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Phytochemical, Toxicological, and Anti-Hyperglycemic Evaluation of *Pennisetum purpureum* in Sprague-Dawley Rats

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Abstract

Many herbal extracts have been used for preventing and managing diabetes. In recent times it was documented that the herbal extracts in traditional Indian medicine are clinically effective in treating sugar imbalances associated with diabetes mellitus. Although it was considered that several bioactivities and phytochemicals have been attributed and it confirmed in terms of their toxicological profile and anti-diabetic activity. Herbal formulation and extract of the plant part of elephant grass plays an important role for the treatment of various diseases and disorders such as inflammation, pain, ulcer, cancer, bacterial infections, and fungal infections. Ethanolic extract of *Pennisetum purpureum* which was prepared by using 50%v/v and their fractions were prepared by using liquid-liquid extraction technique. There was quantitative estimation of gallic acid analyzed by HPTLC technique. The extract has been found safe at high dose through oral acute toxicity study. Antihyperglycemic activity was performed on Sprague-Dawley rats by inducing the diabetes through Streptozotocin. Gallic acid was quantitatively estimated in methanolic fraction of extract and was found to be 0.13%w/w. Extract showed positive response in the treatment of diabetes which was confirmed after performing histopathology of liver and pancreas. Finally in this study, it was found that *P. purpureum* showed no toxicity and ameliorative changes in the blood glucose level, antioxidant level and biochemical parameters viz. triglycerides, total cholesterol, high density lipoprotein, low density lipoprotein and very low-density lipoprotein.

Keyword: *Pennisetum purpureum*, Diabetes mellitus, Antihyperglycemic, Biochemical, Acute toxicity, Histopathology

Introduction

The long-term complications of ineffectively or untreated diabetes include nephropathy, retinopathy, and peripheral neuropathy. This metabolic disorder is exemplified by symptomatic intolerance of glucose as well as fluctuations in protein and lipid metabolism. Moreover, diabetic patients have an increased risk of stroke and cardiovascular disease (1)**.** Hence, there is a strong need for effective and safe oral antihyperglycemic agents that offer the physician a broader range of options to prevent treat and manage diabetes. Many herbal extracts or derivatives have been documented in traditional

Indian medicine (TIM) to be clinically effective in treating sugar imbalances associated with diabetes mellitus.

Pennisetum purpureum (Elephant grass, family Poaceae) is important silage in the tropical and subtropical region. The young shoots and leaves are eaten by humans and can be cooked to prepare stews and soups (2). Leaf and stem infusions are used for its diuretic properties. The plant possesses several pharmacological activities including antifungal activity, antimicrobial activity (3), antioxidant activity, cytotoxic activity, anti-inflammatory activity (4), antiulcer activity (5), analgesic activity, and antimalarial activity (6).

Phytochemical analysis of *P. purpureum* revealed the presence of alkaloids, cyanogenic glycosides, flavonoids, oxalates, phytates, saponins, and tannins. Some bioactive phytochemicals have been isolated from *P. purpureum* methanolic extract includes dihydrocapsaicin, ethyl iso-allocholate, 2-hydroxy-4-methoxybenzaldehyde , 1,2-benzenediol , ergost-5-en-3-ol, stigmast-5-en-3β-ol, 1,2-Dihydroxybenzene, Ethyl iso-allocholate, 4-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1–1-butenyl)−2cyclohexen-1-one, 5-Butylpyridine-2-carboxylic acid, 2,3-Di-O-phenylboranediyl-alpha-D-mannofuranose, 2-Hydroxy-4-methoxybenzaldehyde, 2,4a,5,8a-Tetramethyl-1,2,3,4,4a,7,8,8aoctahydronaphthalen-1-ol, Chlolest-4-en-3-one, Ergosta-4,22–dien-3-one, Stigmast-4-en-3-one, Cholest-4-ene-3,6–dione, N-methyl-adamantane acetamide, 3,5-Bis(1,1-dimethylethyl)−4-hydroxy-2–4cyclohexadien-1-one, and 4-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1–1-butenyl)−2cyclohexen-1-one (7). Although several bioactivities and phytochemicals have been attributed to this plant in the literature, none of them have been confirmed in terms of their toxicological profile and anti-diabetic activity. Therefore, the study aims to justify the use of this plant as an anti-diabetic agent through preclinical study.

