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Original Article

Brassinin Exhibits Anti-Diabetic Activity against Streptozotocin-induced Diabetes Mellitus in Experimental Rats

Bixia Xu[1,*], Abdullah A. Alarfaj[2], Abdurahman Hajinur Hirad[2], Nandakumar Natarajan[3], Petchi Iyappan[4], Samer Hasan Hussein Al Ali[5]

[1]Teaching and Research Section of Internal Medicine, Zhaoqing Medical College, No.6, Xijiang South Road, Duanzhou District, Zhaoqing City, Guangdong Province, Zhaoqing City, Guangdong Province, CHINA.
[2]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, SAUDI ARABIA.
[3]Department of Cellular and Molecular Biology, Center for Biomedical Research, The University of Texas at Tyler Health Science Center, Tyler, TEXAS, USA.
[4]Faculty of Medicine, Bioscience and Nursing, School of Bioscience, Mahsa University, Saujana Putra, MALAYSIA.
[5]Department of Chemistry, Faculty of Science, Isra University, Amman, JORDAN.

ABSTRACT

Background: Diabetes mellitus is among the most serious public health problems worldwide, whose incidence is steadily increasing and is now posing a global epidemic danger. Major secondary complications are associated with diabetes that impacts the normal functioning of major organs such as the pancreas, liver, kidney, and eye and is characterized by a high rate of inflammation, oxidative stress, and apoptosis. Indole phytoalexins such as Brassinin exhibit a variety of biological properties, including antimicrobial, oviposition stimulant, antitumor, and cytotoxic. **Materials and Methods:** The 35mg/kg STZ was intraperitoneally injected to the rats for stimulating the diabetes. Then rats were treated with 25mg/kg of brassinin. The Glibenclamide was used as a positive control. The impact of Brassinin on water and food uptake, body weight, and kidney and liver weight were assessed. The levels of blood glucose, insulin, and glycosylated Haemoglobin (HbA_{1c}), hepatic marker, carbohydrate metabolic enzymes, antioxidants, and inflammatory markers in untreated and treated rats were examined. The histological examination of the pancreas, kidney, and liver were also performed to understand the salutary properties of the Brassinin. **Results:** Brassinin and Glibenclamide treatment remarkably decreased the glucose and HbA_{1c} levels in diabetic rats, while the insulin levels were substantially elevated. They also increased the antioxidant enzymes in the STZ-stimulated rats and considerably decreased the inflammatory marker and hepatic marker enzyme levels. Histological observations established the protective potential of Brassinin on diabetes-associated injury in the pancreas, liver, and kidney. **Conclusion:** It can be inferred that Brassinin is an antihyperglycemic, antioxidant, and anti-inflammatory compound that protects the liver, kidney, and pancreas during the onset of diabetes.

Keywords: Brassinin, Glycosylated haemoglobin, Oxidative stress, Inflammation, Glibenclamide.

Correspondence:
Dr. Bixia Xu
Teaching and Research Section of Internal Medicine, Zhaoqing Medical College, No. 6, Xijiang South Road, Duanzhou District, Zhaoqing City, Guangdong Province, 526020, CHINA.
Email: surither@hotmail.com

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INTRODUCTION

Diabetes Mellitus (DM) is a non-contagious metabolic disorder characterized by an elevated blood glucose level that persists for a prolonged period of time (hyperglycemia); the condition develops when ß cell destruction by the immune system prevents the pancreas from producing sufficient insulin (Type 1) or when the body itself acquires resistance to insulin (Type 2).[1] According to International Diabetes Federation, around 10.5% of the world population is living with the disease, and this percentage might rise to 12.2% by 2045. Moreover, an alarming number of 541 million adults are estimated to be at high risk for type 2 diabetes.[2]

In diabetes, persistent hyperglycemia leads to an elevated free radical formation owing to protein glycosylation and glucose auto-oxidation, which induces oxidative stress.[3-5] As a result, secondary complications such as retinopathy, which might lead to vision loss, peripheral neuropathy, which increases the possibility of foot ulcers and amputations, nephropathy, which might lead to kidney failure, and autonomic neuropathy, which results in gastrointestinal and cardiovascular symptoms along with sexual dysfunction, can develop.[6-8] This impacts the normal functioning

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of major organs including the pancreas, liver, kidney, and eye.[9] In addition to these issues, diabetes individuals frequently have abnormalities in their lipoprotein metabolism and are typically hypertensive.[10] Rigorous and cautious glycemic management with safe and effective treatment agents with antidiabetic and antioxidant potential is, therefore, strongly encouraged to alleviate the risk of oxidative stress-related diabetes complications.

The constituents of different plant extracts are known to possess varying levels of bioactivity, which may be helpful in protecting different organs from persistent hyperglycemia as well as oxidative stress.[11] Fresh vegetables and fruits release phytoalexins upon exposure to stress or infection to protect themselves.[12] Indole phytoalexins such as Brassinin are found extensively in cruciferous plants like Chinese cabbage, Brussels sprouts, and cauliflower, and exhibit a variety of biological properties, including antimicrobial, oviposition stimulant, antitumor, cytotoxic, and an inhibitor of indoleamine 2,3-dioxygenase.[13-15] Although Brassinin exhibits such potent bioactivities, its anti-diabetic activity has not yet been studied. Hence, the current work focuses on discovering the salutary properties of brassinin against STZ-stimulated diabetes in rats by studying the biochemical and histopathological parameters.

MATERIALS AND METHODS

Materials

Brassinin, Streptozotocin, and Glibenclamide were acquired from Sigma-Aldrich, USA. All the other required chemicals were obtained with high analytical categories.

Animal housing and dietary treatments

The procurement of adult healthy Sprague-Dawley rats (180-200g) was from the Institutional Animal House post obtaining ethical clearance from the Institutional Animal Ethical Committee. The rat acclimatization was performed in a strictly hygienic laboratory by maintaining the temperature at 25±1°C and relative humidity at 55±5 for 7 days and a light-dark cycle for 12 hr. After acclimatization, rats were fed with regular diet and clean water was available at all times. The rat cage was changed every 3 days whereas the bedding was changed every single day. Care1 was taken to handle the rats as much as possible, and all of the experimental methods used in the current investigation were authorized by the ethics committee.

Diabetes Induction

Diabetes was induced in the animals with the help of the Streptozotocin (STZ). The animals were injected intraperitoneally with 35mg/kg STZ drug in 0.1M citrate buffer (pH 4.4). After 7 days of the STZ challenge, the level of blood glucose was checked to confirm the diabetes onset. The rats with an estimated blood glucose level greater than 11mmol/l were chosen in the current experimental investigation.

