Impact of Betanin on Diabetes Induced Experimentally by Streptozotocin and Associated Histopathological Changes in Albino Adult Rats

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ABSTRACT

Background: The endocrine problem diabetes mellitus is the most prevalent condition. Over time, the heart and blood arteries become among the tissues and organs that are negatively impacted by this medical disease. Beets' anti-inflammatory, antioxidant, anti-tumor, high pressure-lowering, neuroprotective, and immune-modulating advantages are equivalent to those of pharmaceuticals.

Aim of the work: Analyzing the effects of betanin on experimentally generated diabetes and related Histopathological Alteration in Adult Albino Rats.

Material and Methods: Rattus norvegicus albino adult males were separated into four equal groups, as follows: Group I functioned as the control group, Group II was the diabetic group, Group III was the normal plus betanin, and Group IV was the diabetic group that had been given betanin therapy. The samples of blood have been collected at the completion of the study's period to be investigated blood glucose levels, glycated haemoglobin, serum insulin, alanine amino transferase (ALT), aspartate amino transferase (AST), lipid profiles, malondialdehyde (MDA), tumour necrosis factor alpha (TNFα), catalase, nuclear factor Kapp, lactate dehydrogenase, interleukin 1 and interleukin 6. Additionally, liver and pancreas samples were taken for a histopathology investigation.

Results: The drug streptozotocin (STZ) substantially increased levels of blood sugar, glycated haemoglobin, cholesterol, alanine amino transferase, aspartate amino transferase, malondialdehyde, TNF, lactate dehydrogenase, nuclear factor kappa, IL-1, and IL-6 while substantially reducing levels of serum insulin and catalase. All levels substantially improved after receiving betanin treatment.

Conclusion: Due to its antioxidant properties, betanin has a preventive impact on diabetic rats.

Keywords: Betanin; Streptozotocin; Histopathological; Albino; Diabetes.

INTRODUCTION

Diabetes mellitus (DM), an extremely complicated long-term disorder, is distinguished by an illness of persistently elevated glucose levels spurred on by irregularities in the function of insulin, production, or both, and it also causes a variety of deterioration in the absorption and utilization of carbohydrates, lipids, and proteins **[(1)].**

Diabetes mellitus (DM), which is among the most essential medical conditions, has adverse effects that degrade the patient's quality of life. Nowadays, a lot of people are struggling with DM. The increased prevalence and seriousness of complications related to diabetes brought on by insufficient care and high blood sugar levels that persist are the key contributors to the rising rates of illness and death seen among people with this disease., which results in disturbances in metabolic functions like sugar, lipid, and protein utilization. The primary pathogenic risk factors for diabetes mellitus have all been identified as inflammation, oxidative stress, inflammatory factors, and immunological reactions, although the actual etiopathogenesis of the condition is still not entirely known **[(2)].**

Diabetic microangiopathy develops because of the degenerative alterations that DM led to thickening the vascular basement membrane, produces in the microvasculature **[(3)].** In the family Chenopodiaceae, Beta vulgaris is a plant. Beetroot (betanin), often known as table garden beetroot, is a purple root vegetable., which comes in a variety of grown forms and is the most well-known, is one of its distinguishing characteristics. Raw, baked, boiling, or juice extraction are all acceptable ways to consume beets. Red beets were fantastic when grilled pickled, in dishes such as salads or made into soup, making them a popular meal in many countries in Eastern and Central Europe **[(4)].**

Beta vulgaris, also known as betanin, represents one of the greatest cultivated vegetables in the world. Running efficiency and training endurance can both be improved by beetroot **[(5)].**

Additionally, it is employed to manage hypertension **[(6)]** and possesses antibacterial, antiradical, or cytotoxic effects **[(7)].** As a result, it might have hepatoprotective and anti-diabetic effects **[(8)].**

The conclusion of **Abedimanesh et al. [(2)]** is reinforced by findings of the current research, which suggested that betanin that is had a protective impact contrary to diabetes outcomes in the diabetic rats induced by STZ. The mechanisms explaining this finding involve diminished high blood sugar levels, hyperlipidemia, and tests for liver function, as well as enhanced liver and tissue from the pancreas functionality. It has been discovered that the mechanisms through which betanin produces these encouraging effects are the nuclear factor kappa B, sirtuin-1, and 5′ adenosine monophosphate activated protein kinase (AMPK), respectively. This investigation's goal was to assess how beetroot affected

Received: 02/07/2022
Accepted: 22/09/2022

adult albino rats' experimentally induced diabetes and related histopathological changes.

