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Current Nutrition & Food Science, **2017,** *13,* **1-6**

RESEARCH ARTICLE

Antihyperglycemic Effects of Formulation of Spray Dried Fruit Juice of *Emblica officinalis* in Streptozotocin Induced Diabetic Rats.

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ARTICLE HISTORY

Received: March 12, 2016
Revised: October 02, 2016
Accepted: October 12, 2016

DOI: 10.2174/1573401312666161017143215

Abstract: ***Introduction*:** The present investigation was carried out to study antihyperglycemic activity of formulation prepared by spray-dried powder of fruit juice of *E. officinalis* (SDF) on animal model of type 1 diabetes.

***Methods*:** Hyperglycemia was produced by streptozotocin 45 mg/kg i.v. and formulation was administered orally (100 mg/kg) for 28 days to diabetic rats. At the end of 28 days various biochemical parameters such as serum glucose, insulin, $AUC_{glucose}$, $AUC_{insulin}$ and lipid profile were estimated.

***Results*:** STZ induced rats showed signs and symptoms of diabetes such as body weight loss, polydipsia, polyuria, polyphagia, treatment with formulation produced slight improvement in these symptoms. Treatment with formulation to diabetic rat produced significant decrease in serum glucose, $AUC_{glucose}$, triglyceride, cholesterol, LDL cholesterol, VLDL cholesterol. However, insulin, $AUC_{insulin}$ and serum high density lipoprotein level were not significantly affected after treatment. Treatment also produced reduction in malonaldehyde levels and increased antioxidant enzymes levels in diabetic rats.

***Conclusion*:** Thus, formulation of *E. officinalis* significantly improved glucose and lipid dysfunction and oxidative stress in diabetic status. The mechanism of its antidiabetic activity may be either increase in peripheral glucose uptake, reduced insulin resistance or antioxidant property of formulation.

Keywords: Antidiabetic, antihyperlipidemic, antioxidant, fruit juice, oxidative stress.

INTRODUCTION

Diabetes is among the top five chronic diseases in the developed world and is gaining significance in developing countries. In diabetes there is derangement in carbohydrate, lipid and protein metabolism and the development of long term complications such as microangiopathy, macroangiopathy, atherosclerosis, retinopathy, neuropathy, cardiomyopathy and autonomic neuropathy [1-3]. Diabetes is also linked with increased incidence of morbidity and mortality due to long and short term complications.

Hypoglycemic agents have been reported to produce serious side effects while, herbal preparations are considered to be associated with lesser side effects [4]. Also synthetic drugs possess antidiabetic activity but do not have antioxidant activity and antioxidant plays a key role in treatment of both types of diabetes and associated complications. Therefore the search for more effective and safe antihyperglycemic agents has become an area of current research and WHO has approved the use of traditional medicines as a part of health programme. Various Ayurvedic formulations like Trasina [5], Hyponidd [6], Cogent db [7], tincture of punchparna [8], Diasulin [9], Triphala [10] and Diamed [11] containing *E. officinalis* as one of the ingredients have been reported to produce benificial effect in hyperglycemia in various studies.

Phytochemical investigations of fruits of *Emblica officinalis* (family Euphorbiaceae) have reported to contain abundant amount of polyphenolic constituents such as gallotanins, chebulagic acid, putrajivain A, elacocarpusin, emblicanin A and B, punigluconin, pedunculagin, corilagin, furosin and geraniin [12, 13]. Gallotannins present in many of plants reported to have antihyperglycemic, antioxidant and lipid lowering activities [14-16]. In phytochemical analysis the concentration of total polyphenol was found to be 23-27 %w/v by HPTLC. Thus, from all these previous reports we can speculate that gallotannins present in fruits of *E. offici-*

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1573-4013/17 $58.00+.00

nalis may be beneficial for antidiabetic and antihyperlipidemic activities in form of formulation.

As mentioned above polyphenol present in various plants have been reported to possess antidiabetic, antihyperlipidemic and antioxidant activities. Thus, the objective of the present investigation was to investigate effect of formulation of spray dried fruit juice for antidiabetic, antihyperlipidemic, antioxidant acitvities in STZ-induced type-1 rats.