Experimental Design and Sampling

For the experiment, there were five groups of six rats each divided arbitrarily. First was the control group, which consisted of the rats that were intraperitoneally injected with only 0.5% of DMSO. The second was the Diabetic induced group, where the rats were intraperitoneally injected with 35mg/kg STZ drug. The third group were induced with diabetes and subsequently treated with Brassinin (25 mg/kg bwt in saline). The fourth group were induced with diabetes and subsequently treated with Brassinin (50 mg/kg bwt in saline). The fifth group of animals were treated with the standard antidiabetic drug Glibenclamide (0.1 mg/kg bwt in saline) after diabetes induction. For 45 days, Glibenclamide and Brassinin (in 0.5% DMSO) were orally administered once every day in the morning. After the treatment completion, the rats were sacrificed and the blood was acquired to conduct a biochemical investigation and kidney, liver, and pancreas tissues were harvested to carry out histopathological studies.

Food intake measurement and body weight analysis

Throughout the experimental duration, measurements of water and food intake, and variations in body weight were recorded on a regular basis for each group of rats. After 45 days, all of the rodents in the group were fasted overnight before being sacrificed under anesthetic conditions (24 mg/kg b. wt. of ketamine intramuscularly). Both with and without anticoagulant, blood was drawn into collecting tubes. The kidney and liver were promptly taken out, rinsed in the chilled buffer to eliminate any blood and weighed. Later, 10% of homogenate was prepared with 0.1 M Tris-HCl buffer and subjected to centrifugation for 10 min at 1000 g. Biochemical parameters were evaluated using the isolated supernatants.

Estimation of plasma blood glucose, glycosylated haemoglobin, and insulin levels

At the completion of continuous diabetes induction and Brassinin or Glibenclamide treatment, fasting blood samples were obtained from the control and treated rats. HbA_{1c} measurements were performed on whole blood, and glucose levels were estimated with plasma samples. The blood glucose levels were estimated using rat-specific glucose ELISA kit (Crystal Chem, USA) and the blood HbA_{1c} levels were estimated by Mouse HbA_{1c} Assay Kit (Crystal Chem, USA). The insulin levels of all the groups were measured at end of the treatment by a commercially available ELISA kit obtained from Sigma Aldrich, USA. The collected blood samples were subjected to insulin estimation by following the manufacturer's guidelines and the absorbance was determined at 450nm.

Estimation of hepatic marker enzyme levels

The liver marker enzymes such as Aspartate Aminotransferases (AST), Alanine Aminotransferases (ALT), and Alkaline Phosphatase (ALP) in control and treated rodents were

measured by using kits procured from Sigma Aldrich, USA. The experiments were conducted by complying with the guidelines of the manufacturer.

Estimation of hepatic carbohydrate metabolic enzyme levels

The levels of liver carbohydrate metabolic enzymes such as Hexokinase (HK), Glucose-6-Phosphatase (G6P), and Fructose-1,6-Bisphosphatase (F16BP) were estimated in the liver supernatants. Hexokinase enzyme level was estimated as per the method explained by Brandstrup *et al.* (1957).[16] The G6P levels were estimated as per the protocol mentioned by Baginski *et al.* (1974)[17] and the experiment established by Gancedo and Gancedo, (1971)[18] was utilized to estimate the levels of fructose-1,6-bisphosphatase enzyme.

Quantification of antioxidant status in the pancreas

The levels of pancreatic antioxidants including CAT, GPx, GSH, and SOD were evaluated in control and experimental rats. The CAT activity was estimated as per the protocol of Aebi. (1984).[19] Spectrophotometric monitoring of the H_2O_2 degradation process was carried out at 240 nm for 1 min, and the activity was quantified as mol H_2O_2/min/mg protein. The technique of Leopold and Wolfgang, (1984)[20] was used to measure Glutathione Peroxidase (GPx) activity. The units of the activity were represented as moles of oxidized GSH/min/mg protein. The methodology outlined by Ellman, (1959)[21] was used to test GSH levels. The total GSH concentration was represented as µg/g of tissue, with the absorbance at 412 nm being the measuring wavelength. Superoxide dismutase activity was assessed with the help of nitro tetrazolium blue reduction protocol as explained by Al Batran *et al.* (2013).[22]

Estimation of pancreatic inflammatory markers

The levels of pancreatic inflammatory markers like TNF-α, IL-6, and IL-1β in all the groups were measured by a commercially available ELISA kit procured from Abcam, USA. The experiment was conducted as per the manufacturer's guidelines. The experiment was conducted in triplicates and the absorbance was measured at 450nm. The final values were calculated by using the standard curve with known concentrations of the standard.

Histopathological analysis

The liver, pancreas, and kidney tissues of all the five study groups, i.e., Control, Diabetic-induced, Diabetic-induced and Brassinin-treated and Diabetic-induced Glibenclamide treated rats, were subjected to histopathological analysis. Initially, the tissues were fixed with formalin (10%) for 24 hr. The tissue was then fixed in paraffin wax, segmented with the help of a rotary microtome to achieve 3-5 µm thickness, and then stained with eosin and hematoxylin. The stained slides were then subjected to photo microscopic observation with a light microscope (Olympus).

Statistical analysis

The data obtained with the experimental analyses were further subjected to SPSS software ver.17 analysis. The ANOVA test and post hoc test were utilized for comparison between the different groups. Significance was assessed at p-value <0.05 (confidence interval 95%).

RESULTS

Impact of Brassinin on levels of water and food intake, bodyweight, and relative weight of kidney and liver in experimental rats

The food and water uptake analysis, bodyweight and liver and kidney weight in untreated and treated animals was evaluated and the findings are depicted in Figure 1. There was a considerable decline in their bodyweight, whereas the amount of food and water ingestion as well as the kidney and liver weight were higher in diabetic rats than in control rats. Brassinin and Glibenclamide treatment resulted in augmentation in the body weight and diminution in the water and food intake levels. The weight of the kidney and liver also evidenced an increase upon Brassinin and Glibenclamide treatment.

Impact of Brassinin on blood glucose, glycosylated haemoglobin, and insulin in experimental rats

The blood glucose, HbA_{1c}, and insulin levels in control and treated rats are shown were evaluated. Following diabetes induction, the rats exhibited a substantial elevation in glucose and HbA_{1c} levels, suggesting the onset of hyperglycemia as compared to the untreated rats (Figure 2). The rats with diabetes demonstrated a marked reduction in insulin levels compared with the untreated animals, implying a typical diabetic situation. Treatment with Brassinin and Glibenclamide remarkably declined the blood glucose and HbA_{1c} levels and caused an elevation in the insulin levels as compared with untreated diabetic animals, indicating a preventive effect of Brassinin on hyperglycemia.

Impact of Brassinin on hepatic marker enzyme levels in experimental rats

The AST, ALP, and ALT in the experimental rats were measured. A substantial elevation in AST, ALP, and ALT activities in diabetic rats and a substantial decrease in all Brassinin-administered rats could be observed (Figure 3). Moreover, the levels of these liver enzymes did not significantly vary between the Diabetic + Glibenclamide and untreated control group. Overall, the attenuation of the negative impact of diabetes on the liver enzymes by Brassinin was best observed.

