

## Effect of DCI with Pumpkin Seed Alcohol Extract and Metformin on Liver Function and Histological Changes after Type 2 Diabetes Mellitus

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### Abstract

The study aimed to know the content of both the aqueous and alcoholic extracts of DCI and to study the effect of pumpkin seed extract containing the highest percentage of DCI and the well-known medical treatment metformin on the liver in rats with type 2 diabetes. The concentration of pumpkin seed extract was 400 mg/kg and the concentration of metformin was 250 mg/kg. DCI was diagnosed by HPLC for the aqueous and alcoholic extracts. The level of liver enzymes was also measured after sacrificing the rats and withdrawing blood directly from the heart and removing the liver and placing it in a 10% formalin solution for histological study. The results showed that the alcoholic extract contained the highest percentage of DCI compared to the aqueous extract, as well as the superiority of the alcoholic extract of pumpkin seeds at a concentration of 400 mg/kg over metformin. The results of the histological study of the liver indicated the ability of DCI to improve liver functions and protect its tissue from complications of type 2 diabetes and the ability of the alcoholic extract of pumpkin seeds to restore the tissue to its normal state.

**Keywords:** DCI, HPLC, Pumpkin seed, Liver, T2DM, Diabetes

### Introduction

A large number of advantages are associated with herbal antidiabetic medicines compared with pharmaceutical drugs. Easy availability, low toxicity, raw consumption, easy accessibility, cheap cost and few side effects increase the popularity of herbal medicines[1]. Pumpkin seeds contain many biologically active compounds such as flavonoids, phytosterols and squalene, which makes them a raw material for pharmaceutical and food industries[2]. Pumpkin seeds have the ability to lower triglyceride levels, protect the liver, anti-hypercholesterol and anti-type 2 diabetes[3]. Pumpkin seed oil is an important source of phenolic compounds that have attracted significant scientific interest due to their potential health benefits (because they contain functional hydroxyl groups capable of eliminating free radicals and are well suited to

reduce the risk of many oxidative diseases [4]. Pumpkin seeds contain (DCI) known for its anti-diabetic properties [5]. DCI reduces liver enzymes AST and ALT and also preserves liver tissue in diabetic rats [6]. DCI also reduces liver fat and enhances adiponectin synthesis, which contributes to improving the metabolism of fat in the liver [7]. Type 2 diabetes is characterized as a complex disease, and it is also described as insulin resistance to glucose by cells such as fat cells and muscle, and the presence of insulin resistance leads to the release of a greater amount of insulin by normal  $\beta$  cells, and decreased insulin compensation may lead to hyperglycemia that causes gradual damage to  $\beta$  cell function and worsening insulin resistance [8]. Insulin resistance is a prominent feature of diabetes Type 2 diabetes and a major determinant of

cardiovascular risk, insulin resistance is characterized by a decreased response of certain tissues, including muscle, liver, and adipose tissue. Insulin plays a critical role in regulating and reducing circulating FFA levels, and this is of great importance as elevated essential fatty acids have been linked to insulin resistance and disturbances of glucose homeostasis [9]. Metabolic disorders leading to obesity, insulin resistance, hyperlipidemia, hypertension, and fatty liver are important risk factors for type 2 diabetes.[14] Many chemicals have been used over the years to induce diabetes in laboratory animals in order to study diabetes or to test various therapeutic approaches, and one of the most widely used chemical drugs is alloxan.[15] It is a cytotoxic glucose analogue.[16] Alloxan has two pathophysiological pathways: it can selectively inhibit glucose-induced insulin secretion by inhibiting glucokinase and it can generate reactive oxygen species (ROS) via cyclic oxidation-reduction reactions, dialuric acid.[13] Metformin (1,1-dimethylbiguanide hydrochloride) is the most widely used glucose-lowering agent and has become the first-line drug for individuals newly diagnosed with type 2 diabetes (T2DM)[14]. At the molecular level, the mechanisms of action appear to vary depending on the dose of metformin used and the duration of treatment. Initial studies have shown that metformin targets liver mitochondria [15]. Hence, the idea of this research came about and aimed to study the content of both the aqueous and alcoholic extracts of DCI and to study the effect of pumpkin seed extract and metformin on the liver.

#### Materials and methods

##### -1 Alcoholic extraction method

The alcoholic and aqueous extracts were prepared according to the method [16]

##### -2 DCI estimation in pumpkin seed extracts

DCI was estimated in aqueous and alcoholic pumpkin seed extracts by HPLC according to the method [17].

