Prevention of diabetes-induced myocardial dysfunction in rats using the juice of the *Emblica officinalis* fruit

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Normalization of hyperglycemia, hyperlipidemia and oxidative stress is an important objective in preventing diabetes-induced cardiac dysfunction. The present study investigated the effects of the fruit juice obtained from *Emblica officinalis* on myocardial dysfunction in diabetic rats. Diabetes was induced by streptozotocin (STZ), and the rats were treated with *E officinalis* fruit juice for eight weeks. Injection of STZ produced loss of body weight, polydypsia, polyphagia, hyperglycemia, hypoinsulinemia and dyslipidemia. It also produced hypertension, bradycardia, hypertrophy and myocardial functional alterations associated with an increase in serum lactate dehydrogenase and creatinine kinase-MB levels. Treatment with the fruit juice not only prevented STZ-induced loss of body weight, increases in water and food intake, increases in serum glucose levels and disturbed lipid profile, but also an increase in serum lactate dehydrogenase and creatinine kinase-MB levels, and increased myocardial hypertrophy and cardiomyopathy. There was an increase in the area under the curve (AUC) for glucose, and a decrease in $AUC_{insulin}$ was observed in diabetic rats; treatment decreased $AUC_{glucose}$ but not $AUC_{insulin}$ or hyperinsulinemia. There was a decrease in antioxidant enzyme levels (in superoxide dismutase, reduced glutathione and catalase) in diabetic hearts, which could be improved by treatment with fruit juice. The present data suggest that fruit juice may be beneficial for the treatment of myocardial damage associated with type 1 diabetes mellitus. The activity of *E officinalis* fruit juice can be attributed to the concentration of polyphenol present.

Key Words: *Antidiabetic; Antihyperlipidemic;* Emblica officinalis*; Left ventricular hypertrophy; Myocardial dysfunction; Oxidative stress*

Diabetes mellitus increases the risk of cardiomyopathy and heart failure independent of underlying coronary artery disease (1). The pathogenesis of diabetes-induced cardiomyopathy involves metabolic derangements, such as hyperglycemia and hyperlipidemia, that produce glycation of interstitial proteins, such as collagen and, in turn, lead to myocardial stiffness and impaired contractility (2-4). There may be a decrease in insulin sensitivity associated with autonomic dysfunction, hypertension and left ventricular (LV) hypertrophy (5,6). An increase in oxidative stress contributes to the characteristic morphological and functional abnormalities that are also associated with diabetic cardiomyopathy (7). Various therapeutic strategies for the prevention or delay in the development of the above-mentioned complications include effective glycemic control, prevention of hyperlipidemia and oxidative stress.

The fruit of the *Emblica officinalis* (family: Euphorbiaceae) plant, commonly known as 'Amla' or the Indian gooseberry, has been reported to contain constituents with variable biological activity. Phytochemical investigations of the *E officinalis* fruit show that it has a high concentration of polyphenol with low and high molecular weight gallotannins such as L-malic acid 2-O-gallate; mucic acid 2-O-gallate; corilagin chebulagic acid; putrajivain A; elacocarpusin; mucic acid 1-O-galloyl-β-D-glucose; mucic acid 6-methyl ester 2-O-gallate; mucic acid 1,4-lactone 2-O-gallate; mucic acid 1-methyl ester 2-O-gallate; mucic acid 2-O-gallate; mucic acid 1,4-lactone 6-methyl ester 2-O-gallate; mucic acid 1,4-lactone 3-O-gallate; mucic acid 1,4-lactone 3,5-di-O-gallate; emblicanin A and B; punigluconin; pedunculagin; methyl gallate; corilagin; furosin and geraniin (8,9). Many such polyphenols are known to be present in a number of medicinal plants and are reported to have glucose-lowering (10), insulinomimetic (11), lipid-lowering (12-14), and antioxidant (15,16) and cardioprotective properties (17,18). It is possible that polyphenols present in *E officinalis* possess antidiabetic, antihyperlipidemic and antioxidant activities, which may be effective in preventing or delaying the development of cardiomyopathy. The present investigation was undertaken to examine whether chronic treatment with *E officinalis* fruit juice has beneficial effects on glycemia, lipidemia and cardiac dysfunction in the streptozotocin (STZ)-induced type 1 diabetic rat.