Materials and Method

Material

The plant was collected and authenticated in the Botanical Survey of India, Prayagraj and the voucher specimen number BSI/CRC/2021-22/448 was submitted in the department. The ethanolic extract of *P. Purpureum* was prepared by using 50%v/v ethanol at 55^0 C by hot percolation method.

Preparation of sample

Four fractions were prepared by using liquid-liquid extraction technique considering 100gm of extract in each solvent viz. chloroform, ethyl acetate, methanol and water.

Phytochemical screening and TLC

The plant extract was qualitatively analyzed by TLC using Toluene: ethyl acetate: formic acid: methanol (6:1.5:1.5:1v/v/v/v). PPEE showed (03) spots at 365nm whereas PPMF showed (04) spots at 254nm. Further, PPEE and PPMF were subjected to preliminary phytochemical screening for the identification of various constituents by using standard procedures (8).

Quantitative estimation of gallic acid in methanolic fraction

The standard solution of gallic acid was prepared by mixing 5mg gallic acid in 20 ml methanol.10 µl of the solution was spotted on HPTLC plate and developed at 365nm. 100mg of methanolic fraction was mixed with 5ml methanol. 10 µl of the solution was spotted on HPTLC plate and developed at 365nm using mobile phase Toluene: ethyl acetate: formic acid: methanol (6:1.5:1.5:1v/v/v/v).

Animals

Sprague-Dawley rats, both sexes weighing 190-220 g, were used in the study. All animals were fed with standard pellet diet and water *ad libitum* throughout the study. The research protocol was approved by IAEC of Unit-

ed Institute of Pharmacy, Prayagraj with approval number UIP/IAEC/March-2023/19.

Acute oral toxicity

The procedure for study was followed as per OECD 423 guidelines (9). A single dose of the extract (300 mg/kg, 2000 mg/kg and 5000 mg/kg body weight) was given orally to all animals (three each) and monitored continuously for any adverse effects till 14 days.

Induction of diabetes in experimental animals

The rats were fasted for overnight but were allowed water *ad libitum*. Diabetes was induced in rats by intraperitoneal injection of STZ (60 mg/kg body weight) except group - Ist (10).. After two days of STZ administration, rats with blood glucose level more than 220mg/dl were considered diabetic and used in the study.

Design of experiment

The experimental rats were divided into seven groups (n=6). Group 1 represents normal group receives saline orally. Group 2 represents diabetic control group received saline only. Group 3 represents standard drug metformin (100mg/kg) treated diabetic rats. Group 4 and group 5 received plant extract (PPEE) (100mg/kg b.w and 200g/kg b.w) respectively while group 6 and group 7 received methanolic fraction (PPMF) (100mg/kg b.w and 200g/kg b.w) respectively. The oral administration of saline, extract, and fractions was carried out for a duration of three weeks using an oral gavage tube on a daily basis. Blood samples were collected from the retro orbital puncture on Day 0, Day 7, Day 14, and Day 21 to measure the blood glucose level. The glucometer (SugarScan Thyrocare Technologies Limited, Mumbai, India) was used to determine the blood glucose level. The values of the treated groups were compared to those of the standard group, which was treated with Metformin. The animals were euthanized by intraperitoneal administration of high dose of thiopentone sodium and the liver, kidney, and pancreas were exposed and perfused with cold saline phosphate buffer (pH 7.4) for histopathological examination.

Biochemical parameters

Parameters including total cholesterol, Triglyceride, HDL, LDL, VLDL were assessed in all groups by using diagnostic kits (Erba Mannheim, Mumbai, India).

In vivo antioxidant activity in diabetic rats

Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) were estimated as per previously reported method (11).

Histopathology

Histopathology analysis was performed as per previously reported standard method (12). The histopathological slides were examined and photographs were captured with a digital stereomicroscope (Olympus, B061, USA).

