

HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITIES OF *PIPER CHABA* IN ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

Diabetes mellitus (DM) is a systemic metabolic disorders with several major health complications affecting both the quality and the length of life. The purpose of this study is to appraise the antidiabetic and antihyperlipidemic activities of the *Piper chaba* plant extract. Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of Alloxan (80 mg/kg body weight) in a 0.1 M sodium citrate buffer (pH 4.5). The animals were divided into five groups with five animals in each and treated with both the acetone and ethanol extract of *P. chaba* at dose 100 mg/kg and 200 mg/kg body weight respectively. The results showed that *P. chaba* plant extract decrease

blood glucose level significantly ($p < 0.001$ to $p < 0.01$) and also regulated parameters of lipid profile levels (TG, TC, LDL, VLDL, and HDL) significantly ($p < 0.001$ to $p < 0.01$) in experimental model of diabetes mellitus. The levels of serum SGPT, SGOT and CRP were also adjusted to the normal level notably ($p < 0.001$ to $p < 0.01$) by the oral administration of *P. chaba* plant extract. The results obtained from this study indicate that the acetone and ethanol extract of *P. chaba* contains bioactive substances with hypoglycemic potency.

KEYWORDS: Diabetes, antidiabetic, *P. chaba*, lipid profile.

1. INTRODUCTION

Noninsulin-dependent diabetes mellitus (NIDDM) is a common disease of the endocrine system caused by the reduced secretion of insulin by the pancreatic langerhans β - cell or by the lowering of insulin resistance due to excessive absorption of glucose. Diabetes has become a serious health challenge over the world, with a major impact on the population of

developing countries due to the absence of curative and affordable interventions. Although, both genetic and environmental factors, such as obesity and lack of exercise appear to play a part **Tierney and Papadakis. (2002)**. The disease has been considered as one of the major health concerns worldwide today **Stolar et al., (2008)**. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly diet. Diabetes is the fourth leading cause of death in most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialized nations **Pradeepa and Mohan. (2002)**. India leads the world today with the largest number of diabetics in any given country. Common complications of diabetes are retinopathy, angiopathy, neuropathy etc **Amos et al., (1997), Bajaj and Madan. (1993)**. Diabetes mellitus is a major global health problem, affecting 415 million adult people, accounting for 5 million deaths in 2015, 1 person die every six seconds from diabetes and 46% people with diabetes remain undiagnosed. The global annual cost of diabetes is more than USD 650 billion and by 2040 the number of affected people is expected to increase to 642 million globally **IDF (2015)**. In Bangladesh, a meta-analysis showed that the prevalence of diabetes among adults had increased substantially, from 4% in 1995 to 2000 and 5% in 2001 to 2005 to 9% in 2006 to 2010 and the prevalence will be 13% by 2030 **Saquib et al., (2012)**. It has been also reported that diabetes tends to increase low-density lipoprotein cholesterol and decrease high-density lipoprotein cholesterol levels in blood that leads to coronary occlusions and blocks. Many drugs are commercially available for the treatment of DM but long term use of these drugs causes some side effects. Therefore scientists are looking for herbal medicines having least or no side effect and low toxicity **Michael (2007), Chen et al., (2001)**. Therefore it is required to find out new drugs for the treatment of diabetes mellitus with maximum efficiency and least side effects. Many plants have been found with antidiabetic activity experimentally **Wang et al., (2013)**.

Piper chaba is a flowering vine in the family Piperaceae that is native to South and Southeast Asia. *P. chaba* is called (**Chui Jhal**) or (**Choi Jhal**) in the Khulna-Jessore region of Bangladesh. It is a creeper plant that spreads on the ground. It may also grow around large trees. The leaves are oval-shaped and about 2 to 3 inches long. The flowers are monoecious and blossom during the monsoon. The fruit looks similar to other varieties of long pepper, with an elongated shape that can grow up to 3 inches long. The fruit is red when ripe, which turns dark brown or black when dry. Almost all parts of this plant are used in traditional medicine for the treatment of ulcers, psoriasis, anemia, piles, jaundice, vitiligo, hemorrhage,

diabetes, convulsion, hepatitis, dysentery, biliousness and purgative. There is no sufficient data available on the pharmacological properties of the *P. chaba* fruits in Bangladesh. That's why this study was undertaken to evaluate the anti-diabetic and antihyperlipidemic properties of *P. chaba* plants extract.