##### -2 Nutritional experiment

##### -1-2 Experimental animals

24 mature male Norwegian laboratory rats, 10 weeks old and weighing 220-200 g, were transferred from one of the animal houses in Hilla city to be worked on in the animal house of the College of Science, University of Kufa. The animals were left for two weeks to acclimatize before starting the experiment.

##### -2-2 Animal groups

The 24 animals were divided into 4 groups, 6 animals per group, with similar weights as shown:

-1 The first group: The negative control group was given a physiological saline solution.

-2 The second group: The positive control group was injected with alloxan at a concentration of 90 mg/kg as a single dose and remained untreated with food and water provided normally.

-3 The third group: Injected with alloxan at a concentration of 90 mg/kg and treated with pumpkin seed alcoholic extract at a concentration of 400 mg/kg.

-4 The fourth group: Injected with alloxan at a concentration of 90 mg/kg and treated with metformin treatment at a concentration of 250 mg/kg.

The duration of the experiment was 60 days divided into 30 days of feeding with a fatty feed, then injecting with alloxan and giving the animals the pumpkin seed alcoholic extract and metformin for 30 days via a Stomach tube. At the end of the experiment, body weight was measured, and then the rats were anesthetized

before sacrifice using an anesthetic mixture of 0.5 ml ketamine and 0.1 ml xylazine [18]. Blood samples were collected from the hearts of the rats and placed in a gel tube (biochemical tube) and then the centrifuge device to the blood serum and the serum divided in Eppendorf tubes and the pancreas was placed in (10%) formalin for histological study.

### -3-2 Biological tests

Liver enzymes AST and ALT The enzymatic method was adopted using a dedicated analytical kit from Biomerieux mentioned in [19].

### -4-2 Histological study

Histological sections of the liver were prepared. Where the sacrifice was made and the organs were treated according to [20]

### -5-2 Statistical analysis

The statistical program Gentat V.12.10 was used to analyze the data to study the effect of different treatments on the studied

characteristics according to the completely randomized design (CRD), and the significant differences between the means were compared using Duncan's multiple range test (DMRT).

## Results and discussion

### D-chiro inositol-1 (DCI)

Table (1) shows the percentage of DCI in the aqueous and alcoholic extracts, where the highest percentage in the alcoholic extract was 2.136 mg/100 g, while the lowest percentage in the aqueous extract was 0.547 mg/100 g. Studies have shown that DCI is the most effective substance in treating type 2 diabetes, as it improves insulin resistance and reduces fat deposition in the liver (Cheng et al., 2019). On this basis, the percentage of DCI in pumpkin seed extracts (aqueous and alcoholic) was estimated in this study, and the alcoholic extract was selected in the nutritional experiment.

**Table (1) D-chiro inositol percentages mg/100 g in the alcoholic and aqueous extracts of pumpkin seeds**

Alcoholic extract	Aqueous extract	Component
2.136 mg/100 g	0.547 mg/100 g	D-chiro inositol

### -2 Biological

Figure (2) shows the effect of the alcoholic extract of pumpkin seeds, metformin and DMSO on the liver enzymes AST and ALT. The results of the figure showed a significant increase ( $P < 0.05$ ) in the level of AST and ALT in the positive control group C+, which amounted to 171.3, 65.96 (unit/L) respectively, compared to the negative control

### tests

group C-, which amounted to (115.4, 32.11) (unit/L) respectively. This result is higher in AST and lower in ALT than what was reported in [22], [23], as these studies showed that alloxan causes liver diseases by disrupting metabolic processes and causing liver enlargement or liver cell enlargement. This enlargement stimulates the endoplasmic reticulum to produce a larger amount of