MATERIALS AND METHODS

The Sixty adult albino male rats (Rattus norvegicus) implemented as the study's model were donated by Nile Pharmaceuticals Company, Cairo, Egypt. These rats had a weight range of 125 to 165 grammes. They lived in metal enclosures (20- 30- 20 cm for each of the five rats) with adequate ventilation, a comfortable temperature, and access to water and commissary rat chow meal.

They had the opportunity to acclimatize in the experimental room for 14 days prior to the trial's start. The rats were grouped into the following four equal groups, each composed of 15 rats.

- **Group I (Control group):** 0.5 ml of distilled water was given to normal rats for a period of four weeks while they consumed a commissary rat chow diet.

- **Group II (Diabetic group):** For four weeks, In addition to a solitary I.P. administration of sixty milligrams per kilogram of total body weight of streptozotocin, which was administered to initiate diabetes in the rats, each of the rats in this category obtained 0.5 ml of distilled water per day [(9)].

- **Group III (betanin plus the control group):** For four weeks, normal rats were given an oral dose of betanin diluted in 0.5 ml of distilled water at a rate of 250 mg/kg body weight [(10)].

- **Group IV (Diabetic betanin -treated group):** 0.5 ml of distilled water with 250 mg of betanin per kilogram of body weight was administered orally for four weeks after group II diabetics were induced.

Drugs:

To induce diabetes, A vial of STZ containing 100 mg of STZ powder received from Sigma Pharmaceuticals Company, and 10 ml of saline was mixed with it for generating a ten ml STZ mixture. Following an infusion of sixty milligrams per kilogram of body weight of STZ in a fifty millimeters of citrate buffer, the animals were permitted access to a solution of 5% glucose for the duration of the night. These rats had been evaluated for diabetes using the glucometer (Aquo-Check, Roche) after a period of 72 hours. Animals who were chosen for further study had overnight blood glucose levels that were higher than 190 mg/dl [(9).] In May 2021, Betanin leaves were bought in the Damietta region of Egypt from a regional traditional store. The leaves were rinsed with water from the tap and crumbled for extraction at Mansoura University's college of pharmacy's pharmacology department lab. With the use of distilled water and a muslin cloth, the juice was drained from the betanin, and the shaft was thrown away. After being extracted from 5 kg of beets, 1.5 L of juice was recovered, which was then placed in an airtight container and let 20 to 30 minutes to settle. The acquired juice has been freezing dried and is ready for usage. Until it was time to use it, the refrigerated sample has been preserved at 0 degrees Centigrade [(10)].

For anesthesia, ethyl ether (Analar, Nile Pharmaceutical). All rats were placed in an aesthetic box stuffed with ether vapour at the conclusion of the experimental period after being fasted for the previous night. Applying liquid ether to cotton wool at the base of the box on a regular basis kept the ether vapor present. To measuring blood glucose [(9)], and glycated hemoglobin levels [(9),] blood was taken from the retro-orbital plexus using a heparinized capillary tube.

Blood was centrifuged at 5000 revolutions per minute for 10 minutes to extract serum after being allowed to clot. alanine amino transferase, Aspartate amino transferase, high density lipoprotein**,** triglyceride, total cholesterol, low density lipoprotein, serum insulin level [(9),] malondialdehyde [(11),] catalase [(12),] tumor necrosis factor alpha [(11),] lactate dehydrogenase [(12),] nuclear factor kappa [(11)] and interleukin 1 and interleukin 6 [(12).]

Tissue-extract preparation

The liver and pancreas were taken out and fixed in 10% formalin solution for histological examination. The General Pathology Department at Al- Azhar University in Damietta produced paraffin blocks for the tissue samples, collected various sections, stained slides with hematoxylin and eosin (H and E) stains, and examined the findings under a light microscope.

Ethical Approval

Institutional Research Board approved the study protocol (IRB00012367-23- 7 - 042) of Al-Azhar University's Damietta Faculty of Medicine. This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of science, Al-Azhar University, Cairo, Egypt.