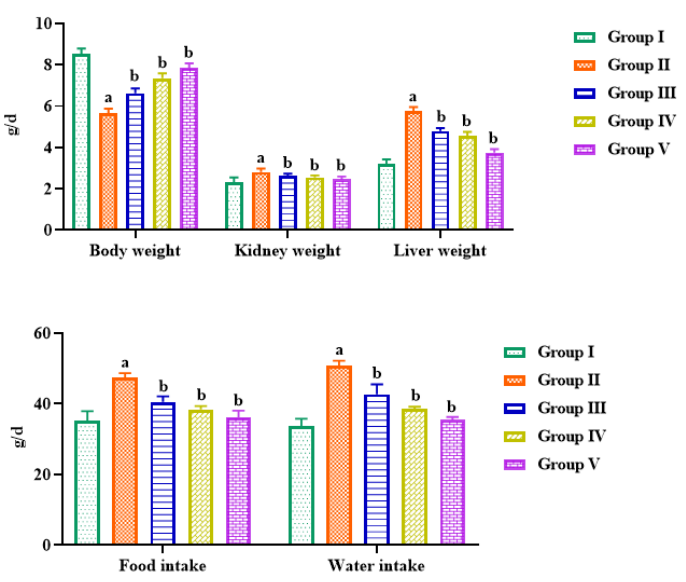

Figure 1: Impact of Brassinin on water and food intake, bodyweight and liver and kidney weights in untreated and treated rats. The animals were grouped into Control, Diabetic induced, Diabetic induced Brassinin treated (25 and 50 mg/kg) and Diabetic induced with Glibenclamide treatment (0.1 mg/kg). A) Bodyweight, liver and kidney weight of rats B) Amount of food and water uptake. The findings are interpreted as mean±SD of 6 rats in each set with a significance level of * p<0.01 from group I and # p<0.05 from group II.

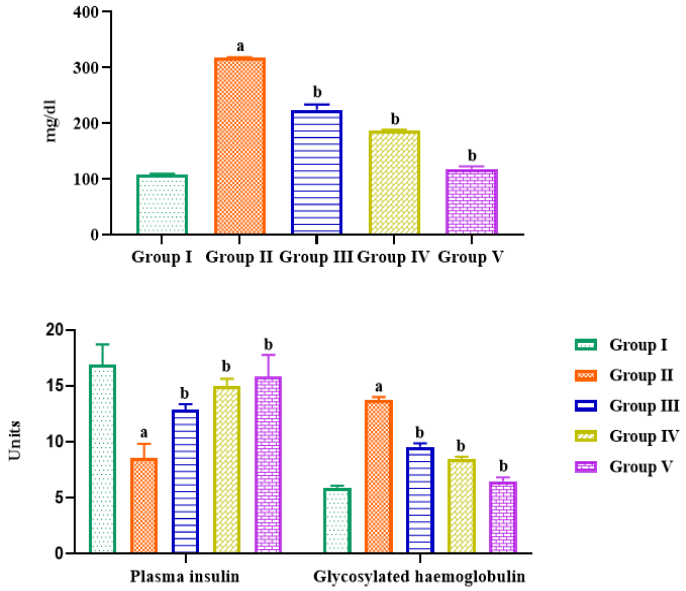

Figure 2: Impact of Brassinin treatment on blood glucose, insulin and glycosylated hemoglobin levels of untreated and treated rats. The animals were grouped into Control, Diabetic induced, Diabetic induced Brassinin treated (25 and 50 mg/kg) and Diabetic induced with Glibenclamide treatment (0.1 mg/kg). A) Blood glucose level of rats B) Insulin and glycosylated hemoglobin level of rats. The findings are interpreted as the mean ± SD of 6 rats in each set with a significance level of * p<0.01 from group I and # p<0.05 from group II.

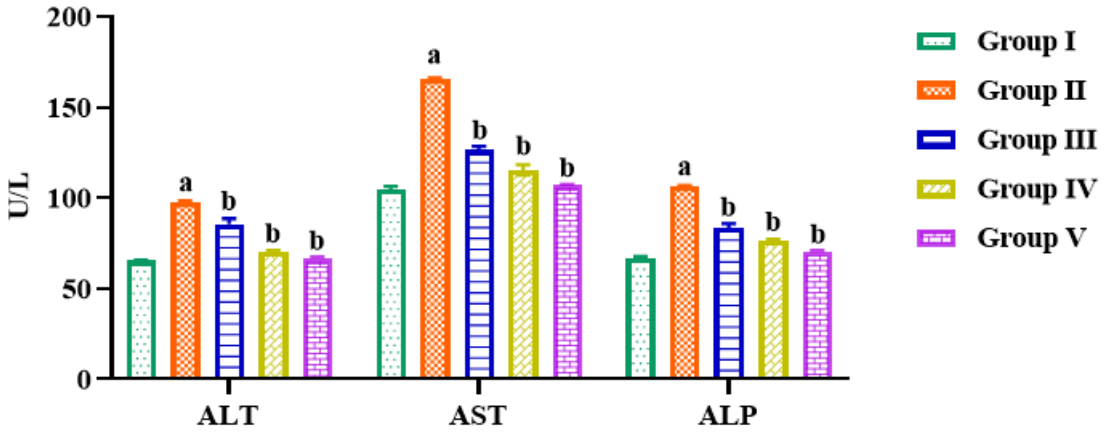

Figure 3: Impact of Brassinin treatment on the levels of liver marker enzymes AST, ALT, and ALP in serum of control and treated rats. The animals were grouped into Control, Diabetic induced, Diabetic induced Brassinin treated (25 and 50 mg/kg) and Diabetic induced with Glibenclamide treatment (0.1 mg/kg). The activities of AST, ALT, and ALP were measured. The findings are interpreted as the mean±SD of 6 rats in each set with a significance level of * p<0.01 from group I and # p<0.05 from group II.

Impact of Brassinin on liver carbohydrate metabolic enzyme activities in experimental rats

The hepatic carbohydrate metabolic enzymes including HK, F16BP, and G6P produced in the liver of experimental rats was evaluated and the findings are represented in Figure 4. The diabetic liver of the rats demonstrated a considerable diminution in HK activity and a substantial elevation in G6P and F16BP activities. However, the modifications in HK, G6P, and F16BP activities were returned to the standard range in the Brassinin and Glibenclamide-treated rats, suggestive of their excellent anti-diabetic nature.