enzymes proportional to the cell size. As a result, these enzymes can leak out of liver cells, highlighting the harmful effects of alloxan on liver tissue, and thus Protein synthesis is impaired, and protein degradation increases, leading to damage to liver cells characterized by poor membrane permeability. When these enzymes escape from the cytoplasm into the bloodstream, their activity levels in serum increase significantly. The results also indicated a significant decrease ( $P<0.05$ ) in the activity of liver enzymes AST and ALT in the groups with type 2 diabetes and treated with pumpkin seed extract at a concentration of 400 mg/kg, which amounted to (117.3, 31.46) (unit/L), respectively, compared with the positive control group, which amounted to (171.3), 65.96) (unit/L), respectively. Liver enzymes decreased significantly with increasing concentration of the alcoholic extract dosed to experimental animals. It has been proven that pumpkin seed extract, especially pumpkin seed oil, leads to a significant reduction in the levels of elevated liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of liver damage [24]. [25] The results showed a significant decrease ( $P<0.05$ ) in the activity of liver enzymes AST and ALT for the T4 group with type 2 diabetes treated with metformin at a dose of 250 mg/kg, which amounted to (132.2, 45.08) (unit/liter) respectively, compared to the positive control group, which amounted to (171.3), 65.96) (unit/liter) respectively, and this result is less than what was reported in [26]. Metformin regulates the metabolism and reduces oxidative stress in the liver, and leads to a reduction in elevated liver enzymes AST and ALT. Metformin also reduces glucose formation in the liver, which leads to a reduction in blood sugar and a reduction in the metabolic burden on the liver [27]. The results showed a significant decrease in the level of liver enzymes AST and ALT in all dosed groups compared to the positive control treatment, which reached 171.3, 65.96 (unit/liter) respectively, where the T3 group, the group treated with the alcoholic extract of pumpkin seeds at a concentration of 400 (mg/dl), significantly outperformed the rest of the groups, where the level of liver enzymes reached (117.3, 31.46) respectively (unit/liter).

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**Table(2) The effect of the alcoholic extract of pumpkin seeds and metformin on liver enzymes**

T2	T1	G+	G-	test
132.2b	117.3a	171.3c	115.4a	AST
45.08b	31.46a	65.96c	32.11a	ALT

significant differences at the probability level ( $P<0.05$ ) according to Duncan's test

-Values are expressed as the arithmetic mean of six replicates

-The presence of different lowercase letters between groups indicates the presence of

-C- represents the negative control treatment, C+ represents the positive control treatment, T1 represents the treatment treated with alcoholic extract of pumpkin seeds 400 mg/kg,

and T2 represents the treatment treated with metformin

#### -2Histological study of the liver

The results of the histological study of the negative control group G- indicated normal liver tissue and the hepatocytes were arranged radially and the nuclei took the normal shape, as the results showed the presence of cells with two nuclei as in Figure (1.)

The results of the histological study of the G+ positive control group infected with diabetes due to alloxan indicated the presence of clear pathological changes, which were evident in the occurrence of clear hepatic steatosis and degeneration in the liver tissue, the dissolution and explosion of the cytoplasm in the liver cells, and severe congestion in the hepatic portal vein with the exudation of inflammatory cells around the blood vessel, with the expansion and disappearance of some hepatic sinusoids and hepatic cells, as in Figure (2), respectively. This result was consistent with [28], as alloxan causes morphological changes in the liver, as a result of oxidative stress ROS and the increase of free radicals with high blood sugar, which leads to the occurrence of oxidative wounds, which leads to the formation of blood clots as well as the expansion of the sinusoids as a result of increased pressure on the hepatic portal vein due to high blood sugar [24], [29]. The results of the histological study in the groups of rats induced with diabetes due to alloxan and

treated with pumpkin seed extract T3 at concentrations of (400) mg/kg indicated that The structure of the liver tissue is normal with no pathological lesion or abnormal structure in the liver tissue. The liver portal area also appears in a normal tissue pattern, as well as the liver sinusoids area and the liver cells have a normal appearance and there is no destruction in the liver tissue as in Figure (3). This result is shown as stated in [24], where it was proven that pumpkin seed oil has antioxidant effects. Eating pumpkin seeds protects the liver, resists changes, and reduces oxidative stress and liver damage [25], [30]. The results of the histological study of the liver tissues of the rats in the group infected with diabetes due to alloxan and treated with metformin T2 showed that the structure of the liver tissue is normal with no pathological lesion or abnormal structure in the liver tissue. The liver portal area also appears in a normal histological pattern, as well as the liver sinusoids area and the hepatic cells have a normal appearance and there is no destruction in the liver tissue as in Figure (4). This result is consistent with Almuttairi, (2023), where he showed that metformin leads to a significant reduction in the elevation of liver enzymes in rats infected with diabetes, which indicates an improvement in liver function. It also reduces oxidative stress by increasing the secretion of antioxidants and reducing lipid oxidation [31.]