Statistical analysis of data

Data entry and analysis were performed out utilizing the statistical software known as the Statistical Package for Social Sciences, or SPSS, version "24". Each result's mean and standard deviation were evaluated. We compared the means of the various groups. A post hoc analysis known as least significant difference (LSD) was carried out to discover significantly different mean values. P values under 0.05 were used to decide whether the disparity was significant.

RESULTS

Results of the current research showed that initiation of diabetes led to significant rises in the overall mean value of blood glucose level, glycated

haemoglobin level, serum triglyceride, cholesterol, low density lipoprotein, alanine amino transferase, aspartate amino transferase, lactate dehydrogenase, malondialdehyde, Nuclear factor kappa, tumor necrosis factor alpha, lactate dehydrogenase, nuclear factor kappa, IL-6 and IL-1 in diabetic group (Group II) correlated with a substantial reduction in serum insulin, HDL, and catalase levels in addition to a mean body weight value **(Table I).**

Betanin was administered to diabetic rats, led to substantial rise of serum insulin, body weight, serum HDL and catalase levels associated with significant drop in the overall mean value of blood sugar level, glycated haemoglobin level, serum TG, cholesterol, LDL, ALT, AST, lactate dehydrogenase, MDA, Nuclear factor kappa, TNF alpha, nuclear factor kappa, lactate dehydrogenase, IL-6, IL-1 and TNF levels **(Table II).**

Table (I): Relation of STZ and studied parameters.

Groups Parameters	Group I	Group II	Group III
Body weight	**221.65±7.82**	**107.09±8.99***	**225.2±7.89**
Blood Glucose (mg/dl)	**98.86±9.89**	**331.76±22.37***	**99.65±8.72**
Glycated hemoglobin (%)	**5.23±0.42**	**10.9±0.56***	**4.32±0.31**
Serum insulin (µIU/L)	**10.98±0.72**	**3.7±0.51***	**10.34±0.28**
TGs (mg/dl)	**91.5±3.99**	**113.45±4.88***	**90.78±3.99**
Cholesterol (mg/dl)	**98.8±5.68**	**129.56±5.22***	**97.9±4.76**
LDL (mg/dl)	**39.25±5.36**	**74.48±5.61***	**39.55±4.67**
HDL (mg/dl)	**40.24±2.57**	**30.99±2.1***	**38.78±1.98**
ALT (U/L)	**22.35±1.68**	**50.11±4.87***	**20.98±2.56**
AST (U/L)	**25.68±2.38**	**68.6±5.23***	**24.85±2.98**
Lactate dehydrogenase (U/L)	**147.6±5.65**	**251.2±6.65***	**144.8±7.28**
Catalase (mg/dl)	**35.3±3.88**	**25.61±1.35***	**33.54±2.02**
MDA (nmol/l)	**20.04±2.86**	**46±2.09***	**23.05±1.23**
Nuclear factor kappa	**0.99±0.25**	**2.37±0.47***	**0.92±0.07**
IL-6 (Pg/ml)	**3.71±0.56**	**7.12±0.87***	**3.98±0.04**
IL-1 (Pg/ml)	**2.08±0.38**	**5.58±0.87***	**2.21±0.37**
TNF (Pg/ml)	**52.86±2.56**	**97.05±3.56***	**51.27±4.62**

*** Significant compared to group I.**

Table (II): Relation of betanin and studied parameters.

GroupsParameters	Group II	Group IV
Body weight	**110.81±7.25**	**144.2±6.58***
Blood Glucose (mg/dl)	**331.23±23.25**	**220.35±16.2***
Glycated hemoglobin (%)	**9.68±0.36**	**6.21±0.27***
Serum insulin (µIU/L)	**4.02±0.58**	**7.09±0.71***
TGs (mg/dl)	**114.75±4.8**	**94.28±4.08***
LDL (mg/dl)	**74.94±5.86**	**57.29±5.99#**
LDL (mg/dl)	**74.12±5.85**	**57.18±5.61#**
HDL (mg/dl)	**30.91±1.86**	**33.8±2.87#**
ALT (U/L)	**50.24±3.55**	**40.25±2.05#**
AST (U/L)	**68.29±4.76**	**46.78±2.84#**
Lactate dehydrogenase (U/L)	**252.2±6.28**	**199.89±7.24#**
Catalase (mg/dl)	**25.86±2.08**	**28.56±1.75#**
MDA (nmol/l)	**44.02±2.09**	**29.94±3.56#**
Nuclear factor kappa	**2.79±0.74**	**1.71±0.09#**
IL-6 (Pg/ml)	**7.12±0.77**	**6.38±0.91#**
IL-1 (Pg/ml)	**5.08±0.78**	**4.01±0.28#**
TNF (Pg/ml)	**97.35±5.21**	**75.38±4.69#**

