Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Study Of The Therapeutic Efficacy Of Pumpkin Seed In Improving The Liver's Activity In Rats With Stz-Induced Diabetes

Mawloud A. M. Lateef[1]; Haitham L. Abdulhadi[2] and Loay H. Ali[3]

[123]Department of Biology, College of Education for Pure Sciences, University Of Anbar, Anbar, Ramadi, Iraq

Corresponding Author: maw21u1009@uoanbar.edu.iq

hatemloay81@uoanbar.edu.iq

Cite this paper: Mawloud A. M. Lateef; Haitham L. Abdulhadi and Loay H. Ali(2024 Study Of The Therapeutic Efficacy Of Pumpkin Seed In Improving The Liver's Activity In Rats With Stz-Induced Diabetes.*Frontiers in Health Informatics, 13* (3), 5336-5351

Abstract

The effect of alcoholic pumpkin seed extract (Cucurbita pepo L.) on metabolic risk, oxidative stress, inflammation, renal function indices, liver death, and histopathological changes in experimentally induced diabetes was investigated. In diabetic rats maintained in an experiment, researchers examined the effects of alcoholic pumpkin seed extract (Cucurbita pepo L.) on liver mortality, renal function indicators, oxidative stress, inflammation, metabolic risk, and histological changes.Male rats were given a single injection of streptozotocin (STZ; 40 mg/kg, i.p.) to cause diabetes. Three months later, the rats received an every-other-day dose of 200 mg/kg of pumpkin seed extract. After receiving pumpkin seed extract treatment, diabetic rats' HbA1c and hyperglycemia improved, as did their insulin, HOMA-IR, and liver function enzyme levels (AST, ALT, and ALP). Additionally, pumpkin seed extract enhanced serum indicators and liver function enzyme activities, normalized serum GSH, CAT, and SOD levels, and decreased elevated levels of MDA and H2O2. These results are supported by histopathological analyses of liver sections, which demonstrate the beneficial effects of pumpkin seeds. According to the current research, pumpkin seeds shield the liver from oxidative stress brought on by diabetes. In summary, pumpkin seeds have a great deal of therapeutic promise for the management of diabetes, including the treatment and control of its liver-related problems.

Keywords: *Diabetes, Pumpkin, Apoptosis, Oxidative stress, Liver, STZ.*

Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood sugar (Hyperglycemia) and altered energy metabolism caused by a relative or complete deficiency in insulin secretion, a lack of insulin action, or both. It is a chronic disease that occurs when the pancreas is incompetent to produce enough insulin or the body cannot use the produced insulin effectively. Insulin is a hormone that controls the amount of glucose in the blood. Persistent high blood sugar in diabetes causes various biochemical and structural changes in the body's cells, tissues, and organs. These changes explain the widespread complications of diabetes that can occur in many body organs, including the liver, kidneys, eyes, nerves, blood vessels, etc. [1]. Medicinal plants have been a suitable alternative to these materials. This trend was reinforced by the World Health Organization (WHO)

Expert Committee report on diabetes, which recommended conducting more studies and research on conventional methods used for treating diabetes [2]. Thus, the interest of many researchers in this aspect has focused on identifying the most important plants that reduce blood sugar levels, which are used in particular by those working in the field of folk medicine. Other researchers investigate the histotoxicity of several of these commonly used plants.

The study aims

In the current study, we studied one of the plants known to those working in folk medicine, which is the pumpkin plant (*Cucurbita*), using the alcoholic extract of its seeds to study its therapeutic effectiveness in reducing blood sugar in male mice with diabetes and improving liver cell functions by studying some chemical and histological aspects.

Materials and methods

Preparation of extracts

The alcoholic extract of *Cucurbita pepo* was prepared using a Soxhlet extractor device, taking 25 g of powder and adding 500mL of absolute ethanol alcohol. The solvent was absorbed for 10min, with a 20:1 ratio between the substance and solvent. The device was operated at boiling temperature for 2.5–3h. The extracted solution was dried in an oven at 40–50°C and collected in a sterilized vial for use [3].

Chemicals used:

Sigma Chemical (St. Louis, MO) supplied the streptozotocin (STZ). The highest grade was used for all other compounds.

Initiation of diabetes

A single intraperitoneal dose (i.p) of STZ (40 mg/kg body weight) dissolved in 0.01 M citrate buffer, pH 4.5, was injected to induce diabetes in male laboratory rats. [4, 5]. 48 hours following the injection of STZ, the blood glucose level was measured with a glucose monitor set (Accu-Chek Active- Germany) . After recording the blood sugar results, male rats with a value higher than 250 mg/dL were considered to be diabetic animals.

Experimental Animals

This study was conducted in the animal house of the Department of Biology/ College of Education for Pure Sciences / University of Anbar. The study comprised 40 adult male albino rats of the Sprague Dawley, aged 3 to 4 months and weighing 300 to 320 g. The experiment needed three months to complete. The animals were divided into four groups, each with ten rats, based on comparable weight categories. The first group, Control, was orally dosed with 5 mL/kg distilled water daily; the second group, Pumpkin, was orally dosed with the alcoholic extract of pumpkin seeds at a concentration of 200 mg/kg [6] every other day for three months at a dose of 2 ml and the third group, STZ, was injected intraperitoneally (IP) with STZ at a concentration of 40 mg/kg in a single dose (0.1ml) and left without treatment. It was given water and food normally and was considered a positive control group.

Sample collection

After the experiment was completed, all experimental animals were anesthetized with ether. Blood was obtained by heart puncture, then the blood was drawn and placed in white tubes free of EDTA, the blood was left to clot

and then separated by centrifugation at 3000 rpm for 10 minutes, and the filtered serum was then stored in the freezer at -20°C.

Biochemical tests:

Utilizing kits provided by Biosystems(Spain), BioSource Europe S.A. manufactory and Spectrum(Egypt) respectively, the serum levels of glucose, insulin, and HbA1c were measured.

Malondialdehyde (MDA) concentrations were determined in accordance with the manufacturer's instructions (Dokki Giza, Egypt) in order to evaluate lipid peroxidation. The content of glutathione (GSH) and the activity of the enzymes catalase (CAT) and superoxide dismutase (SOD) were measured. Biochemical tests are carried out in test tubes to determine the amounts of the liver enzymes aspartate aminotransferase activity (AST) and aspartate aminotransferase activity (ALT).