Impact of Brassinin on the levels of antioxidants in the pancreas of experimental rats

The effect of Brassinin treatment on pancreatic antioxidants like CAT, GPx, SOD, and GSH was assessed and the findings are depicted in Figure 5. A remarkable depletion in all the

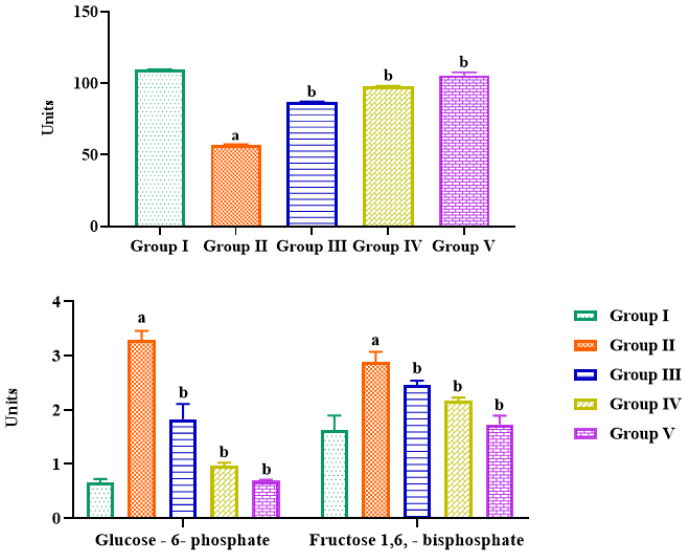

Figure 4: Brassinin on activities of HK, F16BP, and G6P in control and treated rats. The animals were grouped into Control, Diabetic induced, Diabetic induced Brassinin treated (25 and 50 mg/kg) and Diabetic induced with Glibenclamide treatment (0.1 mg/kg). (A) HK and (B) G6P, and F16BP. The findings are interpreted as the mean±SD of 6 rats in each set with a significance level of * p<0.01 from group I and # p<0.05 from group II.

antioxidant levels was exhibited in the pancreatic diabetic rats in contrast to that of untreated rats. Treatment with Brassinin and Glibenclamide, however, substantially prevented the reduction in antioxidant levels relative to that of the untreated diabetic group, indicating the restorative potential of Brassinin to near normal levels.

Impact of Brassinin on pancreatic inflammatory marker levels in experimental rats

The effect of Brassinin on the status of pancreatic IL-6, TNF-α, and IL-1β was determined. A substantial ($p < 0.05$) elevation in all the inflammatory markers was noticed in the pancreas of diabetic rats, indicative of inflammation. Treatment with Brassinin and Glibenclamide considerably reduced the marker levels compared to that of the untreated diabetic group, suggesting the anti-inflammatory potential of Brassinin (Figure 6). Also, the levels of these markers returned to the normal range in the Brassinin and Glibenclamide administered diabetic rats.

Effect of Brassinin on the histology of pancreas, kidney, and liver of experimental rats

The histological characteristics of the pancreas, kidney, and liver were examined to understand the impact of Brassinin and Glibenclamide on the organs of diabetic rats. The liver of the untreated control group had typical microscopic architecture made up of Pacini hexagonal tiny lobes. However, the liver structure in the diabetic group showed central vein congestion and disruption of the hepatic architecture. Brassinin treatment was able to effectively counteract the STZ's liver injuries. With respect to the kidney structure, it was disorganized and

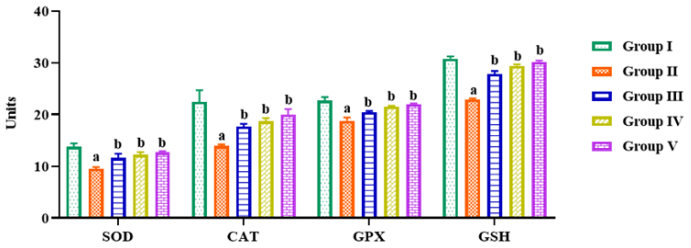

Figure 5: Antioxidant potential of Brassinin on pancreatic antioxidant levels in untreated and treated rats. The rats were grouped into Control, Diabetic induced, Diabetic induced Brassinin treated (25 and 50 mg/kg) and Diabetic induced with Glibenclamide treatment (0.1 mg/kg). The levels of GSH, GPx, SOD, and CAT were examined. The findings are interpreted as the mean±SD of 6 rats in each set with a significance level of * p<0.01 from group I and # p<0.05 from group II.

the bowman's capsule space also shrunk in the diabetic rats. Brassinin-treated groups exhibited remarkable protection of the renal tissue against STZ-induced damage. In the pancreas of diabetic animals, the exocrine glands experienced vacuolation of cells lining pancreatic sacs, and the size and cell count of their endocrine islets of Langerhans significantly decreased. The treatment with Brassinin and Glibenclamide was able to reverse this change and exhibit normal histoarchitecture (Figure 7).

DISCUSSION

Hyperglycemia, a characteristic of the multi-factorial disease, Diabetes, causes excessive urination, compensatory polydipsia, severe fluid ingestion, unknown weight loss, nephropathy, neuropathy, retinopathy, and vascular difficulties. It interferes with the functioning of several essential organs, which might result in serious consequences.[23] Therefore, if we want to develop effective treatments, it is crucial to research how effective agents affect the aforementioned complications. As a result, the present investigation focused on the impact of a naturally occurring phytoalexin, Brassinin, on the attenuation of diabetes-associated complications in STZ-stimulated SD rats. A standard drug, Glibenclamide, was employed to compare the anti-diabetic effect displayed by Brassinin.

In this investigation, diabetes was stimulated in SD rats by administering a pancreotoxic substance called STZ, which damages the β-cells present in the islets of Langerhans, which significantly reduces the amount of insulin secreted and results in blood glucose enhancement. In addition to increased liver glycogen breakdown, gluconeogenesis, and glucose synthesis, these effects are principally introduced by decreased glucose entry into muscle and adipose and peripheral tissue.[24] Upon STZ administration, a substantial elevation in blood glucose and HbA_{1c} and a reduction in insulin were observed, and these outcomes are in concordance with previous investigations.[25-27]

The characteristic features of diabetic rats include weight loss, muscular atrophy, severe hair loss, scaling, cataracts, elevated food and water intake, dehydration, polyuria, etc., In this study,

STZ-stimulated diabetic rodents had significantly lower body weights. Considering that diabetic rat cells cannot make use of glucose for energy production because insulin secretion and action are reduced, weight loss could be ascribed to increased employment of protein and fat. Additionally, increasing protein catabolism to offer amino acids for carrying out gluconeogenesis leads to muscle as well as weight loss.[28] The amount of water and food ingested was much higher in the diabetic group, which may be related to a diminished capacity of the tissues to use glucose, leading to augmented levels of glucose elimination through urine, which continuously stimulates the consumption of excess food and liquids.

