Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 4, 2015

Original Article

ANTIDIABETIC AND HYPOLIPIDEMIC EFFECT OF FICUS RACEMOSA PETROLEUM ETHER EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS

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Received: 20 Jan 2015 Revised and Accepted: 15 Feb 2015

ABSTRACT

Objective: The present study was undertaken to evaluate the antidiabetic, hypolipidemic and toxic effects of petroleum ether extract of FR (PEFR) using streptozotocin (STZ) induced diabetic rats.

Methods: Diabetes was induced by administration of STZ (50 mg/kg) intraperitonially (i. p.) to albino rats. PEFR was administered once in a day for a period of seven days at doses of 100, 200 and 300 mg/kg according to body weight. Blood glucose and body weight changes were measured at different (1st, 3rd, 5th, and 7th) days of experiment. Serum lipid profile (TC, TG, LDL, VLDL, and HDL) and serum hepatic biomarker enzymes (SGOT and SGPT) levels were measured, and various antioxidant parameters in liver and pancreas were also determined at the end of experiment.

Results: Our results collectively suggested that oral administration of PEFR significantly reduced blood glucose level and restored body weight. This extract also reduced serum cholesterol, triglycerides, LDL, VLDL and improved HDL as compared with diabetic control group, signified hypolipidemic action. It increased glutathione and various enzyme levels (catalase and superoxide dismutase) in the pancreas at the same time. Various oxidative stress parameters like thiobarbituric acid reactive substances and protein carbonyl levels in liver were decreased after PEFR administration with respect to diabetic control rats.

Conclusion: PEFR possessed antidiabetic, antioxidant and hypolipidemic activities in STZ induced diabetic rats, which supported the use of FR as a food supplement for future drug design perspective.

Keywords: *Ficus racemosa*, Streptozotocin, Antidiabetic, Hypolipidemic.

INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic disorders, distinguished by increased blood glucose level due to either inadequacy of insulin production or insulin resistance in the body. Deficiency of insulin results in impaired glucose, protein and lipid metabolism [1]. The pervasiveness of DM is anticipated to increase by 42% in developed and by 170% in developing countries in 2025 [2]. In defiance of the presence of lots of synthetic drugs, herbal drugs are prudent due to their less side effects and pronounced efficacy. Alkaloids, triterpenoids and steroids reported to have antidiabetic activity still ancient years [3] and at least 800 plants are available in nature for curing diabetes [4]. The most acceptable procedure for diabetes testing is streptozotocin (STZ) induced rat model where liver and pancreatic tissues are damaged due to generation of reactive oxygen species [5].

According to World Health Organization, there were 1.71 billion diabetic patients in the year 2000 and this is evaluated to increase up to 3.66 billion by 2030, mainly attributable to their life style and eating habits [6, 7]. Many synthetic drugs are available in the market for treatment but they have imperfection due to adverse effects. Therefore, it is necessary to investigate and explore the plant origin hypoglycemic agents to get the better safety profile. A present survey revealed that 70% diabetic patients used plant based medication to consummate their basic health requisite related to diabetes [8]

The natural product, *Ficus racemosa (*FR) belongs to family Moraceae, widely distributed all over India, China, Australia and Southeast Asia. Many bioactive chemical constituents had been isolated from different parts of this plant [9]. Plant leaves contain various types of alkaloids, tannins, sterols and flavonoids. FR leaves reported to possess hepatoprotective, anti-inflammatory, and antibacterial activities [10-12]. β-sitosterol and stigmasterol had been isolated from bark of FR, had potent antidiabetic activity [9] and this compound is present in leaves also. This information recommended that leaves might have antidiabetic activity due to the presence of β-sitosterol. β-sitosterol is phytosterol which can be extracted from non-polar (petroleum ether) solvent [13]. Therefore, the main emphasis of this study was to evaluate the antidiabetic activity of petroleum ether extract of FR (PEFR) in STZ model using albino wistar rats. Furthermore, the role of PEFR in curing STZ induced oxidative stress and hyperlipidemia had also been assessed in this study.

MATERIALS AND METHODS

Plant material and preparation of extract

The fresh leaves of FR were collected during the month of July from Lucknow, Uttar Pradesh, India and authenticated by Department of Horticulture, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow. The plant materials were air dried under shade, powdered and extracted with petroleum ether (60^0-80^0C) with Soxhlet apparatus by successive solvent extraction method. Finally, the extracted samples were evaporated by using rotary vacuum evaporator. The final yield was 12% and petroleum ether extract of FR (PEFR) was used for further studies.