Pathological examination

Animals were sacrificed after the experiment, and tissue samples from Liver was collected, dissected, and immediately fixed in 10% formalin for 24 h, dehydrated in a succession of graded alcohols, clarified in xylene, and encapsulated in paraffin, Tissue sectioning was done at 3µm thickness and stained with hematoxylin and eosin (H&E) for histological evaluation [7]. All sections were inspected and photographed using OLYMPUS CX43 microscope and a microscope-adapted OLYMPUS SC52 camera.

Statistical analysis

Using GraphPad Prism version 6.01, the results were expressed as means and standard error of mean (±SEM). Student's t test was utilized to determine statistical differences between two groups, whereas one-way analysis of variance followed by Tukey's test was used for multiple comparisons. A P value of < 0.05 was considered statistically significant.

Ethical approval

The experimental protocol was approved in accordance with Order (179 on July 2, 2023) and issued by the Ethical Committee for the Care and Use of Laboratory Animals in the Department of Biology, College of Education for Pure Sciences, University of Anbar

Results

Body weight and blood sugar

The results of the current study showed a significant decrease in the weight of the experimental animals in the group injected with STZ (diabetes group) compared to the weights of the control group.Over the course of several months, diabetic rats given 200 mg/kg of pumpkin seed extract orally every other day shown a notable gain in body weight in comparison to rats treated with STZ diabetic (Fig. 1a); they were still far lower than the control group, though. Conversely, A significant increase in blood glucose levels was also observed in diabetic male rats, according to the results shown in the figure attached to the study compared to diabetes rats in the control group (Fig. 1b).

Fig. 1: Effects of pumpkin seed extract and streptozotocin (STZ) on blood serum glucose (a) and animal weight (b) in rats across several groups during experiment period. (n = 10).

Blood sugar, insulin, and HbA-1c levels

Dosing by pumpkin seed extract

Dosing rats in the second healthy group throughout the experiment did not affect their glucose concentration, insulin, and glycated glucose. Likewise. Moreover, MDA levels did not change, as did liver function enzymes (AST, AST). On the contrary, significant increases in serum antioxidants (GSH, SOD, and CAT) were recorded.

Diabetic rats administered orally with pumpkin seed extract showed significant improvement at the end of the trial period (Table 1) compared with the diabetic group, but the level was lower than that of the control group. STZ injection significantly elevated blood glucose and HbA-1c levels after diabetes induction. In addition, these rats had significantly lower insulin levels and higher HOMA-IR than controls (Table 1). Compared to rats treated with STZ, treatment with pumpkin seed extract during the diabetes experiment markedly decreased HbA-1c and blood glucose levels. Our results showed that diabetic male rats given orally with alcoholic extract of pumpkin seeds showed a non-significant increase in insulin levels and a decrease in HOMA-IR levels when compared with the group injected with STZ, however the outcomes were essentially the same as the control levels..

Table 1: Rats in various groups were given streptozotocin (STZ) and pumpkin seed extract (Pumpkin) to see how they affected the rats' blood serum glucose (mg/dl) and insulin levels (pg/ml) as well as the percentages of HbA1c and HOMA-IR at the end of experiment period .

Serum markers of liver function

When compared to normal control animals, the concentration of liver function enzymes (AST, ALT, and ALP) in the blood of STZ-induced diabetic rats increased significantly (Table 2). After receiving pumpkin seed extract treatment, the liver function and enzyme levels in diabetic rats were much lower than in non-diabetic animals and returned to normal.

	Control	Pumpkin	STZ	STZ + Pumpkin
Glucose	**96.74a ±15.69**	**93.18a ±9.052**	**474.3b ±21.99**	**98.33a ±12.44**
Insulin	**100.6a ±3.252**	**94.84a ±7.226**	**39.98b ±4.059**	**98.71a ±8.278**
HbA-1c	**5.100a ±0.511**	**5.240a ±0.498**	**11.34b ±0.654**	**5.320a ±0.517**
HOMA-IR	**21.98a ±2.055**	**21.20a ±2.101**	**46.67b ±3.345**	**23.79a ±1.786**

Values represent mean ± standard error. The different letters indicate the presence of significant differences at a significant level (P<0.05) between the groups. Similar letters indicate that there are no significant differences at a significant level (P<0.05) between the groups (n = 10).

Table 2: Impacts of pumpkin seed extract (Pumpkin) and streptozotocin (STZ) on the activity of liver function enzymes (AST, ALT, and ALP); (U/L) in rats' serum across several groups.

	Control	Pumpkin	STZ	STZ + Pumpkin
AST	**28.51a ±2.575**	**28.48a ±2.685**	**65.25b ±3.652**	**31.55a ±3.778**
ALT	**19.27a ±3.091**	**18.05a ±3.241**	**57.96b ±3.686**	**23.50a ±3.315**
ALP	**62.40a ±6.238**	**61.70a ±6.527**	**149.1b ±8.322**	**68.13a ±6.266**

Values represent mean ± standard error. The different letters indicate the presence of significant differences at a significant level (P<0.05) between the groups. Similar letters indicate that there are no significant differences at a significant level (P<0.05) between the groups (n = 10).

Oxidative damage and protective agents:

The hearts of STZ-induced diabetic rats showed a substantial rise in lipid peroxidation product levels (Fig. 2). Furthermore, when pumpkin seed extract was administered three months after the induction of diabetes, the serum of STZ-treated rats noted significant reductions in glutathione, SOD and catalase activities, and levels that were comparable to control levels (Fig. 2).

