Pharmacogn. Res.
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ORIGINAL ARTICLE

Antidiabetic and Antidyslipidemic Activities of Hexane/Ethyl Acetate/Methanol Fractions of *Paspalum scrobiculatum* Linn. Grains in High-Fat Diet and Streptozotocin-Induced Diabetic Rats

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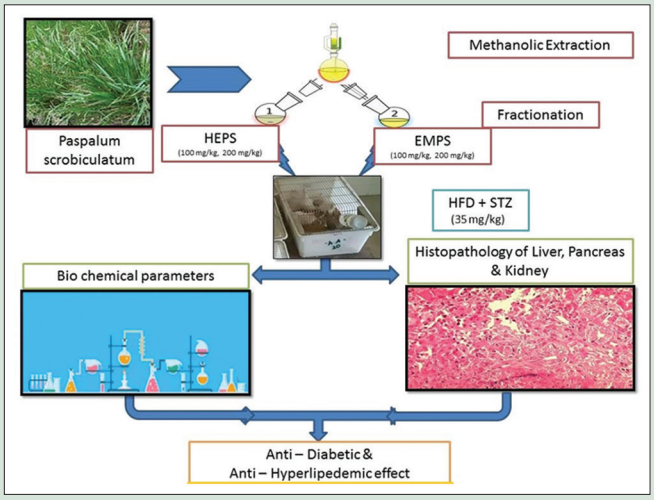
ABSTRACT

Background: Dietary habits and sedentary lifestyle are the major causes of increasing obesity, which in turn causes diabetes and cardiovascular problems. With this alarming increase, there is a need for alternative therapies to treat diabetes and hyperlipidemia. **Objectives:** To evaluate antidiabetic and antidyslipidemic effects of fractions of *Paspalum scrobiculatum* grains in rats. **Materials and Methods:** Different doses of Hexane: Ethyl acetate fraction of *P. scrobiculatum* (HEPS) and Ethyl acetate: Methanol fraction of *P. scrobiculatum* (EMPS) fractions of *P. scrobiculatum* were administered to the high-fat diet (HFD) and streptozotocin (STZ) (35 mg/kg)-induced diabetic rats with glibenclamide at 20 mg/kg body weight (b.w) as a standard reference. Biochemical and histopathological changes were assessed after 5 weeks of treatment. **Results:** Daily oral treatment with HEPS and EMPS each at 100 and 200 mg/kg b.w for 5 weeks was found to be significant in the reduction of plasma glucose levels, HbA_{1c}, increased plasma insulin levels, and normalized lipid profile and liver function parameters in HFD and STZ-induced diabetic rats. In addition, degenerative histopathological changes seen in liver, kidney, and pancreas of diabetic rats were found to be normalized in HEPS and EMPS as comparable to that of glibenclamide-treated rats. **Conclusion:** The obtained results suggest that HEPS and EMPS fractions of *P. scrobiculatum* grains possess promising antihyperglycemic and antidyslipidemic potential without apparent toxic effects, which may prove the claimed use of the plant in treatment of diabetes and dyslipidemia.

Key words: Antidiabetic, antidyslipidemic, high-fat diet, *Paspalum scrobiculatum*, streptozotocin

SUMMARY

- High-fat diet and streptozotocin (35 mg/kg)-induced diabetic rat model with glibenclamide as a standard reference was used to evaluate the antidiabetic and antihyperlipidemic activity of HEPS and EMPS (100 and 200 mg/kg b.w) for 5 weeks
- HEPS and EMPS significantly reduced plasma glucose levels and HbA_{1c}, increased plasma insulin levels, and normalized lipid profile and liver function parameters. In addition, degenerative histopathological changes seen in liver, kidney, and pancreas of diabetic rats were found to be normalized in HEPS and EMPS as comparable to that of glibenclamide-treated rats
- EMPS fractions of *P. scrobiculatum* grains possess promising antihyperglycemic and antidyslipidemic potential than HEPS without apparent toxic effects and are good candidates for future research.



Abbreviations Used: EMPS: Ethyl acetate:methanol (2:8) fractions of *Paspalum scrobiculatum* grains; HEPS: Hexane:ethyl acetate (1:3) fraction of *Paspalum scrobiculatum* grains; IR: Insulin resistance; DM: Diabetes mellitus; FBG: Fasting blood glucose; BGL: Blood glucose levels; H and E: Hematoxylin and eosin; IAEC: Institutional Animal Ethics Committee; OGTT: Oral glucose tolerance test; HbA_{1C}: Glycosylated hemoglobin; ALP: Alkaline phosphatase; AST: Aspartate transaminase; ALT: Alanine transaminase; TC: Total cholesterol; TG: Triglycerides; VLDL: Very low-density lipoprotein; LDL: Low-density lipoprotein cholesterol.