Experimental animals

Healthy adult albino rats (125 – 150g) were used for the study and obtained from CSIR-CDRI, Lucknow (protocol was approved by Institutional Animal Ethical Committee, approval no. UIP/IAEC/2014/FEB/10). Rats were housed in polypropylene cages in standard environmental conditions (temperature 25±5^0C, relative humidity 55±10%). All the animals were acclimatized in laboratory condition for 7 days. The rats were fed on a standard pellet diet and had free access to water during acclimatization.

Induction of diabetes

Hyperglycemia was induced in albino rats by the single dose of STZ (50 mg/kg, intraperitonially) reconstituted in normal saline after overnight fasting. On 5th day after STZ administration, the blood sample was collected through tail vein puncture and blood glucose level was measured using one touch select Glucometer (Johnson & Johnson, India) strips. Rats with fasting blood glucose level 250 mg/dl were considered for hyperglycemic condition [1, 14].

Experimental design

Albino wistar rats were randomly divided into six groups (n=6). Group I served as normal control and received vehicle orally (N control) (0.25% carboxy methyl cellulose [CMC], 1 ml/kg body weight). Group II served as diabetic control, received 0.25% CMC (1 ml/kg body weight) (D control). Group III, IV, V and VI were given glibenclamide (G, 10 mg/kg), PEFR (100 mg/kg), PEFR (200 mg/kg) and PEFR (300 mg/kg) orally, respectively. All these doses were administered after 5th day of STZ administration (except N control) and were given for seven days. Body weight and blood glucose were measured with strips on 1st, 3rd, 5th, and 7th day of treatment. On 8th day, blood was collected for further biochemical estimation, animals were sacrificed by cervical decapitation and organs like the pancreas and liver were dissected out and rinsed with ice cold saline and stored at-20°C for further studies.

Biochemical estimation of blood glucose, liver glycogen level and serum lipid profile

Blood glucose level was measured by one touch select glucometer strips. Liver glycogen level was estimated by using anthrone method [15]. Other estimations such as high density lipoprotein (HDL), total Triglycerides (TG) and total cholesterol (TC) in serum were also measured spectrophotometrically by using lipid profile kit (Erba Diagnostics, India). Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were calculated using Friendewald's Formula [16].

LDL (mg/dl) = TC – HDL – (TG/5)

VLDL (mg/dl) = TC – HDL – LDL

Determination of oxidative parameters

The oxidative parameters like thiobarbituric acid reactive substances (TBARS) [17], protein carbonyl (PC) [18] were measured in liver. Other parameters like, superoxide dismutase (SOD) [19], tissue catalase (CAT) [20], glutathione (GSH) [21] level were estimated in pancreatic tissue in the similar experiment.

Determination of liver function test

Liver function biomarkers like aspartate aminotransferase (AST), alanine aminotransferase (ALT) were also measured in serum using commercially available kit from Recombinogen Pvt. Ltd, India [22].

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5.0 (San Diago, CA, USA). All results were expressed as mean±standard deviation (SD). The data was analyzed by one-way ANOVA (analysis of variances) followed by Bonferroni multiple comparison test. For biochemical estimations, statistical significance differences were considered with respect to D control ($^{a}P<0.001$, $^{b}P<0.01$, $^{c}P<0.05$).

RESULTS

Antihyperglycemic effect of PEFR

Changes in blood glucose level in all groups were tabulated in table 1. Fasting blood glucose level of the normal control group were 89.75±6.39 mg/dl in 7 days of study, while there was the significant increase in blood glucose level of D control group (303±9.93mg/dl) in the similar experiment. G treated group caused significant reduction of blood glucose level from 275.75±8.77 to 178.75±6.02 mg/dl. Oral administration of PEFR (100, 200, 300 mg/kg) showed a significant reduction of blood glucose level as compared with D control group. PEFR (300 mg/kg) exhibited maximum hypoglycemic effect with reduction of glucose concentration from 290 to 205 mg/dl as compared to other doses of PEFR.

Effect of PEFR on body weight in diabetic rats

As depicted in table 2, Body weight of normal control group increased as compared with D control. D control group showed maximum percentage (-12.2%) of weight loss till the end of experiment. Treatment with PEFR (100, 200 and 300 mg/kg) exhibited improved body weight as compared with D control group. Maximum improvement (5.51%) was observed in PEFR (300 mg/kg) dose as compared to lower two dosages.

Table 1: Effect of PEFR on blood glucose level (mg/dl) on STZ induced diabetic rats.

