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Diabetes ameliorative effect of solvent extract of *Bauhinia acuminata* bark in streptozotocin induced diabetic male albino rats

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Abstract

Diabetes is a lifestyle illness that is on the rise and is linked to long-term metabolic problems. This study uses Wistar strain albino male rats as experimental diabetic model animals to assess the anti-diabetic and anti-oxidative effects of hydro-methanol (3:2), hydro-ethanol (3:2), and aqueous extracts of *Bauhinia acuminata* bark. Rats were divided into control, diabetic, and extract-treated diabetic groups. Following diabetes induction by streptozotocin injection, rats were administered the respective extracts for 28 days at a dose of 20 mg/0.5 ml distilled water per 100 g body weight. Fasting blood glucose levels were monitored. Muscular glycogen levels, toxicity indicators (GOT, GPT), oxidative stress markers (catalase, SOD, TBARS), carbohydrate metabolic enzyme activities (hexokinase, glucose-6-phosphatase), and histological features of the pancreas were also evaluated. All assessed parameters showed significant improvement in extract-treated diabetic groups compared to vehicle-treated diabetic controls. Among the extracts, the hydro-ethanol (3:2) extract exhibited the most pronounced restoration of glycemic balance, oxidative stress markers, and enzymatic activities, along with evidence of beta-cell regeneration. These findings suggest that the hydro-ethanol extract of *Bauhinia acuminata* bark possesses potent anti-diabetic and anti-oxidative properties, offering promising therapeutic potential for the management of diabetes and its associated metabolic disturbances.

Keywords: Anti-diabetic; Anti-oxidative; *Bauhinia acuminata*; Histological view of pancreas

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1. Introduction

Diabetes is a hormonally-driven illness that is marked by hyperglycemia and deviation in the catabolism of carbohydrate, lipid, and polypeptide, where the release of insulin from the beta cells of pancreas is limited and/ or the insulin activity is relative (Mukhtar et al., 2020). At present, diabetes become an epidemic throughout the world, 537 million people are affected and the prevalence is higher in nations with poor and moderate incomes because of the changes in people's food style, lifestyle, genetic and environmental factors (Sun et al., 2022). Diabetes causes serious damage and dysfunction to the renal system, heart, eyes, blood circulation and periphery nervous system. Excessive free radicals generation through glucose auto-oxidation, HbA1C formation, and polyol pathway activation produce oxidative stress development, are the key responsible factors for severe chronic comorbidities associated with diabetes (King and Loeken, 2004).

Insulin injections and some effective oral anti-diabetic medications, have varying pharmacokinetic and pharmacodynamic characteristics. They facilitate cellular glucose uptake through different mechanisms, such as beta cell generation, peroxisome proliferator-activated receptor γ stimulation, insulin receptor mediated GLUT-4 translocation, beta cell-specific ATP-dependent K^+ channel inhibition, and activation of incretins. However, long-term use of those drugs is not safe and produces various side effects like edema, weight gain, allergic reactions, sleeping problems, lactic acidosis, constipation, folic acid, and cyano-cobalamin mal-absorption, nausea, headache, vomiting, bloating, night time diarrhoea and very commonly the drug resistance (Stein et al., 2013). Therefore, efforts to find hypoglycemic medications that are safe and more effective have a long demand in community.

Worldwide about 80% of population use a traditional medical system based on natural remedies (Yuan et al., 2016). Over 6000 plants are said to be utilized in traditional, folk, and herbal remedies in India that amounts to over 75% of the third world nation's medical requirements (Parveen et al., 2020).

Herbal plant *Bauhinia acuminata*, also renowned as Shet Kanchan, belonging to the family Fabaceae. It often thrives in slightly sour soil across India and possesses notable pharmacological properties, including antibacterial, anti-diarrheal, and membrane-stabilizing properties (Roy et al., 2017; Sebastian and Sophy, 2020). The in-vitro anti-diabetic properties of this plant extract has been established (Singharoy et al., 2024). However, more in-depth in-vivo research to unfold the mechanism of action of the extract along with genomic mode of action are not yet accessible which is indisputable for drug development.