Through gluconeogenesis or glycogenolysis, the liver and kidneys synthesize the majority of endogenous glucose. Owing to impaired pancreatic functioning and decreased tissue glucose absorption, endogenous glucose production was increased in diabetic rodents, resulting in hyperglycemia.[25] The liver has a special activity in the metabolism of glucose and is important

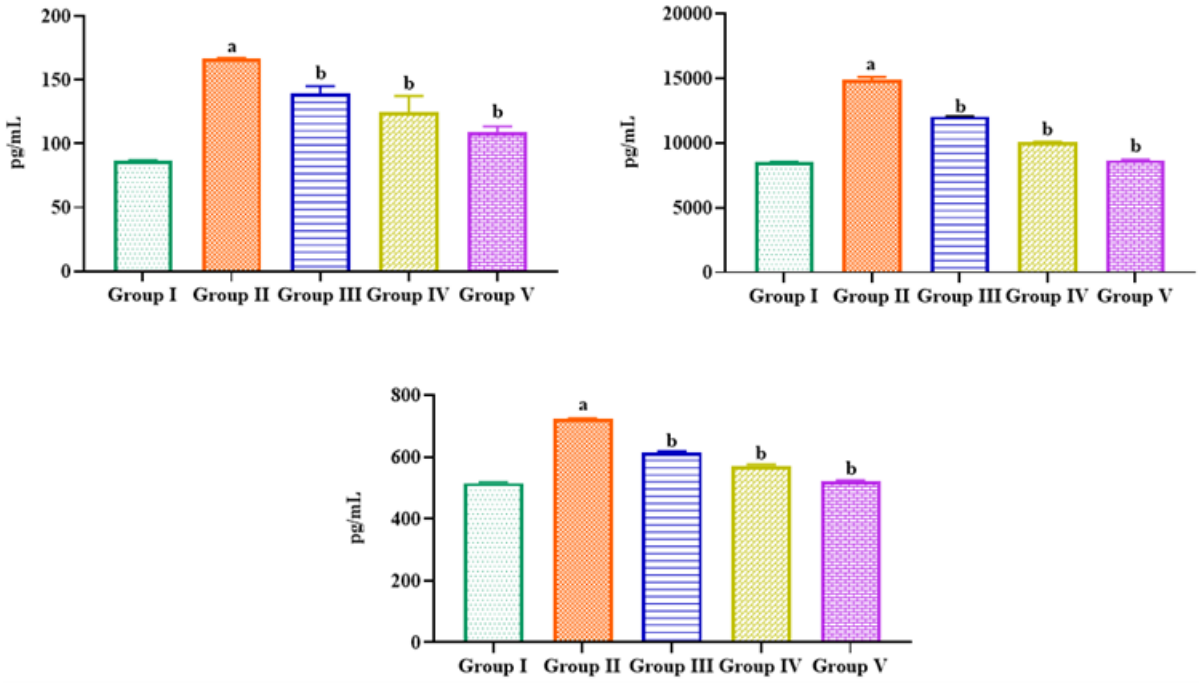

Figure 6: Effect of Brassinin on pancreatic inflammatory markers TNF-α, IL-6, and IL-1β levels in control and treated rats. The animals were grouped into Control, Diabetic induced, Diabetic induced Brassinin treated (25 and 50 mg/kg) and Diabetic induced with Glibenclamide treatment (0.1 mg/kg). (A) TNF-α, (B) IL-6 and (C) IL-1β. The findings are interpreted as the mean±SD of 6 rats in each set with a significance level of * $p<0.01$ from group I and # $p<0.05$ from group II.

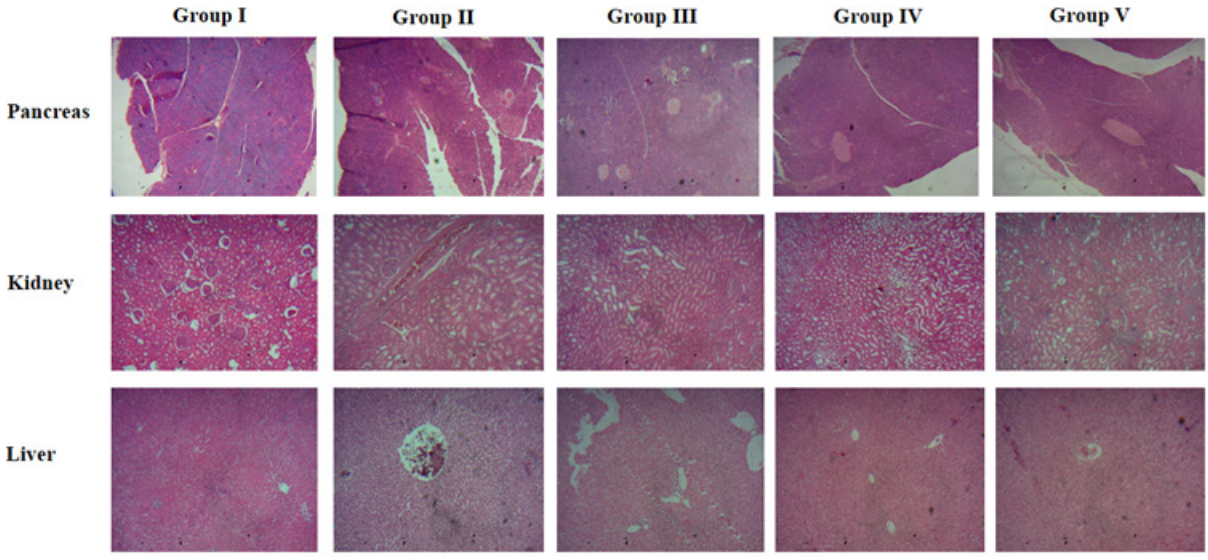

Figure 7: Effect of Brassinin treatment on histoarchitecture of pancreas, kidney, and liver tissues in control and treated rats. A hematoxylin and eosin stained image of formalin-fixed tissues was subjected to light microscopic analysis.

for maintaining systemic glucose homeostasis. The kidneys also take part in the blood sugar regulation process.[29] In this study, Brassinin treatment markedly decreased the elevated glucose concentration. The reduction of glucose level by Brassinin could be ascribed to the recovery of pancreatic islet cells, as described in earlier studies by other natural compounds.[27] In rats with diabetes triggered by STZ, insulin production is diminished.[30] Our diabetic rodents also displayed decreased plasma insulin levels. However, plasma insulin levels dramatically increased after Brassinin treatment, further confirming its anti-diabetic nature. The anti-diabetic potential of Brassinin can be ascribed to the presence of dithiocarbamate ester in its structure, which has been proven to exhibit anti-hyperglycemic activity in previous reports.[31,32]

HbA_{1c} is one of the most suitable indicators for monitoring long-term glycemic control in diabetes individuals. Due to the glycosylation of haemoglobin in diabetic individuals, HbA_{1c} levels increased, and this rise in concentration was correlated positively with blood glucose levels. In diabetic conditions, the high blood glucose concentration interacts non-enzymatically with Hb. As a result, the HbA_{1c} increases.[33] A substantial elevation in HbA_{1c} concentration was noticed in diabetic animals in this study. Diabetes-prone rats treated with Brassinin exhibited lower HbA_{1c} concentrations. Brassinin's anti-hyperglycemic action by improving glycemic control has been clearly demonstrated with this finding.