METHODS

The fresh fruit of *E officinalis* were purchased from Gaziabad (India), and authenticated by the Department of Pharmacognosy, LM College of Pharmacy, Gujarat University (India). The juice from the fruit was prepared in the laboratory; standardization of the juice was performed through estimation of the polyphenolic content, as per the method described by Gao et al (19). STZ was purchased from Sigma-Aldrich (USA), and the glucose GOD-POD kit, as well as the triglyceride and total cholesterol kits were purchased from Span Diagnostics Ltd (India). The radioimmunoassay kit for rat insulin was obtained from Bhabha Atomic Research Centre (India). The cholesterol-high density lipoprotein (HDL) kit was purchased from Lab-care Diagnostics Pvt Ltd (India), and the lactate dehydrogenase (LDH) and the CK-MB kits were purchased from Bayer Diagnostics Ltd, India. Other chemicals used were of analytical reagent grade.

Male Wistar rats weighing 200 g to 250 g were obtained from the animal facility of the Zydus Research Centre (India). They were maintained under standard environmental conditions (12 h light/dark cycle at 20°C to 25°C, and controlled humidity) and provided with feed and purified water ad libitum. All experiments and protocols described in the present study were approved by the Institution of Animal Ethics Committee, and are in accordance with guidelines as per the *Guide for the Care and Use of Laboratory Animals*. Permission was obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Induction of diabetes and treatment protocol

Diabetes was induced by a single-tail vein injection of STZ (50 mg/kg intravenously) dissolved in normal saline. The control animals were injected with equal volumes of the vehicle. Forty-eight hours after STZ injection, animals showing glycosuria (>2%) were considered to be

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TABLE 1
Effect of *Emblica officinalis* fruit juice on the general features of nondiabetic control and diabetic rats

Parameters	Nondiabetic control	Diabetic control	Nondiabetic treated with fruit juice	Diabetic treated with fruit juice
Body weight after treatment, g	214.8±0.38	168.6±2.45	200.40±0.63	199.80±0.77*
Food intake, g/animal/day	23.16±0.39	43.49±0.83	22.51±0.46	35.66±0.87*
Water intake, mL/animal/day	24.88±0.43	59.73±1.35	29.30±0.69	47.88±1.66*

*Data presented as mean ± SEM. *Significantly different from diabetic control rats (P<0.05)*

diabetic. Animals were divided into four groups: normal control, diabetic control, normal animals treated with *E officinalis* fruit juice (1 mL/kg/orally/day) and diabetic animals treated with fruit juice (1 mL/kg/orally/day). Treatment was started three days after STZ injection, and was given daily for eight weeks. Food intake, water intake and body weight gain were measured. Mean and systolic blood pressures and heart rate were measured noninvasively by tail-cuff method using the Harward blood pressure monitor in all the above-mentioned groups at the end of the eight-week treatment period.

At the end of the eight-week treatment period, the animals were kept on an overnight fast, and the blood samples were collected. The blood was allowed to clot for 30 min at room temperature and then centrifuged at 5000 rpm for 20 min. The serum was separated and stored at –20° C until analysis was complete. Serum samples were analyzed spectrometrically (Shimadzu UV-1601, Japan) for serum glucose, triglyceride, total cholesterol, HDL-cholesterol, LDH and CK-MB using their respective kits. Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated from the total cholesterol, triglyceride and HDL values. Serum insulin was estimated by radioimmunoassay technique using a gamma counter (Riastar, Packard, USA).

Oral glucose tolerance test

The same animals were subjected to an oral glucose tolerance test (OGTT). To perform the OGTT, the animals were orally administered 1.5 g/kg of glucose, and blood samples were collected from the tail vein under light ether anesthesia before (ie, 0 min) and 30 min, 60 min and 120 min after oral glucose administration. Samples were analyzed for glucose and insulin. Plotting glucose concentration versus time results in a curve showing the rise and fall in glucose and insulin levels with time, and is expressed as an integrated area under the curve for glucose and insulin ($AUC_{glucose}$, $AUC_{insulin}$).