2. MATERIALS AND METHODS

2.1. Collection of plant material and authentication: The mature *Piper chaba* plants were collected from Daulatpur, Khulna, Bangladesh and authenticated by Dr. A.H.M. Mahbubur Rahman, Professor Department of Botany University of Rajshahi, Bangladesh.

2.2. Preparation of extract: The plants were first washed with clean water to remove adhering dirt and sorted to fresh and mature plants. After complete drying, the entire portions were grinded into a coarse powder by a grinding machine (FFC-15, China) and stored in an airtight container for further use. For each solvent about 80 gm of the powdered material was taken in separate clean, round bottomed glass bottle and soaked in 400 ml of solvent. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The resulting extracts were filtered through Whitman No.1 filter paper. Afterwards, the solvents were evaporated under reduced pressure at 39⁰C using rotary evaporator. At last, the residues were kept in small sterile bottles under refrigerated conditions until used.

2.3. Chemicals: Alloxan was purchased from Sigma chemical Co. All others chemical were used in analytical grade and were acquired from commercial sources.

2.4. Animal care: Swiss albino mice were selected as experimental animal to carry out this study. Mice weighting about 20-25 gm were collected from the International Cholera and Dysentery Disease Research, in Dhaka, Bangladesh (ICDDR). They were individually housed in polypropylene cages in well-ventilated rooms (temperature 25 ± 2oC; humidity 55 ± 5% with 12h light/dark cycle), under hygienic conditions. Mice were allowed free access to standard dry pellet diet and water.

2.5. Induction of diabetes: Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of Alloxan (80 mg/kg body weight) in a 0.1 M sodium citrate buffer (pH 4.5). The age-matched control mice received an equivalent amount of citrate buffer. Food and water intake were closely monitored daily after alloxan administration. The

development of hyperglycemia in mice was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood, 48 hours after Alloxan administration, with a portable glucometer (Accu-Chek, Roche, Germany). The animals with fasting blood glucose level \geq 11.0 mmol/L with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered as diabetic and included in the study.

2.6. Experimental groups: After one-week acclimatization period, the animals were divided into five groups with five animals in each. The mice grouping were as follow:

Group-1 (Normal control): Mice feed with standard pellet diet and water

Group-2 (Diabetic control): Diabetic mice without treatment.

Group-3 (Positive control): Diabetic mice were treated by Glibenclamide at dose of 5 mg/kg body weight.

Group-4 (Treated-1): The diabetic mice treated with acetone extract of *Piper chaba* at a dose of 100 mg/kg body weight for 21 days.

Group-5 (Treated-2): The diabetic mice treated with acetone extract of *Piper chaba* at a dose of 200 mg/kg body weight for 21 days.

Group-6 (Treated-1): The diabetic mice treated with ethanol extract of *Piper chaba* at a dose of 100 mg/kg body weight for 21 days.

Group-7 (Treated-2): The diabetic mice treated with ethanol extract of *Piper chaba* at a dose of 200 mg/kg body weight for 21 days.

2.7. Collection of blood: Blood samples from all groups were collected on day 1, 5, 10, 15 and 21 in a fasting state from the tail vein by 26 G needle and syringe. At the end of experiment period (21 days), mice were sacrificed after overnight fasting. Mice were anesthetized with chloroform and blood was collected from the heart. The serum was separated by allowing blood samples left for 15 minutes at temperature of 25°C then centrifuged at 3000 rpm for 20 minutes, then kept in plastic vials at -80°C until the experiments were performed.

2.8. Measurement of biochemical parameters: Blood glucose level was measured by glucose oxidase peroxidase method. Plasma concentrations of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL, CRP, SGPT, SGOT, were measured using a quantification kit (Linear chemicals, Barcelona, Spain) by automatic Bio-analyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

2.9. Statistical analysis: All values were expressed as Mean \pm Standard Deviation. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's t test using SPSS software of 16 version. $p < 0.05$ were considered to be statistically significant.