MATERIALS AND METHODS

Materials

Plant Material

The fresh fruits of *E. officinalis* were procured from Ghaziabad (Madhya Pradesh, India). Authentication was done by the Department of Pharmacognosy, L. M. College of Pharmacy, Gujarat University, Ahmedabad and voucher specimen was deposited (LM/2007/SP/07). Juice from fruits was prepared and juice was further subjected to a spray drying. The spray dried powder was used for preparation of tablet by dry granulation technique. Formulation was prepared by Rajsha Pharmaceuticals (Ahmedabad, India).

Chemicals

STZ and standard compound gallic acid were procured from Sigma Chemicals (St.Louis, USA). Diagnostic kits such as glucose, triglyceride, total cholesterol, High density lipoprotein (HDL)-cholesterol kits were purchased from Accucare Diagnostics Pvt. Ltd (Vadodara, India). The chemicals used in present study were of analytical grade.

Preparation of Spray-Dried Powder from fruit Juice of *E. officinalis*

The preparation of fruit juice was carried out by cutting fruits into small pieces by removing seeds. Fruit pieces were weighed and grinded in mixer grinder. Fresh juice was obtained by compression and grinding and the juice was filtered and the filtrate was concentrated under vacuum until the total solid content exceeded more than 30 %. Each one kg of fruit produced 750ml of juice yield of spray dried powder was 33% w/v. The concentrated juice was further subjected to a spray drying to obtain the spray dried powder. The spray dried powder was used for the preparation of tablet by dry granulation technique.

Preparation of Formulation

Spray dried powder was mixed with mixture of 10 % water and 90% Isopropyl Alcohol until Lumpy mass was obtained. The mass was passed through 8# Sieve. Granules were dried at 45` C for 3-4 hours in tray drier. Granules were again passed through 12#Sieve in shifter or granulator. The granules were lubricated with 2% Talcum and 1% Magnesium Stereate in mass mixture for 15 mins. Granules were compressed with high speed tablet compressor (CADMACH C-300).

Experimental Animals

Male Sprague Dawley rats (250-300gm) were acquired from Torrent Research Centre, Ahmedabad, India. All experiments and protocols were approved by Institutional Animal Ethics Committee (IAEC) with protocol no (IPS/PCOL/CON10-11/2001). Animals were maintained under 12h light/dark cycle, temperature (20-25°C) and controlled humidity (40-50%). Pelleted feed and purified water was provided *ad libitum*.

Experimental Protocol

Animals were divided into four groups: normal control, normal treated, diabetic control and diabetic treated with formulation (100mg/kg, p.o). Diabetes was induced by STZ (45mg/kg, i.v.) dissolved in citrate buffer (pH3.5). After 48hr of STZ injection, animals having urine glucose level more than 2% was included in study. After three days of STZ injection, oral administration with SDF (100mg/kg/day) was started and it was given for the period of six weeks. Body weight gain and food and water intake were accessed weekly.

Blood Sampling and Biochemical Analysis

At the end of treatment, blood samples were collected and serum samples were stored at -70°C until analysis was carried out. All the samples were analyzed for serum glucose, triglyceride, total cholesterol, HDL-cholesterol using diagnostic kits and UV-Visible spectrophotometer (Shimadzu UV-1601, Japan). Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated based on triglyceride and HDL values. The same animals were subjected to oral glucose tolerance test (OGTT) by method previously described by Goyal *et al.* [17].

Biochemical Parameters

Estimation of glucose was carried out by GOD/POD method described by Trinder [18], Serum insulin was determined by radioimmunoassay. Estimation of serum cholesterol and serum triglyceride was carried out by enzymatic method by Allain *et al*.; Bucolo and David [19, 20]. Estimation of serum HDL-Cholesterol by the Phosphotungstate method, was mentioned by Lopes *et al*. [21].

Oral Glucose Tolerance Test

OGTT was performed after an overnight fasting [22]. The animals were orally administered with 1.5 g/kg of glucose and blood samples were collected from the tail vein under light ether anesthesia before *i.e.* 0 min and 30, 60 and 120 min after oral glucose administration. Samples were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 25 min and analyzed for glucose and insulin as explained earlier. Plotting the glucose or insulin concentration versus time gives a curve showing rise and fall in glucose and insulin levels with time after an oral glucose load. Comparison of such curves gives only a vague idea about alterations in insulin-mediated glucose disposal and insulin release in response to oral glucose load. Therefore, results were expressed as integrated area under the curve (AUC) for glucose and insulin. This was calculated by applying trapezoid rule [$AUC = (C_1 + C_2)/2 \times (t_2 - t_1)$] and changes in glucose and insulin concen-

Table 1. Effect of treatment of SDF on general features of diabetic rats.