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DOI: 10.4103/pr.pr_6_20

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INTRODUCTION

Diabetes mellitus (DM) is a complex, chronic ailment necessitating continuous medical care with multifactorial risk-reduction approaches beyond glycemic control.[1] Chronic obesity and the associated fat deposition in the tissues lead to metabolic disorders, especially advancement of insulin resistance (IR) and type-2 DM. Dietary composition has been related to the pathogenesis of IR, particularly a high intake of dietary fats.[2,3] Diabetes caused 4.2 million deaths and at least USD 760 billion dollars in health expenditure in 2019.[4] In this scenario,

Cite this article as: Reddy GJ, Reddy KB, Reddy GV. Antidiabetic and antidyslipidemic activities of hexane/ethyl acetate/methanol fractions of *Paspalum scrobiculatum* linn. grains in high-fat diet and streptozotocin-induced diabetic rats. Phcog Res 2020;12:230-5.

Submitted: 08-Feb-2020 **Revised:** 15-Apr-2020
Accepted: 17-Apr-2020 **Published:** 14-Aug-2020

diabetes has become a leading problem to be tackled by the health-care systems worldwide. Despite tremendous advances in medicine, there is still no cure.[5] Therefore, it is an urge to develop natural therapeutic alternatives to treat diabetes and metabolic disorders.

Paspalum scrobiculatum Linn. Grains (family *Poaceae*, synonyms *Poaceae polystachyum, Poaceae commersonii* Lam, commonly called as kodo millet) has been found to possess flavonol, quercetin, and phenolic acids such as cis-ferulic acid, vanillic acid, syringic acid, p-hydroxy benzoic acid, and melilotic acid. The oil consists of oleic acid, stearic acid, and palmitic acid.[6] Ayurvedic texts such as Charak Samhita and Sushruta Samhita have reported the usefulness of these grains for the management of DM.[7,8] Therefore, the present study aims to evaluate antidiabetic and antidyslipidemic potential of hexane:ethyl acetate fraction of *P. scrobiculatum* grains and ethyl acetate:methanol fraction of *P. scrobiculatum* grains in high-fat diet (HFD) and streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

STZ was bought from M/S Sigma-Aldrich, St. Louis, MO, USA. Glibenclamide was received as a gift from M/S Cipla Limited, Mumbai, India. All the biochemical studies were performed using kits from M/S ERBA Diagnostics, Mumbai 400072, India, and Span Diagnostics Ltd., M/S Surat, India. All the chemicals used were of analytical grade.

Collection of plant material and preparation of fractions

P. scrobiculatum grains were collected (voucher no. 2032, dated; 02/09/2017) and processed as described earlier.[9] Methanolic extract of *P. scrobiculatum* grains was prepared by soxhlet extraction. It was filtered and concentrated using Buchi Rotavapor R-200. The resultant extract was fractionated using column chromatography on silica gel and eluted with n-hexane, ethyl acetate, and methanol in their increasing order of polarity to yield 2 different fractions, viz., hexane:ethyl acetate (1:3) fraction of *P. scrobiculatum* grains (HEPS) and ethyl acetate:methanol (2:8) fraction of *P. scrobiculatum* grains (EMPS).

Experimental animals

Male Sprague-Dawley rats, weighing 180–200 g, were procured from Sri Venkateswara Enterprises, Bengaluru. They were accommodated in standard cages, fed with normal pellet diet and tap water *ad libitum,* and were placed in a room maintained at a temperature of 24°C ± 2°C, with 40 ± 5% relative humidity and 12 h light/dark cycle. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Sri Venkateshwara College of Pharmacy, Chittoor, India, with reference No. 11-18/IAEC/CPCSEA/PO/SVCP/2017.

Determination of acute toxicity

Two groups of Sprague-Dawley rats ($n = 6$) selected by random sampling techniques were employed in this study. The animals were fasted overnight providing only water. Then, the fractions of HEPS and EMPS dissolved in tween-80 suspension were administered orally at the dose of 2000 mg/kg by intragastric tube and observed periodically for 2 days for the gross behavioral changes and mortality. These observations were continued further for 14 days for any signs of overt toxicity.[10]

Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) was executed in overnight fasted normal rats. The rats were allocated into six groups of six ìn each.[11]

- Group I: Normal Control rats received 1 ml normal saline p. o.(*per os*)
- Group II: Glibenclamide treated (20 mg/kg) p. o
- Group III: HEPS-100 mg/kg b.w. p.o
- Group IV: HEPS-200 mg/kg b.w. p. o
- Group V: EMPS-100 mg/kg b.w. p.o
- Group VI: EMPS-200 mg/kg b.w. p.o

30 min after the respective treatment, glucose 2 g/kg was administered orally to all the groups of rats. Tail blood glucose levels (BGL) were measured with the help of glucometer at 0, 30, 60, 90, and 120 min of glucose administration.

