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Modulation of diabetes and associated complications using ethanolic extract of green fruit of *Ficus racemosa Linn.* in alloxan induced rats

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Abstract: The present study was undertaken to explore the antidiabetic potentials of ethanolic extract of unriped fruits of *ficus Recemosa* L. (EEFFR) on Alloxan-induced diabetic rats. Hyperglycemia induces the generation of free radicals which can affect antioxidant defenses and induces oxidative stress leading to diabetes associated complications. The diabetes was induced by intraperitoneal injection of ice-cold aqueous Alloxan monohydrate at dose of 150 mg/kg body weight. After a daily single oral administration of the EEFFR at 400 mg/kg for 28 days starting from study protocol, the blood glucose, serum glutamate pyruvate transferase (SGPT), serum glutamate-oxaloacetate transaminase (SGOT), total cholesterol (TC) and triglyceride (TG), total insulin, glycosylated hemoglobin (HbA1c) and C-reactive protein (CRP), superoxide dismutase (SOD) levels were assessed. The results obtained from the study suggested that administration of EEFFR at 400 mg/kg significantly reduced the blood glucose, SGPT, SGOT, TC, TG and CRP, SOD, total insulin and glycosylated hemoglobin. The results obtained were comparable to that of standard glibenclamide. The TC, TG and CRP, HbA1c, SOD and total insulin serum level was significantly altered in diabetic control group but, it was significantly decreased in extract treated groups and standard glibenclamide treated group. During this study protocol, we found that novelty alteration in level of total insulin and HbA1c. The finding obtained suggested that EEFFR act at cellular level modulating the pathways responsible diabetes and associated complications. The results obtained suggest that the EEFFR significant potential for management of diabetes and associated complications.

Key words: SGPT, SGOT, CRP, HbA1c and SOD.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder which results, from absolute insulin deficiency or insulin resistance or inadequate secretion of insulin. Insulin is required for normal metabolism of glucose, fat, protein and lipid. Insulin dependent diabetes mellitus or Type I caused by autoimmune destruction of beta cells of pancreas causing absolute insulin deficiency [1]. In Type II diabetes there is insulin resistance or inadequate secretion of insulin from beta cells of pancreas. The principle outcome of both forms of diabetes is hyperglycemia. Sustained hyperglycemia is responsible for is responsible for oxidative stress, impaired antioxidant balance, glycosylation and altered structural proteins and abnormal metabolism of proteins, lipids and carbohydrate leading to microvascular complications such as diabetic nephropathy, retinopathy, neuropathy and macrovascular complications cardiomyopathy[2]. The prevalence rate of diabetes in South East Asia and especially in India increasing day by day than rest of world. The prevalence rate for type 2 diabetes is increasing sharply from 19.4 million to 80.4 million in 2030 which, would of half of the world [3]. WHO have recommended evaluation and scientific validation of traditional herbal medicine system for management diabetes as they are safe, devoid of side effect least toxic and more effective than synthetic oral hypoglycemic drugs. Wide variety herbal species all over the globe are known to have hypoglycemic, hypolipidemic, and both pharmacological actions [4]. Even presence potent

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synthetic hypoglycemic agent in health care system, discovery of novel antidiabetic oral agent from natural herbal system remains attractive because they contains active phytopharmaceutical that have an alternative, effective and safe pharmacological effect on diabetes mellitus. *Ficus Recemosa* L. is an evergreen large spreading with 15-18 M height belonging to family Moraceae found most commonly in warmer part of Asia, South Africa, Australia and America. The various part of ficus recemosa such as leaves, stem bark, roots, fruits, trunk bark and latex are used in Ayurveda. In Ayurveda all parts of *Ficus recemosa* L. have claimed high therapeutic potentials for the treatment of inflammatory disorders, jaundice, dysentery, diarrhea, biliary disorders, and diabetes etc. [5,6].

Material and Methods

The green unriped green fruits of Ficus recemosa L. were collected from Osmanabad districts of Maharashtra in month of May-June month. Herbarium authenticated at Manjara charitable trust Ayurvedic medical college Latur voucher specimen no. MCT/AMC 2018/201 and Botanical Survey of India Pune, where voucher specimen is deposited.

Preparation of plant extract.
The fruits were shed dried for two days at room temperature. 1 kg shed dried fruits were grinded and converted to powder form. The powder is passed through sieve no.40 and stored in airtight container. 150 gm powder is taken and refluxed and extracted by using soxhlet extraction apparatus using ethanol as solvent for 24 hrs. The alcoholic portion was transferred in rota-vapor (temp of bath 50°C, RPM – 110,) until complete drying of alcoholic extract occurs which was dark brownish green. The percentage yield of extracts was 5%. Ethanolic extracts were prepared in 2% gum acacia solution for oral administration.