Parameter	Normal Control	Normal Treated with SDF	Diabetic Control	Diabetic Treated with SDF
Body weight after treatment (g)	248.9±6.18	250.0±5.33	209.23±5.62*	225.23±5.73
Food intake (g/animal/day)	31.24±7.22	30.31±6.21	49.53±7.32*	36.58±2.82
Water intake (ml/animal/day)	24.33±6.25	24.48±5.24	42.18±9.26*	37.33±8.22

Values are expressed as Mean ± S.E.M
*- significantly different from normal control ($p< 0.05$)

Table 2. Effect of treatment of SDF on glucose and insulin of diabetic rats.

Parameter	Normal Control	Normal Treated with SDF	Diabetic Control	Diabetic Treated with SDF
Serum glucose (mg/dl)	96.23±4.2	95.12±4.98	399.2±10.5*	209.13±5.52$^{\#}$
Serum insulin (μU/ml)	19.15±1.04	17.75±0.85	11.23±1.03*	13.32±1.32
$AUC_{glucose}$ (mg/dl.min)×10^3	13.32±0.57	12.73±0.44	47.56±1.88*	35.24±1.95$^{\#}$
$AUC_{insulin}$ (μU/ml.min)×10^3	6.55±0.28	6.85±0.26	2.22±0.17*	3.02±0.23

Values are expressed as Mean ± S.E.M
*- significantly different from notmal control ($p< 0.05$)
$^{\#}$- significantly different from diabetic control ($p< 0.05$)

trations over 120 min during OGTT were expressed as $AUC_{glucose}$ (mg/dl.120min) and $AUC_{insulin}$ (μU/ml.120min) respectively.

Estimation of Oxidative Stress Markers

At the end of experiment animals were sacrificed and liver was isolated, weighed, homogenized. The homogenate was used for the estimation of antioxidant parameters. Decomposition of H_2O_2 in presence of catalase was estimated by Aeibi *et al*. A Homogenate was added to buffered substrate containing H_2O_2. The decrease in the absorbance was read at 240 nm for 2.5 min at an interval of 15 sec [23]. Reduced glutathione (GSH) was estimated by the method of Moron. The homogenate was mixed with trichloroacetic acid. The mixture was mixed with disodium hydrogen phosphate. The 5, 5'-dithiobis-2-nitrobenzoic acid was added just before measuring the absorbance at 412 nm [24]. SOD was diagnosed by the method described by Mishra and Fridovich. Liver homogenate was mixed with EDTA and Epinephrine. The optical density of formed adrenochrome was read at 480 nm for 3 min at an interval of 30 sec [25], Estimation of MDA levels was carried out by the method described by Slater and Sawyer where homogenate was mixed with sodium dodecyl sulfate, freshly prepared of thiobarbituric acid (TBA) (1%W/V). The intensity of pink color developed was read against blank at 532 nm [26]. All parameters were expressed per protein content and was measured as per the method described by Lowry *et al*. [27].

Statistical Analysis

The results were analyzed using one-way ANOVA followed by Tukey's test of multiple comparison. The value of $p<0.05$ was considered as statistically significant.

RESULTS

General Features of Experimental Animals

STZ administration produced loss of body weight, increase in food intake, and water intake in rats which are cardinal signs and symptoms of type 1 diabetes. Chronic administration of SDF produced slight protection against loss of body weight, polydipsia, and polyphagia in diabetic rats (Table **1**).

Serum Glucose, Insulin

STZ induced diabetic rats were found to produce significant increase in glucose levels with a corresponding hypoinsulinaemia. Treatment with SDF produced significant ($p<0.05$) reduction in elevated serum glucose and $AUC_{glucose}$ levels by the treatment with SDF. STZ induced diabetic rats also produced significant increase in serum insulin levels in diabetic rats but treatment with SDF did not produce any significant change in insulin levels as compared to diabetic rats (Table **2**).