3. RESULTS

3.1. Effects of PCA and PCE on blood glucose level: In *in vivo* antidiabetic assay, PCA and PCE were administered to evaluate the blood glucose level of Swiss albino mice. The plant extract produced significant changes in the blood glucose level in Alloxan-induced diabetic mice showed in Table 1. The administration of plant extract showed significantly ($p < 0.01$ to $p < 0.001$) reduction of blood glucose level in both concentration of 100 mg/kg and 200 mg/kg body weight respectively compared to diabetic control mice. This level of reduction was very close to positive control mice (here 5mg/kg body weight Glibenclamide is used as standard). In 5th to 21st days, PCA at both doses (100 mg/kg and 200 mg/kg body weight) lowered the blood glucose level by 1.04% - 52.80% and 6.80% - 62.35% and PCE at both dose (100 mg/kg and 200 mg/kg body weight) lowered the blood glucose level by 8.14% - 48.65% and 10.64% - 58.17% respectively.

3.2. Effects of PCA and PCE on lipid profile level: Fig. 1 showed the serum lipid profile levels of Total cholesterol (TC), Triglycerides (TG), LDL, VLDL, HDL and hyper cholesterol of control and Alloxan-induced diabetic mice. All lipid except HDL parameters showed in significantly different at $p < 0.001$. Reduction of Total Cholesterol (TC) level was 21.54 % (in 100 mg/kg body weight) and 31.39 % (in 200 mg/kg body weight) observed in *P. chaba* acetone extract (PCA) and 12.63% (in 100 mg/kg body weight) and 24.26 % (in 200 mg/kg body weight) observed in *P. chaba* ethanol extract (PCE) treated diabetic mice group whereas in positive control group reduction was 36.78 %. In 21 days observation the treatment groups showed significant decrease ($p < 0.001$) of total cholesterol compared with the diabetic control group. The Levels of triglyceride in the diabetic group were increased after 21 days of alloxan induction.

Serum triglyceride of treatment group of mice was lower than that of diabetic control. In Fig.1 the diabetic control group showed an increase in LDL levels higher than the (normal) control group. LDL level was significantly reduced ($p < 0.001$) with *P. chaba* treatment at 39.08 % (in 100 mg/kg body weight of PCA), 76.37 % (in 200 mg/kg body weight of PCA) and 22.65 % (in 100mg/kg body weight of PCE) and 38.23 % (in 200mg/kg body weight of PCE) respectively. VLDL level was significantly reduced ($p < 0.001$) with *P. chaba* treatment

at 22.28 % (in 100 mg/kg body weight of PCA), 32.12 % (in 200 mg/kg body weight of PCA) and 19.04 % (in 100mg/kg body weight of PCE), 29.3% (in 200mg/kg body weight of PCE) respectively. Whereas the HDL level increased by 20.16% (in 100 mg/kg body weight of PCA), 43.8% (in 200mg/kg body weight of PCA) and 20.98% (in 100mg/kg body weight of PCE) and 37.67 % (in 200 mg/kg body weight of PCE) respectively. Glibenclamide (5 mg/kg body weight) treated mice showed the reduction of TG by 38.50 %, LDL by 77.53%, VLDL by 34.75% and an increase in HDL by 39.71% respectively.

3.3. Effects of PCA and PCE on serum SGPT, SGOT and CRP level: Serum SGPT and SGOT levels were increased significantly ($p < 0.001$ to $p < 0.01$) in diabetic mice compared to the normal mice and these were also compensated considerably ($p < 0.001$) by the oral administration of PCA, PCE and glibenclamide (Fig. 2). The percent of lowering of SGPT level by *Piper chaba* both acetone and ethanol extract from diabetic control groups were 12.83 % to 26.51 % and 10.90% to 19.99% at dose 100 mg/kg and 200 mg/kg body weight respectively whereas 36.44 % for glibenclamide. The reduction of SGOT level was highly significant ($p < 0.001$) for *Piper chaba* both acetone and ethanol extract at 14.90 % to 32.66 % and 8.77 % to 24.24% at dose 100 mg/kg and 200 mg/kg body weight respectively, whereas 30.49 % for glibenclamide.

