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Assessment of the Antidiabetic Potential of Piper Chaba Stem Extract in Streptozotocin-Nicotinamide-Induced Diabetes in Rats

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KEYWORDS

Piper Chaba stem; type II diabetes; antidiabetic; in vitro; in vivo; streptozotacin-nicotinamaide.

ABSTRACT:

Introduction: *Piper chaba*, belongs to the family Piperaceae and is an inhabitant of West Bengal, India. The unripe fruits show various pharmacological activities due to the presence of Piperine. There is no previously reported work on the antidiabetic activity of the *P. chaba* stem.

Objective: The present research work is carried out to examine the antidiabetic potential of *P. chaba* stem extract in both *in vitro* and *in vivo* diabetic models.

Methods: In this research work, the methanolic extraction of the *P. chaba* stem was done using a cold maceration process. α-Amylase and α-glucosidase inhibition assays were performed to assess the *in vitro* antidiabetic activity. Further, *in vivo* antidiabetic activity was studied using a streptozotocin-nicotinamide-induced diabetic model in rats.

Results: The methanolic extract of *P. chaba* stem (PCME) shows good antidiabetic potential in both α- amylase (IC50:108.89 µg/ml) and α- glucosidase (IC50: 127.28 µg/ml) as compared to their respective standard. Further, the methanolic extract showed no acute toxicity in the rat before the experiment. The chronic study of the PCME extract in two different doses (200 mg/kg b.w. & 400 mg/kg b.w.) and standard oral hypoglycemic drug Metformin (70 mg/kg b.w.) after induction of diabetes using STZ-nicotinamide injection followed up for 21 days, shows a significant lowering in the rise blood glucose level and it also helps to maintain the uncontrollable weight loss in diabetic rats. The PCME regulates the serum parameters in the diabetic group which helps in increasing haemoglobin and other blood counts. In addition, the abnormal levels of lipid peroxides and glutathione in the liver tissues were restored to those of the control rats. These results show that PCME possesses good antioxidant and antidiabetic activities.

Conclusion: PCME showed potent antidiabetic properties against STZ-nicotinamide induced diabetic rats.

1. Introduction

Natural products have gained a lot of attention these days as possible therapeutic agents for the treatment of various metabolic disorders, including hypertension, diabetes mellitus, obesity, and dyslipidemia (1). Insulin resistance (type 2 diabetes) or inadequate insulin production (type 1 diabetes) causes diabetic mellitus, a metabolic disorder. In type 2 diabetes, the individual develops resistance to insulin hormones, leading to increased blood glucose levels (2). The World Health Organization has recognized type 2 diabetes as a major global health concern. The World Health Organization reports that this illness affects more than 176 million individuals globally (3). As a result, it is one of the world's leading causes of death and disability. Moreover, some studies suggest that increasing antioxidant uptake and regulating digestive enzymes help combat diabetes and its associated problems (4, 5). Although marketed synthetic medicines like insulin, biguanides, sulfonylureas, and alpha-glucosidase inhibitors can effectively manage diabetes when taken regularly, they may exhibit unfavorable side effects that cause severe hypoglycemia (6). As a result,

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there is a need for research into more potent antidiabetic drugs. The WHO has suggested traditional medicine as a viable choice for diabetes management due to its extensive history, effectiveness, and lack of adverse effects (5, 7, 8).

2. Objectives

The *Piper chaba* belongs to the Piperaceae family and naturally occurs in Singapore, Indonesia, Bangladesh, Sri Lanka, and some places in India, such as Kerala, West Bengal, and Tripura. In this plant family, Piperaceae 9 is the most abundant compound (9). *P. chaba*, commonly known as Chui Jhal or Choi Jhal, is a common spice herb in India and Bangladesh. This plant has been widely used in traditional medicine because of its numerous important biological activities (10). This plant contains various phytochemicals such as saponins, glycosides, flavonoids, terpenoids, alkaloids, and phenols, which exhibit medicinal properties that help treat different diseases. *Dried, mature, unripe fruits of P. chaba, are widely used as carminatives, antidiarrheals, expectorants, and oxytocides in traditional medical systems.* Studies have shown that *P. chaba* fruit extract has numerous uses, such as hepatoprotective, anti-inflammatory, cytotoxic, anti-tumor, chemo-preventive, and immunomodulatory (11). *P. chaba* fruits show excellent antioxidant potential (12). The dried roots of *P. chaba* have antimicrobial and antidiabetic activities (13). The objective of this research work is to evaluate the *in vitro* antidiabetic activity of *P. chaba* stem extract. Additionally, we conducted an in vivo antidiabetic study using this *P. chaba* stem extract. The *P. chaba* stem has not yet received any scientific reports. So, this research work aims to explore the antidiabetic activity of the *P. chaba* stem.

3. Materials and methods

3.1 Chemicals and materials

Analytical-grade solvents and chemicals were all used in the process. We bought streptozotocin, nicotinamide, and acarbose (≥ 95%) from Sigma-Aldrich (Bangalore, India). Other chemicals, such as 4-nitrophenyl-αD-glucopyranoside, α-amylase, and α-glucosidase, were obtained from Merck Life Sciences Private Limited (Mumbai, India). 10% formalin was acquired from Himedia, while dinitro salicylic acid (DNSA), sodium dihydrogen phosphate, sodium hydrogen phosphate, and soluble starch were procured from SRL (Mumbai, India). Sigma Aldrich provided the standard medication, metformin hydrochloride (≥ 95%). We bought normal saline and water for injections from a nearby medical supply store.