Isolated heart experiment

Three to four days following OGTT, the animals were euthanized and the hearts were isolated and mounted as per the Langendorff heart technique. The hearts were perfused with Chenoweth-Koelle buffer (119.8 mmol/L NaCl, 5.6 mmol/L KCl, 2.88 mmol/L $CaCl_2$, 4.5 mmol/L $MgCl_2$, 3.8 mmol/L $NaHCO_3$ and 5 mmol/L glucose), and were continuously infused with 95% O_2 and 5% CO_2 carbogen. The responses were recorded using a transducer attached to the students' physiographs. At the end of the study, the hearts were blotted with filter paper to remove excess water and the remaining extraneous tissues were removed. The weight of the hearts were noted to calculate the index of hypertrophy as wet left ventricular weight to body weight (LV/BW) and heart weight to body weight (HW/BW), and were subjected to assessment of antioxidant parameters and estimation of LV collagen and protein content.

Assessment of oxidative stress-related markers in the heart

Heart tissues were finely sliced and homogenized in chilled tris buffer. The homogenates were centrifuged and the clear supernant was used for estimation of various antioxidant parameters such as superoxide dismutase (SOD), catalase, lipid peroxidation or malondialdehyde (MDA), and reduced glutathione (GSH). SOD was determined by the Misra and Fridovich (20) method, catalase was determined by the Aebi (21) method, and GSH was determined according to the method described by Moron et al (22). MDA formation was determined using the Slater and Sawyer (23) method. Antioxidant activity in the liver was expressed in terms of protein content, which was measured as per the method reported by Lowry et al (24).

Statistical analysis

Values were expressed as mean ± SEM. The results were analyzed using one-way factorial ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered to be statistically significant.

RESULTS

Effect of *E officinalis* fruit juice on general features and biochemical parameters

STZ administration produced the cardinal signs of diabetes such as loss of body weight and increase in food and water intake compared with control animals. Chronic treatment with *E officinalis* fruit juice prevented the loss of body weight and elevated food and water intake significantly (P<0.05) in diabetic rats (Table 1). Diabetic rats were found to exhibit significant hyperglycemia and hypoinsulinemia, with hypertriglyceridemia and hypercholesteremia. Treatment with *E officinalis* fruit juice also produced an increase in serum LDL and VLDL cholesterol levels and a decrease in HDL cholesterol levels in diabetic rats. Additionally, it produced a significant decrease in the elevated serum glucose, triglyceride, total cholesterol, LDL cholesterol and VLDL cholesterol levels of diabetic rats. There was a slight increase in insulin and HDL cholesterol levels at the same dose, but it was not statistically significant (Table 2).

OGTT

Administration of glucose produced a significant increase in $AUC_{glucose}$ of the diabetic control group compared with that of the normal control group. Treatment with *E officinalis* fruit juice produced a significant (P<0.05) decrease in $AUC_{glucose}$ of diabetic rats compared with that of the diabetic controls (Table 2).

Effect on hemodynamic parameters

Mean blood pressure was found to be increased, and heart rate and force of contraction were found to be decreased in diabetic rats compared with the control animals. Chronic treatment with *E officinalis* fruit juice showed a significant (P<0.05) increase in heart rate and force of contraction and a decrease in blood pressure compared with diabetic control animals (Figure 1).

Cardiac hypertrophy index

The ratio of wet LV/BW and HW/BW served as an index of cardiac hypertrophy. The LV/BW, HW/BW, LV collagen and protein content were found to be significantly increased in diabetic rats. Treatment with *E officinalis* fruit juice significantly (P<0.05) decreased the LV/BW ratio, left ventricular collagen and protein content (Figure 2).