 # Significant compared to group II.

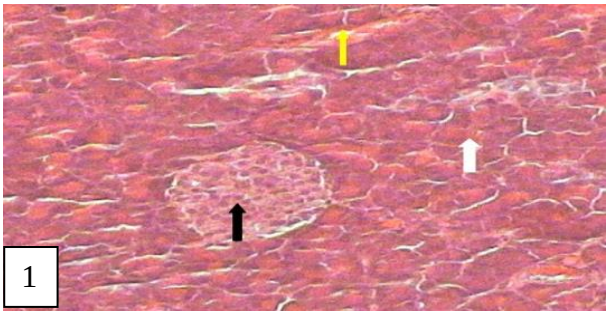

Fig. (1): Photo micrograph of cross section through pancreas of control group shows, normal pancreatic islets, pancreatic acini, and blood vessels are depicted in a slice of the pancreas with black, white, and yellow arrows, respectively. **(H&E, X :400).**

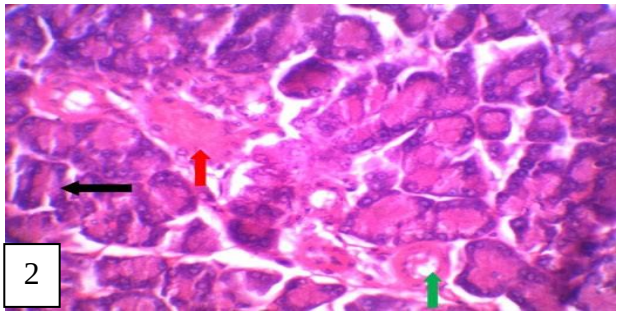

Fig. (2): Photo micrograph of cross section through pancreas of the diabetic group's shows, pancreatic islets (pointed by the red arrow), normal pancreatic acini (represented by the black arrow), and normal pancreatic blood arteries (represented by the green arrow) all exhibit deformation and atrophy. **(H&E, X :400).**

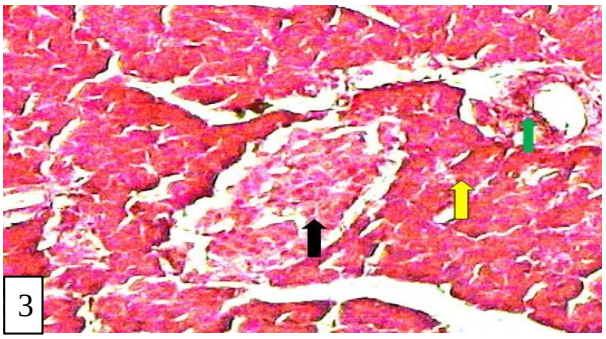

Fig. (3): Photo micrograph of cross section through pancreas of control plus betanin group's pancreas portion group shows, the control plus betanin group's pancreas portion demonstrates normal pancreatic islets and acini black and yellow arrows, respectively) and normal blood arteries (green arrow). **(H&E, X :400).**

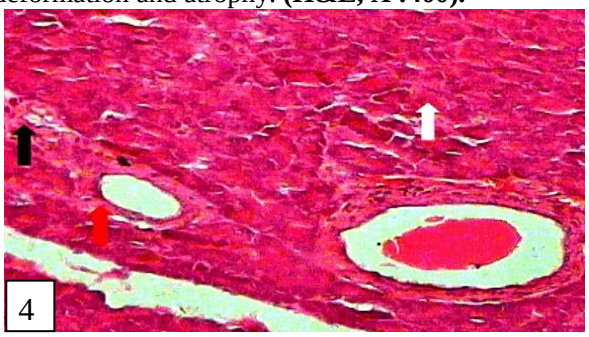

Fig. (4): Photo micrograph of cross section through portion of the pancreas from the diabetic betanin treatment group exhibits better pancreatic islets were atrophied (black arrow), normal pancreatic acini and normal blood arteries (red and white arrow, respectively). **(H&E, X :400).**