The study has been taken for effective extraction of phyto-ingredients through hydro-ethanol (3:2), hydro-methanol (3:2) and aqueous solvent of *Bauhinia acuminata* bark extract and assess their potentiality to manage diabetes and related complications in STZ induced experimental diabetic animals.

2. Methods

Chemicals and reagents

Every chemicals referred to analytical class. Methanol (99%) was obtained from Thermo Fisher, India; G-6PD (>98%) was purchased from Tokyo Chem, India; NADP (95%), ATP (99%), α-ketoglutarate (99%), alanine (99%) were purchased from Sisco, India; Pyrogallol (98%), TBA (98%) were procured from SD Fine Chem Ltd, India; TCA (99%), aspartic acid (99%), PNPG (98%) were obtained from LOBA Chem, India; and hydrogen peroxide (30%) was taken from Merck Ltd, Germany.

Plant material

Bauhinia acuminata, also known as Shet Kanchan or Dudh Kanchan in the local dialect. The bark portion was taken from the naturally maturing Kanchan plant at West Midnapore, West Bengal, during the summer months. The plant part was verified by Botany department, Vidyasagar University (verification no: Bauhinia acuminata/BIO/08/22/VU). Using tap water, the barks were scrubbed and washed properly to get rid of dirt. The barks were processed into a coarse powder using an electric grinder after being let to dry in the shade for about two weeks.

Extraction procedure

Hydro-methanol, hydro-ethanol and aqueous solvents were used for the extraction of secondary metabolites present to the *Bauhinia acuminata* bark. Hydro-methanol and hydro-ethanol solvents at a ratio of 3:2 were used as the menstrum. About 75 g of the powdered plant material was macerated with 1 litre distilled water only for aqueous extract and for hydro-methanol and hydro-ethanol solvents, 75 g powdered material was macerated in 600 mL distilled water and 400 mL methanol and ethanol respectively in a 5-litre air-tight glass container. For two days, the slurry was left at room temperature, shaken, and stirred every two hrs. Filtration of the extract was done. To acquire the extract in dry powder form, the filtrated solvent extract was concentrated at 42°C under reduced pressure (0.08 MPa) in a rotary evaporator and then lyophilized (Maiti et al., 2018).

Animal care

Thirty male Wistar strain genetically inbreed albino rats were bought from a registered animal vender, Kolkata (voucher no- 1828PO/BT/S/15/CPCSEA). Every rat weighed around 100$\pm$10 g and had an approximate age of 1.5 months. The rats were possessed normal glycemic status. Fasting level blood glucose was within 70–80 mg/dl. Before beginning the experiment, the rats were allowed to acclimatize to their new surroundings for ten days. Rats were kept in cages (6 animals/ cage) constructed of propene polymer with a broad square mesh to avoid coprophagy nature. Rats were housed with good ventilation, maintained 25$\pm$2 °C temperature, relative moisture content of 50–60%, and a cycle (12:12 hr) of light and dark throughout the experiment. Food and purify water were freely available to the animals.

Ethical statement

According to the standards set forth by the Committee for Control and Supervision of Experiments on Animals (CCSEA), all conditions pertaining to rats were followed. The ethical issue number was VU/IAEC/CPCSEA/9/7/2022 for this experiment.

Diabetes (type 1) induction

Following acclimatization period, twenty-four rats (weight 120–130 g) were fasted overnight (8–12 hrs). To induce diabetes in these rats, streptozotocin (Sigma Aldrich, Germany) was injected intramuscularly once at a dose of 4 mg/100 μl in 100 (mM) citrate buffer (4.5-pH) / 0.1 kg body weight (Sarkar et al., 2019). Streptozotocin (STZ), a glucosamine nitrosourea classed compound that particularly inhibits the activity of aconitase, causes DNA alkylation, NO (nitric oxide) induced oxidative stress formation and pancreatic β cells destruction (Furman, 2015). On the 7th day after receiving an intramuscular STZ injection, blood was drawn from the caudal vein of the experimental rats in a fasting state to confirm diabetes development. Rats with glucose level between 250 and 350 mg/dl were denoted as diabetes model animals for this investigation.