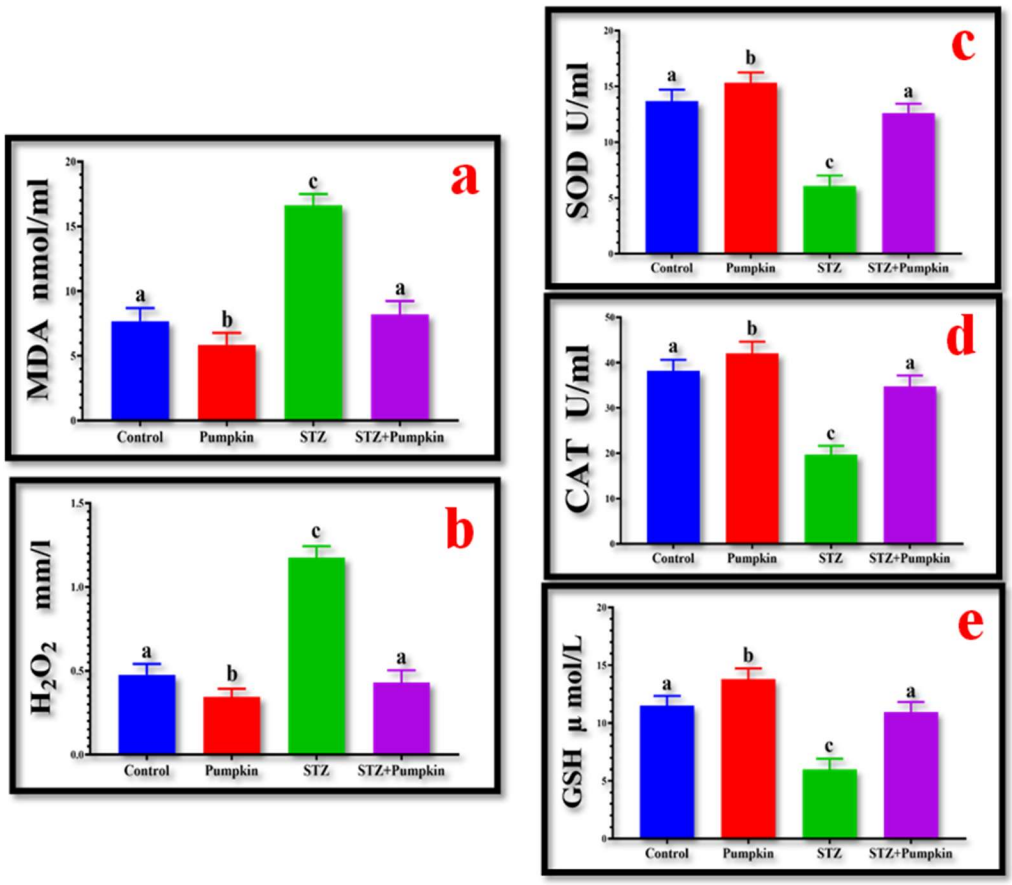

Figure 2 shows that MDA (nmol/ml) (a), (H2O2) (mml/l) (b), SOD (U/ml) (c), CAT (U/ml) (d) and GSH (µ mol) /L) (e) were affected in the serum of rats of different groups by streptozotocin (STZ) and pumpkin extract. Values represent mean ± standard error. The distinct letters signify the existence of noteworthy distinctions between the groups at a significant level (P<0.05). Similar letters denote the absence of significant differences between the groups (n = 10) at a significant level (P<0.05).

Histological study

Liver histological examination

The results of the microscopic examination of the liver of male rats in the first group (Fig. 4), showed the normal structure of the liver tissue, as the liver lobule shows the central vein lined with squamous endothelial cells supported by the basement membrane, and around the vein wall were found groups of multi-sided liver cells (hepatocytes) with large spherical nuclei and acidic cytoplasm with the presence of small blood sinusoids between the hepatocyte groups in which there are some Kupffer cells. The results of the microscopic examination of the liver of male rats in the first group (Fig. 4), showed the normal structure of the liver tissue, as the hepatic lobule shows the central venule lined with squamous endothelial cells supported by the

basement membrane, and around the vein wall were found groups of multi-sided liver cells (hepatocytes) with

large spherical nuclei and acidic cytoplasm with the presence of small blood sinusoids between the hepatocyte groups in which there are some Kupffer cells.

Fig. 5 shows the histology examination's findings of the second group treated with the alcoholic extract of pumpkin seeds at a concentration of 200 mg/kg, in which the tissue composition appears similar to the control group, as the liver tissue contained a wide-bore central vein lined with endothelial cells based on the basement membrane and the vein wall was continuous with the surrounding blood sinusoidal network. The liver cells appeared as regular, well-structured rows. Each cell has a nucleus or two dark chromatin spherical nuclei. The

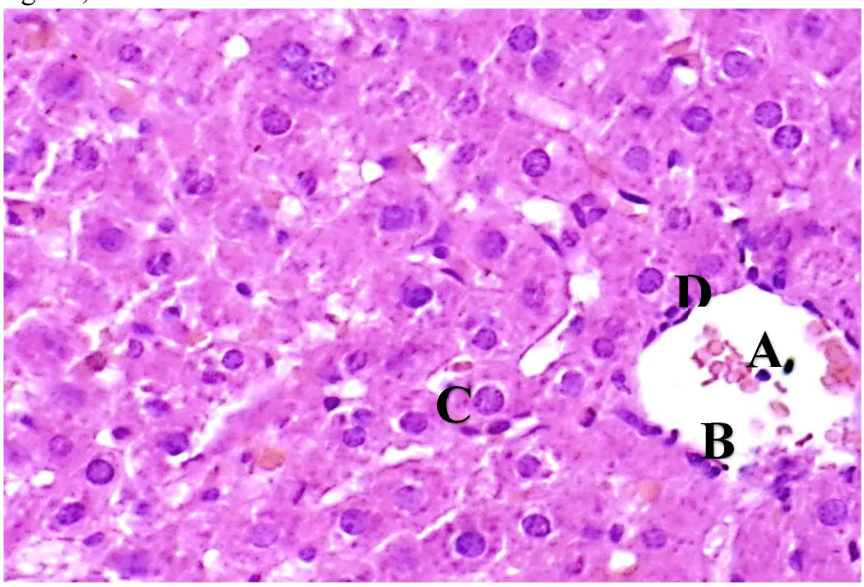

Fig. 4 - A cross-section of the liver of a control rat showing the normal tissue structure, the presence of a central venule (A) lined with endothelial cells on the basement membrane (B), polygonal hepatocytes with spherical nuclei (C), small blood sinusoids containing Kupffer cells (D), H & E, (40X).

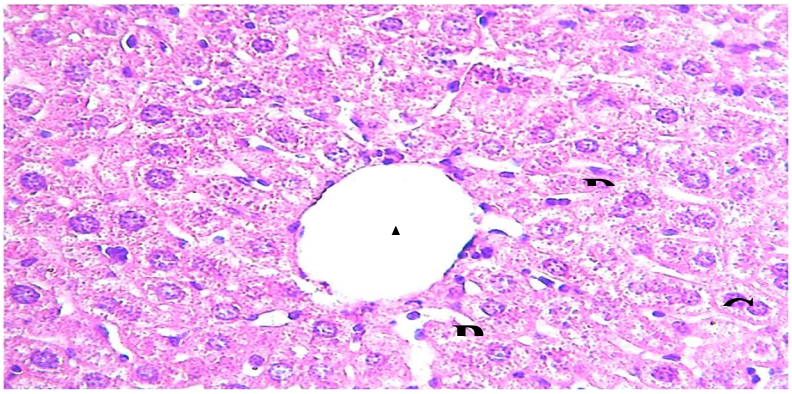

Fig. 5 - Cross section of a rat liver treated with pumpkin. The large-bore central venous is observed (A), the network of blood sinusoids containing Kupffer cells (B),

blood sinusoids contained phagocytic Kupffer cells. The results are consistent with several studies that showed

that orally dosing rats with an alcoholic extract of pumpkin seeds did not show any harmful effects on the tissue structure of the liver [8], This could be caused by the presence of the essential elements and minerals in pumpkin seeds along with their high concentration of active chemicals that function as antioxidants.