3.2. Plant collection and extraction

We collected the *P. chaba* plant from West Bengal, India. The Botanical Survey of India, located in Shibpur, India, prepared and authenticated the herbarium. We registered the voucher specimen with the number. The collected plant part was shed, dried, and powdered. Then 250 g of powder was taken in a conical flask, and 800 mL of methanol was added. We kept the mixture for 7 days to facilitate the cold maceration process. After that, the mixture was filtered, and the filtrate solvent was evaporated by using a rotary vacuum evaporator. At last, the dried methanolic extract (PCME) of this plant was stored at 4 °C for further analysis.

3.3. In vitro antidiabetic activity

3.3.1. α-amylase inhibition assay

We evaluated the 'α-amylase inhibition of methanolic extract based on previously published work (14). By employing the α-amylase inhibitory assay and comparing it to the reference drug Acarbose, the antidiabetic activity of the methanolic extract was examined. Phosphate buffer (pH 6.9, sodium chloride concentration of 0.06 M) was used to prepare the PCME solution with serial dilutions (100–1,000 µg/mL). From the dilution of the extract solution, 500 µL was pipetted, and 500 µL of α-amylase (13 U/mL) was added. We then incubated the mixture for 30 minutes at 37 °C. After that, the mixture was mixed with 500 µL of a 1% starch solution and further incubated for 20 minutes at 37 °C. 1 mL of 3,5-dinitrosalicylic acid (DNSA) was added to stop the reaction. The absorbance at 500 nm was measured using an ultraviolet-visible (UV-vis) spectrophotometer, where phosphate buffer was used as a blank. We carried out three repetitions of the experiment. We then calculated a molecule's half-maximum inhibitory concentration (IC50) to determine its efficiency in reducing biological or biochemical function. % Inhibitory activity = [(A0 - A1)/A0] x 100, where A0 = absorbance of the control and A1 = absorbance of the sample. We used the half-maximal inhibitory concentration (IC50) to represent the inhibitory action.

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3.3.2. α-Glucosidase inhibition assay

For the α-glucosidase inhibition assay of the methanolic extract, a series of serial dilutions were used. In a test tube, mix 250 µL of 100 mM potassium phosphate buffer (pH 6.8), 150 µL of 5 mM 4-nitrophenyl-D-glucopyranoside, 50 µL of different PCME concentrations, and 150 µL of α-glucosidase (0.1 unit/mL in 10 mM potassium phosphate buffer). This mixture was incubated at 37 °C for 30 minutes, and then 600 µL of 200 mM Na2CO3 was added to stop the reaction further in the mixture. Then the absorbance was measured at 400 nm by using a UV-visible spectrophotometer. % Inhibitory activity = [(A0 - A1)/A0] x 100, where A0 = absorbance of the control and A1 = absorbance of the sample. We calculated the half-maximal inhibitory concentration (IC50) using the percentage of inhibitory activity.

3.4. In vivo antidiabetic activity

3.4.1. Animals for in vivo studies

We selected forty male mature rats in good health from the Wistar Albino strain, weighing between 150 and 300 g, for the anti-diabetic study. We purchased them from the authorized vendor (Chakraborty Enterprise, Kolkata, India; Regd. No. 1443/PO/Bt/s/11/CPCSEA). The animals were housed in polypropylene cages (Tarsons, India) with bedding made of paddy husk and standard laboratory conditions (20 ± 2 °C, 50 ± 15% relative humidity, and a 12-hour light-dark cycle). Rats had unrestricted access to water and a standard diet. The Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) at the University of North Bengal in West Bengal, India, approved the study (IAEC/NBU/2023/67). Before the study, all animals went through a two-week acclimatization period.

3.5. Acute toxicity studies in rats

We tested the acute oral toxicity of PCME on male Wister albino rats by OECD (Organisation for Economic Cooperation and Development) Guideline 423. In each group, there were two groups of three rats. As the control group, Group I received distilled water, whereas Group II received PCME (2000 mg/kg) orally once only. We routinely checked each animal every 4 hours after 14 days for signs of general toxicity and behavioral abnormalities. The body weight, food intake, and number of deaths were all recorded for these rats.

3.5.1. Oral glucose tolerance test (OGTT) in diabetic rats

We evaluated the hypoglycemic effects of methanolic extract in a rat model using the modified method of Reza et al. (15) . We conducted the experiment with four groups of six rats each. Rats in the standard group received metformin (70 mg/kg b.w.), while those in the control group received only distilled water (10 mL/kg b.w.). Two different groups received PCME treatments at dosages of 200 and 400 mg/kg b.w. Before the trial, we starved all the animals for a full night. We monitored the fasting blood glucose concentration, and then administered the test sample orally, followed by a 30-minute rest period for the animals. Next, we administered a glucose solution (2 g/kg b.w.) orally to each of the four groups. We drew blood from the rat tail vein at 0, 30, 60, and 120 minutes after the glucose administration. Finally, we used an Accu-Check electronic glucometer to determine the blood glucose levels.