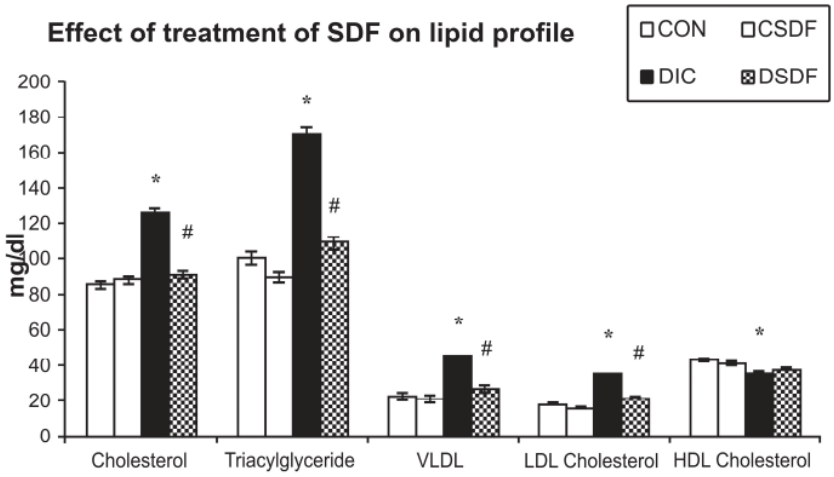

Fig. (1). Effect of treatment of SDF on lipid profile. Each bar represents Mean ± SEM of 6 animals. CON-normal control. COD-normal control animals treated with SDF, DIC-diabetic animals. DID-diabetic animals treated with SDF. ∗ - Significantly different from normal control animals. (P<0.05), #-Significantly different from diabetic group. (P<0.05).

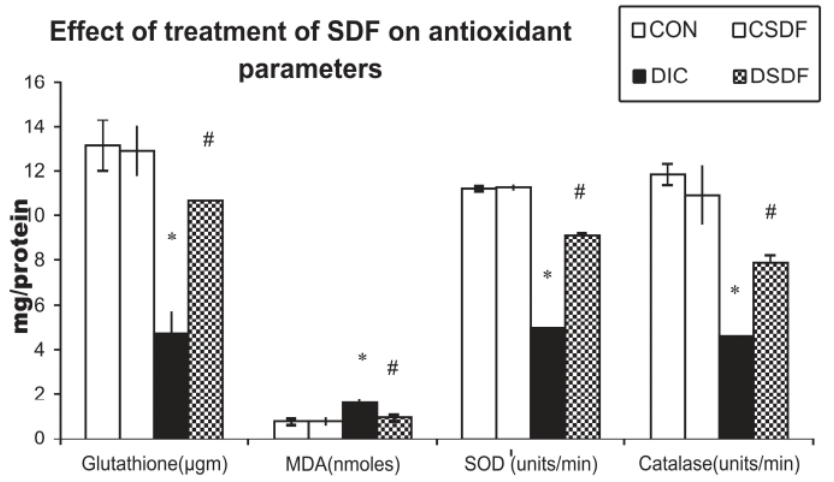

Fig. (2). Effect of treatment of SDF on antioxidant parameters. Each bar represents Mean + SEM of 6 animals. CON- normal diabetic control animals. COD- normal control animals treated with SDF, DIC-diabetic animals, DID-diabetic animals treated with SDF. ∗-Significantly different from normal control animals (P<0.05), #- significantly different from diabetic control (p< 0.05).

Oral Glucose Tolerance Test

In our study it was observed that significant increase in $AUC_{glucose}$ produced in diabetic animals in after glucose load. Treatment with SDF significantly reduced elevated $AUC_{glucose}$ of diabetic animals. $AUC_{insulin}$ of diabetic animal was also significantly decreased and treatment with SDF produced significant change in $AUC_{insulin}$ of diabetic rats as compared to diabetic control animals (Table **2**).

Serum Lipid Profile

Serum levels of all lipids like triglyceride, total cholesterol, LDL-cholesterol and VLDL-cholesterol were found to be increased significantly (p<0.05) in disease control rats as compared to normal control animals by administration of STZ, while, HDL-cholesterol was found to be decreased significantly. Chronic administration with SDF produced a significant improvement in lipid profile in diabetic rats. The increase in HDL-cholesterol levels was observed in treatment groups. However, increase in HDL-cholesterol levels were not found to be statistically significant. Treatment of normal rats with SDF did not produce any significant alteration on lipid profile (Fig. **1**).