Experimental design

The rats were divided into five groups:

Control: The rats were allowed to give intramuscular injection of citrate buffer (0.1 ml/100 gram body weight, ph-4.5). After 8 th day of injection, they were orally treated with 0.5 ml distilled water/100 gram body weight for 28 days.

Diabetes: The rats were allowed to give single intramuscular injection of STZ (4 mg/ 0.1 ml citrate buffer (pH 4.5)/ 100 g body weight for diabetes development. Then after 8 th day of injection, they were orally treated with 0.5 ml distilled water/100 gram body weight for 28 days.

Hydro-methanol extract treated group: Following the eighth day of STZ injection, the diabetic rats in this group were gavaged 20 mg of powdered *Bauhinia acuminata* bark hydro-methanol extract/0.5 ml distilled water/100 g body weight each day at 11–12 hours of fasting for the next 28 days.

Hydro-ethanol extract treated group: After diabetes induction, on the 8 th day of STZ injection, this grouped rats were fed hydro-ethanol extract of *Bauhinia acuminata* extract at the dose of 20 mg/0.5 ml distilled water/100 g body weight each day at 11–12 hours of fasting for the next 28 days.

Aqueous extract treated group: The rats were treated with 20 mg aqueous extract of *Bauhinia acuminata* bark/0.5 ml distilled water/100 g body weight after diabetes induction from 8 th day of injection to 28 days of treatment duration.

The experiment was followed for 28 days. On 29 th day of treatment period, all the rats were sacrificed under euthanasia, collected the relevant organs and preserve at -20 °C for biochemical tests.

Parameters

Fasting Blood Glucose (FBG) – For FBG, one drop of blood was syringed from the caudal vein present to the tail portion of rats to the On call® Plus blood glucose test strip [glucose oxidase (<25 IU), mediator (<300 μg)], insert the strip to the On call® EZ II glucometer and the reading was expressed in mg/dl (Mitra et al., 2020).

Glycogen level – The amount of glycogen in skeletal muscle was estimated according the standardized protocol. After homogenizing the tissues (100 mg/ml) in 80% ethanol, centrifuged at 8000 rpm, for 20 mins. The dried residual part was then treated with 5 ml of distilled water and 6 ml of 52% perchloric acid, extracted for 20 minutes at 0°C, and then centrifuged again (8000 rpm, 15 minutes). The supernatant (0.2 ml) part was diluted to 1 ml after centrifugation and 4 ml of anthrone reagent was added to each labelled sample tube. The tubes were boiled for 8 minutes, cooled at room temperature, and the absorbance at 630 nm was measured. Glycogen content was measured in μg of glucose per milligram of tissue (Maiti et al., 2005).

Hexokinase – Liver, and skeletal (quadriceps) muscles tissues were used to assess the activity of hexokinase. According to the standard process, the said tissue homogenate (require 50 mg tissue and 1 ml PBS) was added with assay mix [contain glucose (0.0037 M), $MgCl_2$ (0.0075 M), thioglycerol (0.011 M), HEPES (0.045 M) (pH-7.5)], 10% glucose-6-phosphate dehydrogenase solution, 2.5% NADP, and 22 mM ATP, forwarding to analysis on spectrophotometer and recorded the absorbance at 340 nm (Dey et al., 2024).

Glucose-6-phosphatase (G-6-phosphatase) – According to standard method, G-6-phosphatase kinetics in liver, and skeletal (quadriceps) muscle were assessed by spectrophotometer and the O.D was read at 340 nm. In this protocol, tissue homogenate (50 mg/ml PBS) was added to 0.1 M glucose-6-phosphate (substrate), 0.5 M maleate buffer and 10% TCA (reaction ending agent) (Das et al., 2024).

Catalase – The activity of catalase was assessed as per the standard protocol utilising the liver and kidney tissues. After the homogenization of target tissue samples at the concentration of 50 mg target tissue per ml 0.05 (M) tris-chloride, centrifugation was completed. The isolated supernatants were properly mixed with 30% H_2O_2 solution and measured the O.D at 240 nm by spectrophotometer (Mondal et al., 2024).