Studying the pathological alterations in the liver throughout hyperglycemia for the regulation and management of diabetes complications has generated interest due to the abundant incidence of liver disease in the diabetic population.[3] By converting the metabolic pathway intermediates, the hepatic enzyme activity of ALT and AST establish the connection between protein and carbohydrate metabolism.[34] The findings demonstrated that all liver enzymes were considerably elevated in diabetic rats that were untreated, and these findings are analogous to various reports.[35,36] However, the Brassinin-treated groups of rats were effectively protected from the hepatic enzyme changes. Increased blood AST, ALT, and ALP activities are utilized as indicators for acute liver toxicity and as signs of hepatic lesions. The increased levels of hepatic enzymes are triggered by damage to the hepatocyte membrane due to diabetic toxicity and the leaking of these enzymes into the circulation.[37]

The hexokinase enzyme present in the liver plays a considerable part in glucose utilization and glycogen synthesis; in diabetes, its activity is decreased but is recovered by the introduction of insulin.[38] In this study, diabetic rats' livers showed a noticeably decreased level of HK activity. The discovery is in line with previous research, and it may be explained by a lower insulin level, diminished glucose absorption in the body, and increased blood sugar levels.[39,28] The HK activity was considerably elevated in the diabetic rat liver following Brassinin treatment. This elevation may be caused by Brassinin, which triggers the delivery of insulin from the residual pancreatic β-cells. By enhancing the HK activity of the liver, Brassinin improved glucose homeostasis and augmented glucose metabolism.[25]

The glucose homeostasis enzymes, F16BP and G6P, which are mostly present in the liver and kidney, are essential for gluconeogenesis. The current study discovered a substantial enhancement in G6Pase and F16BP activities in the liver of STZ-stimulated diabetic rats, which could be because of insulin diminution. Enhanced G6Pase and F16BP activities trigger the production of glucose.[40] The bioactive substances that occur in Brassinin may be responsible for the reversal of G6P and F16BP activities to a state that is close to normal after receiving Brassinin treatment.[32]

According to research on oxidative stress and diabetes complications, the onset of pathological abnormalities associated with hyperglycemia is accelerated mainly by oxidative stress.[41] In order to combat oxidative stress, which is the primary factor contributing to the emergence of complications, it is crucial to increase both enzymatic and non-enzymatic antioxidants in the body.[42] While catalase and glutathione peroxidase enzymes catalyze the breakdown of H_2O_2 to produce oxygen and water, SOD is involved in the catalysis of superoxide radical degradation. The reduction of Glutathione disulfide to GSH, which is necessary for preventing oxidative stress, is catalyzed by glutathione reductase.[43,44] The prolonged hyperglycemia-induced ROS generation that results in the depletion of hepatic antioxidants may be the cause of the decline in the functions of liver antioxidants in diabetic rats, and the reduced activity has been established in several investigations.[45,46] However, this trend seemed to reverse when Brassinin was administered. Therefore, Brassinin's anti-peroxidative potential to inhibit the H_2O_2 levels was confirmed.

Chronic oxidative stress has been shown to stimulate transcription factors that cause inflammation and apoptosis.[45] The pathophysiology of DM is influenced by inflammation-associated regulators i.e., IL-6, C-reactive protein, and TNF-α. One of the primary cytokines produced during inflammatory reactions is TNF-a, which can trigger signalling pathways of cell death, cell survival, inflammatory reactions, and cellular proliferation. A number of mechanisms have been suggested involving inflammation-related stress, including ROS and NO in the pathogenesis of diabetes in addition to β-cell impairment. The apoptosis of β-cells is greatly influenced by inflammation-associated molecules.[46,47] In the group II rats, elevated levels of IL-6, TNF-α, and IL-1 β proteins were observed in the pancreas, as noticed in earlier works.[48,49] The brassinin administration in the diabetic rats with Brassinin demonstrated a considerable decline in the status of these inflammatory mediators, suggestive of its anti-inflammatory potential.

As a result of diabetes, secondary complications primarily target organs like the liver, pancreas, and kidney, and the histological alterations of these organs have already been reported in prior research.[50,51] The liver of the untreated control group had typical microscopic architecture made up of Pacini hexagonal tiny lobes that were positioned around the major vein. The liver structure in the diabetic untreated group showed central vein congestion and disruption of the hepatic architecture, which might lead to necrosis and severe damage. Brassinin treatment was able to effectively counteract the STZ's liver injuries. The morphology after Glibenclamide administration resembled the control structure that was not provided with any treatment. Compared to the non-diabetic group, the kidneys of the diabetes-induced rats showed architectural modifications. With the kidney structure being disorganized and the renal vein dilating, Bowman's capsule space also shrunk. Brassinin-treated groups exhibited remarkable protection of the renal tissue against STZ-induced damage. The morphology resembled the non-diabetic control structure when Glibenclamide was administered.

Normal exocrine and endocrine characteristics may be observed in H&E-stained pancreatic sections from control animals, which also reveal acinar structures and islets of Langerhans. Exocrine glands of diabetic rats experienced vacuolation of cells lining pancreatic sacs, and the size and cell count of their endocrine islets of Langerhans significantly decreased. Brassinin treatment of diabetic rats revealed minor degenerative alterations in exocrine glands as well as an apparent enhancement in the islets of Langerhans size with normal architecture. These findings concurred with the outcomes of earlier kinds of literature,[52,53] and they may be explained by redox status instability, inflammation, and apoptosis. Overall, in the current study, Brassinin not only reduced fasting blood glucose levels but also increased pancreatic antioxidant status and lowered inflammation, protecting β-cells from additional impairment.

CONCLUSION

In conclusion, diabetes treatment with a therapeutic dose of Brassinin is a key strategy for strengthening the body's antioxidant defense system, which in turn prevents hyperglycemia, oxidative stress, and inflammation. This approach also helps to protect against complications caused by diabetes at the earliest stage of organ damage. This is the first investigation of Brassinin's potential for protection against the progression of diabetes and the related secondary problems in experimental animals. Furthermore, additional studies are still needed in the future to understand the underlying molecular mechanisms of the therapeutic potentials of the brassinin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

STZ: Streptozotocin; **DM:** Diabetes Mellitus; **AST:** Aspartate aminotransferases; **ALT:** Alanine aminotransferases; **ALP:** Alkaline phosphatase; **HK:** Hexokinase.

SUMMARY

- In diabetes, persistent hyperglycemia leads to an elevated free radical formation owing to protein glycosylation and glucose auto-oxidation, which induces oxidative stress.
- Brassinin not only reduced fasting blood glucose levels but also increased pancreatic antioxidant status and lowered inflammation, protecting β-cells from additional impairment.
- Brassinin's potential for protection against the progression of diabetes and the related secondary problems in experimental animals.