3.5.2. Induction of Type 2 Diabetes

To induce type 2 diabetes in laboratory animals that have been starved overnight, intraperitoneally (i.p.) streptozotocin (STZ) (60 mg/kg body weight) diluted in 0.1 M citrate buffer (pH 4.5) prepared with sterile water was given. We administered nicotinamide (100 mg/kg) 15 minutes later. We identified rats with blood glucose levels higher than 200 mg/dL as type 2 diabetic rats after seven days and chose them for the study.

3.5.3. Experimental design

The animals were divided into five groups, where six animals were present in each group (n = 6).

Group I: normal, healthy rats that received only distilled water (2 mL/kg, p.o.) continuously for 21 days.

Group II: diabetic control rats that received only distilled water (2 mL/kg, p.o.) continuously for 21 days.

Group III: diabetic rats that received PCME (200 mg/kg, p.o.) continuously for 21 days.

Group IV: diabetic rats that received PCME (400 mg/kg, p.o.) continuously for 21 days.

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Group V: diabetic rats that received the standard drug Metformin (Met) (70 mg/kg, p.o.) continuously for 21 days. We administered a distinctive treatment to the animals for 21 days. We used a single-touch glucometer to test the fasting blood glucose levels on days 1, 7, 14, and 21 after drawing blood from the tail vein. On days 1, 7, 14, and 21 of the experiment, we measured body weight while all animals, including the healthy control group, were fasting.

3.5.4. Evaluation of serum parameters

We assessed the effects of giving the methanolic extract to rats with diabetes after the 21st day of animal sacrifice. We measured several serum parameters to evaluate the effects of the extracts on diabetic animals compared to the control group, normal healthy animals, and the standard metformin group. These included alkaline phosphatase (ALP), α-amylase, γ-glutaraldehyde, creatinine, serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT). Standard enzyme-linked immunosorbent assay (ELISA) kits were used, following the instructions included in the kit.

3.6. Assessing oxidative stress markers in liver tissues

3.6.1. Liver homogenate preparation

We made a 10% liver homogenate using the earlier methodology to evaluate oxidative stress in the liver. In short, 10 mL of cold phosphate buffer solution (pH 7.4) at 4 ºC was used to homogenize liver tissues (1 g). The mixtures were homogenized, and then they were centrifuged for 10 minutes at 4 ºC at 12000 rpm. Then the supernatant that was produced was collected, and the following oxidative stress markers were detected:

3.6.2. Determination of Catalase

The Abei et al. protocol was followed when evaluating the activity of catalase (CAT). We measured changes in absorbance at 240 nm using a UV-VIS spectrophotometer (UV-1780, Shimadzu, Japan) after one minute. An absorbance change of 0.01 units/min was defined as one CAT unit.

3.6.3. Determination of reduced glutathione

We used G. L. Ellman's (1959) [61] method to measure the levels of reduced glutathione (GSH). The yellow-colored solution's absorbance was immediately measured at 412 nm using a SPECTRO Star Nano UV/vis plate reader (BMG LABTECH, Germany) in comparison to a blank reference.

3.6.4. Determination of Lipid Peroxidation

We measured the malondialdehyde (MDA) content in the liver using the standard protocol to determine liver peroxidation. We measured the thiobarbituric acid reactive substrates (TBARS) calorimetrically to determine the amount of liver lipid peroxidation. The clear supernatant's absorbance was then measured at 535 nm using a SPECTRO Star Nano UV/vis plate reader (BMG LABTECH, Germany) against a blank reference.

3.6.5. Histopathological study of the liver

We preserved a portion of the liver for further processing in a 10% formalin solution for histopathological evaluation. The paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin using a microtome. Subsequently, the sections stained with Zeiss light microscopy were examined with a 40X magnification lens to gain a micro-level understanding of the study's impact. Additionally, we took tissue images to identify injuries in those specific organs.

3.7. Statistical analysis

The mean standard error was utilized to represent the data and determine the overall significance of the study. We performed one-way ANOVA analysis in vivo experiments using GraphPad Prism 4.0 to evaluate the effects of different animal treatments. We conducted multiple comparisons between different treatments at a p-value of less than 0.05.

4. Results and discussion

3.1 α-Amylase activity

α-Amylase is a vital pancreatic enzyme that significantly speeds up the digestion of carbohydrates. By blocking α-amylase, the digestibility of carbohydrates is inhibited, ultimately leading to a reduction in blood glucose levels (16, 17). The activity of the α-amylase enzyme decreased in PCME, as shown in Fig. 1.a. It appears that the enzyme inhibition is dose-dependent, even though the extract's concentration has an impact. The IC_{50} values for PCME (108.89 µg/mL) were slightly lower than those for standard acarbose (89.34 µg/mL) in terms of inhibitory activity. The PCME has good α-amylase inhibiting

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activity compared to acarbose (standard), ultimately lowering the diabetic risk and helping to regulate hyperglycemia (18).