In Fig. 6, the STZ group appears with significant tissue changes observed, represented by the presence of hepatocyte hypertrophy, cells in the stage of apoptosis, and enucleation of some cells, with the appearance of other hepatocytes that appeared in a normal polygonal manner, in addition to the presence of hyperplasia of the Kupffer cells, which were a large size between the blood sinusoids. In addition, the presence of rupture of the basement membrane and sloughing of some endothelial cells, with inflammatory infiltration of white blood cells surrounding the central venule, as well as cytoplasmic vacuolation of some hepatocytes.

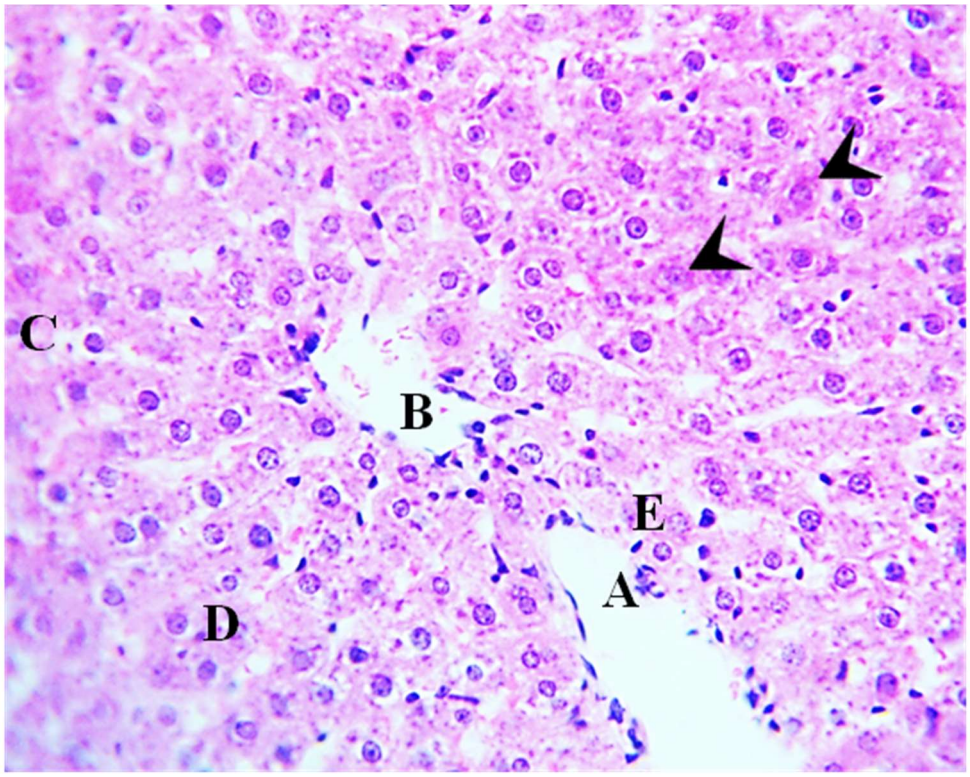

Fig. 6 - Cross section of STZ-treated rat liver. Liver lobule with endothelial cell sloughing and basement membrane rupture in central vein (A) Inflammatory leukocytes (B) Hepatocyte cytoplasm rupture (C) Hepatocytes radially

A study reported that rats treated with STZ showed evident tissue changes that may lead to liver damage [9], the generation of free radicals as a result of STZ injection and the elevated MDA concentration, which support

the theory that free radical production is what causes the degenerative effects in the liver cells in our investigation, as the latter are characterized by their ability to interact with the phospholipids of hepatocytes membranes, It causes a number of membrane-degrading processes that result in the lysosome membranes being destroyed and the mitochondrial membrane losing some of its vitality, which activates the Apoptosis enzymes, and this was proven in the physiological aspect results of the study.

Microscopic examination results for the liver tissue of experimental diabetic rats that were orally dosed with the alcoholic extract of pumpkin seeds showed simple tissue changes, such as the liver tissue containing some degenerated cells with degenerated nuclei; however, most of the tissue cells appeared normal and had large spherical nuclei. The blood sinusoids showed a slight tissue of Kover cells, and the central venule showed a slight blood congestion surrounded by some white blood cells, Fig. 7.

The evident improvement in liver tissue may be due to the therapeutic role of pumpkin seed extract. It contains many highly effective antioxidant compounds, such as phenols and flavonoids, that enhance and maintain the liver cell membranes of the study animal groups, indicating that the extract under study is free from toxic effects and also inhibits oxidative stress damage by eliminating free radicals and preventing fat oxidation.