REFERENCES

1. Franklin BE, Crisler SC, Shappley R, Armour MM, McCommon DT, Ferry RJ. Real-time support of pediatric diabetes self-care by a transport team. Diabetes Care. 2014;37(1):81-7. doi: 10.2337/dc13-1041, PMID 23959568.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, *et al*. IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119. doi: 10.1016/j.diabres.2021.109119, PMID 34879977.
3. Antu KA, Riya MP, Mishra A, Sharma S, Srivastava AK, Raghu KG. *Symplocos cochinchinensis* attenuates streptozotocin-diabetes induced pathophysiological alterations of liver, kidney, pancreas and eye lens in rats. Exp Toxicol Pathol. 2014;66(7):281-91. doi: 10.1016/j.etp.2014.05.004, PMID 24912748.
4. Muruganathan U, Srinivasan S, Vinothkumar V. Antidiabetogenic efficiency of menthol, improves glucose homeostasis and attenuates pancreatic β-cell apoptosis in streptozotocin–nicotinamide induced experimental rats through ameliorating glucose metabolic enzymes. Biomed Pharmacother. 2017;92:229-39. doi: 10.1016/j.biopha.2017.05.068, PMID 28549291.
5. Chitra PS, Chaki D, Boiroju NK, Mokalla TR, Gadde AK, Agraharam SG, *et al*. Status of oxidative stress markers, advanced glycation index, and polyol pathway in age-related cataract subjects with and without diabetes. Exp Eye Res. 2020;200:108230. doi: 10.1016/j.exer.2020.108230, PMID 32931824.
6. Dal Canto E, Ceriello A, Rydén L, Ferrini M, Hansen TB, Schnell O, *et al*. Diabetes as a cardiovascular risk factor: an overview of global trends of macro and micro vascular complications. Eur J Prev Cardiol. 2019;26(2_suppl):25-32. doi: 10.1177/2047487319878371, PMID 31722562.
7. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019;62(1):3-16. doi: 10.1007/s00125-018-4711-2, PMID 30171279.
8. Tayanloo-Beik A, Roudsari PP, Rezaei-Tavirani M, Biglar M, Tabatabaei-Malazy O, Arjmand B, *et al*. Diabetes and heart failure: multi-omics approaches. Front Physiol. 2021;12:705424. doi: 10.3389/fphys.2021.705424, PMID 34421642.
9. Maresch CC, Stute DC, Alves MG, Oliveira PF, de Kretser DM, Linn T. Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review. Hum Reprod Update. 2018;24(1):86-105. doi: 10.1093/humupd/dmx033, PMID 29136166.
10. Kothari V, Bornfeldt KE. A new treatment strategy for diabetic dyslipidemia? Diabetes. 2020;69(10):2061-3. doi: 10.2337/dbi20-0035, PMID 32958608.