Assessment of antihyperglycemic effect of *Paspalum scrobiculatum* fractions in high-fat diet and STZ-induced diabetic rats

Male Sprague-Dawley rats (180–200 g) were fasted for 12–14 h. Type 2 diabetes was induced by HFD (25% protein, 17% carbohydrate, and 58% fat) for a period of 2 weeks. The composition and preparation of the HFD was as per Srinivasan *et al.*[12] The animals were then injected with STZ at a dose of 35 mg/kg b.w intraperitoneally. STZ was dissolved in 0.01 M citrate buffer, pH 4.5 which was freshly prepared[13] (0.1 M sodium citrate with 3 parts of 0.1 M citrate acid, pH adjusted to 4.5 by using 1 N NaOH). Normal control rats ($n = 6$) were provided with normal pellet diet (Amrut Rodent Diet-Hypro, Krishna Valley Agro LLP., Pune, India) *ad libitum* and were injected with 1 ml/kg b.w of 0.01M citrate buffer, pH 4.5. STZ-administrated rats were given with 10% glucose solution after 6 h for the next 24 h to prevent hypoglycemia. After 72 h, rats manifesting marked hyperglycemia (fasting blood glucose [FBG] >250 mg/dl) were used for the study using glibenclamide 20 mg/kg b.w p. o. as standard reference.

After successful induction of diabetes, the animals were distributed into Six groups with six rats in each group and treated daily as follows for 5 weeks.

- Group I: Normal pellet diet + 1 ml tween 80 suspension p. o
- Group II: HFD + STZ-induced rats + 1 ml tween 80 suspension p. o
- Group III: HFD + STZ-induced rats + glibenclamide 20 mg/kg body weight p. o. dissolved in 1 ml normal saline
- Group IV: HFD + STZ-induced rats + HEPS-100 mg/kg b.w. p.o
- Group V: HFD + STZ-induced rats + HEPS-200 mg/kg b.w. p.o
- Group VI: HFD + STZ-induced rats + EMPS-100 mg/kg b.w. p.o
- Group VII: HFD + STZ-induced rats + EMPS-200 mg/kg b.w. p.o.

Freshly prepared solutions dissolved in 1 ml tween-80 suspension were administered every day orally at noon (2.00 PM). As rats are nocturnal feeders, overnight fasting is equivalent to 24 h fasting. Therefore, fasting was initiated on the morning of blood sampling, i.e., from 6.00 AM to 2.00 PM (8 h fasting time). FBG levels were estimated weekly on days 1, 7, 14, 21, 28, and 35.

Biochemical studies

At the end of the study (on 36th day), all the fasted animals were sacrificed under light ether anesthesia by cervical decapitation and blood was collected by bleeding of carotid artery in tubes with and without anticoagulant to study the biochemical parameters such as serum insulin[14] and glycosylated hemoglobin (HbA_{1c}),[15] liver glycogen,[16] hepatic function markers such as serum alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT),[17] total protein, total cholesterol (TC),[18] triglycerides (TG),[19] very low-density lipoprotein (VLDL), and low-density lipoprotein cholesterol (LDL)[20] using commercially available kits.

Histopathological studies

Liver, kidney, and pancreas were washed immediately with saline and fixed in 10% phosphate-buffered formalin. Paraffin-embedded specimens were cut into 5 µm-thick sections and stained with hematoxylin and eosin following standard protocols.[21]

Statistical analysis

All information was presented as mean ± standard deviation (n = 6). Results were analyzed by Microsoft Excel Spread Sheet for Windows (2010). The differences between treated and untreated groups were assessed by one-way analysis of variance followed by independent sample t-test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Acute toxicity study

No adverse effects, no mortality, and no change in the behavioral or autonomic responses of all the rats were detected during the period of study, even after acute oral administration of 2 g/kg p. o. of HEPS and EMPS. Therefore, according to the OECD guidelines, 200 mg/kg extract dose that is 1/10th of 2000 mg/kg dose was selected as high dose and 100 mg/kg as low dose to study the *in vivo* antidiabetic activity.

