

## Effects of Cinnamon (*Cinnamomum zeylanicum*) extract on liver damage of diabetic mice

**Khaleel I. Rashid - Ali S. Majeed**

(Al-Farabi University)

**Meriam F. Hameed**

(Al-Nukhba University College)

**Sarah K Yunis**

(Scientific Research Commission)

### Abstract:

This study was conducted in October/2023 in the College of Medicine–Baghdad University laboratories to determine the effects of Cinnamon (*Cinnamomum zeylanicum*) extract on blood glucose levels and liver damage of induced diabetic mice by alloxan. Cinnamon is utilized for its potential anti-diabetic and antioxidant properties which made it a subject of selection in this study which concerning with liver health.

This study results revealed that the treatment with 200 mg/kg of body weight showed significant reduction in blood glucose levels after 3 to 12 days. This reduction revealed that the Cinnamon extract possesses hypoglycemic activity in comparison to the untreated mice (control). The Cinnamon-treated diabetic group of mice showed normal hepatic architecture which was similar to that of untreated group, they have few PAS positive granules, and most of the hepatocytes were studded with PAS positive granules. The cinnamon-treated diabetic group showed that hepatocytes were studded with bluish granules, while those of untreated diabetic group showed fewer bluish granules in the cytoplasm. It was observed the accumulation of lipid droplets in the cytoplasm of hepatocytes of diabetic group. This change was reminiscent to the formation of fatty liver due to the increasing of fatty acids into the liver induced by hypo-insulinemia. It was observed a reduction in glycogen in the liver cells of diabetic group as compared to the Cinnamon-treated group which shows that Cinnamon extract stimulated protein synthesis by increasing the number of ribosomes, and improving the insulin resistance. The results showed that Cinnamon have hypo-protective role in diabetic mice and give promising for further studies.

**Key words:** Cinnamon, Diabetes, Liver, Alloxan.

## Introduction

Cinnamon (*Cinnamomum zeylanicum*) is a genus of evergreen trees and shrubs belonging to the family Lauraceae, it is one of the most important spices used daily by people all over the world. Traditionally cinnamon is used for flavoring food in Asian countries and in preparations a wide range of pharmacological due to the various phytochemical compounds that found in its extracts. Cinnamon has shown beneficial effects in both in vitro and in vivo studies for the treatment of diabetes. It has the potential to inhibit enzymes involved in glucose absorption and increase glucose uptake at the cellular level, leading to improved glycemic control and insulin release. Histological findings suggest that cinnamon extract has a therapeutic and (Yusra et al., 2018; Anas et al., 2019).

Cinnamon extracts have antioxidant properties that can help reduce oxidative stress in the liver (Sanjay et al., 2019). Cinnamic acid in Cinnamon was found for treatment of DNA damage in liver cells of diabetic. Treatment with cinnamic acid (CA) was shown significantly decrease of DNA damage in kidney cells of diabetic rats, indicating a potential protective effect on the kidneys. Cinnamon extracts contain catalpol isolated use to reduce inflammation, which may help prevent kidney damage in diabetes. Cinnamon can help reduce cholesterol and blood sugar levels. (Sanjay et al., 2019).

*Cinnamomum burmannii* is known for its anti-inflammatory and renoprotective properties. Cinnamaldehyde, Cinnamic acid, and Cinnamate, in cinnamon can help alleviate oxidative stress, inflammation, hyperglycemia, gut dysbiosis, which are common complications in CKD (Evi et al., 2020).

Cinnamon aqueous extract has shown protective effects kidney damage by reducing serum markers of kidney function, restoring antioxidant enzyme activity, and reducing apoptosis in renal tissue. (Gehad et al., 2021)

It also works as an effective complementary medicine to normalize the metabolism and clearance processes in diabetes mellitus (Navid et al., 2021). In addition, cinnamon has been shown a protective effect against the toxic effects of ciprofloxacin on the kidney (Moayad et al., 2022).