Statistical Analysis

The statistical analysis of all pharmacological analysis was performed using software Graph Pad prism version 9.1.2 using Two-way ANOVA through Newman-keuls test. The values are represented as mean ± S.D. (n=6)

Results

Preliminary phytochemical screening

Phytochemical screening of PPEE ensure the presence of bioactives viz. alkaloids, phenolic compounds, steroids, flavonoids, tannins whereas PPMF shows presence of Tannic acid, Phenolic compounds, steroids, flavonoids, alkaloids, amino acids and proteins.

HPTLC fingerprinting analysis

The qualitative HPTLC fingerprint analysis of PPEE was carried considering mobile phase as Toluene: ethyl acetate: formic acid: methanol (6:1.5:1.5:1v/v/v/v). The four spots at different rf value viz. 0.23 (39.95%), 0.43

(17.11%), 0.56 (36.59%), and 0.61 (6.35%) were obtained as shown in figure 1. The amount of gallic acid in PPMF by quantitative HPTLC method was found to be 0.13 %w/w.

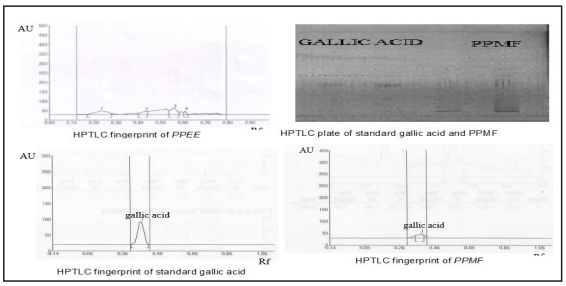

Figure 1: HPTLC finger printing analysis of PPEE, PPMF and standard Gallic acid

Acute oral toxicity

The extract (PPEE) didn't show any mortality even at higher dose 5000 mg/kg b.w and found completely safe. Therefore, for further pharmacological study, two doses 100 and 200 mg/kg body weight have been selected.

Antihyperglycemic activity

By the end of treatment, A decline in blood glucose value was seen in PPMF treated rats (100mg/kg and 200mg/kg) as (224.21± 2.75-122.78±1.25) and (224.21±2.75 – 108.33±4.03) ($p<0.001$) respectively; in PPEE treated rats (100mg/kg) & (200mg/kg) as (224.21±2.75 – 141.66±3.01) & (224.21±2.75 – 137.85±7.02) ($p<0.001$) respectively; whereas metformin reduces blood glucose value (224.21±2.75 to 102.33±3.07) ($p<0.001$). The results were moderately significant shown in figure 2.

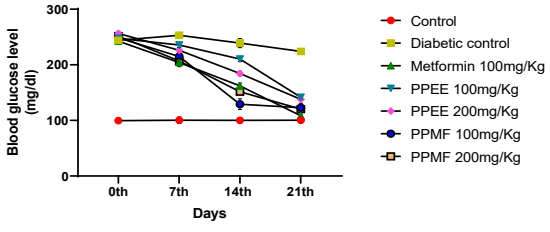

Figure 2: Antihyperglycemic effect of PPEE and PPMF

Effect of PPEE and PPMF on lipid profile

Figure 3 revealed that on oral administration of PPEE (100 mg/kg) showed the significant amelioration in biochemical parameters as compared to diabetic group viz. TG (102.16 ± 4.16; $p<0.01$), TCh (120.66 ±2.58) HDL (20.66 ± 2.56), LDL (50.45±4.87) and VLDL (25.33 ±2.16) levels in diabetic rats. While in case of PPEE 200mg/kg treated animals, shows remarkable improvement in lipid profile as TG (96.45±6.23), TCh (106.85±8.54) HDL (20.25±6.80), LDL (48.5 ±3.67) and VLDL (23.87±3.21) levels in diabetic rats. Oral administration of methanolic fraction PPMF 100 mg/kg reveals diminution in TG level (81.99±8.25; $p<0.001$), Tch (89.25±9.22; $p<0.001$), LDL (46.98 ±2.85), and VLDL (21.96±3.41) in diabetic animals whereas enhancement in the level of HDL (21.45±6.23; $p<0.001$).