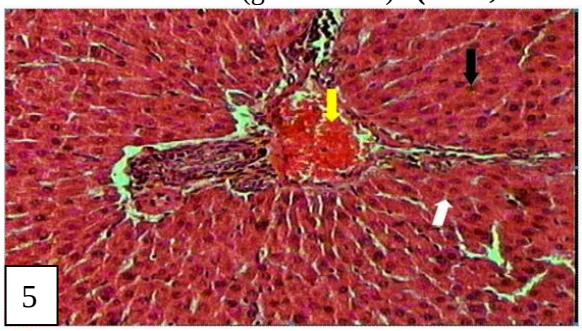

Fig. (5): Photo micrograph of cross section through liver control group, shows normal hepatocytes, blood sinusoids and central vein (white, black, and yellow arrows, respectively. **(H&E, X :400).**

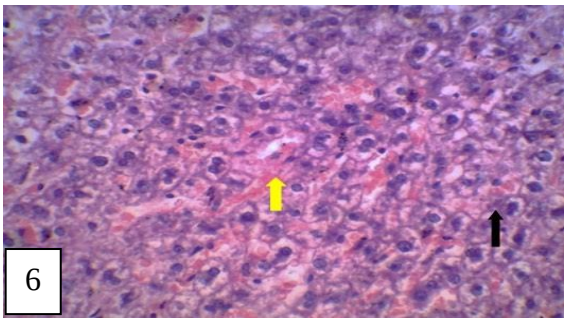

Fig. (6): Photo micrograph of cross section through liver with diabetes shows, hepatocytes in a section of the liver with diabetes have noticeable vacuolations (black arrow) and engorged blood vessels (yellow arrow). **(H&E. X :400).**

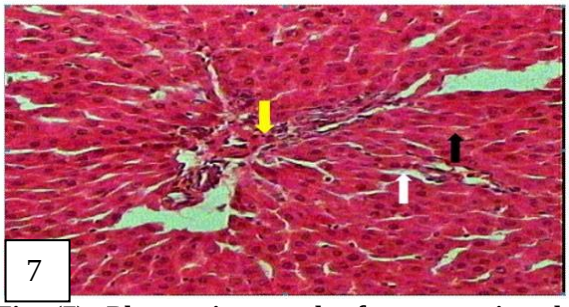

Fig. (7): Photo micrograph of cross section through liver of the control betanin group shows, hepatocytes (white arrow), blood sinusoids (black arrow), and the central vein (yellow arrow) are all normal in the cross-section of the liver. **(H&E, X :400).**

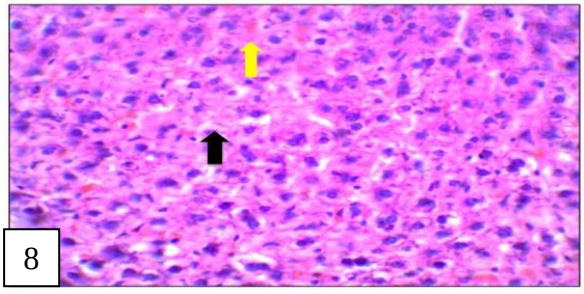

Fig. (8): Photo micrograph of cross section through liver of the diabetic betanin treatment group shows, few hepatocyte vacuoles (black arrow) and clogged blood vessels (yellow arrow) are visible in the section. **(H&E, X :400).**

DISCUSSION

The current study's findings demonstrated that when compared to normal, STZ injection significantly decreased the body weight of diabetic rats. These findings corroborated those of **Yang and Hyung-Sub** [13] who found that streptozotocin-induced diabetes in rats caused a considerable drop in body weight that was caused by an absolute or relative insulin deficit or by a decrease in protein synthesis in all organs.

In rats with untreated diabetes, **Soliman** [14] noted a substantial loss of body weight. Degradation of proteins and lipids or catabolism could be to blame for this loss. Thus, enhanced catabolic processes led to a loss of muscle mass, which in diabetic rats is a major contributor to weight loss.