Super-Oxide Dismutase (SOD) – In a 3.0 ml cuvette, 20 μl aforementioned tissue (liver and kidney) supernatants [50 mg/ml PBS] were combined with 2 ml PBS and 10 mM pyrogallol (20 μl) to assess the activity of SOD. The O.D was taken from spectrophotometer at 420 nm (Khajuria et al., 2018).

Quantification of Thiobarbituric Acid Reactive Substances (TBARS) – Ice cold PBS was used to homogenise hepatic and kidney tissues at 50 mg/ml concentration, and 0.5 ml homogenate was mixed with 2 ml thiobarbituric acid-trichloroacetic acid (TBA-TCA) mixture and 0.5 ml of 0.9% NaCl. The mixture was centrifuged at 10000 g for 5 min at 4°C. The prepared supernatant was collected and used in a spectrophotometer to quantify TBARS at 540 nm. The outcome was stated as nM/mg of tissue (Dhibi et al., 2011).

Measurement of GOT and GPT activities – In this assay, 1 ml substrate solution [(1.33 g aspartate + 15 mg α-ketoglutarate)/20.5 ml PBS (pH-7.5) for GOT, and (1.78 g alanine + 30

mg α-ketoglutarate)/20 ml PBS (pH-7.5) for GPT activity assessment] was mixed with 0.2 ml hepatic or renal tissue homogenate (50 mg/ml PBS) separately. Then 1 ml of 1 mM DNPH was added, and the O.D was determined at 520 nm wavelength in the spectrophotometer. The enzymatic activities of GOT and GPT were expressed as Unit/mg of tissue (Mitra et al., 2019; Das et al., 2023).

Histological study

The dissected pancreas was immediately given into the Bouins fixative. After 14 hrs of fixation, tissues were cut and shaped accordingly, dehydrated with graded alcohol in an upward manner, and remove the excess alcohol with xylene solution. The tissues were then embedded in paraffin wax and section cutting was done in 5.0 μm. Hematoxylin and eosin were used for tissue staining purposes after deparaffinization. The slides were observed under the light microscope (Haligur et al., 2012).

Statistics

The data were expressed as "Mean $\pm$ SEM" because the mean represents the average numerical response of each group, while SEM indicates the precision of the estimated mean. Prior to analysis, the data were checked for normality and were found to follow a normal distribution. Since the data were continuous and normally distributed, parametric statistical tests were appropriate. Therefore, ANOVA followed by "Multiple Comparison Student's two tail 't'-test" was applied to compare differences between experimental groups (Sokal and Rohlf, 1997).

3. Results and Discussion

Streptozotocin is a very popular chemical used for diabetes induction in animal model through the specific destruction of insulin producing pancreatic beta cells and excess ROS generation to the animal body (Matsunami et al., 2011). In diabetic condition, the level of FBG was significantly increased (Figure 1A) whereas the glycogen level was decreased (Figure 1B). The major mechanism of insulin mediated glucose regulation was hampered. The hydro-methanol, hydro-ethanol and aqueous extract treatment causes significant decrease to the level of FBG and restoration of the muscle glycogen that may be due to production of more insulin from pancreatic beta cells or the generation of beta cells itself that improve glucose metabolism (Oh, 2015).

Hexokinase, the primary enzyme for the first irreversible step of glycolysis, significantly decreased in diabetic condition (Figure 2A) whether G-6-phosphatase, the final enzyme of gluconeogenesis was markedly increase (Figure 2B) (Elsadek and Ahmed, 2022). Treatment with different solvent extracts for 28 days resulted in a significant restoration of hexokinase activity, suggesting improved insulin sensitivity and enhanced glycolytic flux. The increased hexokinase activity promotes efficient trapping and utilization of glucose within cells, thereby lowering circulating glucose levels. Simultaneously, the reduction in G-6-phosphatase activity indicates suppression of gluconeogenesis, effectively halting excess glucose production. These