Effect on serum LDH and CK-MB

Diabetic rats showed a significant increase in serum CK-MB activity compared with control rats. Administration of *E officinalis* fruit juice significantly decreased the diabetes-induced increase in serum CK-MB activity in diabetic animals. Serum LDH levels of diabetic animals were also significantly increased compared with control animals. Administration of *E officinalis* fruit juice to diabetic rats significantly

TABLE 2
Effect of *Emblica officinalis* fruit juice on biochemical parameters of nondiabetic control and diabetic rats

Parameters	Nondiabetic control	Diabetic control	Nondiabetic treated with fruit juice	Diabetic treated with fruit juice
Serum glucose, mmol/L	6.62±0.33	25.02±1.23	5.50±0.31	14.47±0.94*
Serum insulin, pmol/L	318.8±30.0	161.8±25.4	292.9±28.0	202.6±26.0
$AUC_{glucose}$, mmol/L·min ×10^3	6.75±0.27	29.64±1.09	6.57±0.24	15.26±1.37*
$AUC_{insulin}$, pmol/L ×10^3	39.57±1.67	22.78±1.88	40.28±2.92	25.90±1.46
Serum triglyceride, mmol/L	0.91±0.12	2.23±0.22	0.97±0.10	1.33±0.10*
Serum cholesterol, mmol/L	2.24±0.13	3.22±0.13	2.11±0.13	2.62±0.1*
HDL cholesterol, mmol/L	0.98±0.09	0.82±0.05	0.98±0.08	0.98±0.11
LDL cholesterol, mmol/L	0.98±0.04	0.82±0.14	0.98±0.11	0.98±0.16*
VLDL cholesterol, mmol/L	0.42±0.06	1.02±0.10	0.45±0.05	0.61±0.04*

*Data presented as mean ± SEM. *Significantly different from diabetic control rats (P<0.05). AUC Area under the curve; HDL High-density lipoprotein; LDL Low-density lipoprotein; VLDL Very low-density lipoprotein*

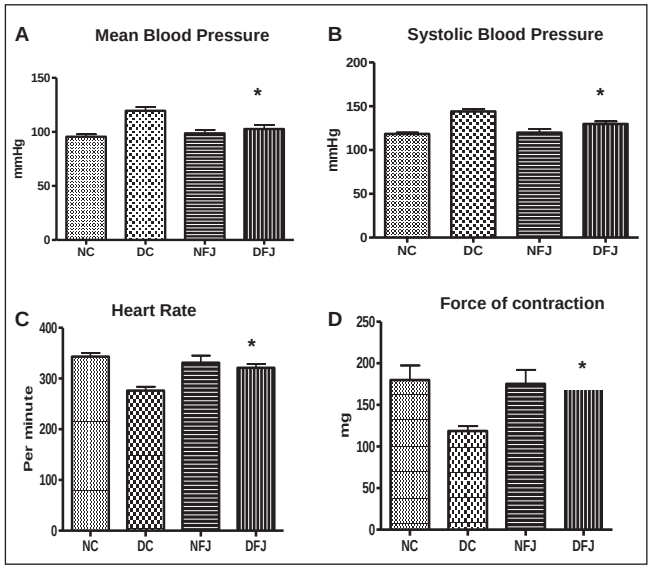

Figure 1) *Effect of* Emblica officinalis *fruit juice on hemodynamic parameters of nondiabetic control and diabetic rats. Each bar represents the mean ± SEM of six animals. DC Diabetic control; DFJ Diabetic animals treated with* E officinalis *fruit juice 1 mL/kg orally; NC Nondiabetic control; NFJ Normal animals treated with* E officinalis *fruit juice 1 mL/kg orally. *Significantly different from diabetic control rats (P<0.05)*