11. Efferth T, Koch E. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. Curr Drug Targets. 2011;12(1):122-32. doi: 10.2174/138945011793591626, PMID 20735354.
12. Hong T, Ham J, Song J, Song G, Lim W. Brassinin inhibits proliferation in human liver cancer cells via mitochondrial dysfunction. Cells. 2021;10(2):332. doi: 10.3390/cells10020332, PMID 33562611.
13. Pedras MSC, Zheng QA, Sarma-Mamillapalle VK. The phytoalexins from Brassicaceae: structure, biological activity, synthesis and biosynthesis. Nat Prod Commun. 2007;2(3). doi: 10.1177/1934578X0700200315.
14. Chripkova M, Drutovic D, Pilatova M, Mikes J, Budovska M, Vaskova J, *et al*. Brassinin and its derivatives as potential anticancer agents. Toxicol *In vitro*. 2014;28(5):909-15. doi: 10.1016/j.tiv.2014.04.002, PMID 24747292.
15. Yin WM, Cao XB, Li SX, Zhang F, Guan YF. Brassinin inhibits proliferation and induces cell cycle arrest and apoptosis in nasopharyngeal cancer C666-1 cells. Arab J Chem. 2022;15(9):104018. doi: 10.1016/j.arabjc.2022.104018.
16. Brandstrup N, Kirk JE, Bruni C. The hexokinase and phosphoglucoisomerase activities of aortic and pulmonary artery tissue in individuals of various ages. J Gerontol. 1957;12(2):166-71. doi: 10.1093/geronj/12.2.166, PMID 13416554.
17. Baginski ES, Foà PP, Zak B. Glucose-6-phosphatase. In: Methods of enzymatic analysis. Academic Press. 1974:876-80.
18. Gancedo JM, Gancedo C. Fructose-1,6-diphosphatase, phosphofructokinase and glucose-6-phosphate dehydrogenase from fermenting and non-fermenting yeasts. Arch Mikrobiol. 1971;76(2):132-8. doi: 10.1007/BF00411787. PMID: 4324161.
19. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984;105:121-6. doi: 10.1016/s0076-6879(84)05016-3, PMID 6727660.
20. Leopold F, Wolfgang AG. Assays of glutathione peroxidase. Methods Enzymol. 1984;105(93):05013-8.
21. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. doi: 10.1016/0003-9861(59)90090-6, PMID 13650640.
22. Al Batran R, Al-Bayaty F, Jamil Al-Obaidi MM, Abdualkader AM, Hadi HA, Ali HM, Abdulla MA. *In vivo* antioxidant and antiulcer activity of *Parkia speciosa* ethanolic leaf extract against ethanol-induced gastric ulcer in rats. PLoS One. 2013;8(5):e64751. doi: 10.1371/journal.pone.0064751. PMID: 23724090.
23. Lin Y, Sun Z. Current views on type 2 diabetes. J Endocrinol. 2010;204(1):1-11. doi: 10.1677/JOE-09-0260, PMID 19770178.
24. Hamza RZ, Al-Eisa RA, El-Shenawy NS. Efficacy of mesenchymal stem cell and vitamin D in the treatment of diabetes mellitus induced in a rat model: pancreatic tissues. Coatings. 2021;11(3):317. doi: 10.3390/coatings11030317.
25. Sureka C, Elango V, Al-Ghamdi S, Aldossari KK, Alsaidan M, Geddawy A, *et al*. Ameliorative property of *Sesbania grandiflora* on carbohydrate metabolic enzymes in the liver and kidney of streptozotocin-induced diabetic rats. Saudi J Biol Sci. 2021;28(7):3669-77. doi: 10.1016/j.sjbs.2021.05.002, PMID 34220217.
26. Abdulwahab DA, El-Missiry MA, Shabana S, Othman AI, Amer ME. Melatonin protects the heart and pancreas by improving glucose homeostasis, oxidative stress, inflammation and apoptosis in T2DM-induced rats. Heliyon. 2021;7(3):e06474. doi: 10.1016/j.heliyon.2021.e06474, PMID 33748504.
27. Al Nohair SF, Ahmed SS, Ismail MS, El Maadawy AA, Albatanony MA, Rasheed Z. Potential of honey against the onset of autoimmune diabetes and its associated nephropathy, pancreatitis, and retinopathy in type 1 diabetic animal model. Open Life Sci. 2022;17(1):351-61. doi: 10.1515/biol-2022-0039, PMID 35480484.
28. Srinivasan S, Sathish G, Jayanthi M, Muthukumaran J, Muruganathan U, Ramachandran V. Ameliorating effect of eugenol on hyperglycemia by attenuating the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. Mol Cell Biochem. 2014;385(1-2):159-68. doi: 10.1007/s11010-013-1824-2, PMID 24078031.
29. Jiang S, Young JL, Wang K, Qian Y, Cai L. Diabetic-induced alterations in hepatic glucose and lipid metabolism: The role of type 1 and type 2 diabetes mellitus (Review). Mol Med Rep. 2020;22(2):603-11. doi: 10.3892/mmr.2020.11175. PMID: 32468027.
30. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res. 2007;125(3):451-72. PMID 17496368.
31. Mollazadeh M, Mohammadi-Khanaposhtani M, Valizadeh Y, Zonouzi A, Faramarzi MA, Kiani M, *et al*. Novel coumarin containing dithiocarbamate derivatives as potent α-glucosidase inhibitors for management of type 2 diabetes. Med Chem. 2021;17(3):264-72. doi: 10.2174/1573406416666200826101205, PMID 32851964.
32. Deshpande TA, Martínez-Málaga J, Priefer R. Dithiocarbamates as potential PTP1B inhibitors for diabetes management. Results in Chemistry. 2022;4:100372. doi: 10.1016/j.rechem.2022.100372.
33. Sundaram R, Naresh R, Ranadevan R, Shanthi P, Sachdanandam P. Effect of iridoid glucoside on streptozotocin induced diabetic rats and its role in regulating carbohydrate metabolic enzymes. Eur J Pharmacol. 2012;674(2-3):460-7. doi: 10.1016/j.ejphar.2011.10.039, PMID 22094064.
34. Chamundeswaramma KV, Das P, Rathnamma VV, Philip GH. Activity levels of Aspartate Transaminase (AAT) and Alanine Transaminase (ALAT) in freshwater fish *Labeo rohita* exposed to deltamethrin. Ecol Environ Conserv. 2010;16(2):145-8.
35. Cheng D, Zhang X, Xu L, Li X, Hou L, Wang C. Protective and prophylactic effects of chlorogenic acid on aluminum-induced acute hepatotoxicity and hematotoxicity in mice. Chem Biol Interact. 2017;273:125-32. doi: 10.1016/j.cbi.2017.06.013, PMID 28623112.
36. Singh D, Lawrence K, Singh S, Ercisli S, Choudhary R. *In-vivo* hyperglycemic, antioxidant, histopathological changes, and simultaneous measurement of kaempferol verified by high-performance thin layer chromatography of *Setaria italica* in streptozotocin-induced diabetic rats. Saudi J Biol Sci. 2022;29(5):3772-90. doi: 10.1016/j.sjbs.2022.03.009, PMID 35844408.
37. Nna VU, Bakar ABA, Mohamed M. Malaysian propolis, metformin and their combination, exert hepatoprotective effect in streptozotocin-induced diabetic rats. Life Sci. 2018;211:40-50. doi: 10.1016/j.lfs.2018.09.018, PMID 30205096.
38. Elsadek MF, Ahmed BM. Effect of sakuranin on carbohydrate-metabolizing enzyme activity modifications in streptozotocin-nicotinamide-induced diabetic Wistar rats. Saudi J Biol Sci. 2022;29(3):1402-6. doi: 10.1016/j.sjbs.2021.11.035, PMID 35280595.
39. Prasath GS, Subramanian SP. Modulatory effects of fisetin, a bioflavonoid, on hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in hepatic and renal tissues in streptozotocin-induced diabetic rats. Eur J Pharmacol. 2011;668(3):492-6. doi: 10.1016/j.ejphar.2011.07.021, PMID 21816145.
40. Sundaram R, Nandhakumar E, Haseena Banu H. Hesperidin, a citrus flavonoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. Toxicol Mech Methods. 2019;29(9):644-53. doi: 10.1080/15376516.2019.1646370, PMID 31345080.
41. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm J. 2016;24(5):547-53. doi: 10.1016/j.jsps.2015.03.013, PMID 27752226.
42. Lubrano V, Balzan S. Enzymatic antioxidant system in vascular inflammation and coronary artery disease. World J Exp Med. 2015;5(4):218-24. doi: 10.5493/wjem.v5.i4.218, PMID 26618108.
43. Deponte M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochim Biophys Acta. 2013;1830(5):3217-66. doi: 10.1016/j.bbagen.2012.09.018, PMID 23036594
44. Nna VU, Abu Bakar AB, Md Lazin MRML, Mohamed M. Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin-induced diabetic rats. Food Chem Toxicol. 2018;120:305-20. doi: 10.1016/j.fct.2018.07.028. PMID: 30026088.
45. Giribabu N, Eswar Kumar K, Swapna Rekha S, Muniandy S, Salleh N. *Vitis vinifera* (Muscat Variety) seed ethanolic extract preserves activity levels of enzymes and histology of the liver in adult male rats with diabetes. Evid Based Complement Alternat Med. 2015;2015:542026. doi: 10.1155/2015/542026, PMID 25852767.
46. Oyenihi OR, Brooks NL, Oguntibeju OO. Effects of kolaviron on hepatic oxidative stress in streptozotocin induced diabetes. BMC Complement Altern Med. 2015;15(1):236. doi: 10.1186/s12906-015-0760-y, PMID 26179065.
47. Zhang Z, Ding Y, Dai X, Wang J, Li Y. Epigallocatechin-3-gallate protects pro-inflammatory cytokine induced injuries in insulin-producing cells through the mitochondrial pathway. Eur J Pharmacol. 2011;670(1):311-6. doi: 10.1016/j.ejphar.2011.08.033, PMID 21925162.
48. Chandirasegaran G, Elanchezhiyan C, Ghosh K, Sethupathy S. Berberine chloride ameliorates oxidative stress, inflammation and apoptosis in the pancreas of streptozotocin induced diabetic rats. Biomed Pharmacother. 2017;95:175-85. doi: 10.1016/j.biopha.2017.08.040, PMID 28843149.
49. Roh SS, Kwon OJ, Yang JH, Kim YS, Lee SH, Jin JS, *et al. Allium hookeri* root protects oxidative stress-induced inflammatory responses and β-cell damage in pancreas of streptozotocin-induced diabetic rats. BMC Complement Altern Med. 2016;16:63. doi: 10.1186/s12906-016-1032-1, PMID 26888412.
50. Mohammadi J, Naik PR. The histopathologic effects of *Morus alba* leaf extract on the pancreas of diabetic rats. Turk J Biol. 2012;36(2):211-6. doi: 10.3906/biy-1008-51.
51. Lafraxo H, Bakour M, Laaroussi H, El Ghouizi A, Ousaaid D, Aboulghazi A, *et al*. The synergistic beneficial effect of thyme honey and olive oil against diabetes and its complications induced by alloxan in Wistar rats. Evid Based Complement Alternat Med. 2021;2021:9949056. doi: 10.1155/2021/9949056, PMID 34594393.
52. Nurdiana S, Goh YM, Ahmad H, Dom SM, Syimal'ain Azmi N. Noor Mohamad Zin NS, Ebrahimi M. Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with *Ficus deltoidea* and vitexin. BMC Complement Altern Med. 2017;17(1):1-17.
53. Balamash KS, Alkreathy HM, Al Gahdali EH, Khoja SO, Ahmad A. Comparative Biochemical and Histopathological Studies on the Efficacy of metformin and Virgin Olive Oil against streptozotocin-induced Diabetes in Sprague-Dawley Rats. J Diabetes Res. 2018;2018:4692197. doi: 10.1155/2018/4692197, PMID 30581871.

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