Groups	1st day	3rd day	5th day	7th day
N Control	89.75±6.39	92.75±3.86	94.23±3.44	94.51±2.38
D Control	282.50±10.34	286.08±8.08	293.75±9.18	303.09±9.93
D+G (10 mg/kg)	275.75±8.77	254.5±9.38[a]	220.25±11.44[a]	178.75±6.02[a]
D+PEFR (100 mg/kg)	290.06±5.54	288.11±4.65	281.37±5.11	278.33±2.54[a]
D+PEFR (200 mg/kg)	295.5±7.74	281.27±7.50	260.75±2.87[a]	223.12±5.47[a]
D+PEFR (300 mg/kg)	290.33±4.56	270.54±4.67[b]	240.38±5.52[a]	205.13±5.42[a]

Data represented as mean±SD. Statistically significant differences were observed between D control and G/PEFR groups (100, 200 and 300 mg/kg) [one way-ANOVA followed by Bonferroni multiple comparison test; $^{a}p<0.001$, $^{b}p<0.01$]

Table 2: Effect of PEFR on body weight (gm) on STZ induced rats.

Groups	1st day	3rd day	5th day	7th day	% Change in body weight
N Control	145.08±2.01	148.02±6.05	156.23±9.08	168.34±4.09	15.80
D Control	147.21±7.12	142.13±4.09	132.33±6.10	129.19±3.10	-12.20
D+G (10 mg/kg)	145.25±3.07	147.34±6.14	151.31±7.16[a]	157.18±2.00[a]	8.27
D+PEFR (100 mg/kg)	144.15±7.03	145.41±4.15	145.32±6.03[b]	146.23±3.04[a]	1.38
D+PEFR (200 mg/kg)	146.17±5.11	147.35±3.08	149.20±2.07[a]	151.09±3.18[a]	3.42
D+PEFR (300 mg/kg)	145.27±7.22	148.25±6.10	149.19±3.07[a]	153.08±5.19[a]	5.51

Data represented as mean±SD. Statistically significant differences were between D control and G/PEFR groups [one way-ANOVA followed by Bonferroni multiple comparison test; $^{a}p<0.001$, $^{b}p<0.01$]

Effect of PEFR on glycogen content and lipid profile in diabetic rats

Glycogen content in liver is an important parameter to measure hypoglycemic effect of drugs. Oral administration of PEFR at various doses showed statistically significant increase of glycogen content in liver with respect to D control group. The glycogen reduction effect of PEFR at 300 mg/kg (30.34±2.11 mg/gm) was comparable to standard control (G group, 31.01±1.13 mg/gm) (table 3).

In the present study, we observed that cholesterol level was significantly elevated in D control group (193±12.05 mg/dl) as compared to normal control group 78.75±5.12 mg/dl. The treatment with PEFR (200 and 300 mg/kg) showed significant reduction in cholesterol level (157.5±6.45 and 150.33±2.43 mg/dl) as compared

with D control group after 7 days of treatment. Similar trends were observed for TG, LDL and VLDL where we found that there was a significant reduction of all these parameters with respect to D control group (table 3). Treatment with PEFR significantly reversed the TG level from 160 to 134 mg/dl where the effect was comparable to standard. Similarly, significant attenuation was observed in the case of LDL from 134 to 91 mg/dl in the dose dependent manner after PEFR treatment. Moreover, PEFR and G groups showed significant reduction in VLDL level as compared with D control group. Opposite trend was observed for HDL level where treatment with PEFR and G (glibenclamide) improved HDL level as compared to D control group (table 3).

Table 3: Effect of PEFR on glycogen content in liver and lipid profile in serum (TG, TC, HDL and LDL) on STZ induced rats.

Groups	Glycogen (mg/gm)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
N Control	39.46±2.62	78.75±5.12	59.5±6.55	38.75±3.30	28.1±1.36	11.9±0.29
D Control	17.03±1.78	193±12.05	160.5±6.75	26.75±3.5	134.15±2.19	32.1±1.08
D+G (10 mg/kg)	31.01±1.13[a]	142.5±4.20[a]	126.25±4.75[a]	35.75±2.21[a]	81.5±2.08[a]	25.25±1.96[a]
D+PEFR (100 mg/kg)	22.45±2.23[a]	180±8.97	160±4.98	26.55±2.37	121.45±1.39[a]	32.09±1.73
D+PEFR (200 mg/kg)	29.82±1.91[a]	157.5±6.45[a]	140±2.58[a]	28.5±3	101.45±1.98[a]	28.1±1.55[a]
D+PEFR (300 mg/kg)	30.34±2.11[a]	150.33±2.43[a]	134.66±3.35[a]	32.11±2.55[c]	91.29±1.06[a]	26.6±1.18[a]