Phytochemical screening.
The preliminary Phytochemical study was carried out to detect the various chemical constituent such bioflavonoids, glycosides, alkaloids, tannins and other various Phytochemical constituents in the extract.

Chemicals and reagents.
All the chemicals and reagent used in present studies were of analytical grade, the diagnostic kits used for the estimation of SGPT, SGOT, lipid profile, CRP, HB_{A1C} were obtained from Span Diagnostic, India. Rat insulin ELISA kit Crystal Chem Germany. Alloxan monohydrate from Sigma - Aldrich India and glibenclamide is received as gift sample from Wockhardt pharmaceutical.

Acute toxicity and dose fixation study.
The acute toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) 401 guidelines. Different doses (50–2000 mg/kg, p.o.) of EEFFR were given to six mice and observed continuously at every hour for 24 hrs. For any physical signs of toxicity, behavioral such as writhing, gasping, palpitation, and mortality up to 14days [7,8].

Animals.
Healthy male and female rats weighing 250 g and 300 g were procured from Wockhardt Research Centre, Aurangabad (Maharashtra, India) and maintained in standard animal cages at a temperature of 25 ± 1◦C and relative humidity of 50–60% with 12-hour light and dark cycle. The animal experiments were carried out CPCSEA guidelines, India. The experimental protocol was approved by the Institutional Animal Ethics Committee (CPCSEA/IAEC/P-ceutics-37/2018-19/147). During the animal experiments, animal were fed with standard pellet diet and free water ad libitum.

Induction of diabetes and experimental design.
Diabetes was induced by intraperitoneal injection of ice-cold aqueous Alloxan monohydrate at a dose of 150 mg/kg body weight in animals fasted overnight. After 48 hours, blood samples were collected from retro-orbital puncture and the blood sugar level was determined by glucometer (Accu Check, Johnson and Johnson, India). The animals showing blood sugar level more than 225 mg/dL were considered as hyperglycemic and selected for the studies. After distribution of animals into different groups all the animals have given the two units of 1.5 unit of insulin for five days two reduce the mortality rate and stabilizes the animals. After seven days all the animals received the treatment as per study protocol [2,9]. After seven days of induction of diabetes, all groups have given the treatment of extract and standard orally for 28 days At the end of experimental study biochemical parameters such antioxidant parameters such as SOD and lipid profile, SGPT, SGOT, C reactive protein, total insulin level and (HB_{A1C}) were estimated from the serum of Alloxan induced diabetic rats. The animals are randomly divided

into following groups containing six animals in each group (*n* = 6) as follows.

- Group I.   Normal control receiving potable water
- Group II. Diabetic control receiving potable water.
- Group III.   Standard   diabetic   receiving glibenclamide 10 *mg /kg*.
- Group V. Diabetic receiving EEFFR 400 *mg/kg*.

Biochemical estimations.
The SGPT and SGOT were determined by Reitman and frankel colorimetric method. TC was estimated by the method of using a modified Triender method. The serum (0.01 ml) was added to 1ml of standard reagent and incubated at 37°C for 5 min; the absorbance was measured at 500 nm. The content was expressed as mg/dl. TG was determined using glyceryl phosphate oxidase method. To 0.01 ml of the serum, 1ml of standard reagent was added and incubated at 37°C for 5 min; the absorbance was measured at 500 nm [10, 11, and 12]. The content was expressed as mg/dl. C-reactive protein is measured by colorimetric principle [13]. Total insulin is measured as per the procedure given in Rat insulin ELISA kit by Crystal Chem Germany. HB$_{A1C}$ measured as per reference [14]. SOD level in serum was estimated as per

reference no [15]. SOD level was expressed as mU of SOD/mg protein.

Statistical analysis.
Statistical analyses of all the results were performed by one-way ANOVA followed by Dunnet's multiple comparison test by using graph pad instat 3 software. Results were expressed as mean ± S.E.M. Statistical significance level was set at p < 0.01.

Results
Phytochemical analysis.
Preliminary phytochemical analysis of crude extract of EEFFR revealed that presences of flavonoid, tannins, glycosides, alkaloids saponins and triterpenoids and phenols.

Effect of EEFFR on blood glucose level.
During   this   study   protocol,   we   observed hypoglycemic effect of EEFFR in Alloxan diabetic rats illustrated **Table 1.** The group treated with EEFFR   at   400   mg/kg   showed   significant hypoglycemic activity on day 7th, 14th, 21st and 28th day when compared to diabetic control group. The standard group received glibenclamide 10 mg/kg which significantly reduce the blood glucose level during study period.