3.2. α-Glucosidase activity

The α-glucosidase enzyme inhibitors slow down the breakdown of complex carbohydrates and stop the rise in postprandial glucose levels (19). When intestinal α-glucosidase is stopped, the rate of oligosaccharide hydrolytic cleavage slows down. This lets the lower

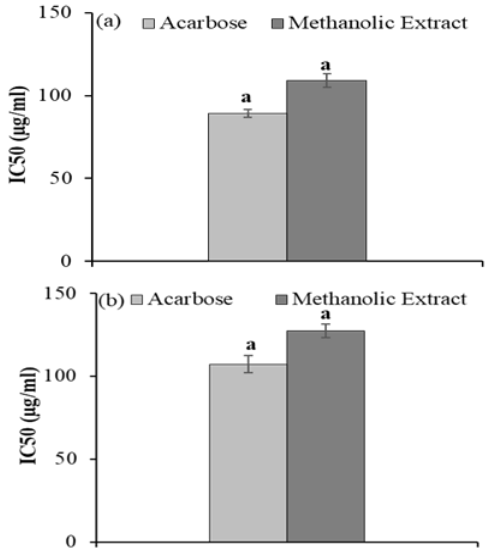

Figure 1. (a) α-Amylase and (b) α-glucosidase inhibition assay of PCME and standard. Data are mean ± SD of six animals for each group. Values with different letters are significantly different ($p < 0.05$), as shown by the Tukey test.

small intestine start breaking down carbohydrates. This broadening of the digestive process generally delays the pace of glucose absorption into the circulation. This is one of the most effective ways to reduce the blood glucose rise that occurs after meals, which helps prevent the development of issues related to late-life diabetes (20). Fig. 1.b shows how well PCME inhibited α-glucosidase compared to the standard. The sample PCME (127.28 µg/mL) significantly inhibited the enzyme compared to the standard acarbose (IC50: 107.24 µg/mL), making it a good drug for diabetes.

3.3. Acute toxicity study

The PCME acute toxicity experiment reported no mortality after 72 hours at a dose of 2000 mg/kg. As far as we know, if the oral LD_{50} of a pharmaceutical product or chemical is more than 1,000 mg/kg, it can be considered low-hazardous and safe (21). At the selected dosages, there were no toxicity or negative effects and no behavioral changes in the rats. Finally, after administration with PCME, no fatal side effects were found in any of the group's experimental rats.

3.4. Oral glucose tolerance test (OGTT)

We performed the OGTT in normal rats at 0, 30, 60, 90, and 120 minutes to measure the glucose contents (Fig. 2.a). Before glucose loading, the rats' blood glucose levels were similar, with no differences found. The normal and diabetic groups reached their maximal glucose levels 60 minutes following the oral glucose challenge (Fig. 2.a). When compared to diabetes control (Fig. 2.a), PCME treatment before glucose loading significantly reduced the rise in blood glucose levels at 90 minutes ($P<0.001$) and 120 minutes ($P < 0.01$). The percentage of glucose in the blood changed by 16.35%, 28.30%, and 37.11% for metformin (70 mg/kg), PCME (200 mg/kg), and PCME (400 mg/kg) after 60 minutes compared to the group that had been given a vehicle (Fig. 2.a).

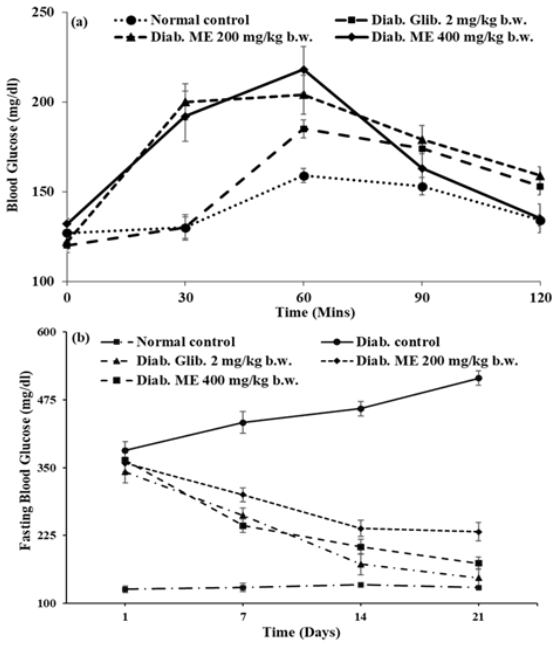

Figure 2. (a) Oral glucose tolerance test in normal rats and (b) fasting blood sugar level in diabetic rats. Values are mean ± SD (n=6 animals).

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To maintain a normal plasma glucose level, glucose is utilized. The ability to use glucose through systemic body circulation is defined as glucose tolerance which is measured by OGTT. OGTT can diagnose pre-diabetes and diabetes (22). The result is supported by previously published works (23, 24).