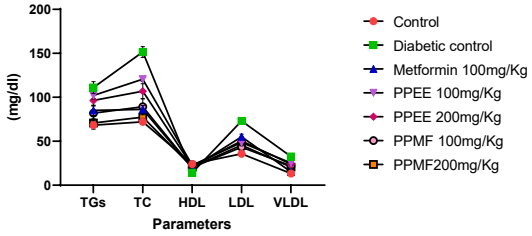

Figure 3: Effect of PPEE and PPMF on biochemical parameters

Effect of PPEE and PPMF on antioxidant parameters

Table 1 shows significant reductions in GSH, GPx, SOD, and catalase enzymes in the diabetic control rats as compared to normal control rats. After 21 days treatment with 200 mg/kg of PPEE and PPMF, there is significant increase in GSH, GPx and CAT level in liver viz. 74.23 to 98.12 and 74.23 to 109.42; 5.42 to 6.48 and 5.42 to 8.62; and 31.45 to 47.32 and 31.45 to 78.2 respectively ($p<0.001$). From the results, it confirms that oral administration of PPEE and PPMF ameliorates oxidative stress in diabetic experimental animals.

Table 1: Effect of extract and fraction on antioxidant enzyme activities in STZ diabetic rats

Parameters	Normal control	Diabetic control	Metformin 100mg/kg	PPEE 100mg/ kg	PPEE 200mg/ kg	PPMF 100 mg/kg	PPMF 200 mg/kg
	GSH (mM of DTNB conjugated/ mg protein)						
Liver	129.67 ± 2.6	74.23 ± 1.5 ^{z}	124.78± 2.4^{c}	82.46± 2.2^{a}	98.12± 3.3^{c}	102.42± 3.6^{c}	109.42± 2.36^{c}
Kidney	118.77 ± 2.3	46.17 ± 2.4^{z}	88.18 ± 3.45	62.15± 3.5	69.38± 2.7	78.84± 5.34^{c}	92.84 ± 2.54
	GPx (mg glutathione consumed/ min/ mg protein)						
Liver	9.46 ± 0.91	5.42 ± 0.88 ^{z}	7.10 ± 0.21^{b}	5.87± 0.76^{b}	6.48± 0.44^{c}	7.85± 0.62^{c}	8.62± 0.95^{c}
Kidney	7.36 ± 0.14	4.98 ± 1.01^{y}	6.58 ± 0.25^{a}	5.18± 0.14^{a}	5.90± 0.26^{c}	6.89± 0.48^{c}	7.38± 0.6^{4}c
	CAT (mmol of H_2O_2 consumed/ min/ mg protein)						
Liver	71.25 ± 2.17	31.45± 1.71^{z}	83.5 ± 0.97^{c}	44.32± 2.5^{b}	47.32± 3.1^{b}	63.7± 0.97^{c}	78.2 ± 0.97^{c}
Kidney	38.33 ± 1.22	52.01± 1.58^{z}	28.8± 1.29^{c}	21.26± 1.0^{a}	21.56± 1.2^{a}	22.46± 1.5^{c}	25.7± 1.49^{c}
	SOD (U/ min/ mg/ Hb)						
Erythrocytes	6.35 ± 0.17	3.45 ± 0.28 ^{z}	4.29± 0.24^{b}	5.98± 0.66^{b}	5.42± 0.40^{c}	6.1 ±0.70^{b}	6.78 ±0.83^{c}

The values represent the means ± S. D. for six rats per group. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 compared to diabetic group. ^{z}p<0.001 compared to normal group. SOD: superoxide dismutase; GSH: Glutathione; GPx: glutathione peroxidase; CAT: Catalase

Histopathological investigation

Liver

Normal hepatic cells were observed in group (4a). In group II (4b) diabetic rats, shows degeneration and inflammatory infiltrate. In group III (4c), the hepatocytes portal tracts and central veins come out to be normal. In group IV (4d) lessened degeneration and necrosis is visible. In group V (4e) inflammatory infiltrate and degeneration of cells is seen. In group VI (4f) and VII (4g) normal architecture of liver cells are regained.