Effect of HEPS and EMPS on oral glucose tolerance test in normal rats

Oral glucose loading (2 g/kg) to the rats produced significant changes in BGL after 30 min in all the groups of rats, confirming the induction of hyperglycemia. There was a significant elevation in BGL in normal control rats After 30 min of glucose loading. The rats treated with glibenclamide, HEPS and EMPS, at 100 and 200 mg/kg showed a significant reduction in BGL from 30 min after glucose loading. The results are depicted in Table 1.

Effect of long-term treatment of HEPS and EMPS on blood glucose levels in high-fat diet and STZ-induced diabetic rats

The effect of oral treatment with HEPS and EMPS at different doses on FBG level is presented in Table 2. Out of all the rats administered with STZ, four rats were found to be dead and three rats were eliminated from the study due to insufficient or severe hyperglycemia. Diabetic control rats had 2–5-fold increase in FBG levels throughout the study period in comparison to normal rats ($P < 0.0005$). Daily oral treatment with HEPS and EMPS at 100 and 200 mg/kg b.w made a significant reduction in FBG level, detected from the day 7 of the treatment compared to diabetic control rats ($P < 0.05$). Furthermore, at the end of the experiment, treatment with EMPS at 100 and 200 mg/kg b.w produced significant drop ($P < 0.0005$) in BGL, which was comparable to that of glibenclamide-treated rats, whereas HEPS-treated rats showed less significant ($P < 0.005$) effect in comparision to EMPS group.

Effect of HEPS and EMPS on biochemical parameters

Diabetic control rats shown significant reduction in plasma insulin levels compared to those in normal rats, after 35 days of study. The experimental results were presented in Table 3. The plasma insulin levels of diabetic-treated rats with EMPS and glibenclamide were significantly enhanced ($P < 0.0005$) when compared to that of diabetic control rats, whereas HEPS showed a less significant effect ($P < 0.005$). There was a significant increase in $HbA1_c$ levels of diabetic control rats than in normal rats. On treatment with HEPS and EMPS at both the tested doses, there was a significant decrease in $HbA1_c$ levels. Total proteins in diabetic control rats were significantly lesser than in normal rats and on treatment with the HEPS and EMPS significant upsurge was seen as comparable to that of glibenclamide-treated rats. Diabetic control rats shown significantly lower levels of liver glycogen than that of normal rats and on treatment with HEPS and EMPS, significant rise in the liver glycogen levels was observed. ALP, AST, and ALT showed significantly elevated activities in diabetic control rats when compared to the normal group. Treatment with glibenclamide, HEPS and EMPS each at a dose of 100 and 200 mg/kg b.w for 35 days Significantly reduced ALP, AST, and ALT, in a dose-dependent manner, EMPS showed a more significant effect ($P < 0.0005$).

Effect of HEPS and EMPS on hyperlipidemia

Oral treatment with HEPS and EMPS on TC, TG, HDL, VLDL, and LDL cholesterols, in all groups of rats, was assessed at the end of the study as presented in Table 4. The serum TC, TG, LDL, and VLDL cholesterol levels were found to be increased significantly in diabetic control rats ($P < 0.005$) when compared to normal rats, while the HDL cholesterol levels were significantly lessened in diabetic control rats. Treatment with HEPS and EMPS showed a significant reduction in the TC, TG, LDL, and VLDL cholesterol levels in a dose dependent manner. The results of EMPS at both low dose and high dose were comparable to that of the glibenclamide group ($P < 0.0005$), whereas HEPS presented less significant effect ($P < 0.005$). Further, there was a significant increase in HDL levels in diabetic-treated rats compared to those in diabetic control rats and was more effective in comparison with glibenclamide group. Daily oral treatment with HEPS and EMPS for 5 weeks normalized the above lipid parameters in the diabetic rats to almost normal levels.

Effect of HEPS and EMPS on histopathological analysis

Histopathological study of liver of normal rat presented normal hepatocytes with central vein and sinusoid space, while the diabetic

Table 1: Effect of fractions of *Paspalum scrobiculatum* grains on OGTT in normal rats

Groups	Blood glucose levels (mg/dL) at time intervals (min)				
	0	30	60	120	180
Normal control	99.17±7.4	166.67±15.8	145.33±11.5	108.17±10.2	101.83±12.8
Glibenclamide 20 mg/kg	95.17±5.3	132.00±5.8*	122.33±5.2*	109.33±4.3	98.83±3.4
HEPS 100 mg/kg	98.6±4.8	147.3±6.3*	119.2±6.5*	112.7±5.6	101.8±5.3
HEPS 200 mg/kg	94.3±5.1	138.1±7.7*	125.1±5.7*	107.2±4.8	98.2±4.7
EMPS 100 mg/kg	89±4.0	145.9±8.3*	120.7±6.3*	114.8±3.8	97.6±5.2
EMPS 200 mg/kg	92.6±5.6	134.5±8.9*	113.3±5.0*	102.5±4.3	94.0±4.6