Table 1. Effect of Ethanolic extract EEFFR on blood glucose level on Alloxan induced diabetic rats.

Groups	Dose/ treatment	Effect on blood glucose level (Day)			
		7	14	21	28
Normal control	Normal saline (2*ml/kg*)	106 ± 1.96**	106 ±3.3**	103± 1.9**	98 ± 2.5**
Diabetic control	Tap water (2*ml/kg*)	280± 3.3	286 ± 2.11	286 ± 1.2	285± 2.4
EEFFR	400 *mg/kg*	281 ± 1.7**	204 ±2.7**	208 ± 1.5**	202 ± 0.7**
Glibenclamide	10 *mg/kg*	243±4.8 **	188 ±3.1**	189 ±1.1**	193±0.71**

Values are expressed as mean ± S.E.M; *n*=6, ** *P* < 0.01 significant from diabetic control

Table 2. Effect of Ethanolic extract EEFFR on TC, TG, and HDL level on Alloxan induced diabetic rats.

Parameters	Day	Normal control 2 *ml/kg* N. saline	Diabetic control 2 *ml/ kg* tap water	Standard 10 *mg/kg*	EEFFR 400 *mg/kg*
TC	7th D	99 ±1.1	101±0.9	97±1.4	98±2.3
	28th D	94±0.4**	196±1.3	122 ±1.1**	136±0.7**
TG	7th D	87.1±1.2	92.6±1.12	85±1.1	87±1.7
	28th D	85±2.7**	146±1.07	105.5±0.9**	106 ±1.8**
HDL	7th D	58±0.5	52±0.7	55±0.8	54±08
	28th D	62.3±0.9**	44±1.2	62±0.6**	53.2±0.7**

Values are expressed as mean ± S.E.M; *n*=6, ** *P* < 0.01 significant from diabetic control. D = days

Effect of EEFFR on serum lipid profile
Lipid abnormalities is most commonly found in Alloxan   and   streptozotocin   induced   diabetic model. TC, TG and were determined on day first and at end of experimental study to find out the significant changes in lipid profile after treatment

with EEFFR extract and standard glibenclamide. We found that, on day first of experimental study there were no notable and significant changes in lipid profile but, at end of study it has been observed that treatment of EEFFR for 28th day significantly alters the lipid abnormalities. The

results obtained were comparable to standard group and normal control when compared diabetic group as shown table no.2.

Effect of EEFFR on serum SGOT and SGPT.
The SGOT and SGPT activities are known to be cytosolic marker enzymes indicating hepatocellular damage, they released into the

blood after hepatocellular membrane damage. Therefore, increased level of SGOT and SGPT is an indicator of hepatic damage. During this study protocol, we found that there is significant alteration in level of SGOT and SGPT in diabetic animals. The treatment of EEFFR significantly reduced the level of SGOT and SGPT at the 28th day.

Table 3. Effect of EEFFR on serum SGOT and SGPT.

Parameters	Day	Normal control 2 *ml/kg* N. saline	Diabetic control 2 *ml/ kg* tap water	Standard 10 *mg/kg*	EEFFR 400 *mg/kg*
SGOT	7th D	53.3±0.6**	66±1.5	61±0.9**	64.3±0.9**
	28th D	53.1±0.7**	115±1.6	73.3±0.8**	80±2.6**
SGPT	7th D	17±0.80**	34.5±1.3	23±0.4**	24±0.5**
	28th D	20±0.5**	46.5±0.7**	29.8±0.7**	34.6±0.6**

Values are expressed as mean ± S.E.M; *n*=6, ** $P < 0.01$ significant from diabetic control.

Effect of EEFFR on serum level of insulin, glycosylated HB, CRP and SOD.
Estimation of total insulin was done at the end of experiment. It has been observed that administration of EEFFR for 28th days at a dose 400 mg significantly increased the serum insulin levels of SOD (285.5±16) whereas, SOD level in untreated diabetic animals was significantly decreased. Chronic hyperglycemia were observed in Alloxan induced diabetic rats, which results into glycosylation of structural protein. In this study it was noted that increased in level of glycosylated hemoglobin (8.5±0.4), while in group treated standard and EEFFR significantly decreased the

level of it (5±0.2 and 5.8±0.4) respectively. CRP is an independent inflammatory marker and it is associated with both forms diabetes. CRP level was significant higher in untreated diabetic rats as compared to normal control. Administration of EEFFR and standard significantly reduced the level of CRP as compared to diabetic control. Alloxan induces oxidative stress and generation free radicles causing decreased antioxidant enzymes. In our study it was observed that there is significant decreased level of SOD in untreated diabetic groups in contrast to it, there is significant increased antioxidant enzyme in EEFFR and standard treated groups.