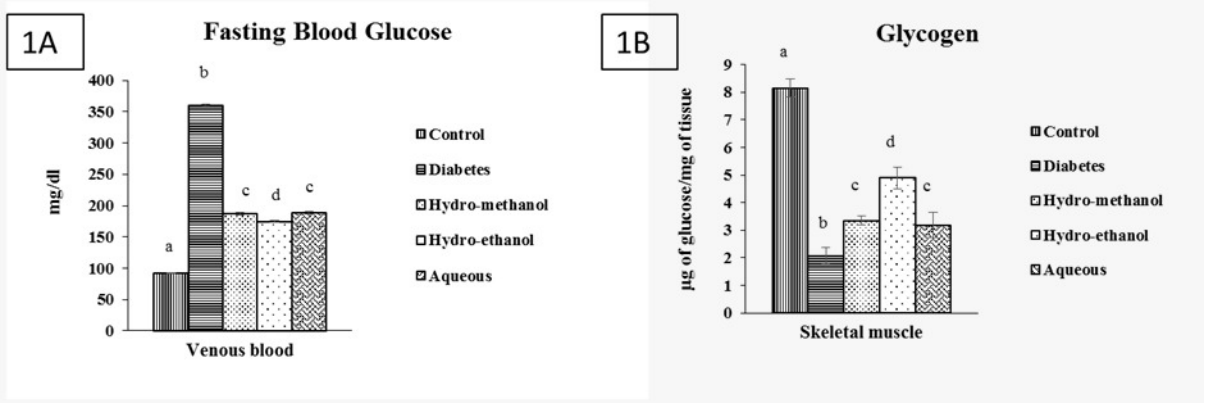

Figure 1: Effect of hydro-methanol, hydro-ethanol and aqueous extract of *Bauhinia acuminata* bark on the management of fasting blood glucose and glycogen store in male albino diabetic rats. Values were expressed as Mean ± SEM, $n = 6$. ANOVA followed by "Multiple Comparison Student's Two tail 't'-test". Bars with different superscripts for FBG (a, b, c, & d) and muscle glycogen (a, b, c, & d) differ from each other significantly, $P < 0.05$.

coordinated effects reflect improved regulation of carbohydrate metabolism and highlight the potential of the extracts in restoring metabolic enzyme balance under diabetic conditions.

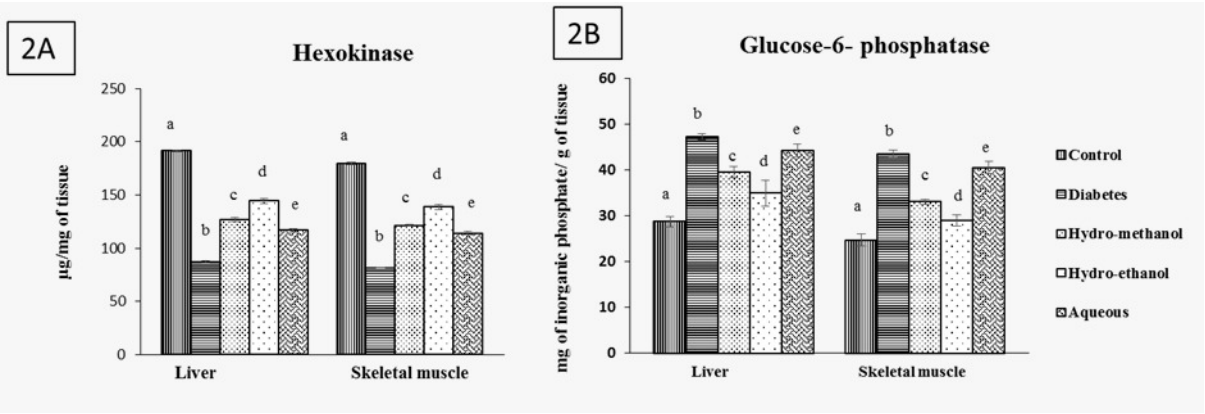

Figure 2: Effect of hydro-methanol, hydro-ethanol and aqueous extract of *Bauhinia acuminata* bark on the activities of hexokinase, glucose-6-phosphatase (liver, skeletal muscle) in male albino diabetic rats. Values were expressed as Mean ± SEM, $n = 6$. ANOVA followed by "Multiple Comparison Student's Two tail 't'-test". Bars with different superscripts for each parameters (a, b, c, d, & e) differ from each other significantly, $P < 0.05$.