CRP is a potent marker of hepatic and cardiovascular diseases, which is increased in diabetic condition. The administration of PCA, PCE and glibenclamide reduced the CRP level significantly ($p < 0.001$ to $p < 0.01$) compared to the diabetic control mice, MCUF administration at 100 and 200mg/kg body weight doses lowered the CRP level by 33.21% and 39.92% respectively.

FIGURE AND TABLE

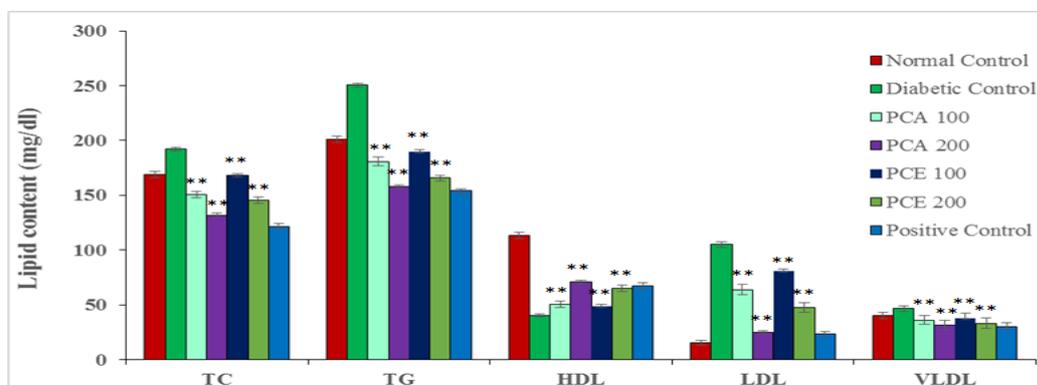


Fig. 1: Effects of acetone and ethanol extract of *P. chaba* on lipid profile of diabetic mice after 21 days treatment.

Results were expressed as mean \pm standard deviation (n=5). Biochemical parameters of lipid profile in the treated mice were significantly different from diabetic control group at $**p<0.001$ and $*p<0.01$ respectively.

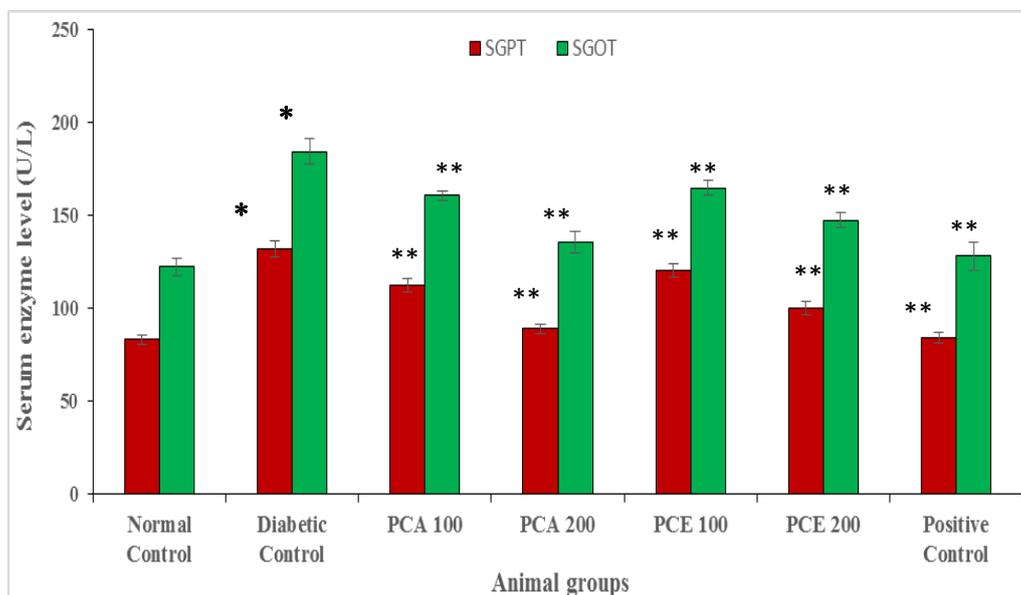


Fig. 2: Effects of acetone and ethanol extract of *P. chaba* on serum SGPT and SGOT level in diabetic mice after 21 days treatment.

