were obtained from the central animal house of Kasturba Medical College, Mangalore. They were housed in individual cages under natural light and dark cycles at a temperature of 28 ± 4ºC, given a standard pellet diet and water *ad libitum* and were acclimatized for 3 days before the beginning of the experiment. The animals were randomized to the following groups containing 6 rats each: group I, normal control; group II, diabetic control; group III received glibenclamide, 5 mg/kg, by gavage; groups IV, V, and VI received *E. jambolana* seed powder, 250, 500, 1000 mg/kg by gavage, respectively. Since the seed powder was poorly soluble in water, a suspension was prepared in 2% gum acacia. All animals were deprived of food for at least 12 h but had free access to drinking water. Animals in group I received citrate buffer intraperitoneally. Animals in groups II, III, IV, V, and VI received STZ, 50 mg/kg, in freshly prepared 10 mM citrate buffer, pH 4.5, intraperitoneally. Animals were kept fasted for 3 h after injection, returned to their cages and given food and water *ad libitum*. The mean weight of the animals in each group was recorded on days 1, 5, 10, 15, and 20. Daily fluid intake was recorded and mean fluid intake by the animals in each group was tabulated on days 1, 5, 10, 15, and 20. On day 5 after STZ administration, fasting blood glucose (FBS) was measured (5) and the glucose tolerance test (GTT) performed after an overnight fast. Blood was collected from the inner canthus of the eye using capillary tubes. Only animals whose FBS was more than thrice the normal value were included in the study (N = 6 per group). The GTT was performed after a glucose challenge of 2.5 g/kg body weight given orally by gastric intubation. Blood glucose was measured at 30-, 60- and 120-min intervals. Glibenclamide and *E. jambolana* were given

intragastrically once a day at a fixed time interval each day for 15 days. The animals were fasted 30 min before and after treatment to ensure maximum bioavailability. Animals in the untreated groups were force-fed 2% gum acacia suspension. On day 20, after STZ injection, FBS and GTT were performed after an overnight fast. On day 21, fasting animals were sacrificed by cervical dislocation and liver glycogen was estimated with anthrone reagent (6).

Statistical analysis

Kruskal-Wallis one-way analysis of variance was used to compare the mean changes in metabolic parameters of the 6 groups. When a significant difference was detected between groups, the multiple comparison criterion was used for pairwise group comparison. ANOVA for repeated measures or Friedman's test was used to compare the differences between the five periods of observation. The level of significance was set at $P < 0.05$.

The experimental protocol was approved by the Institutional Ethics Committee.

Results and Discussion

Table 1 summarizes the effect of STZ and treatments on mean body weight and fluid intake. A statistically significant increase in body weight was observed on days 10 and 15 of treatment with glibenclamide and *E. jambolana*, with 500 and 1000 mg/kg body weight.

Table 2 summarizes the results of the GTT. The FBS was increased up to 4.5 times the normal control value on day 0 of treatment in groups II, III, IV, V, and VI, and was significantly reduced in all treatment groups 15 days after treatment. The peak blood glucose level observed in all groups occurred at 30 min. In the normal control and treatment groups, 15 days after treatment, the blood glucose levels were reduced to nearly fasting levels at 2 h. However, in the diabetic control and treatment groups, at day 0 of treatment, the blood glucose levels remained elevated at 2 h. On day 0 of treatment the difference between fasting and peak blood glucose levels ranged from 65 to 100 mg/dl in groups II, III, IV, V, and IV, and was only 25 mg/dl in group I. On day 15 of treatment the difference between fasting and peak blood glucose levels was 53 mg/dl in group III, 50 mg/dl in group IV, 38 mg/dl in group V, and 36 mg/dl in group VI, and was 26 mg/dl in group I and 78 mg/dl in group II. In groups IV, V, and VI the percent reductions in pre-treatment versus post-treatment fast-

Table 1. Effect of *Eugenia jambolana* seed powder on body weight (g) and fluid intake (ml/24 h) of streptozotocin-diabetic rats.