Values are given as mean±SD. n=6. *P<0.05 treated group compared with normal control group. EMPS: Ethyl acetate: Methanol fractions of *P. scrobiculatum* grains; HEPS: Hexane:ethyl acetate fraction of *P. scrobiculatum* grains; HEPS: Hexane:ethyl acetate (1:3) fraction of *P. scrobiculatum* grains; SD: Standard deviation; OGTT: Oral glucose tolerance test

Table 2: Effect of fractions of *Paspalum scrobiculatum* grains on blood glucose levels (mg/dL) in high-fat diet and streptozotocin-induced diabetic rats.

Groups	Blood glucose level (mg/dL)					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Normal rats	77.67±2.5	81.33±1.9	88.00±1.8	83.50±2.4	81.50±1.9	89.00±2.4
Diabetic control	325.67±6.2$^{\#}$	340.83±9.2$^{\#}$	341.67±8.5$^{\#}$	367.17±9.7$^{\#}$	388.67±8.5$^{\#}$	398.33±8.4$^{\#}$
Glibenclamide 20 mg/kg	319.00±7.4	190.67±8.4*	163.00±6.5*	149.33±6.3**	136.50±4.4**	121.17±5.8**
HEPS 100 mg/kg	331.4±6.08	254.39±5.4*	238.05±6.2*	216.41±6.4*	190.37±5.6*	166.05±6.3*
HEPS 200 mg/kg	327.7±7.3	248.24±6.6*	225.30±6.9*	208.03±6.4*	183.40±7.1*	153.27±6.5*
EMPS 100 mg/kg	315.80±8.1	206.57±6.9*	185.47±7.3*	159.29±6.5*	145.56±7.6**	110.37±6.7**
EMPS 200 mg/kg	320.08±6.4	191.23±7.5*	178.64±6.2*	142.53±7.6**	134.82±5.5**	98.25±6.7**

Results are expressed in mean±SEM, n=6; $^{\#}P$<0.0005 diabetic control compared to normal control; **P<0.0005 treated group compared to diabetic control; *P<0.005 treated group compared to diabetic control. EMPS: Ethyl acetate: Methanol fractions of *P. scrobiculatum* grains; HEPS: Hexane:ethyl acetate fraction of *P. scrobiculatum* grains; HEPS: Hexane:ethyl acetate (1:3) fraction of *P. scrobiculatum* grains; SEM: Standard error of mean

Table 3: Effect of fractions of *Paspalum scrobiculatum* grains on biochemical parameters in high-fat diet and streptozotocin-induced diabetic rats

Groups	Plasma insulin (µIU/ml)	HbA_{1c} (%)	Total protein (g/dL)	Liver glycogen (mg/g)	ALP (mg/dL)	AST (IU/L)	ALT (IU/L)
Normal control	17.52±0.3	4.92±0.19	4.57±0.3	39.57±3.05	184.50±5.9	81.17±6.8	22.50±2.6
Diabetic control	5.37±0.4$^{\#\#}$	8.98±0.28$^{\#}$	8.28±0.9$^{\#}$	15.80±3.53$^{\#\#}$	495.50±7.6$^{\#\#}$	157.50±7.05$^{\#}$	46.67±2.2$^{\#}$
Glibenclamide 20 mg/kg	15.13±0.6**	5.67±0.7**	5.25±0.3*	34.75±3.2**	251.33±9.1**	92.17±6.5*	23.58±1.9**
HEPS 100 mg/kg	9.01±0.70*	7.55±0.16*	6.86±0.6*	27.46±3.7*	366.57±8.2*	126.67±5.1*	33.62±2.7*
HEPS 200 mg/kg	10.53±0.94*	7.06±0.21*	6.01±0.8*	28.70±3.4*	329.24±7.3*	118.56±6.0*	35.31±2.9*
EMPS 100 mg/kg	13.82±0.76**	6.15±0.13**	6.16±0.7*	32.15±2.69**	287.27±8.0**	99.34±5.5*	26.18±2.1**
EMPS 200 mg/kg	15.04±0.34**	5.83±0.09**	5.83±0.5*	35.07±2.9**	256.29±6.7**	86.29±6.6*	24.39±2.5**