Results are expressed as mean \pm standard deviation (n=5). $**p<0.001$ compared with normal control (NC) group; $**p<0.001$ and $*p<0.01$ compared with diabetic control group.

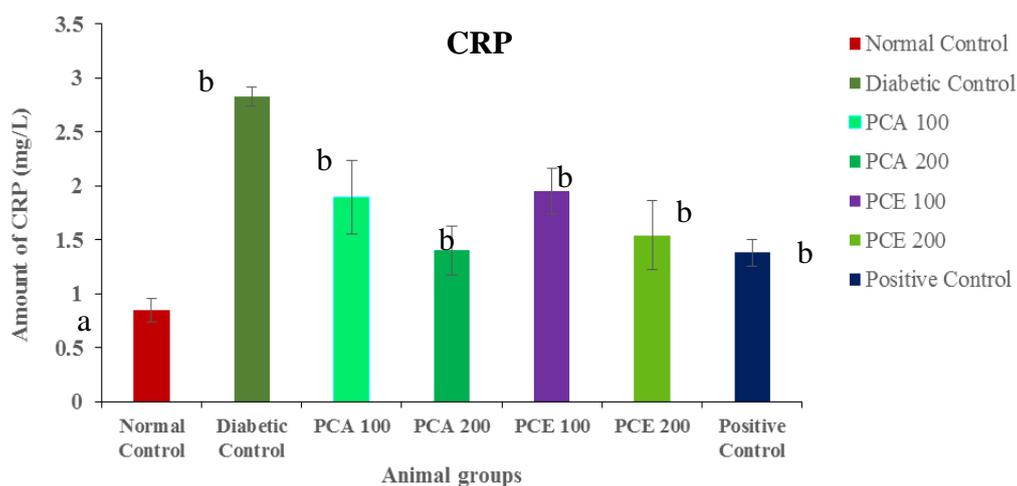


Fig. 3: Effects of acetone and ethanol extract of *P. chaba* on serum CRP level in diabetic mice after 21 days treatment.

All values are expressed as mean \pm standard deviation (n=5). $^ap<0.001$ compared with normal control group; $^bp<0.01$ compared with diabetic control group.

Table 1: Effects of acetone and ethanol extract of *P. chaba* on blood glucose level in alloxan-induced diabetic mice.

| Groups of animals | | Serum glucose concentration (mmol/L) | | | | |
|-------------------|---------|--------------------------------------|------------------------|-------------------------|------------------------|--------------------------------------|
| | | Day 1 | Day 5 | Day 10 | Day 15 | Day 21 |
| NC | | 6.03±2.53 | 5.56±3.46 | 5.97±1.62 | 6.94±2.65 | 5.86±4.28 |
| DC | | 19.4±4.52 ^a | 19.1±3.02 ^a | 20.32±4.02 ^a | 22.7±3.18 ^a | 26.7±7.98 ^a |
| PC | | 19.2±7.60 | 15.3±3.20 ^b | 12.3±3.18 ^c | 11.3±3.18 ^c | 9.1±2.05 ^c |
| Treatment | PCA 100 | 20.7±1.76 | 18.9±1.76 ^b | 14.1±8.68 ^b | 13.9±2.67 ^b | 12.6±7.96 ^c |
| | PCA 200 | 20.3±0.91 | 17.8±3.91 ^b | 14.9±0.77 ^b | 10.5±1.20 ^c | 10.3±1.06 ^c |
| | PCE 100 | 22.1±0.67 | 20.3±0.67 ^b | 18.9±1.76 ^b | 16.3±4.52 ^b | 13.4±5.37 ^c |
| | PCE 200 | 26.3±2.06 | 23.5±2.06 ^b | 20.7±5.55 ^b | 18.6±1.67 ^c | 11.4 ^b ±4.17 ^c |

All values are expressed as mean ± standard deviation (n=6). ^a $p < 0.001$ compared with general control group; ^b $p < 0.01$ and ^c $p < 0.001$ compared with diabetic control group.

4. DISCUSSION

Our results show that the intraperitoneal administration of alloxan to mice significantly increase blood glucose levels three days after injection, as well as decreased body weight. In addition, other diabetes-related signs were observed. These results agree with previous observations that have employed this model and that also report loss of body weight and increased blood glucose level **Junod et al., (1969), Montano et al., (2010)**.