Group/Treatment	Days after STZ injection (days after treatment)									
	0 days		5 days (0 days)		10 days (5 days)		15 days (10 days)		20 days (15 days)	
	Body weight	Fluid intake	Body weight	Fluid intake	Body weight	Fluid intake	Body weight	Fluid intake	Body weight	Fluid intake
I Normal control	188 ± 4	38 ± 2	191 ± 4	38 ± 2	193 ± 4	38 ± 2	195 ± 4	37 ± 2	198 ± 4	39 ± 2
II Diabetic control	182 ± 8	36 ± 2	155 ± 6[a]	38 ± 2	148 ± 8[a]	43 ± 2	143 ± 9[a]	46 ± 2	139 ± 8[a]	50 ± 2
III Diabetic + glibenclamide 5 mg/kg	189 ± 6	35 ± 2	163 ± 6	38 ± 2	170 ± 5	40 ± 2	172 ± 5[b]	39 ± 2	178 ± 5[b]	38 ± 2
IV Diabetic + EJ 250 mg/kg	182 ± 11	28 ± 2	166 ± 11	30 ± 2	165 ± 9	33 ± 2	161 ± 10	33 ± 2	154 ± 11	33 ± 2
V Diabetic + EJ 500 mg/kg	183 ± 6	29 ± 2	165 ± 3	34 ± 2	169 ± 4	36 ± 2	170 ± 5[b]	33 ± 2	171 ± 5[b]	35 ± 2
VI Diabetic + EJ 1000 mg/kg	174 ± 8	30 ± 2	157 ± 6	32 ± 2	162 ± 7	32 ± 2	162 ± 8[b]	31 ± 2	166 ± 7[b]	31 ± 2

Data are reported as means ± SEM for groups of 6 animals each. EJ = *Eugenia jambolana*. STZ = streptozotocin.
[a]$P < 0.001$ compared to normal control; [b]$P < 0.001$ compared to diabetic control (Kruskal-Wallis analysis of variance).

Table 2. Hypoglycemic effect of *Eugenia jambolana* seed powder on blood glucose (mg/dl) of streptozotocin-diabetic rats.

Group	Days after STZ injection (days after treatment)								Percent fall in blood glucose	
	5 days (0 days)				20 days (15 days)				20 days (15 days)	
	Fasting	Glucose tolerance test			Fasting	Glucose tolerance test			Fasting	Peak level in GTT
		30 min	60 min	120 min		30 min	60 min	120 min		
I	50±7.1	75±9.5	66±8.7	60±7.7	54±6.8	80±9.6	70±10.7	65±9.1		
II	243±11.1[a]	340±10.6[a]	295±9.5[a]	280±8.8[a]	277±12.6	355±10.1	330±12.8	300±10.1		
III	264±10.6[a]	350±11.3[a]	320±9.7[a]	310±9.7[a]	122±12.6[b]	175±11.6[b]	140±12.6[b]	130±10.7[b]	-54	-50
IV	258±13.7[a]	345±12.6[a]	315±10.6[a]	285±9.6[a]	225±14.5[b]	275±11.1[b]	250±12.1[b]	230±10.5[b]	-13	-20
V	247±10.7[a]	330±10.8[a]	290±10.7[a]	275±9.1[a]	172±12.8[b]	210±10.8[b]	200±13.6[b]	180±11.5[b]	-30	-36
VI	267±13.3[a]	335±10.1[a]	310±10.8[a]	290±9.6[a]	144±14.6[b]	180±10.9[b]	160±14.7[b]	155±11.6[b]	-46	-46

See Tables 1 and 3 for explanation of groups and treatments. Data are reported as means ± SEM for groups of 6 animals each. EJ = *Eugenia jambolana*. STZ = streptozotocin.
[a]P < 0.001 compared to normal control; [b]P < 0.05 compared to diabetic control (Kruskal-Wallis analysis of variance).

Table 3. Effect of *Eugenia jambolana* seed powder on liver glycogen of streptozotocin-diabetic rats.

Group	Treatment	Liver glycogen (µg/g of tissue)
I	Normal control	90 ± 6.6
II	Diabetic control	30 ± 7.6
III	Diabetic + glibenclamide 5 mg/kg	65 ± 6.5[a]
IV	Diabetic + EJ 250 mg/kg	35 ± 5.6[a]
V	Diabetic + EJ 500 mg/kg	50 ± 6.8[a]
VI	Diabetic + EJ 1000 mg/kg	52 ± 7.5[a]

Data are reported as means ± SEM for groups of 6 animals each on day 21 (15 days after treatment). EJ = *Eugenia jambolana*.
[a]P < 0.001 compared to normal control (Kruskal-Wallis analysis of variance).

ing and peak blood glucose levels were 13, 30, 46 and 20, 36, 46, respectively.

Table 3 summarizes the liver glycogen content. There was a statistically significant increase in liver glycogen in all treatment groups which, however, was not equivalent to the normal control.