3.5. Effects of PCME on FBS and body weight in diabetic rats induced with STZ-Nicotinamide

In this study, the administration of streptozotocin (STZ) elevated the blood glucose level an average of four times in all groups except the normal control group. We also administered the nicotinamide (NA) injection after 15 minutes to prevent severe damage to the pancreatic cells caused by STZ-inoculation. On the 2nd day of the streptozotocin-nicotinamide injection, we selected the rats with a fasting blood glucose level of more than 200 mg/dl for the PCME extract treatment. Fig. 2.b. represents the fasting blood glucose level of the rats in different groups on the 1st, 7th, 14th, and 21st days of the experiment. The results demonstrated a significant reduction in the fasting blood glucose level in type 2 diabetic rats with PCME doses of 200 mg/kg and 400 mg/kg body weight. The maximum reduction of blood glucose level on the 21st day compared to the 1st day for PCME (200 mg/kg) and PCME (400 mg/kg) was found to be 35.10% (p <0.01) and 52.19% (p<0.01). Metformin, the standard oral hypoglycemic drug, showed a maximal reduction of 57.02% (p<0.01) on the 21st day compared to the 1st day. Conversely, we tracked the body weight of these STZ-nicotinamide-induced diabetic rats across all groups on the 1st, 7th, 14th, and 21st days, as presented in Table 1. It shows that the body weight of the diabetic control group was significantly reduced as compared to normal rats (p< 0.05). The diabetic control group lost weight throughout the study. The rats with PCME were administered at dosages of 200 mg/kg and 400 mg/kg, and body weight significantly improved (p<0.05) in comparison to the diabetic control group (Table 1).

Table 1. Changes in the body weight (g) of rats in the five groups during the experimental period of 21 days

	1 Day	7 Days	14 Days	21 Days
Normal control	213 ± 11	222 ± 15	229 ± 13	235 ± 10
Diab. control	231 ± 16	232 ± 13	175 ± 14	168 ± 11
Diab. Glib. 2 mg/kg b.w.	223 ± 15	180 ± 10	195 ± 8	218 ± 5
Diab. PCME 200 mg/kg b.w.	231 ± 11	202 ± 8	200 ± 4	213 ± 7
Diab. PCME 400 mg/kg b.w.	225 ± 9	198 ± 10	207 ± 6	227 ± 8

Data are mean ± SD of six animals for each group.

The results of this study show that, through day 21 of the experiment, the PCME may considerably reduce blood glucose levels while maintaining body weight. Diabetes mellitus has a correlation with dehydration and weight loss (25). In diabetes, weight loss and polydipsia occur due to tissue protein breakdown, which further leads to muscle loss. Because of their bioactive compounds, diabetic rats show a reduction in fasting blood glucose levels and maintain their body weight near normal after treatment with PCME. The bioactive compound in this PCME functions as an antioxidant, suppressing oxidative damage and subsequently reducing hyperglycemia, thereby improving body weight under controlled hyperglycemic conditions (26, 27). The previously published work of different plant-extracted treatments on diabetic rats showed a significant reduction in blood glucose levels, which supported our findings (27-29).

3.6. Assessing the blood's biochemical parameters in diabetic rats given PCME extract

We assessed the biochemical parameters of the blood in diabetic rats given PCME extract. Table 2 presents the assessment of the biochemical characteristics of the blood. The levels of ALP, α-amylase, γ-glutaraldehyde, creatinine, SGPT, and SGOT were significantly lower (p<0.05) in rats that had diabetes caused by STZ-nicotinamide and were treated with standard and PCME at two different dosages (200 mg/kg and 400 mg/kg). Compared to the lower dose of PCME extract (200 mg/kg), higher doses of PCME extract (400 mg/kg) were

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able to regulate blood serum biochemical contents to levels almost identical to those of normal rats. The diabetic control group showed a significant increase in various serum parameters, which was also noteworthy (Table 2). SGOT, SGPT, alkaline phosphatase, creatinine, α-amylase, and γ-glutaldehyde transferase levels go up in

Table 2: Biochemical parameters. (SGPT- Serum glutamic pyruvic transaminase, SGOT- Serum Glutamic Oxaloacetic Transaminase).

	Normal control	Diab. control	Diab. Metformin. 70 mg/kg b.w.	Diab. ME 200 mg/kg b.w	Diab. ME 400 mg/kg b.w.
α- Amylase	63.22±7.36 **	291.93±31.44	79.33±8.19 **	101.07±11.02**	83.56±7.46**
γ-Glutaraldehyde	18.83±3.48**	158.04±16.22	44.91±4.06**	55.93±3.98**	45.35±5.57**
Alkaline phosphatase	186.32±21.43**	374.19±29.04	235.65±14.77**	277.85±13.64*	216.15±23.37**
SGPT	31.32±2.23**	102.47±4.61	44.31±4.81**	53.08±4.18*	48.43±3.87*
SGOT	16.75± 2.51**	88.03± 6.78	27.67± 2.21**	41.72± 3.02**	29.19± 2.58**
Creatinine	19.33± 3.13**	61.64± 4.83	37.05± 3.85**	52.58± 4.93**	39.95± 2.54**

Data are mean ± SD of six animals for each group. *Indicates statistical significance of $p < 0.05$ when compared each row the diabetic control group. This may be due to increased enzyme liberation from tissues, especially the liver, oxidative stress, or the formation of advanced glycosylation end-products (30). On the other hand, the treated PCME group rats showed a decrease in serum levels of SGPT, SGOT, and alkaline phosphatase, as evidenced by the hepatoprotective effects. It is also evident that the PCME extract has a similar effect to the standard drug metformin.