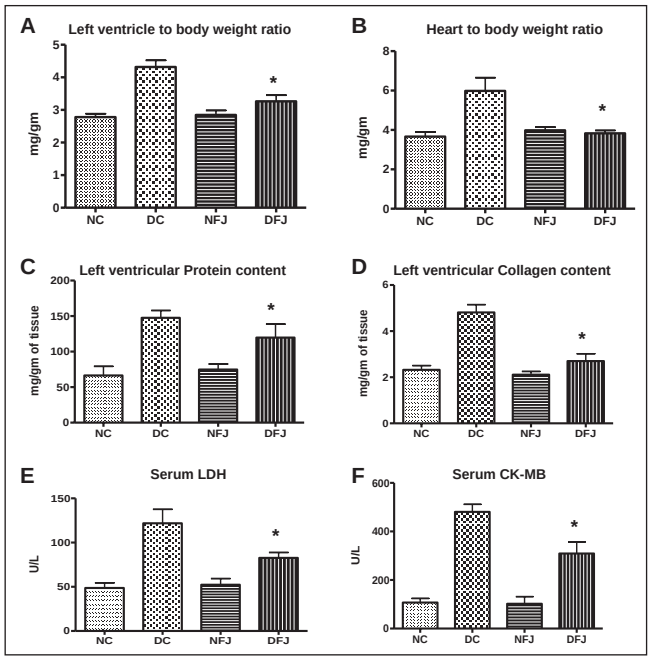

Figure 2) *Effect of* Emblica officinalis *fruit juice on serum lactate dehydrogenase (LDH), creatine kinase-MB, and morphological parameters of nondiabetic control and diabetic rats. Each bar represents the mean ± SEM of six animals. DC Diabetic control; DFJ Diabetic animals treated with* E officinalis *fruit juice 1 mL/kg orally; NC Nondiabetic control; NFJ Normal animals treated with* E officinalis *fruit juice 1 mL/kg orally. *Significantly different from diabetic control rats (P<0.05)*

(P<0.05) attenuated LDH serum activity level compared with diabetic control animals (Figure 2).

Effect on antioxidant parameters in the heart

STZ diabetic rats were found to have decreased SOD, GSH and catalase enzyme levels in the heart compared with control rats. Treatment with *E officinalis* fruit juice produced a significant increase in these enzyme levels. STZ diabetic rats were also found to exhibit a significant increase in MDA levels in the heart compared with control rats. Treatment with *E officinalis* fruit juice produced significant (P<0.05) decreases in MDA levels (Table 3).

DISCUSSION

STZ diabetic rats were found to exhibit significant hyperglycemia and hypoinsulinemia with hypertriglyceridemia and hypercholesteremia. Treatment with fruit juice produced significant decreases in elevated serum glucose, triglyceride, total cholesterol, LDL cholesterol and VLDL cholesterol levels in diabetic rats. The results of the present study showed that there was a decrease in the glucose level without a significant increase in insulin output, indicating that treatment with *E officinalis* fruit juice produced increased sensitivity of peripheral tissue to insulin or direct insulin-like effects. The results of the OGTT further support these findings.

Several studies have suggested that carbohydrate and lipid metabolic abnormalities, such as hyperglycemia and hyperlipidemia, may contribute to the development of cardiac dysfunction in diabetes mellitus (25). A significant reduction in myocardial glucose supply and utilization has been observed in isolated diabetic cardiomyocytes, which could be the primary injury in the pathogenesis of this specific heart muscle disease (26,27). Therefore, it is necessary to increase glucose utilization or the rate of glucose transport in the diabetic heart. Thus, the study suggests that diabetes results in progressive, marked changes in the myocardium that can be prevented by treatment with fruit juice either by increasing glucose utilization or glucose transport in the diabetic heart.

Earlier studies have shown that in STZ diabetic rats, hyperglycemia is associated with hypercholesterolemia and hypertriglyceridemia (26). Abnormalities in lipid metabolism have been demonstrated in cardiomyopathy in which the rate of free fatty acid uptake by myocardium is inversely proportional to the severity of the myocardial dysfunction (28); correction of lipid abnormalities can improve heart function in

TABLE 3
Effect of *Emblica officinalis* fruit juice on antioxidant parameters of nondiabetic control and diabetic rats

Parameters	Nondiabetic control	Diabetic control	Nondiabetic treated with fruit juice	Diabetic treated with fruit juice
SOD, units/min/mg protein	12.1±1.28	4.81±0.34	11.61±1.40	9.44±0.82*
Catalase, units/min/mg protein	12.29±0.71	5.43±0.46	11.73±0.99	9.24±1.01*
MDA, nmol/mg protein	0.58±0.08	1.86±0.26	0.63±0.15	0.73±0.08*
GSH, µg/mg protein	11.58±0.94	4.39±0.40	11.44±0.96	9.54±0.98*

*Data presented as mean ± SEM. *Significantly different from diabetic control rats (P<0.05). GSH Reduced glutathione; MDA Malondialdehyde; SOD Superoxide dismutase*

STZ diabetic rats (29). Thus, the strategy used to produce improvement in cardiac function is to improve on these metabolic derangements. In the present study, it was found that the oral administration of *E officinalis* fruit juice decreases glucose levels and elevates lipid profiles, which may be a major contributing factor in the improvement of cardiac dysfunction. Polyphenolic compounds are present in a number of medicinal plants that are reported to possess antidiabetic and antihyperlipidemic activity, thus it is possible that polyphenols abundant in *E officinalis* fruit juice (541.3 mg gallic acid equivalent/1 g extract) might be responsible for glucose-lowering and the lipid-lowering effects of fruit juice, which may be responsible for cardioprotective effects.