Data represented as mean±SD. Statistically significant differences were between D control and G/PEFR groups [one way-ANOVA followed by Bonferroni multiple comparison test; [a]p<0.001, [c]p<0.05]

Determination of oxidative stress parameters in liver and pancreas

We measured various oxidative stress related parameters like GSH, SOD, CAT (in pancreas) and MDA, PC (in liver) to evaluate the toxicological parameters in albino rats. As depicted in table 4, it was observed that GSH (reduced) level was found to be decreased in D treated group (~ 29 µM/mg of protein) than N control (~ 46 µM/mg of protein). It was found that GSH level was restored to normal for both G and PEFR treated groups.

When we estimated total TABRS concentration, we observed that TABRS level was higher in D group (~ 0.93 nM/mg of protein) than normal control (~ 0.37 nM/mg of protein, table 4). This concentration again normalized for G and PEFR treated groups. Similar trends were observed for PC assay where we found that PC formation was lower for both positive control and treated groups than D control (table 4).

Separately, we measured liver CAT and SOD enzymes to determine the oxidative based toxicity. CAT is most abundant in the liver which is mainly responsible for the catalytic decomposition of H_2O_2 to oxygen and water. Increase in concentration of H_2O_2 in PEFR treated sample depicted that there was higher amount of CAT enzyme available in the tissue to decompose the H_2O_2 with respect to D control. As shown in table 4, it was observed that SOD enzyme level also increased in PEFR treated groups.

The effect of PEFR on ALT and AST levels (hepatic biomarker enzymes) in serum was also observed during experiment. In the D control rats, serum ALT and AST levels were elevated with respect to N control. The treatment with PEFR and G reduced these enzyme levels (p<0.001) as compared to D control (table 5).

Table 4: Effect of PEFR on SOD, CAT, GSH in pancreas and TBARS and PC in liver on STZ induced rats.

Groups	SOD (Unit/mg of Protein)	CAT mM H_2O_2 decomposed/min/mg of protein	Reduced GSH (µM/mg of Protein)	TBARS (nM of MDA/mg of protein)	PC (µM/mg of protein)
N Control	5.93±1.09	72.16±0.98	46.21±2.87	0.37±0.07	54.76±2.97
D Control	3.42±1.42	51.06±1.27	29.64±1.29	0.93±0.11	137.85±4.63
D+G (10 mg/kg)	7.62±0.87[a]	63.78±1.14[a]	38.02±0.93[a]	0.47±0.04[a]	78.32±4.57[a]
D+PEFR (100 mg/kg)	4.95±0.65	50.11±2.45	29.65±2.05	0.87±0.06	120.54±6.33[a]
D+PEFR (200 mg/kg)	5.01±0.76	58.42±1.63[a]	32.17±1.22	0.71±0.05[a]	104.43±3.68[a]
D+PEFR (300 mg/kg)	5.69±0.48[b]	60.15±3.22[a]	34.13±1.67[a]	0.54±0.03[a]	86.02±3.47[a]

Data represented as mean±SD statistically significant differences were between D control and G/PEFR groups [one way-ANOVA followed by Bonferroni multiple comparison test; [a]p<0.001, [b]p<0.01]

Table 5: Effect of PEFR on AST and ALT in serum on STZ induced rats.

Groups	AST (U/dl)	ALT (U/dl)
N Control	31.45±3.28	52.56±2.60
D Control	75.19±3.71	97.39±2.94
D+G (10 mg/kg)	43.36±1.93[a]	74.05±2.01[a]
D+PEFR (100 mg/kg)	56.47±2.45[a]	90.11±2.65[a]
D+PEFR (200 mg/kg)	50.94±1.62[a]	82.38±1.92[a]
D+PEFR (300 mg/kg)	47.23±2.27[a]	78.43±1.43[a]

Data represented as mean±SD. Statistically significant differences were between D control and G/PEFR groups [one way-ANOVA followed by Bonferroni multiple comparison test; ap<0.001]

DISCUSSION

Plant derived secondary metabolites like alkaloids, flavonoids and triterpenoids reported to acquire good antidiabetic properties. Increasing evidence demonstrated that FR has the capacity to reduce glucose and lipid metabolism. β-sitosterol and stigmasterol obtained from FR, had been reported to possess potent antidiabetic activity [23]. Both these triterpenoids are present in leaves of FR and isolated in petroleum ether as they are very non polar in nature. Hence, the objective of our project is to investigate the antidiabetic and hypolipidemic effects of petroleum ether extract of FR (PEFR) leaves. To achieve this goal, PEFR was administered orally to STZ induced diabetic rats and various parameters like blood glucose, lipid profile and liver glycogen were measured during experiment.