Results are expressed in mean±SEM, n=6; $^{\#}P$<0.005 diabetic control compared to normal group; $^{\#\#}P$<0.0005 diabetic control compared to normal group; *P<0.005 treated group compared to diabetic control; **P<0.0005 treated group compared to diabetic control. ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; HbA_{1c}: Glycosylated hemoglobin; EMPS: Ethyl acetate: Methanol fractions of *P. scrobiculatum* grains; HEPS: Hexane:ethyl acetate fraction of *P. scrobiculatum* grains; SEM: Standard error of mean

Table 4: Effect of fractions of *Paspalum scrobiculatum* grains on biochemical parameters on hyperlipidemia in high-fat diet and streptozotocin-induced diabetic rats

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)
Normal control	92.83±3.12	65.33±3.72	42.41±1.68	13.06±0.74	37.42±1.95
Diabetic control	215.33±6.97$^{\#}$	161.83±5.45$^{\#}$	26.75±1.54$^{\#}$	32.360±1.09$^{\#}$	161.62±4.83$^{\#}$
Glibenclamide 20 mg/kg	105.00±3.89*	83.16±3.43**	36.91±1.42*	16.63±0.68*	52.09±2.01*
HEPS 100 mg/kg	167.42±5.62*	131.51±5.85*	31.71±2.53*	26.45±0.81*	109.81±2.61*
HEPS 200 mg/kg	154.76±6.08*	122.04±5.23*	32.15±2.31*	24.72±0.77*	97.67±2.68*
EMPS 100 mg/kg	114.39±6.27**	89.37±4.28**	38.53±2.04*	17.84±0.63*	58.21±2.63**
EMPS 200 mg/kg	99.82±6.53**	84.64±5.38**	40.88±2.84*	16.90±0.76*	42.37±2.89**

Results are expressed in mean±SEM, n=6; $^{\#}P$<0.005 diabetic control compared to normal group; *P<0.005 treated group compared to diabetic control; **P<0.0005 treated group compared to diabetic control. LDL-c and VLDL-c calculated using Friedewald formula. VLDL-c=TG/5; LDL-c=TC−HDL − (TG/5). TG: Triglyceride; TC: Total cholesterol; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; VLDL-c: Very low density lipoprotein cholesterol; EMPS: Ethyl acetate:Methanol fractions of *P. scrobiculatum* grains; HEPS: Hexane:ethyl acetate fraction of *P. scrobiculatum* grains; SEM: Standard error of mean

control rat revealed extensive hydropic degeneration and necrotic changes in the hepatocytes, with partially rounded, irregularly-edged vacuoles, congestion, inflammatory cell infiltration and sinusoidal dilatation in portal areas as presented in Figure 1-Diabetic control. These findings were found to be significantly lessened in the liver of rats in glibenclamide, HEPS 200 mg/kg, EMPS100 and 200 mg/kg treated groups, while HEPS 100 mg/kg treated rats shown improvement from the degenerative changes. Histopathological sections of liver after 5 weeks of treatment are presented in Figure 1.

The histological view of the kidney in the normal group shown no pathological appearance with normal structure of glomerulus enclosed by Bowman's capsule and renal tubules with no inflammatory changes [Figure 2-normal], whereas the kidney of diabetic control rats presented severe necrosis with hydropic degeneration resulting in shrunken glomerulus, distracted Bowman's capsule and tubular inflammation [Figure 2-diabetic control]. In the kidney sections of rats of HEPS- and EMPS-treated groups, the pathological findings were detected to be significantly reduced as comparable to that of glibenclamide-treated rats Histopathological sections of treated kidneys are shown in Figure 2.

The islets of Langerhans were observed to be large and regular with normal histological architecture in normal group [Figure 3-normal], while diabetic control group exhibited hydropic degenerative and necrotic manifestations with disrupted cellular order [Figure 3-diabetic control]. Degenerative and necrotic changes were normalized with less beta cell granulation in glibenclamide-treated rats [Figure 3, glibenclamide treated]. A significant improvement was observed with islet architecture similar to normal group rats, in HEPS- and EMPS-treated rats at both 100 mg/kg and 200 mg/kg doses. Histopathological sections of pancreas are visualized in Figure 3.