Diabetes also produces various hemodynamic changes such as hypertension, decrease in the force of contraction and bradycardia (24,30). Chronic treatment with *E officinalis* fruit juice showed a significant increase in heart rate and decrease in blood pressure compared with diabetic control animals, which is consistent with previous reports that show that medicinal plants containing polyphenol produce hypertensive effects by decreasing blood pressure and increasing heart rate (31,32).

In the present study, diabetic rats were found to exhibit increased LV weight and HW. Both the ratio of wet LV/BW and HW/BW serve as an index of cardiac hypertrophy. LV dysfunction has also been associated with increased LV/BW and heart weight to body weight (33). In our study, LV/BW and HW/BW were found to be increased in diabetic hearts. This is in agreement with previous reports in which it has been shown that there is an increase in fibrous tissue formation and accumulation of collagen in diabetic rats, causing an increase in the LV weight (34). A correlation with a biopsy study in diabetic patients has also supported these findings in animals (35). Treatment with fruit juice produced an improvement in the wet LV/BW and HW/BW ratio. It also produced decreases in LV collagen and protein content in diabetic rats. Various studies have shown that experimentally induced diabetes causes an increase in collagen formation and accumulation of protein within the interstitium, which in turn, results in anatomical and physiological changes in the myocardium (36). Furthermore, various studies have demonstrated that an increase in LV collagen and protein content may produce cardiac stiffness and fibrosis, resulting in cardiac dysfunction (37,38). Thus, *E officinalis* fruit juice may provide protection against cardiac stiffness and fibrosis in cardiac dysfunction.

Serum LDH and CK-MB activities were found to be increased in STZ diabetic rats, possibly due to myocardial dysfunction because it has been previously reported that serum LDH and CK-MB activities were found to be increased in cardiomyopathy (39). Serum CK-MB and LDH levels were also reported to increase in diabetic patients, and may serve as a marker for cardiovascular risk and cardiac muscular damage (40). In the present study, there was a significant decrease in serum LDH and CK-MB levels observed with treatment of fruit juice, indicating good cardioprotection.

Increased reactive oxygen species (ROS) production in the diabetic heart is a contributing factor in the development and progression of diabetic cardiomyopathy (41). Increased ROS amplifies hyperglycemia-induced activation of protein kinase C isoforms, increased formation of glucose-derived advanced glycation end products, and glucose flux through the aldose reductase pathways (42,43). All of these factors may contribute to the development of cardiac complications in diabetes mellitus. Increased ROS generation may activate maladaptive signalling pathways, which may lead to cell death and could promote abnormal cardiac remodelling, which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy. Thus, the levels of antioxidant enzymes, such as GSH, SOD or catalase, are decreased in the diabetic heart. The strategies either to reduce ROS or augment myocardial antioxidant defense mechanisms might have therapeutic efficacy in improving myocardial function in diabetes mellitus. Treatment with fruit juice increased the levels of endogenous antioxidants and decreased lipid peroxidation in diabetic rats. The phenolic content of *E officinalis* fruit juice is reported to have strong antioxidant properties, which can be beneficial in the prevention of diabetes-induced cardiomyopathy (44,45). In our study, an increase in antioxidant enzyme levels in the heart were observed after treatment with fruit juice in diabetic animals, which can reverse diabetic cardiomyopathy. Thus, diabetics patients who are at an increased risk of cardiac dysfunction may benefit both from an improvement in glucose and lipid homeostasis, as well as from the antioxidant cardioprotective effect of *E officinalis* fruit juice.

CONCLUSION

E officinalis fruit juice treatment ameliorates hyperglycemia, hyperlipidemia, oxidative stress and the development of cardiac dysfunction associated with STZ diabetes.

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