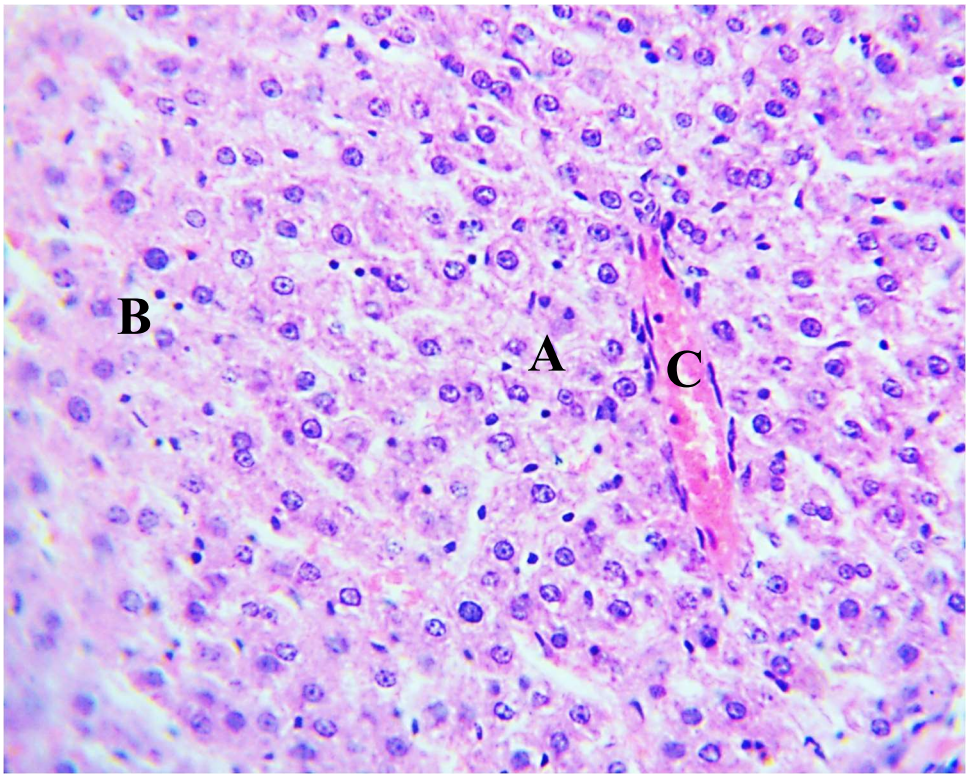

Fig. 7- Cross section of a rat liver treated with pumpkin seed alcoholic extract after injection with STZ. Normal hepatocytes with large spherical nuclei were observed (A), blood sinusoids with little tissue of Kupfer cells (B), and central venule with blood congestion (C). H & E, 40X.

Discussion

Effect of pumpkin seeds extract *Cucurbita pepo* L. on blood and serum glucose levels of rats

The results of the present study showed a significant improvement in glucose levels during and after the experiment in the blood serum of rats injected intraperitoneally with STZ, resulting from dosing with the alcoholic extract of pumpkin seeds. This result is consistent with recent studies that indicated that using pumpkin proteins reduced blood sugar levels by regulating receptors associated with oxidative stress in diabetic rats [10, 11].

Pumpkin seed extract's active components have the ability to move glucose across cell membranes, enhancing its uptake by the body's cells or enabling it to be broken down for energy production. In this context, it is also important to note that the extract used in the current study may have lowered blood sugar through a variety of mechanisms, including reducing the rate at which glucose is absorbed through the intestine, inducing insulin secretion by the remaining pancreatic beta cells, and encouraging the peripheral use of sugar by adipose, muscle, and nervous tissues either directly or indirectly by raising insulin sensitivity or lowering cell resistance to insulin. The findings of our investigation about HOMA-IR tests with a concurrent drop in glucose construction supported this conclusion. [11].

Continuous increase in blood glucose leads to what is called glucose toxicity, which leads to a reduction in the remaining beta cell mass and the accumulation of amyloid, which is similar to fibrils and is in the pancreas called amylin, causing fibrosis of these cells and leading to a deficiency in the insulin hormone. A significant increase in blood glucose levels is also noticed, coinciding with an increase in glycated hemoglobin HbA1c. These results support those obtained by [12, 13]. The reason for this blood glucose increase may be due to the close relationship between the concentration of HbA1c formed and the incidence of diabetes that depends on the concentration of blood glucose that the red blood cells are exposed to during their presence in the bloodstream, which in turn leads to binding to the hemoglobin molecule. Thus, we observe that measuring the concentration of glycated hemoglobin (HbA1c) is an accurate indicator for assessing the level of glucose concentration in the blood [14].

Regarding healthy animals dosed with the alcoholic extract of pumpkin seeds, they showed a slight and insignificant decrease in glucose levels at the beginning of the experiment and even after ending it, which can be attributed to the nature of the active components that this plant possesses, especially flavonoids and terpenes [15, 16], to them may be due this effectiveness of reducing as natural antioxidants and thus protecting beta cells from death through their work in scavenging free radicals.

Effect of pumpkin seeds extract *Cucurbita pepo* L. on serum insulin concentration of rats

Insulin is the primary regulator of the body's economy in storing the three major nutrients, and it also acts to reduce the sugar level in the blood through its essential role in helping glucose enter most of the body's cells.

Taking pumpkin seed extract, with its active ingredients represented by antioxidants, helps a body to better respond to insulin at the cellular level. Moreover, the extract may enhance beta cells by protecting them from oxidative stress resulting from STZ injections and keeping them healthy and effective, in addition to reducing inflammation that has a role in stimulating insulin resistance; thus, it enhances the sensitivity of cells to insulin.

The curative effect of pumpkin seeds may be due to their content of D-chino-Inositol, a vitamin-like

substance (found only in pumpkin seeds) [17] that increases insulin sensitivity and reduces cell resistance to insulin, and also increases the mass of pancreatic beta cells [18]. A study confirmed that pumpkin seeds cause a blood sugar decline, which is similar to the activity of the drug Tolbutamide, a standard drug for diabetics.

Glycosylated hemoglobin (HbA1c)

The study results demonstrated an increase in the glycated hemoglobin (HbA1c) percentage in the group of rats with STZ-induced diabetes compared to the negative and positive control groups, and these results are consistent with many other studies [19, 20]. The study results demonstrated an increase in the glycated hemoglobin (HbA1c) percentage in the group of rats with STZ-induced diabetes compared to the negative and positive control groups. These results are consistent with many other studies [19, 21]. This increase may be due to high blood glucose concentrations resulting from damage in beta cells or insulin resistance (IR). Insulin resistance can progress due to the impairment of B-cells. In other words, a reduction in the mass of beta cells and an accumulation of amylin occur due to glucose toxicity, leading to a deficiency in the insulin hormone. This, in turn, results in a rise in blood glucose levels and an increase in the HbA1c percentage. Consequently, the amount of glucose taken up by the cells decreases, and the production of glucose in the liver through glycogenolysis increases, leading to blood hyperglycemia [22].

STZ destroys most of the B-cells in the pancreatic Langerhans islets, as a result, the secreted insulin is no longer sufficient for the body's glucose degradation, instead, leading to stimulate glycogen breakdown and glucose formation. It can cause the development of insulin resistance (IR) and disruption in the functions of cellular insulin receptors; hence, stopping the cells from receiving glucose and, consequently, the processes of glycogen breakdown and glucose formation from non-carbohydrate sources (gluconeogenesis) would be activated.

At the end of the experiment, treatment with the alcoholic extract of pumpkin seeds led to a significant increase in the insulin level in the rat injected with STZ. These results were also accompanied by a significant decrease in the level of fasting blood sugar, HbA1c and HOMA-IR.