Table 4. Effect of EEFFR on serum level of insulin, glycosylated HB, CRP and SOD.

Groups	Dose/ treatment	*Level of insulin* (*μ*/L)	HBA1c %	CRP (Mg/L)	*SOD* (*mU/mg protein*)
Normal control	Normal saline (1ml/kg)	5.5±0.4**	2.9±0.2**	21.8±0.7**	584.1±1.19**
Diabetic control	Tap water (1ml/kg)	1.6±0.3	8.5±0.4	82.5±0.4**	236±1.08**
Standard	10 mg/kg	3.6±0.4**	5±0.2**	52.5±0.5**	323±1.6**
EEFFR	400 mg/kg	3.4±02**	5.8±0.4**	56.8±1.3**	285.5±16**

Values are expressed as mean ± S.E.M; *n*=6, ** $P < 0.01$ significant from diabetic control. D = days.

Discussion

Diabetes mellitus causes various complications such as metabolic, microvascular and macrovascular complications through various mechanisms such as free radical generation, oxidative stress and inflammation. Thus, it would be worthwhile to find a medicinal plant with anti diabetic properties with antioxidants and inflammatory plant for the treatment of diabetes and associated complications [16]. If such herbs are used a lonely or with adjuvantly allopathic medicine, the complications can be modulated or ameliorated. Alloxan is responsible for destruction

of beta cells by its cytotoxic effect causing non-insulin dependent diabetes [17]. Thus, the present study was undertaken to evaluate the diabetes and associated complication modulating effect of EEFFR on Alloxan-induced diabetic rats. So far, this is the first report of in vivo anti diabetic and associated complication modulating effect green fruit of Ficus recemosa. Result obtained suggested that EEFFR at dose 400 mg/kg showed significant hypoglycemic, hypolipidemic, insulin, CRP, SGOT and SGPT modulating effects. The hypoglycemic effect of EEFFR is comparable to the hypoglycemic effect of glibenclamide, which may indicate that

EEFFR enhances plasma insulin action by increasing pancreatic secretion or reactivity of insulin from residual beta cells of Langerhans islets. The mechanism of EEAP may be similar to glibenclamide, which has an insulin-independent mechanism and insulin releasing effect observed from total insulin. Plant extracts may act by reducing hepatic glucose production, inhibiting intestinal glucose uptake or correcting insulin resistance [18]; however, the possibility that other mechanisms of hypoglycemia cannot be ruled out. Changes in lipid and protein metabolism occurred most commonly in alloxan-induced diabetic rats, as observed by increased TC, TG, HbA1c and decreased HDL levels. Sustained hyperglycemia leads to glycosylation of structural protein leading to increased level of HbA1c. The hypertriglyceridemia observed in alloxan-induced diabetic rats can be attributed to increased absorption and formation of TG, as well as decreased uptake of TG in peripheral tissues. Hypercholesterolemia may be due to an increase in changes in enzyme pathways for cholesterol metabolism or an increase in cholesterol absorption in the diet [19, 20]. Decreased HDL was also observed in diabetes. Our results obtained from EEFFR study shows the significant reduction TC, TG, glycosylated hemoglobin and increased level of HDL. The Possible mechanism of EEFFR is like glibenclamide by increasing insulin production from residual beta cells or activating plasma insulin response in alloxan-induced diabetic rats, increasing the activity of the enzyme lipoprotein lipase and peripheral tissue utilization of cholesterol [20, 21]. SGPT and SGOT are intracellular enzymes released into the bloodstream, and they serve as markers of tissue damage, mainly liver cells and in kidney cells damage. Our results obtained suggest that 28 days of oral administration of EEFFR in our study showed a significant reduction in the activity of these enzymes, and EEFFR exerts hepato protection and lipid metabolism modulating the diabetic associated hepatic and metabolism complications. Alloxan produces reactive oxygen species, which decreases the antioxidant mechanism. As a result of this higher concentration of free radicals in plasma, increased oxidative stress, which is of particular importance in the pathogenesis of various diseases including cardiovascular diseases and diabetes.

CRP is an inflammatory marker independent, which is associated not only with type 1 diabetes but also with type 2 diabetes. [22, 23]. It has been observed that after 28-day administration of EEFFR in diabetic rats induced by alloxan, EEFFR reduces the level of production of CRP and SOD probably by reducing the level of plasma concentration of interleukin-6 and the activities of elimination of free radicals modifying inflammatory and antioxidant pathways.

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