The present study shows that the antidiabetic effect of *E. jambolana* seed powder was better with 500 mg/kg body weight, as shown by an increase in mean body weight, a post-treatment difference between fasting and peak blood glucose levels, the percent reduction in pre-treatment versus post-treatment fasting and peak blood glucose levels, and elevation in liver glycogen values. The seed powder at a dose of 1000 mg/kg body weight did not show a proportional improvement in the above metabolic parameters, suggesting that an increased concentration of active ingredients is not always proportionally beneficial (7,8) and can be associated with adverse effects (4) when used as complementary therapy to established hypoglycemic agents. The hypoglycemic activity of *E. jambolana* seed powder has been detected in studies conducted in places other than the Western Ghats of India. In most studies on seed powder, the dose used was 1 g/kg body weight or higher (7,9), or was administered as a single dose of 200 mg/kg (10), or as fixed multiple doses of 100, 200, or 400 mg (11). The present study clearly defines a dose-response effect on glycemia and liver glycogen for *E. jambolana* seed powder administered according to body weight to STZ-induced diabetic female rats.

HPLC chromatograms of *E. jambolana*

seed powder extracted with dichloromethane showed seven peaks. Tri-terpenoids, tannins, gallic acid, and oxalic acid were detected using suitable chemical tests. Alkaloids were present in small amounts. Similar constituents have been detected in previous studies (12,13). Further studies on the extraction and purification of each component will help identify the active ingredients.

Toxicity studies were carried out as a requirement for Ethics Committee clearance. The animals were observed for 14 days, with no evidence of mortality or abnormalities (Table 4). Blood glucose levels decreased only by 11-15% *vs* the normal controls after the GTT, in contrast to the study of Achrekar et al. (14), in which a 34% decrease in blood glucose levels was observed in normal rats 5 days after treatment with 50 g of seed powder homogenized in 100 ml water. This shows that *E. jambolana* possibly acts as a hypoglycemic agent by increasing insulin levels (14,15) rather than just as an antihyperglycemic agent. Glibenclamide, tested at doses of 0.25, 0.5, and 1.3 mg/kg, did not promote any antihyperglycemic activity in STZ-diabetic rats in the pilot study.

The experimental STZ-diabetes model usually involves type 1 diabetes. However, in the present study, at the dose of STZ used, the model probably involved type 2 diabetes and some functioning ß cells of islets of Langerhans were present because: a) untreated diabetic controls survived in spite of increasing FBS and GTT blood glucose levels as observed on day 15 of treatment; b) a response to treatment was observed in group III without insulin treatment. The measurements of the end points of insulin activity, i.e., FBS and GTT blood glucose and liver glycogen levels, in this study suggest that *E. jambolana* seed promote the release of insulin, a finding also reported by Achrekar et al. (14) after measuring insulin levels *in vivo* and *in vitro*. A dose-dependent *in vitro* reduction in insulin degradation (14) was also seen. Thus, we conclude that the seed powder should be evaluated in a) type 2 diabetes, not as monotherapy on account of inadequate activity (Tables 2 and 3), but complementing established oral hypoglycemic agents, reducing their established dosing regimen which would reduce their side effects, and in b) type 1 diabetes as a treatment complementary to insulin.

Table 4. Effect of a single oral dose of *Eugenia jambolana* seed powder on the glucose tolerance test.

	Blood glucose (mg/dl)			
	Fasting	Glucose tolerance test		
		30 min	60 min	120 min
EJ 2.5 g/kg body weight	48 ± 4.1	72 ± 2.1	65 ± 5	59 ± 6
EJ 5.0 g/kg body weight	46 ± 2.1	68 ± 4.1	61 ± 2.1	56 ± 3

Data are reported as means ± SEM for groups of 6 normal animals each, 14 days after treatment. EJ = *Eugenia jambolana*.

Diabetes is now considered to be a vascular disease. The cost of treating the microvascular component (retinopathy, nephropathy and neuropathy) and controlling the macrovascular component is a serious drain on health resources, particularly in developing countries. It is expected that by the year 2025 India will have 57.2 million diabetics (one sixth of the world total) (16). Besides the prevention strategies proposed (16), the use of cost-effective therapies goes a long way towards the aforementioned goal. The authors contend that patient preferences for therapies are guided by cultural heritage and by the natural environment of the region they live in. The results of this preclinical and toxicity study provide the necessary data for phase 2 clinical trials in type 2 and type 1 diabetes with *E. jambolana* seed powder as an 'add on' therapy to established oral hypoglycemic agents and/or insulin.

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