Efficacy of pumpkin seeds extract *Cucurbita pepo* L. on oxidation balance

Many causes of health problems are associated with the activities of free radicals and diabetes and the role these radicals play in the emergence of disease complications. In both types of diabetes, an increase in generation of active oxygen species (ROS) and the occurrence of oxidative stress has been found. Oxidative stress is the main player in the development of diabetes [23]. Consequently, it is thought that using physiologically active antioxidants as a kind of treatment is a potential way to stop this from happening, as well as any pathological changes or consequences related to diabetes.

Concerning oxidative balance, the results of the present study are also in line with a recent study that reported that hyperglycemia associated with diabetes produces free radicals via glucose breakdown or non-enzymatic protein degradation. These free radicals induce cell membrane destruction and lipid peroxidation in diabetes [24]. Lipid peroxidation in diabetic rats decreased the activities of several antioxidant enzymes, which are functionally important in removing toxic compounds that arise during the incomplete oxidation process. Our results indicate that hyperglycemia elevates MDA and decreases SOD, GSH, and CAT activity. These effects may be due to increased oxidative stress induced by hyperglycemia via excessive production of reactive oxygen species. Our study results are consistent with a study showing that increased ROS levels in diabetic animals

resulted in decreased SOD and GSH levels and increased MDA levels [25], confirming that STZ injection generated oxidative stress. A decrease in the activity of these antioxidants could increase hydrogen peroxide and superoxide anion in biological systems, thus generating hydroxyl radicals leading to lipid peroxidation. The increase in MDA level in the groups treated with STZ is due to its effect on stimulating the enzyme Fatty-acetyl-CoA, which is an essential part of the metabolic processes and oxidation of fatty acids, which leads to an increase in endogenous-origin hydrogen peroxide H_2O_2, which contributes to lipid peroxidation [26]. Recent studies have reported that STZ poisoning causes an increase in lipid peroxidation and loss of Membrane integrity which may be a risk factor for altered lipid metabolism and raised blood lipids [27, 28].

Many pathological conditions caused by oxidative stress, including diabetes, increase the activity of free radicals to an extent that exceeds the ability of antioxidants to scavenge them or remove their products, causing increased lipid peroxidation, increased MDA levels, and loss of cell membrane flexibility [29]. A study exhibited a connection between metabolic control and free radicals in diabetic people. Researchers found that cellular damage in diabetes is associated with an imbalance between the activity of free radicals and antioxidants. As a result, the issues arising from diabetes, particularly the production of free radicals, were considered. Scholars discovered that free radical activities are the cause of most complications of diabetes [30]. Our study's findings suggest that when more hydrogen $H2O_2$ is absorbed into the bloodstream, more oxygen is produced and the tissues experience hyperoxia, or elevated oxygen pressure, which in turn promotes the synthesis of active oxygen molecules.

The increase in the H_2O_2 concentration raises the glutathione level in its oxidized form (disulfide GSSG), which decreases the effectiveness of the antioxidant glutathione peroxidase. The protection, provided by glutathione to cells and tissues against lipid peroxidation, depends on the reconversion of oxidized glutathione GSSG to unoxidized glutathione. This process depends on the enzyme glutathione reductase (GSH-rd) and requires the presence of nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor [31]. Severe oxidative stress was caused in STZ-induced diabetic rats by high MDA levels along with low SOD, CAT, and GSH levels. The augmentation in oxidative stress seen in diabetic rats is due to increased generation of ROS as a result of metabolic disturbances. [32].

The study showed a significant decrease in the concentration of MDA in diabetic rats treated with the alcoholic extract of pumpkin seeds compared to the positive control group. The results of our study are consistent with a study that confirmed that pumpkin seeds have an essential role in reducing oxidative stress caused by tramadol in rats [33]. This effect may be due to the active substances in pumpkin seeds, including phenolic compounds, which act as natural antioxidants and thus play an essential role in reducing oxidative stress and enhancing the activity of antioxidant enzymes that scavenge free radicals and reduce their activity. In STZ-injected rats, treatment with pumpkin seed extract dramatically reduced oxidative stress (H_2O_2 and MDA) and raised antioxidant levels, suggesting the anti-peroxide action of the pumpkin extract under investigation. These findings demonstrate that, in rats with induced diabetes, pumpkin seed extract improves antioxidant status and lowers lipid peroxide; additionally, pumpkin seed extract has a potent antioxidant capacity that shields cells from oxidative stress. The decrease in glutathione levels may be attributed to the increase in oxidative stress in rats with induced diabetes, which leads to the generation of free radicals and then raises the rate at which the non-enzymatic antioxidant glutathione is consumed, the most important in removing free radicals and their products; as it converted to its second inactive form, glutathione disulfide. The correlation between decreased glutathione and increased Malondialdehyde levels in diabetic rats may be due to the stimulation of fatty acyl-

CoA oxidase and the initiation of fatty acid oxidation, which leads to increased endogenous H_2O_2 production, which contributes to the production of lipid peroxidation and altering the permeability of cell membranes, causing endothelial damage through its participation in the oxidative modifications of low-density lipoproteins.

The increase in antioxidant levels in single treatments or combined with Streptozotocin in our present study may be due to the impact of the alcoholic pumpkin seed extract, containing high levels of active compounds, all with strong antioxidant properties [34]. The results of our study showed an increase in the enzymes GSH, SOD, and CAT in the second group compared to the control group, in addition to the fourth group compared to the third group. Also, a-tocopherol (vitamin E) found in pumpkin seed oil improves the activity of antioxidants in an ideal way [35]. It may be due to the microelements content in pumpkin seeds, including selenium, which has a dealing role as an antioxidant, as it is one of the essential elements on which the enzyme glutathione peroxidase (GSH-Px) depends since a deficiency in its concentration leads to diseases resulting from free radicals [36]. The reason may be attributed to the relationship between selenium and vitamin E (the main components in pumpkin seeds) and that they complement each other as they work synergistically, inhibiting the effect of free radicals in fatty acids [17, 37].

Effect of pumpkin seeds extract *Cucurbita pepo* L. on liver functions of male rats with induced diabetes:

The liver, in general, is a major organ of detoxification and the primary site of metabolism. It is subject to various disturbances due to exposure to toxins. When liver cells are damaged, cellular enzymes leak into the bloodstream, which can be measured through blood tests for the main liver enzymes ALT and AST. The simultaneous elevation of their levels in the blood indicates a high probability of liver damage [38]. As a critical clinical indicator of liver function, measuring the levels of liver enzymes in the blood serum is essential for evaluating the condition of hepatocytes. When the cell membrane of liver cells breaks down, it can lead to the release of liver enzymes into the bloodstream.