Furthermore, oxidative stress in chronic diabetic conditions leads to lipid peroxidation (Figure 3C), depletion of reduced glutathione (GSH) levels and reduced activities of catalase and SOD (Figure 3A and 3B), exacerbating tissue damage in vital organs like the liver and kidneys (Dawi et al., 2024). The restoration of antioxidant defense mechanisms upon extract treatment indicates the potential of the phytochemicals to scavenge reactive oxygen species (ROS) and enhance the endogenous antioxidant system. This protective effect may also involve upregulation of gene expression related to antioxidant enzymes and stabilization of cellular redox balance, thereby mitigating oxidative damage and supporting tissue homeostasis in diabetic

conditions (Mihailović et al., 2021).

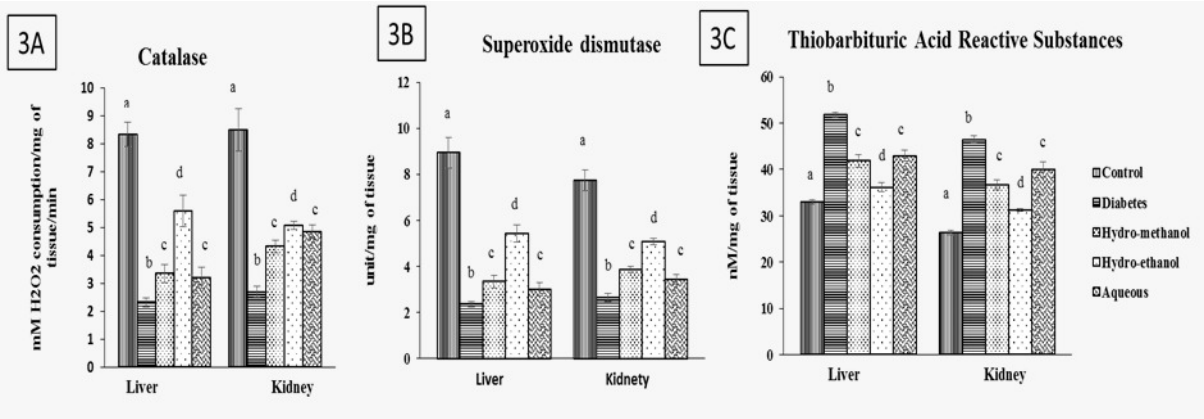

Figure 3: Measurement of the activities of catalase, SOD and TBARS level in liver and kidney tissues after 28 days treatment with the hydro-methanol, hydro-ethanol and aqueous extract of *Bauhinia acuminata* bark. Values were mentioned as mean $\pm$ SEM, $n = 6$. ANOVA followed by "Multiple Comparison Student's Two tail 't'-test". Bars with different superscripts for catalase, SOD and TBARS (a, b, c & d) are differ from each other significantly, $P < 0.05$.

The *Bauhinia acuminata* extracts did not exert any hepatotoxic effect at the tested doses, suggesting its safety and tolerability in vivo. The unchanged levels of these liver enzymes further support the non-toxic nature of the extract (Figure 4A and 4B), reinforcing its potential as a therapeutic agent for long-term use in diabetic managements (Mujeeb et al., 2011).