Effect Antioxidant Parameters in Liver

Liver homogenate of diabetic rats showed decreased antioxidant enzyme levels. SDF treatment showed significant increase in all these enzyme levels in diabetic rats. Diabetic rats have also shown significant increase in MDA levels in diabetic rats which is a characteristic of lipid peroxidation. Treatment with SDF produced significant decrease in MDA levels. Treatment of normal control animals with SDF did not produce any significant change on the MDA levels (Fig. **2**).

DISCUSSION

STZ induced diabetic rats exhibited increased food and water intake and decreased body weight. These may be due to catabolism of fats and proteins in diabetic rats which cor-

relates with earlier findings which showed that polyphagia, polydipsia condition in diabetes are due to excessive catabolism of fats and proteins which leads to weight loss in diabetic rats [28, 29]. Chronic treatment with SDF produced slight decrease in food and water intake and improvement in body weight loss and this could be due to decrease in catabolic reaction produced by treatment.

STZ, induces diabetes by destroying pancreatic β-cell, which results in reduction in endogenous insulin release, which produced decreased glucose uptake by the tissues [30]. In present study, the SDF caused decrease in glucose and $AUC_{glucose}$ level in STZ induced diabetic rats this may be attributed to either increased insulin production by reestablishing the function of β-cell of pancreas or inhibition of glucose absorption from gastrointestinal tract. SDF showed slight increase in insulin and $AUC_{insulin}$ on oral glucose tolerance test. Previous studies have reported that fruits of *E. officinalis* possess glucose lowering effect in type 1 diabetic rats [31]. The results of the study clearly indicate that decreased glucose level without increase in insulin levels with treatment of SDF to diabetic rats may be related to direct insulin like effect or increased insulin sensitivity to peripheral tissue.

In present investigation STZ induced diabetic rats found to produce increase in lipid levels which correlate with earlier clinical and preclinical findings [32, 33]. Treatment of SDF produced significant improvement in lipid levels. Previous studies have reported that fruits of *E. officinalis* possess lipid lowering activities [34, 35]. Phytochemical study of fruits of *E. officinalis* showed the presence of esters of gallic acid in large amount [12, 36, 37]. The possible mechanism for decreased lipid levels could be either insulin releasing or insulin sensitizing activity of gallotanins, because insulin inhibits the hormone sensitive lipases activity in adipocytes, which inhibits the removal of triglyceride and cholesterol because free fatty acids are involved in the inverse relationship between hormone-sensitive lipase activity and expression in adipose tissue [38]. Thus, SDF also played an important role in lipid metabolism.

Numerous investigations have reported that hyperglycemia contributes to an increased formation of free radicals with reduction in antioxidative enzymes like SOD, GSH, catalase [39, 40]. In present investigation, we observed a decrease SOD, GSH, catalase levels in the liver of diabetic rat correlate with previous findings that in diabetes decrease in antioxidant enzymes take place [41]. Chronic administration of SDF produced antioxidant effect by increase in SOD, GSH and catalase levels these could be due to decreased accumulation of superoxide anion. Polyphenolic principal of fruit juice of *E. officinalis* is reported to have strong antioxidant property and this can be beneficial against free radicals generated by hyperglycemia [42]. Thus, antioxidant protects the body against damage produced by reactive oxygen species. STZ diabetic rat also showed increased level of lipid peroxidation. The treatment with SDF decreased lipid peroxidation significantly in diabetic rats indicates protection. The results of study show that the antioxidant effects of SDF may be due to the reduction in lipid peroxidation and increase in antioxidant enzymes by gallotannins present in SDF, Hence, in addition to antidiabetic and antihyperlipidemic effect, SDF also possess antioxidant activity that may be helpful for preventing diabetes associated complications.

CONCLUSIONS

On the basis of above results, it can be concluded that SDF, exerts a significant antidiabetic, antihyperlipidemic and antioxidant effects. The improvement in diabetes-associated lipid and glucose dysfunction is through different mechanisms and phytochemical analysis showed presence of gallotannin 23-27% w/v. These pharmacological actions may be due to presence of gallotannins as an active principles and SDF can be used as supplementary or adjunct treatment for a long term management of diabetes.

CONFLICT OF INTERESTS

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by National Facilities in Engineering and Technology with Industrial Collaboration (NAFETIC) scheme from Department of Science and Technology, New Delhi, India.

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