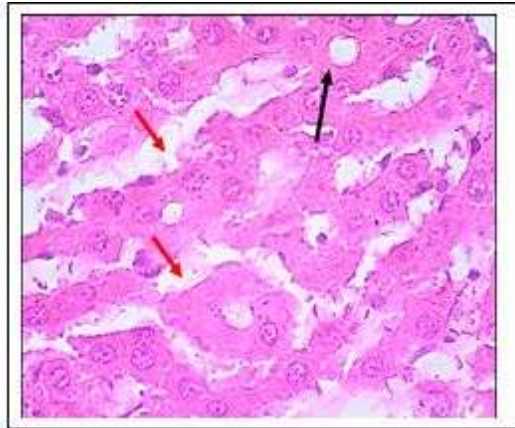


Figure (4) shows a section of the liver tissue of a rat from the metformin T2 group. The hepatic portal region appears in a normal tissue pattern (black arrow), and the hepatic sinusoidal region and hepatic cells have a normal appearance and no destruction in the hepatic tissue (red arrow). The tissue was stained with hematoxylin and eosin, magnification power (40X).

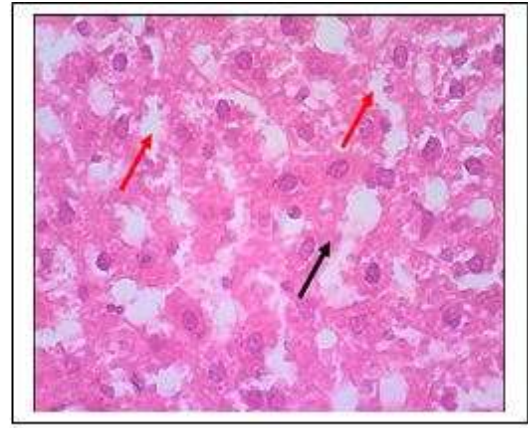


Figure (3) shows a section of the liver tissue of a rat from the T1 group, which was treated with the alcoholic extract of pumpkin seeds at a concentration of 400 mg/kg. The hepatic portal region appears in a normal tissue pattern (black arrow), and the hepatic sinusoids and hepatocytes also appear normal and there is no destruction in the hepatic tissue (red arrow). The tissue was stained with hematoxylin and eosin, magnification power (40X).



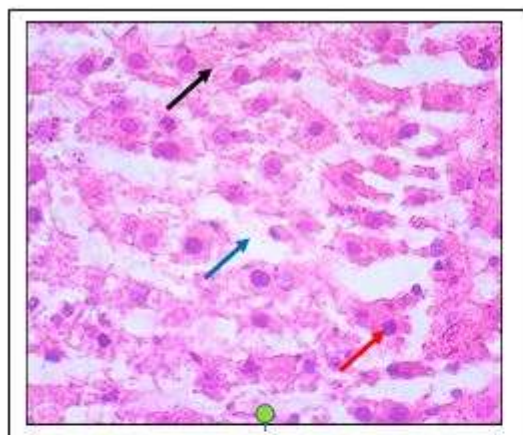


Figure (2) shows a section of the liver tissue of a rat from the G+ positive control group, showing clear rupture in the liver tissue (black arrow) with inflammatory cells exuding around the blood vessel (red indicator) with dilation of the liver sinusoidal spaces and atrophy of the hepatocytes (blue indicator). The tissue was stained with hematoxylin and eosin, magnification power (40X).

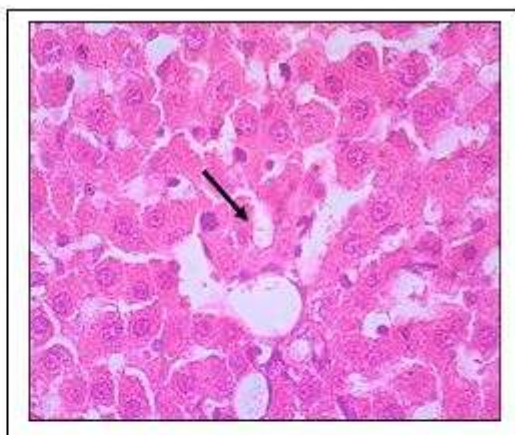


Figure (1) shows a section of the liver tissue of a rat from the G-negative control group (black arrow) indicating the normal liver tissue. The tissue was stained with hematoxylin and eosin, magnification power (40X).

## -5Conclusions

The study concluded that pumpkin seeds contain DCI, which has the ability to alleviate liver complications due to high blood sugar.

Pumpkin seed extract at a concentration of 400 mg/kg outperformed metformin in reducing liver enzymes and improving liver tissue.

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