The current study's findings demonstrated that STZ injection caused the blood glucose level in the diabetic group to significantly increase as contrasted with control group, while glycated haemoglobin levels substantially decreased. These findings concur with those of **Sekkin *et al.*** [15]'s research who reported a single STZ injection results in the onset of diabetes mellitus., which is identified by raised blood sugar, glycated haemoglobin, and drop serum insulin levels. This happened because STZ generated an inflammatory response that resulted in macrophage and later lymphocyte infiltration, damaged the islets cells, destroyed the cells within twenty-four hours, and triggered an immune response. In rats, STZ-induced oxidative damage and persistent inflammation that may result in tissue damage are tightly related.

In the current study, Rats with diabetes exhibited substantially increased cholesterol, triglycerides and also low-density lipoprotein compared to control rats, while having significantly drop high density lipoprotein level. These findings were in line with those of **Saleh and Maged** [16] who discovered that diabetic rats had much higher levels of cholesterol, LDL, and TGs than normal rats reported.

The current investigation shown that, in contrast to normal rats, diabetic rats had significantly higher levels of AST and ALT. These findings concurred with those of **Makena *et al.*** [17] who found that the diabetes group's ALT and AST levels increased more than those of the normal group. They reported diabetes mellitus can cause hepatic damage, which causes glucogenesis and an increase in protein concentrations. The production of keto acids from amino acids is correlated with ALT and AST in DM.

In the current study, diabetic rats' levels of malondialdehyde, lactate dehydrogenase, tumor necrosis factor alpha, interleukin 1 and also interleukin 6 dramatically rise as compared to normal, along with a significant drop in catalase. These findings corroborated those of **Elsawy and Emara** [18] who claimed that increased levels of proinflammatory cytokines, such as interleukin 1, interleukin 6, malondialdehyde, and tumour necrosis factor alpha, were brought on by elevated oxidative stress or a decline in antioxidant defence mechanisms in diabetes mellitus.

Furthermore, the results of the current investigation concur with those of **Edrees *et al.*** [19] who noticed that elevated TNF- levels were found in diabetic rats as a result of oxidative stress, which resulted in aberrant cytokine production, including TNF-, which the liver produces in response to inflammation. According to **ElKomy and Mouafi** [20] oxidative damage is linked to elevated levels of TNF, which in turn promote neutrophil infiltration and the synthesis of nuclear factor kappa B (NFkB). TNF is crucial for the caspases' activation, which induces cell apoptosis. TNF- levels were higher in diabetic rats. This occurred because TNF-'s inflammatory response caused an apoptotic cell in the -cell. A high concentration of inflammatory cells can cause -cell apoptosis, which can result in a drop in the quantity of cells that make insulin or an increase in insulin levels.

In this work, diabetic rats had significantly rise levels of malondialdehyde and lactate dehydrogenase in addition significantly lower levels of catalase. These findings concurred with those of **Lo *et al.*** [21], who found that diabetic rats had higher malondialdehyde levels and drop catalase level. Superoxide anion and peroxide of hydrogen levels developing in biological systems, which in turn produce hydroxyl radicals and cause lipid peroxidation, can be caused by a reduction in the activity of these antioxidants. In order to defend cells from extremely reactive OH radicals, catalase reduces H_2O_2. Hyperglycemia-induced enhanced free radical production is the primary source of oxidative stress in diabetes.

Betanin significantly increased body weight in the current study. The findings supported those of **Choi *et al.*** [22] who claimed that B. vulgaris leaves extract caused a considerable rise in body weight in diabetic rats as a result of B. vulgaris' good anabolic impact through enhancing lipid and glucose metabolism.

In the current investigation, betanin significantly reduced blood glucose levels, and glycated haemoglobin was linked to significantly reduced insulin levels. Due to the pancreatic beta-cells' regeneration, **Ninfali and Angelino** [23] observed that administering Beta vulgaris extract to DM decreased blood glucose levels.

When treated to streptozocin-induced diabetic rats, **Kumar *et al.*** [24] ultimately reached a decision that the ethanol-based extract of betanin might have anti-diabetic properties. They concluded that a six-week betanin juice treatment lowered blood sugar levels by restricting the digestion of carbohydrates, reducing the absorption of glucose in the gastrointestinal tract, and modulating the liver's expulsion of glucose.