This study showed that the acetone and ethanol extract of *P. chaba* (PCA & PCE) significantly diminished blood glucose levels ($p < 0.01$ to $p < 0.001$) in diabetic mice. This hypoglycemic effect is similar to the one reported for other plants **Pérez et al., (2003), Islam (2011), Sasidharan et al., (2011), Gaamoussi et al., (2010)**. Such effect may be explained in part by either a decrease in the rate of intestinal glucose absorption **Hamden et al., (2011), Porchezian et al., (2000), Gupta et al., (2012)**, or an increase in peripheral glucose utilization. In this line, some authors have ascertained increased catabolism of glucose due to GLUT4 translocation to the plasma membrane in muscle and brown adipose cells, with upregulation of the uncoupling protein-1 in brown adipose tissue and hepatic gluconeogenesis **Bera et al., (2010)** causing as a result hyperinsulinemia or enhancement of peripheral glucose utilization **Adeneye et al., (2010), Abeywickrama et al., (2011)**.

Our results demonstrated that TG, TC, VLDL, LDL levels decreased in diabetic mice with the administration of *P. chaba* (PCA & PCE) extract. The hyperlipidemia associated with diabetes may result from an accelerated hepatic triglyceride biosynthesis and the release of VLDL without an increase in its rate of clearance from the blood by lipoprotein lipase, which

is dependent on the insulin/glucagon ratio **Gepts et al., (1981)**. Our current study clearly indicated that the oral administration of PCA and PCE have significantly ($p < 0.01$ to $p < 0.001$) antidiabetic and antihyperlipidemic ($p < 0.001$) activities, which are quite similar to the antidiabetic and hypolipidemic activities of glibenclamide in alloxan induced diabetic mice compare to the diabetic control mice. PCA showed maximum antidiabetic activity was found at dose 200 mg/kg body weight among two doses, where blood glucose level was decreased by 6.80 % - 62.35 % compared to the diabetic control group. PCA and PCE showed maximum hypolipidemic activity. Antihyperlipidemic activity of PCA at dose 200 mg/kg body weight was found almost similar to the antihyperlipidemic activity of glibenclamide in diabetic mice at dose 5 mg/kg body weight. At this dose PCA decreased serum TC level by 31.39%, TG level by 37.03%, LDL level by 76.37%, VLDL level by 32.12% and increased HDL level by 43.8% respectively.

On the other hand, the elevation of serum biomarker enzymes such as SGPT and SGOT has been observed in diabetic mice indicating impaired liver function that may be due to hepatic damage induced by hyperglycemia **Pepato et al., (2004), Kondeti et al., (2010), Dobretsov et al., (2007), Rodrigues et al., (2010)**. In addition, CRP is a marker of systemic inflammation, which is emerging as an independent risk factor for cardiovascular disease **Ridker et al., (1997), Ridker et al., (1998)**. The serum CRP level is elevated in diabetic patients which has been reported previously **Ford (1999)**. In our present study PCA and PCE reduced serum CRP level significantly ($p < 0.001$) in diabetic mice.

Data are preliminary on the hypoglycemic effect of *P. chaba* (PCA & PCE) in alloxan-induced diabetic mice. This study have some limitations: a sample size with five animals in every group, a short period of study, the diabetes model correspond more to a type 1 diabetes than to type 2 diabetes, moreover the active metabolite in the *P. chaba* was not identified. Further studies administering the extract for longer periods of time are necessary.

5. CONCLUSION

This preliminary study confirms the hypoglycemic effect of *P. chaba* (PCA & PCE) together with other beneficial effects in diabetic mice. These results suggest that the acetone and ethanol extract of *P. chaba* (PCA & PCE) may improve the metabolic disruption produced by diabetes. However, further research is needed to gain a better understanding of its potential therapeutic action, the implicated phytochemical constituents and the exact mechanism of action.

Ethical Clearance

This research work was approved by the Institutional Animal, Medical Ethics, Bio-Safety and Bio-Security Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes, and Living Natural Sources, memo no. 118/320-IAMEBBC/IBSc. Institute of Biological Sciences, University of Rajshahi, Bangladesh.

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist and are fully responsible for all experimental works and the content of this article.

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