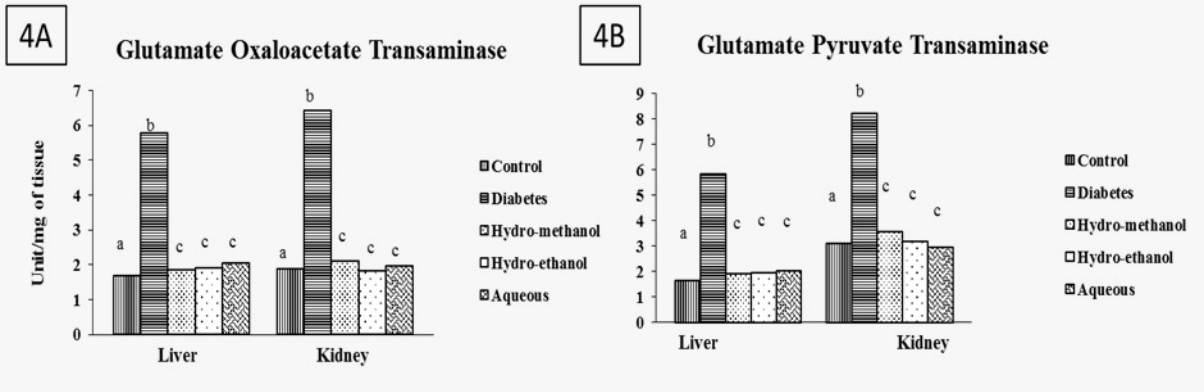

Figure 4: Assessment of the toxicity profiles in liver and kidney tissues after 28 days treatment with the hydro-methanol, hydro-ethanol and aqueous extract of *Bauhinia acuminata* bark. Values were mentioned as mean $\pm$ SEM, $n = 6$. ANOVA followed by "Multiple Comparison Student's Two tail 't'-test". Bars with different superscripts for GOT, GPT (a, b, & c) are differ from each other significantly, $P < 0.05$.

Histological analysis further supported the regenerative effect of the extract on pancreatic beta cells, indicating improved insulin production and glucose metabolism (Figure 5).

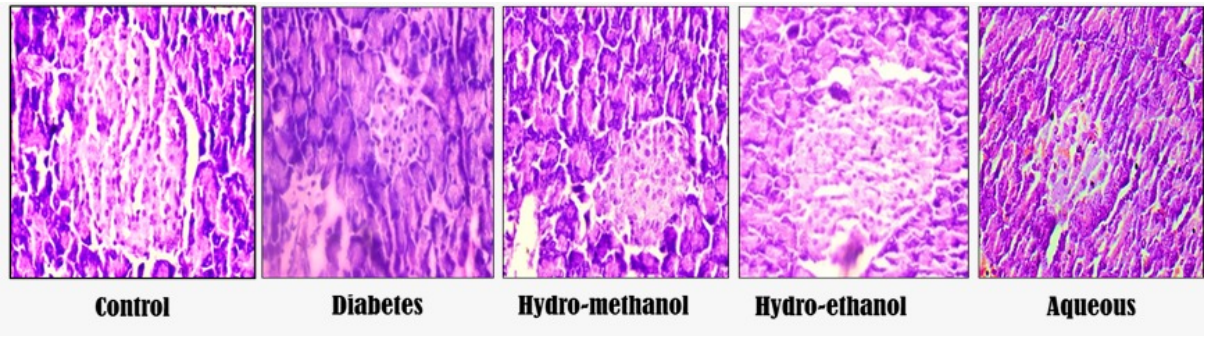

Figure 5: Histology of pancreas: Control: normal histoarchitecture of pancreatic islet, Diabetes: diminution of cell density to the pancreatic islet, Hydro-methanol extract treated group: recovery to the islet size, Hydro-ethanol extract treated group: prominent recovery to the islet size and islet cell population, Aqueous extract treated group: pancreatic islet size recovered comparatively.

4. Conclusions

The study demonstrates that *Bauhinia acuminata* bark extracts possess notable anti-diabetic and antioxidant potential in streptozotocin-induced diabetic rats. The hydro-ethanol extract showed the greatest efficacy in reducing blood glucose, restoring metabolic enzyme activity, enhancing glycogen storage, and improving hepatic and renal antioxidant status without hepatotoxic effects. Overall, it presents a safe and promising natural therapeutic option for diabetes management.

Conflict of Interest

There is no conflict arises during the study.

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