The findings of the present research corroborated those of **Abdul Bari *et al.*** [25] who found that betanin powder consumption significantly reduced cholesterol, total lipids, and liver enzymes in diabetic rats. Beetroot powder consumption also significantly decreased ALT and AST levels, as well as lipid levels. In diabetic rats given betanin therapy, **Nouri *et al.*** [26] discovered that liver enzymes dropped.

Additionally, according to **Iahtisham *et al.*** [27], the antiradical scavenging activity of the extract is what gives betanin its hepatoprotective properties. Due to its abundant supply of polyphenols and flavonoids, including betalains, betanin may have antiradical properties. More proof is in favour of polyphenols' capacity to improve Phase II antioxidant and detoxifying enzymes, which preserve the liver.

Similar to this, **Singh *et al.*** [28] found that consuming betanin juice reduced levels of total cholesterol, LDL, and triglycerides while also dramatically raising HDL levels. Because of the active ingredient betaine, which possesses antioxidant qualities, betanin has a hepatoprotective effect.

According to **Clifford *et al.*** [29] in the same context, betanin reduces cholesterol and oxidised LDL cholesterol, which protects the vascular system and helps to prevent and treat cardiovascular disease. Betanin consumption has a positive physiological effect for atherosclerosis disease, claim **Ninfali and Angelino** [23]. Due to its high betalain content, which decreased homocysteine levels and controlled vascular homeostasis.

A substantial reduction in MDA was seen in the current study after betanin administration. These findings were in line with those of **Fustinoni-Reis *et al.*** [30] who discovered a significant reduction in MDA in diabetes patients receiving betanin treatment. Due to the antioxidant properties of the polyphenols found in betanin, this effect might be the result.

Furthermore, **Albalawi *et al.*** [31] demonstrated that administering betanin extract caused a considerable drop in the levels of NF-kB and TNF- in liver tissue. Oral treatment of betanin ethanol extract for 28 days resulted in significantly lower levels of many inflammation-promoting mediators including as MDA, Il-6, TNF-, and NF-B, as well as significantly higher levels of catalase because of the anti-inflammatory protective action.

Because pro-oxidants are inhibited and the body's natural antioxidant system is stimulated when betanin is consumed, oxidative processes have been shown to be prevented [29]**.** Betanin's capacity to stimulate nuclear factor (erythroid-derived 2)-like 2 and subsequently activate the gene expression of antioxidant enzymes is the cause of the elevated levels of antioxidant enzymes observed after betanin treatment [32]**.**

The histo-pathological analysis of the pancreatic islets in the current study showed that the development of diabetes caused distortion and atrophy, and that betanin treatment corrected the condition of the atrophied islets. My findings were in line with those of **Dhananjayan *et al.*** [33] who reported that streptozotocin -induced diabetes resulted in necrotic alterations and breakdown of pancreatic islets and that betanin supplementation restored the islet cells' histology in the pancreas. Streptozotocin damages pancreatic beta-cells by DNA alkylation by attacking the glucose transporter (GLUT 2) in beta-cells. superoxide radical generation and free radical damage to -cells are related. By partially destroying -cells, free radicals are a significant contributor to the onset of diabetes mellitus [34].

In the current investigation, the administration of betanin reduced the amount of hepatocyte vacuolation that was caused by the induction of diabetes, which resulted in substantial vacuolation of hepatocytes with congested blood vessels. These findings agree with those of **Olumese and Oboh** [10] and **Dhananjayan *et al.*** [33], who found that diabetes brought on by streptozotocin caused hepatocyte vacuolations with broadening sinusoids, and that betanin administration reduced the pathological alterations to the liver and enhanced hepatic architecture.

By simultaneously scavenging reactive oxygen species and activating nuclear factor erythroid 2-related factor 2, betanin reduced liver damage. In vitro scavenging of 2, 2-Diphenyl-1-picrylhydrazyl radicals by betanin has demonstrated the effectiveness of its antioxidants [32].

By conclusion, adult male albino rats' lipid profiles, blood urea, blood creatinine, and blood glucose levels are all significantly enhanced by betanin. It could be because to the drug's hepatoprotective, hypoglycemic, and lipid-lowering effects. These properties of betanin may reduce diabetic mortality and morbidity.

- **Disclosure**: Regarding this study's implementation, authorship, or publishing, the authors state that they have no potential conflicts of interest.
- **Funding**: The authors guarantee that no funding was granted for this work.
- **Author contribution:** In the study, all authors contributed equally.

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