The results of the study showed a rise in activity of liver function enzymes (ALP, AST, and ALT) (Table 2). These results were consistent with those obtained by [39, 40], Thus the enhanced production of free radicals brought on by STZ injections is responsible for the rise in the levels of the liver enzymes under investigation. As the free radicals attack the plasma membranes of liver cells, damaging them, and consequently leakage of these enzymes into the blood [41]. The increase of liver enzymes in the group where diabetes was induced by intraperitoneal injection of STZ is an indicator of the enzyme leakage from the liver cells into the bloodstream as a result of necrosis of those cells, which was confirmed by the results of our study on the histological aspect, (Fig. 6). The insulin deficiency resulting from the destruction of beta cells due to STZ injection causes additional changes in the liver of infected animals due to the increase in oxidative stress resulting from the accumulation of free radicals. This leads to the collapse of liver cells and the accumulation of lipid peroxide in the cell membrane or mitochondrial membranes, weakens the venous flow at the level of the hepatic vein or inferior vena cava, expands sinusitis, and forms inflammatory areas [42].

The increase in the activity of the AST enzyme may be due to the enlargement of the liver cells and the stimulation of the endoplasmic reticulum to produce an enormous amount of the enzyme to match the size of the cell [43]. The aspartate transferase enzyme (AST) is one of the enzymes necessary for biological processes, since it is three times more abundant in the liver than it is in the heart or kidney. A rise in its concentration outside of the liver, particularly in the blood serum, suggests tissue injury or stems from the breakdown of liver tissue as a result of a malfunctioning material metabolism or a reduction in the flow of bile secretion. [44].

The results also indicate a significant decrease in the AST enzyme activity in the group of diabetic rats treated with alcoholic pumpkin seed extract. This result is consistent with the finding of [35]. The reason for this decrease in enzyme activity may be due to the active components found in pumpkin seeds, which act as natural antioxidants such as flavonoids and phenolic acids that analyze and scavenge free radicals and prevent the destruction of cell membranes through oxidation, thus preserving liver tissue from damage and preventing the exit of enzymes into the bloodstream. Our study on the histological aspect confirmed this, as shown in Fig. (7).

References

1. Antoniou, M., et al., *A systematic review of methodologies used in models of the treatment of diabetes mellitus.* Pharmacoeconomics, 2024. **42**(1): p. 19-40.
2. Fattahi Ardakani, M., et al., *Consumption of Medicinal Plants among Patients with Diabetes in Eastern Mediterranean Regional Office Countries: A Systematic Review and Meta-Analysis.* Journal of Nutrition and Food Security, 2024. **9**(1): p. 116-131.
3. R Dabdoub, B., R. Hazim Mohammed, and H. L Abdulhadi, *GANODERMA LUCIDUMATTENUATESANDPREVENTS CCL4-INDUCED HEPATICANDRENALDAMAGE IN SPRAGUE–DAWLEYRATS.* Systematic Reviews in Pharmacy, 2020. **Vol 11** (Issue 12): p.:1704-1709.
4. Abdulhadi, H.L., et al., *Punicalagin protects against the development of pancreatic injury and insulitis in rats with induced T1DM by reducing inflammation and oxidative stress.* Molecular and Cellular Biochemistry, 2022. **477**(12): p. 2817-2828.
5. Chalapathi Rao, N. and R.K. Srivastava, *Petrology and geochemistry of diamondiferous Mesoproterozoic kimberlites from Wajrakarur kimberlite field, Eastern Dharwar craton, southern India: genesis and constraints on mantle source regions.* Contributions to Mineralogy and Petrology, 2009. **157**: p. 245-265.
6. Sharma, A., et al., *Antidiabetic and antihyperlipidemic activity of Cucurbita maxima Duchense (pumpkin) seeds on streptozotocin induced diabetic rats.* Journal of Pharmacognosy and Phytochemistry, 2013. **1**(6): p. 108-116.
7. Suvarna, K.S., C. Layton, and J.D. Bancroft, *Bancroft's theory and practice of histological techniques*. 2018: Elsevier health sciences.
8. Abo Bakr, H.G., et al., *The impact of Pumpkin treatment on TCDD-induced nephrotoxicity in Wister albino rats: biochemical and histopathological studies.* SVU-International Journal of Veterinary Sciences, 2024. **7**(1): p. 71-85.
9. Özsobacı, N.P., et al., *Effect of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker on oxidative stress and metabolism of elements in kidney of STZ-induced diabetic rats.* Archives of Biochemistry and Biophysics, 2024. **751**: p. 109851.
10. Yang, Z., et al., *Study on the mechanisms by which pumpkin polysaccharides regulate abnormal glucose and lipid metabolism in diabetic mice under oxidative stress.* International Journal of Biological Macromolecules, 2024. **270**: p. 132249.
11. Vinayashree, S., et al., *In Vitro Studies of Pumpkin (Cucurbita moschata var. Kashi Harit) Seed Protein Fraction (s) to Evaluate Anticancer and Antidiabetic Properties.* Plant Foods for Human Nutrition, 2024. **79**(3): p. 632-640.
12. Friedman, J.G., et al., *Primary care diabetes assessment when HbA1c and other measures of glycemia disagree.* Primary Care Diabetes, 2024. **18**(2): p. 151-156.