DISCUSSION

The growing trend of DM in the last decade is a global concern and has flagged for the research towards conveying natural therapeutic alternatives to successfully treat diabetes. The present study was performed to assess HEPS and EMPS in HFD and STZ-induced

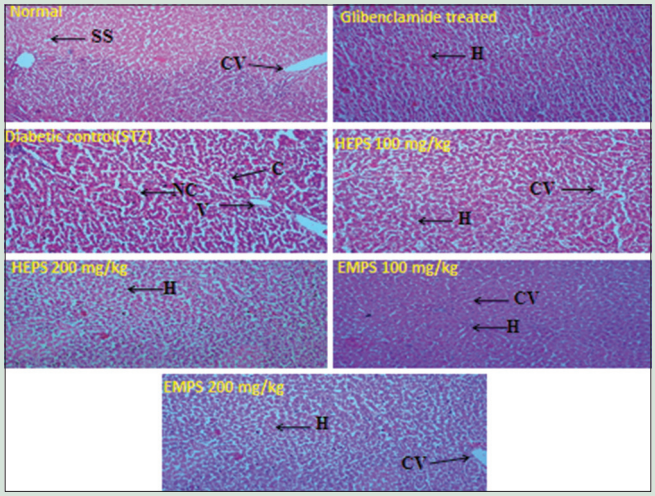

Figure 1: H and E-stained histopathological sections of liver after 5 weeks of treatment. CV = Central vein, SS = Sinusoids space, H = Hepatocyte, C = Congestion, NC = Necrotic Change, V = Vacuolization

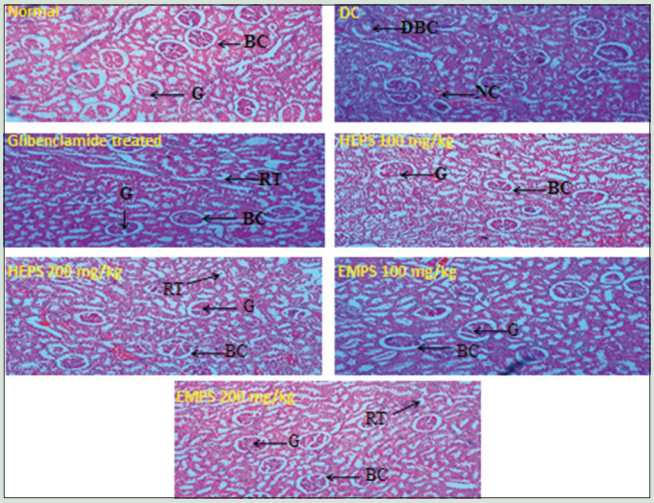

Figure 2: H and E-stained kidney sections after 5 weeks of treatment with HEPS and EMPS. DC = Diabetic control; BC = Bowman's capsule, G = Glomerulus, DBC = Distractive Bowman's capsule, SG = Shrunken glomerulus, RT = Renal tubule. NC = Necrotic changes

diabetic rats. HFD rats may be vulnerable to IR as the receptor cells are blocked by fat deposits and the diabetic action of a low dose of STZ is boosted. Hence, it has been recognized by several researchers that the combinatorial effect of HFD and a low dose of STZ may imitate the development of an ideal model for type 2 DM,[22] which was reproduced in our study.

The OGTT is valuable as it was executed to assess the changes in glucose metabolism under physiological conditions and replicated a postabsorptive state in which the manufacture and release of insulin and its sensitivity are necessary.[23] HEPS- and EMPS-treated rats displayed a significant fall in BGL within 60 min of glucose loading, that implies the beneficial effect of the plant in the avoidance of postprandial hyperglycemia and related complications of diabetes. The results were in agreement with earlier reports on the antiglycemic effect of *P. scrobiculatum*.[24]

It was evident from the present results that hyperglycemia, hypoinsulinemia, and hyperlipidemia were successfully induced in the rats that were fed with HFD and STZ at a dose of 35 mg/kg b.w. These results were consistent with previous studies.[25,26] Chronic treatment with HEPS and EMPS (100 and 200 mg/kg) for 5 weeks remarkably normalized the elevated BGL, decreased HbA_{1c} levels, restored serum insulin, liver biochemical parameters and other dyslipidemic markers, indicating their potent antihyperglycemic and hypolipidemic activity. The possible mechanism for this antihyperglycemic effect could be due to protection of pancreatic β-cells from further atrophy or by the potentiation of insulin secretion from residual β-cells,[27] as was evident from the increased plasma insulin levels in HEPS and EMPS (100 and 200 mg/kg) treated rats. This also infers that the antihyperglycemic actions of *P. scrobiculatum* extract may be due to the insulin like action. The liver glycogen level may be considered as the best marker to assess the antihyperglycemic activity of any drug.[28] The hepatic glycogen levels in HEPS- and EMPS-treated rats at all the tested doses were significantly increased, which may possibly be due to increase in glycogenesis and decrease in glycogenolysis and gluconeogenesis.[29] These results are in accordance with previous reports showing reduced hepatic glycogen content in diabetic models.[30]