3.7. Assessing the oxidative stress marker in diabetic rats given PCME extract

We used the MDA content, GSH level, and CAT activity in our studies to measure oxidative stress in the liver. The results for this marker are shown in Table 3. The low dose (75.04.3 ± 4.98 nmol/gm tissue) and high dose (70.55 ± 2.65 nmol/gm tissue) of PCME significantly ($p<0.05$) decreased the amount of MDA in the tissue compared to the diabetic control (90.53 ± 6.76 nmol/gm tissue). Conversely, we found no significant differences in the MDA content between the rats administered ARME and those given the standard drug metformin (70 mg/kg b.w.). However, the diabetic rats treated with PCME show a significant ($p<0.05$) increase in GSH and CAT levels. These oxidative stress markers act as enzymatic antioxidants in the cells and protect from oxidative damage. The excessive glucose levels in diabetes mellitus can deactivate these antioxidant enzymes by glycating these proteins and generating oxidative stress. Lipid peroxidation also results from the generated oxidative stress (31). The improvement in CAT, GSH, and MDA activities demonstrated the efficacy of PCME in lowering oxidative stress in the liver of diabetic rats. The extract seemed to have provided a protective barrier for the rats' livers because it could return the cellular antioxidant levels in diabetic rats to normal. GSH is an important non-protein thiol present in living organisms and plays a critical role in coordinating the body's antioxidant defense mechanism. Multiple reports have documented serious consequences when a biological system's GSH status changes (32, 33). Diabetes causes a decrease in GSH content; however, the PCME extract significantly maintains normal levels (34). So, it is evident that the PCME extract helps maintain diabetes.

3.8. The histopathology of the rats

Figs. 3.A-3.E presents the liver's histopathological observation, with PCME demonstrating normal parenchyma cell organization in the liver (Fig. 3.A) with hepatocytes arranged in circular form along with sinusoidal cards about portal tract (portal vein, hepatic artery, and bile duct) and central vein. The same condition was observed for diabetic rats treated with Metformin (70 mg/kg b. w.) (Fig. 3.B). A section of the hepatic tissues of the diabetic group is shown in Fig. 3.C

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where distortion in the hepatocyte arrangement around the central vein, periportal fatty infiltration with hepatocyte focal necrosis, sinusoidal congestion around central vein regions, granular degeneration, microvesicular vacuolization, focal necrosis, hyperemia in the sinusoids,

Table 3: Effect of ARE on MDA level, GSH level, and CAT activity in the liver of experimental diabetic rats.

	MDA level (nmol/gm tissue)	GSH level (nmol/gm tissue)	CAT activity (U/min/gm tissue)
Normal Control	48.87 ± 4.07***	0.000610 ± 2.86e-005*** #	26.64 ±1.08***##
Diabetic ARE (200 mg/kg b.w.)	75.04.3 ± 4.98***	0.000271 ± 1.38e-005** ###	14.64 ±1.13*** ###
Diabetic ARE (400 mg/kg b.w.)	70.55 ± 2.65***	0.000432 ± 1.06e-005*** ###	18.84 ± 0.85***##
Diabetic Metformin (70 mg/kg b.w.)	67.12 ± 4.35***	0.000511 ± 2.99e-005***	20.47 ± 0.93***
Diabetic Control	90.53 ± 6.76###	0.000197 ± 1.39e-005###	7.89 ± 0.43###

MDA: Malondialdehyde; GSH: Reduced Glutathione; CAT: Catalase. Analysis was done by One-way ANOVA followed by Dunnett's post hoc test, where '*' indicates statistical difference from the Diabetic Control group, and '#' indicates statistical difference from the Diabetic Metformin (70 mg/kg b.w.) group. All data represented as mean ± SD of 6 animals and inflammation of the portal tract were observed. A portion of the hepatic tissues from the diabetic group of rats treated with PCME (200 mg/kg b. w.) is shown in Fig. 3.D.

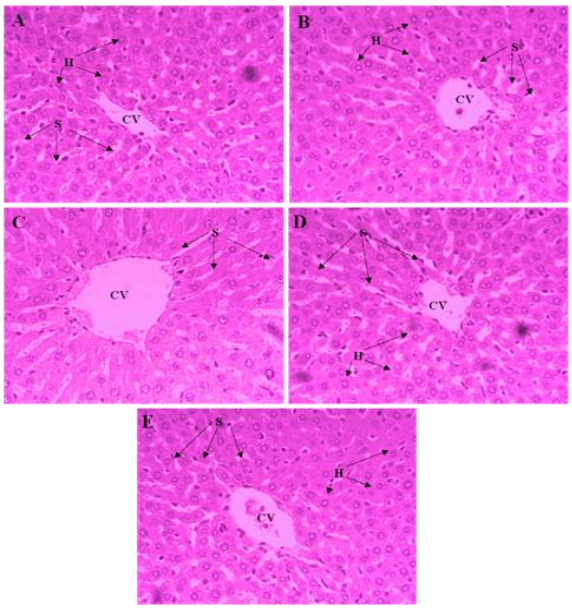

Figure 3. Liver histogram of hematoxylin-eosin staining of hepatic tissues of (A) normal control, (B) Diabetic control, (C) Metformin treated, (DARME(200mg/kg). (200 mg/kg) treated, and (E) ARME (400mg/kg) treated rats. Histograms of hepatic tissue sections were shown at 200× magnification. Sinusoids (S), central vein (CV), and hepatocytes (H).