Our present study promulgated that oral administration of PEFR caused diminution of blood glucose level in the dose and time dependant manner as compared with D group (table 1). Antihyperglycemic consequences of extract could be either attributed to increase plasma insulin level, regeneration of pancreatic β cells or by increasing the peripheral utilization of glucose [23]. Loss of body weight is a common characteristic parameter of diabetes due to excessive protein catabolism for the gluconeogenesis [24]. Body weight consistently reduced in the D group in the similar experiment. Treatment with G and PEFR significantly improved body weight as compared with D control group, due to increased formation of structural proteins (table 2). Glycogen synthesis was altered and reduced in liver which was observed in D control group. Treatment with both G and PEFR significantly improved liver glycogen level in the dose dependant manner (table 3).

Impaired lipid metabolism is another complication, resulted in dyslipidemic condition in diabetes [25]. Elevated TG, TC, LDL and VLDL levels are the primary factors for coronary artery disease and atherosclerosis like complications in diabetes. As depicted in table 3, it was observed that PEFR significantly reduced the TG, TC, LDL and VLDL and improved HDL level as compared to D control. All these results signified that PEFR had good antidiabetic and hypolipidemic effect on STZ induced diabetic rats.

STZ contains free –NO-group which has tendency to release nitric oxide radicals (NO˙) and this radical ultimately generates reactive species (ROS) and free radicals. These free radicals bind with cellular macromolecules and cause toxicity mainly in liver and pancreatic cells [26]. Free radicals are generated during diabetic condition due to nonenzymetic glycation of proteins and glucose degradation [27]. Therefore, it is necessary to investigate the toxicity profile of STZ in this study. While our PEFR extract showed good antidiabetic potential, the question arises whether PEFR had any effect to reduce STZ induced toxicity in pancreas and liver.

The elevation of hepatic TBAR level indicated the enhanced formation of oxidized lipid in D control rats due to over production of ROS. Treatment with PEFR significantly transposed the TBARs level, indicated that extract prevented the lipid peroxidation caused by free radicals. The carboxyl group of protein becomes oxidized due to formation of ROS [28] and converted to PC which is an important marker for oxidative stress. As depicted in table 4, PEFR treated groups formed less PC than D group which were an important indication that free radicals were scavenged during extract treatment.

In the similar experiment, we observed GSH, CAT and SOD levels in liver. GSH is a tripeptide which is most abundant in all tissues including liver. GSH plays a major role in the oxidation-reduction process, resulting in the formation of disulfide glutathione (GSSG) [29] during oxidative damage. Elevation of GSH (reduced) level by PEFR treated group which was an indication of free radical scavenging activity of extract. The antioxidant enzymes, SOD and CAT catalyze dismutation of free radicals and decrease superoxide levels. Treatment with PEFR significantly increased these enzymes, signified protective action of extract (table 4).

Hepatic cells are irreversibly necrotized by STZ and liver enzymes leaked from hepatic cells cytosol to blood stream, resulted in increasing liver enzyme (ALT, AST) concentrations in blood stream [30]. Treatment with PEFR significantly reduced the hepatic enzyme levels as compared to D control group, indicating the hepatoprotective activity of PEFR in STZ induced diabetic rats (table 5). All these observations suggested that PEFR had good antioxidant capacity.

CONCLUSION

In our study, PEFR decreased blood glucose level and improved lipid profile. It also restored hepatotoxicity biomarkers, ALT and AST enzymes. It significantly increased SOD, CAT, and GSH levels and decreased MDA and PC levels, showing cellular protective nature of extract. These results suggested that PEFR have antidiabetic, hypolipidemic and antioxidant activities in STZ induced D rats and it could be good adjutants in pharmacotherapy of diabetes. Moreover, further work is required to explore the cellular and molecular mechanism of action of this extract. Finally, we observed that PEFR had good antidiabetic activity and lesser toxicity potential which might be beneficial for future drug design perspective.

ACKNOWLEDGEMENT

Dr. Sudipta Saha (Corresponding Author) wishes to express his thanks to the University Grants Commission (UGC), New Delhi, India, for providing UGC-MRP Grant [Project No. 42-680/2013(SR)**].**

CONFLICT OF INTERESTS

The author declares that they have no conflict of interest

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