13. Malaba, J., P. Kosiyo, and B. Guyah, *Haemoglobin types and variant interference with HbA1c and its association with uncontrolled HbA1c in type 2 diabetes mellitus.* 2024.
14. Mukherjee, S., et al., *Clinical Risk Assessment and Comparison of Bias between Laboratory Methods for Estimation of HbA1c for Glycated Hemoglobin in Hyperglycemic Patients.* Current Diabetes Reviews, 2024. **20**(7): p. 62-70.
15. Abed, H.M., L.K. Hassan, and H.K.B. Abd Razak, *Identification the Effective Compounds of Pumpkin Seeds and its Possibility of Application in Some Food Products.* Ibn AL-Haitham Journal For Pure and Applied Sciences, 2024. **37**(2): p. 314-326.
16. Wal, A., et al., *Pumpkin Seeds (Cucurbita spp.) as a Nutraceutical Used In Various Lifestyle Disorders.* The Natural Products Journal, 2024. **14**(1): p. 118-137.
17. Yadav, M., et al., *Medicinal and biological potential of pumpkin: an updated review.* Nutrition research reviews, 2010. **23**(2): p. 184-190.
18. Citarrella, R., et al., *Effectiveness of a Food Supplement Based on Glucomannan, D-Chiro-Inositol, Cinnamomum zeylanicum Blume and Inulin in Patients with Metabolic Syndrome.* Nutrients, 2024. **16**(2): p. 249.
19. Hua, J., et al., *Associations of glycosylated hemoglobin, pre-diabetes, and type 2 diabetes with incident lung cancer: A large prospective cohort study.* Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 2024. **18**(2): p. 102968.
20. Akhter, A., et al., *Vitamin D supplementation modulates glycated hemoglobin (HBA1c) in diabetes mellitus.* Archives of Biochemistry and Biophysics, 2024: p. 109911.
21. Chowdhury, J.R., et al., *Glycated hemoglobin (HbA1c) and biofluid-based diagnosis in diabetes: A comprehensive review.* Journal of Electroanalytical Chemistry, 2024: p. 118301.
22. Rishisree, A., et al., *Pomegranate peel, chokeberry leaves and Ironwort extract as novel natural inhibitors of amylin aggregation and cellular toxicity in pancreatic β cells.* Biophysical Chemistry, 2024. **304**: p. 107130.
23. Rais, N., et al., *Oxidative Stress and Diabetes Mellitus: Unravelling the Intricate Connection: A Comprehensive Review.* Journal of Pharmaceutical Research International, 2024. **36**(1): p. 13-30.
24. Ahmad, S. and F.A. Yousuf, *Role of free radicals in normal human physiology*, in *Fundamental Principles of Oxidative Stress in Metabolism and Reproduction*. 2024, Elsevier. p. 17-41.
25. Ilkhomovich, A.N., *The Role of Diabetes Mellitus in the Occurrence and Development of Endothelial Dysfunction.* Journal of Intellectual Property and Human Rights, 2024. **3**(3): p. 129-136.
26. Basha, M.P. and N. Sujitha, *Chronic fluoride toxicity and myocardial damage: antioxidant offered protection in second generation rats.* Toxicology international, 2011. **18**(2): p. 99.
27. Bakir, S.M., et al., *Anti-diabetic and ant-ioxidant of sage tea, Salvia officinalis in male rats exposed to hydrogen peroxide as a source of reactive oxygen species ROS.* Caspian Journal of Environmental Sciences, 2024. **22**(2): p. 453-457.
28. Li, Y., et al., *Extracellular Vesicles Derived from H2O2-Stimulated Adipose-Derived Stem Cells Alleviate Senescence in Diabetic Bone Marrow Mesenchymal Stem Cells and Restore Their Osteogenic Capacity.* Drug Design, Development and Therapy, 2024: p. 2103-2124.
29. Sun, M., et al., *Malondialdehyde and Zinc May Relate to Severity of Microvascular Complications in Diabetes: A Preliminary Study on Older Adults with Type 2 Diabetes Mellitus in Northeast China.* Clinical Interventions in Aging, 2024: p. 1141-1151.

30. Anitha, K., et al., *Association of oxidative stress and diabetes mellitus*, in *Biochemical Immunology of Diabetes and Associated Complications*. 2024, Elsevier. p. 59-73.
31. Liu, X., et al., *Structure of NADPH oxidase from human phagocytes in the activated state.* Nature, 2024. **627**(8002): p. 189-195.
32. Girgih, A., et al., *Free Radicals and Antioxidant Quenching Properties of Plant Phytochemicals in the Management of Oxidative Stress*, in *Plant Food Phytochemicals and Bioactive Compounds in Nutrition and Health*. 2024, CRC Press. p. 202-240.
33. Ekpono, E.U., et al., *Ameliorative Potential of Pumpkin Seed Oil (Cucurbita pepo L.) Against Tramadol-Induced Oxidative Stress.* Dose-Response, 2024. **22**(1): p. 15593258241226913.
34. Hu, Z., et al., *Pumpkin seed oil: a comprehensive review of extraction methods, nutritional constituents, and health benefits.* Journal of the Science of Food and Agriculture, 2024. **104**(2): p. 572-582.
35. Šamec, D., et al., *The potential of pumpkin seed oil as a functional food—A comprehensive review of chemical composition, health benefits, and safety.* Comprehensive reviews in food science and food safety, 2022. **21**(5): p. 4422-4446.
36. Monica, S.J., et al., *Chemical composition of pumpkin (Cucurbita maxima) seeds and its supplemental effect on Indian women with metabolic syndrome.* Arabian Journal of Chemistry, 2022. **15**(8): p. 103985.
37. Kaur, S., et al., *Functional and nutraceutical properties of pumpkin–a review.* Nutrition & Food Science, 2020. **50**(2): p. 384-401.
38. Awad, A.M., et al., *Ameliorative effect of montelukast against STZ induced diabetic nephropathy: targeting HMGB1, TLR4, NF-κB, NLRP3 inflammasome, and autophagy pathways.* Inflammopharmacology, 2024. **32**(1): p. 495-508.
39. Khaled, M., et al., *Apigenin ameliorates genitourinary dysfunction in a type 1 diabetic rat model via Drp1 modulation.* Scientific Reports, 2024. **14**(1): p. 5754.
40. Grucka-Mamczar, E., et al., *Impaired renal function in rats with fluoride-induced hyperglycemia after acute sodium fluoride poisoning*, 2005. **38**(1): p. 48-51.
41. Yang, Y., et al., *Association between oxidative balance score and serum uric acid and hyperuricemia: a population-based study from the NHANES (2011–2018).* Frontiers in Endocrinology, 2024. **15**: p. 1414075.
42. Khan, K. and S. Padyana, *Diabetic nephropathy: Ayur-nutri-pharmaco approach.* Food Safety and Health, 2024.
43. Balekundri, A., E.D. Ahire, and R.K. Keservani, *Plant Metabolites and Vegetables for Diabetes Prevention and Treatment*, in *Plant Metabolites and Vegetables as Nutraceuticals*. 2025, Apple Academic Press. p. 333-360.
44. Stannard, L.M., et al., *Multi-endpoint analysis of cadmium chloride-induced genotoxicity shows role for reactive oxygen species and p53 activation in DNA damage induction, cell cycle irregularities, and cell size aberrations.* Mutagenesis, 2024. **39**(1): p. 13-23.