The increase in the activities of plasma ALP, ALT, and AST and decreased level of total protein in the HEPS- and EMPS-treated rats were observed as compared to diabetic control rats, indicating that some of the detrimental effects of DM may be related to hepatic dysfunction resulting from hepatic necrosis. The leakage of these enzymes from the liver cytosol into the bloodstream could be the possible reason for the increase in plasma liver enzymes. These deleterious effects in diabetic rats were corrected after daily oral administration HEPS and EMPS at the doses of 100 and 200 mg/kg. The results are in agreement with previous literature of plants having liver protective property.[21]

The treatment with the HEPS and EMPS at the doses of 100 and 200 mg/kg was able to correct the serum lipid profile in diabetic rats. The reduction of TC or LDL-C in serum has been reported to lower the risk of coronary heart disease.[31] Protective effects of *P. scrobiculatum* on serum lipid profile may be related to the effect on carbohydrate metabolism or modification of hepatic enzymes. This observation is further supported by improved histopathology of pancreas of HEPS- and EMPS-treated rats [Figure 3].

Histopathological investigation of diabetic control rats reported inflammation, necrosis of the hypatocytes,[32] renal and pancreatic cells, impairment in portal intervals, glomerular atrophy, and tubular necrosis.[33] In this study, HEPS- and EMPS-treated rats exhibited normalization of the necrotic changes in liver, kidney, and pancreas, improvement in the pancreatic islets of beta cells, typical appearance of hepatocytes, portal areas and central vein, and the kidney section demonstrated improvement in the glomerular architecture and decrease in hydropic degeneration and congestion which were comparable to glibenclamide-treated rats (5 mg/kg). These results suggest that administration of HEPS and EMPS at 200 mg/kg may perhaps greatly ameliorate degenerative and necrotic destruction by repairing the cell damage in the liver, kidney, and pancreas associated with development of DM that are produced by STZ administration.[30] Moreover, the results point out that the protective effect of HEPS and EMPS at 200 mg/kg was better than that of HEPS and EMPS at 100 mg/kg.

The biochemical estimations were consistent with the histopathological outcomes, substantiating the protective role of *P. scrobiculatum* in overcoming DM and associated complications. However, EMPS fraction of *P. scrobiculatum* shown better activity than HEPS, both the fractions have potential antidiabetic and antidyslipidemic properties and could be used to prevent the development of secondary macrovascular complications of severe DM.

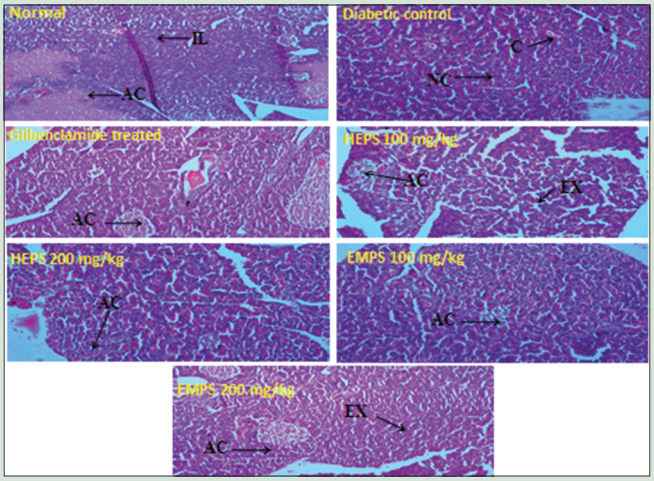

Figure 3: H and E-stained sections of Pancreas after 5 weeks of treatment; EX = Exocrine, IL = Islets of Langerhans, AC = Acinar cells, NC = Necrotic change

CONCLUSION

The current study confirms that daily oral administration of HEPS and EMPS displayed a pronounced antihyperglycemic and antihyperlipidemic effect in HFD and STZ-induced diabetic rats, without any hypoglycemic action in OGTT of normal rats. Further studies to identify the active principles in the fractions of *P. scrobiculatum* as well as elucidating their mechanisms of action would be helpful so as to develop it as a promising antidiabetic and antihyperlipidemic drug.

Financial support and sponsorship

The authors thank Department of Science and Technology (DST), New Delhi, for providing support under FIST Program of DST [SR/FST/COLLEGE-280].

Conflicts of interest

There are no conflicts of interest.

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