It shows a typical arrangement of hepatocytes around the central vein with shortened necrosis, decreased fat buildup, and modest sinusoidal dilation with a decrease in Kupffer cells. Likewise, the hepatic tissues of rats with diabetes treated with PCME (400 mg/kg b. w.) exhibit a comparable hepatocyte organization pattern to the animals in the control group. The liver tissue lesions are developed more slowly because of streptozotocin's diabetogenic propensity. The growth of capillaries and thickness of capillary walls were the main pathogenic changes found in the diabetic liver. The majority of the liver slices from diabetes patients showed increased fibrosis, with plasmacytic infiltrates causing the normal concentric arrangement of hepatocytes around the central vein to be distorted. Additionally, the sinusoids and portal arteries were congested, and the veins were dilated (24, 35).

4. Conclusion

The current study sheds new light on the hepatocyte-protective properties of methanolic extracts of the stem of *P. chaba* in diabetic rats induced by STZ-Nicotinamide. The extract was able to lower the rise in blood sugar as well as stop uncontrollably rapid weight loss. It also shows that the protective effects may be attributed to a decrease in the hepatic tissues' production of oxidants. This extract may have an anti-diabetic mechanism by sensitizing pancreatic insulin secretion or reducing oxidative damage from free radicals. A notable ameliorative potential of PCME was observed by

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maintaining the hepatocytes' structural and functional integrity. Therefore, conventional and modern medicine may use the *P. chaba* plant as an effective source for antidiabetic drug development.

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References

1. Chirumamilla P., Taduri S., 2023. Assessment of in vitro anti-inflammatory, antioxidant and antidiabetic activities of Solanum khasianum Clarke. Vegetos. 36(2), 575 - 582.
2. Giri S., Dey G., Dua T.K., 2022. Antioxidant and Antidiabetic activity of hydroalcoholic flower extract of Woodfordia fruticosa (L.) Kurz. Journal of Drug Delivery and Therapeutics. 12(1), 1 - 6.
3. Pizzino G., Irrera N., Cucinotta M., Pallio G., Mannino F., Arcoraci V., Squadrito F., Altavilla D., Bitto A., 2017. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017, 8416763.
4. Hussain T., Tan B., Yin Y., Blachier F., Tossou M.C., Rahu N., 2016. Oxidative stress and inflammation: what polyphenols can do for us? Oxid Med Cell Longev. 2016, 7432797.
5. Abdulkhaleq L., Assi M., Abdullah R., Zamri-Saad M., Taufiq-Yap Y., Hezmee M., 2018. The crucial roles of inflammatory mediators in inflammation: A review. Veterinary world. 11(5), 627 - 635.
6. Sharma V., Mehdi M., M., 2023. Oxidative stress, inflammation and hormesis: The role of dietary and lifestyle modifications on aging. Neurochem Int. 164, 105490.
7. Singh N., Kesherwani R., Tiwari A.K., Patel D.K., 2016. A review on diabetes mellitus. The Pharma Innovation. 5(7), 36-40.
8. Mohan V., Sandeep S., Deepa R., Shah B., Varghese C., 2007. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res. 125(3), 217-230.
9. Das P.K., Mondal A.K., 2012. A report to the rare and endangered medicinal plants resources in the dry deciduous forest areas of Paschim Medinipur district, West Bengal, India. International Journal of Drug Discovery and Herbal Research. 2(2), 418-429.
10. Hota S., Chatterjee A., 2016. Traditional Knowledge and Use of Medicinal Plants for Gastro-intestinal Disorders by Tribal People in Paschim Medinipur District of West Bengal. International Journal of Pharma Sciences. 6(3), 1571-1576.
11. Tapondjou L.A., Jenett-Siems K., Böttger S., Melzig M.F., 2013. Steroidal saponins from the flowers of Dioscorea bulbifera var. sativa. Phytochemistry. 95, 341-350.
12. Ghosh S., Parihar V., More P., Dhavale D., Chopade B., 2015. Phytochemistry and therapeutic potential of medicinal plant: Dioscorea bulbifera. Medicinal chemistry. 5(4), 160-172.
13. Dahiya P., 2017. Phytochemical investigation and antimicrobial properties of Dioscorea bulbifera tuber. Asian J Pharm Clin Res. 10(12), 317-319.
14. Galani Varsha J., 2017. A comprehensive phytopharmacological review of Dioscorea bulbifera Linn. International Journal of Environmental Sciences & Natural Resources. 4(5), 177-187.
15. Mustafa A., Ahmad A., Tantray A.H., Parry P.A., 2018. Ethnopharmacological potential and medicinal uses of miracle herb Dioscorea spp. J Ayurvedic Herb Med. 4, 79-85.
16. Murugan M., Mohan V.R., 2012. In vitro antioxidant studies of Dioscorea esculenta (Lour). Burkill. Asian Pacific Journal of Tropical Biomedicine. 2(3), S1620-S4.
17. Kumar S., Das G., Shin H-S., Patra J.K., 2017. Dioscorea spp.(a wild edible tuber): a study on its ethnopharmacological potential and traditional use by the local people of Similipal Biosphere Reserve, India. Front Pharmacol. 14(8), 220323.
18. Kundu B.B., Vanni K., Farheen A., Jha P., Pandey D.K., Kumar V., 2021. Dioscorea bulbifera L.(Dioscoreaceae): A review of its ethnobotany, pharmacology and conservation needs. S Afr J Bot. 140, 365-374.
19. Mandal S., Rahman M.L., Das P., Ashraf G.J., Dua T.K., Paul P., Nandi G., Sahu R., Effect of maceration, ultrasound, and microwave-assisted method of extraction on antioxidant activity and phenolic profile of free, esterified, and bound