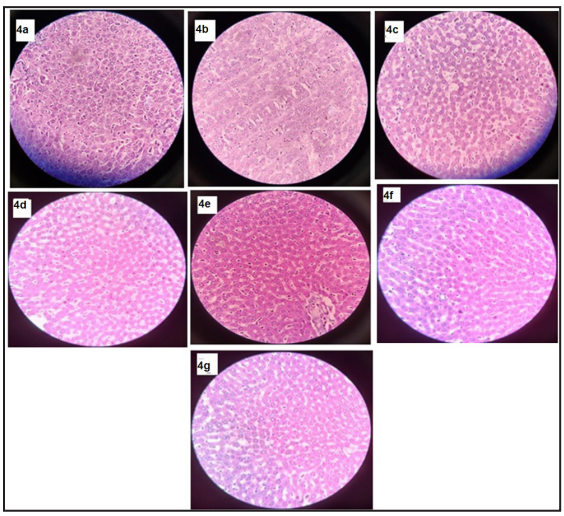

Figure 4: Histopathology of liver

Pancreas

Normal acini, cells and normal cellular islets of Langerhans were observed in group (5a). In group 2 (5b) vacuoles and wider intralobular duct is observed. In metformin group (5c) restoration of normal structure were observed. In group IV (5d) and group V (5e) reduction of vacuoles and thinning of intralobular duct were

observed. Group VI (5f) and group VII (5g) were observed with normal cells and architecture.

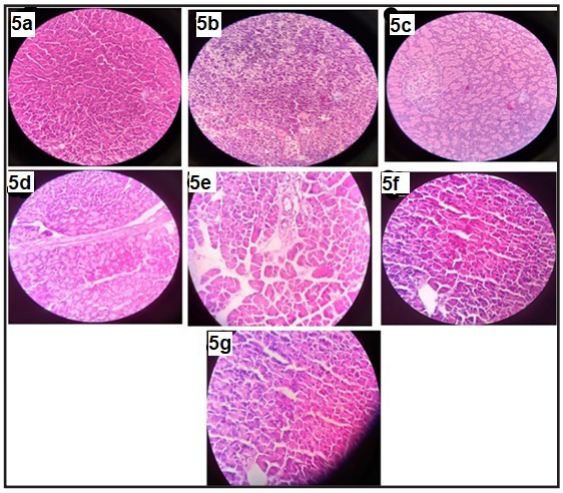

Figure 5: Histopathology of pancreas

Discussion

The traditional use of *Pennisetum purpureum* for its antidiabetic properties prompted the current study. Streptozotocin-induced diabetes was used as a model in Sprague-Dawley rats, which partially damages beta cells and produces type 2 diabetes. The anti-hyperglycemic potential of PPEE and PPMF was investigated in which we observed reduction in elevated blood glucose concentration that might be due to the presence of Gallic acid in extract and fraction. Gallic acid was found to upregulate pAkt, PPAR-γ, and Glut4 that facilitate insulin sensitivity and glucose homeostasis; (13) moreover, the antidiabetic effects of Gallic acid could be mediated via regulation of TNF-α and adipocytokines expression. Gallic acid also improved the function of the β cells by inhibiting caspase-9-related cellular apoptosis (14). Acute toxicity studies showed that PPEE was safe even at higher dose 5000 mg/kg b.w. The test group exhibited a significant decline ($p \leq 0.001$) in serum glucose, serum total cholesterol, and serum triglycerides levels compared to the control group. Free radical generation and antioxidant resistance damage can lead to the oxidation of glucose, glycation of protein, and oxidative degradation of protein glycation. After intake of streptozotocin, oxidative stress increased due to a compromise in the antioxidant system in the diabetic condition (15). Herbal and edible plant that having this polyphenolic compound (Gallic acid) modulated different antidiabetic signaling pathways through its antioxidative potential in diabetic complications, and plays a major role in amelioration of cardiac complications, diabetic nephropathy, and neuropathy (16), moreover preventing oxidative stress induced hepatic and pancreatic injury. Therefore, the study suggests that *Pennisetum purpureum* extract having antioxidant, hypolipidemic and antidiabetic potential.

Conclusion

In this study *Pennisetum purpureum* showed no toxicity and ameliorative changes in the elevated blood glucose level, antioxidant level and biochemical parameters Therefore could be considered as an ingredient in the development of antidiabetic herbal formulations. Further studies might be led to investigate the mode of action of the fraction in interacting with the oxidative and antidiabetic pathways.

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Statement and Declarations

Competing Interests

Author declares that there is no financial interest that are directly or indirectly related to the work submitted for publication.

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