phenolics of Tulaipanji rice. International Journal of Food Engineering 2023(0).

20. Baishya T, Das P, Ashraf GJ, Dua TK, Paul P, Nandi G., Bhattacharya M., Sahu R., 2023. Tissue specific changes of phytochemicals, antioxidant, antidiabetic and anti-inflammatory activities of tea [Camellia sinensis (L.)] extracted with different solvents. Zeitschrift für Naturforschung C. 78(5-6), 235-246.
21. Ashraf G.J., Das P., Dua T.K., Paul P., Nandi G., Sahu R., 2021. High-performance thin-layer chromatography based approach for bioassay and ATR–FTIR spectroscopy for the evaluation of antioxidant compounds from Asparagus racemosus Willd. aerial parts. Biomed Chromatogr 35(12), e5230.
22. Rahman M.L., Mandal S., Das P., Ashraf G.J., Dua T.K., Paul P., Nandi G., Sahu R., 2023. Evaluation of maceration, microwave, ultrasound-assisted extraction methods on free, esterified and bound phenolic profile and antioxidant activity of black rice. Z Naturforsch C. 78(11-12), 389-398.
23. Das P., Ashraf G.J., Baishya T., Dua T.K., Paul P., Nandi G., Singh R.K., Dutta A., Kumar A., Sahu R., 2023. In vitro pharmacological evaluation, phytochemical profiling, and in silico molecular docking of Duabanga grandiflora leaves and flowers. Vegetos. 2023, 1-13.
24. Das P., Ashraf G.J., Baishya T., Dua T.K., Paul P., Nandi G., Sahu R., 2022. High-performance thin-layer chromatography coupled attenuated total reflectance-Fourier-transform infrared and NMR spectroscopy-based identification of α-amylase inhibitor from the aerial part of Asparagus racemosus Willd. Phytochemical Analysis. 33(7), 1018-1027.
25. Guideline IHT, 2005. Validation of analytical procedures: text and methodology. Q2 (R1). 1(20), 05.
26. Sahu R., Mandal S., Das P., Ashraf G.J., Dua T.K., Paul P., Nandi G., Khanra R., 2023. The bioavailability, health advantages, extraction method, and distribution of free and bound phenolics of rice, wheat, and maize: A review. Food Chemistry Advances. 2023, 100484.
27. Aryal S., Baniya M.K., Danekhu K., Kunwar P., Gurung R., Koirala N., 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 8(4), 96-107.
28. Paudel M.R., Chand M.B., Karki N., Pant B., 2015. Antioxidant activity and total phenolic and flavonoid. Botanica Orientalis–Journal of Plant Science. 9, 20-26.
29. Chigayo K., Mojapelo P.E.L., Mnyakeni-Moleele S., Misihairabgwi J.M., 2016. Phytochemical and antioxidant properties of different solvent extracts of Kirkia wilmsii tubers. Asian Pacific Journal of Tropical Biomedicine. 6(12), 1037-1043.
30. Wong S. P., Leong L. P., Koh J.H.W., 2006. Antioxidant activities of aqueous extracts of selected plants. Food Chem. 99(4), 775-783.
31. Chen C., You L-J., Abbasi A.M., Fu X., Liu R.H., Li C., 2016. Characterization of polysaccharide fractions in mulberry fruit and assessment of their antioxidant and hypoglycemic activities in vitro. Food Funct. 7(1), 530-539.
32. Zahrani N.A.A., El-Shishtawy R.M., Asiri A.M., 2020. Recent developments of gallic acid derivatives and their hybrids in medicinal chemistry: A review. Eur J Med Chem. 204, 112609.
33. Alam M., Ahmed S., Elasbali A.M., Adnan M., Alam S., Hassan M.I., 2022. Therapeutic implications of caffeic acid in cancer and neurological diseases. Front Oncol. 12, 860508.
34. Manuja R., Sachdeva S., Jain A., Chaudhary J., 2013. A comprehensive review on biological activities of p-hydroxy benzoic acid and its derivatives. Int J Pharm Sci Rev Res. 22(2), 109-115.
35. Yang D., Wang T., Long M., Li P., 2020. Quercetin: its main pharmacological activity and potential application in clinical medicine. Oxid Med Cell Longev. 2020, 8825387.
36. Gupta G., Siddiqui M.A., Khan M.M., Ajmal M., Ahsan R., Rahaman M.A., Ahmad M.A., Arshad M., Khushtar M., 2020. Current pharmacological trends on myricetin. Drug Res. 70(10), 448-454.