Diabetes is known to affect various organs, including the liver, kidney, and can lead to complications such as inflammation and fibrosis (Tugrul et al., 2022). Cinnamaldehyde, an active constituent of cinnamon, has been shown to protect against liver damage caused by oxidative stress. Cinnamaldehyde, an active constituent of cinnamon, has been shown to protect against liver damage caused by oxidative stress (Lais et al., 2023).

Cinnamon contains cinnamic acid (CA) which has been found to possess pharmacologic properties such as antioxidant, hepatoprotective, antimalarial, and anti-tyrosinase activities. It is also be used as an anti-diabetic agent and an add-on treatment to control glycemic indices in patients (Meysam et al., 2023).

Cinnamaldehyde, Cinnamic acid, and Cinnamate, in cinnamon can help alleviate oxidative stress, inflammation, hyperglycemia, and dyslipidemia. Mahmoud and others had been reported to possess astringent, stimulant, carminative, blood purifier, digestive, antifungal, antiviral, antibacterial, antioxidant, anti-inflammatory, and immunomodulatory properties (Mahmoud et al., 2023).

The aim of this study is to investigate the effect of cinnamon extract on blood glucose level and its effect on liver health of Alloxan-induced diabetes mice.

## Materials and Methods

The study was conducted in October /2024 in the medicine college- laboratories- Baghdad University in Baghdad.

### Plant material

The bark of cinnamon was obtained from the local markets and authenticated by Biotechnology center- Al Nahrain University.

### **Preparation of Cinnamon extract**

The bark was powdered in an electrical blender then ten grams of the powdered cinnamon was mixed with 100 ml of distilled water and kept in a water bath at 60°C for two hours. The extract was diluted with distilled water (1/10-part cinnamon extract: water) and given orally to mice daily (Adel et al., 2016)

### **Experimental animals and diabetes induction**

Healthy 30 albino mice were obtained from the animal house of the College of Medicine-, Baghdad University. The mice's age was 8 weeks, and 25 gm of weight. The animals were housed in clean metallic cages, sterilized with 70% alcohol. The mice were kept with natural 14 hours light, 10 hours dark, at a temperature (24-28) C°. The mice were fed chow and water. The protocol was proved by (Institutional Animal Ethical Committee. 2021.).

The animals were fasted for 24 hours, then diabetes was induced by alloxan as single intraperitoneal injection at a dose of 150 mg/kg of mice body weight in volume of 0.1 ml. (Muhamed et al., 2016) body weight, and normal mice which were given only saline solution. To confirm the diabetes induction, 3 days after alloxan administration. Blood glucose level was measured using glucometer. The mice having fasting blood glucose level above 200 mg/dl were considered diabetic. (Anas et al., 2019). Blood glucose was measured 310 mg/dl which indicate hyperglycemia (120-140 mg/dl) as standard before treatment, and there was 5% mortality in treated mice with alloxan

### **Experimental groups and blood samples collection**

The animals were divided into three groups (Ten mice per each group), and the groups were treated as following: First group, control, normal mice administrated with 0.1 ml distilled water. Second group, diabetic mice administrated with 0.1 ml of alloxan. Third group, diabetic mice administrated with 0.1 ml of Cinnamon extract (200 mg/kg b.w). For 12 days after alloxan injection, blood samples were collected every three days (0, 3, 6, 9, 12) days from the tail vein of the mice and glucose level was measured using glucometer apparatus.

### **Microscopic investigation**

At the final stage of the experiment, the mice were sacrificed under ether anesthesia and complete liver tissues were obtained. The light microscopic study; the liver tissue was fixed in 10 % formalin then embedded in paraffin. Conduction of histological study; Five um sections were taken and stained with Hematoxylin and Eosin, periodic Acid Schiff and Diastase techniques for the demonstration of glycogen in the liver sections and Methylene blue and Eosin for demonstration of endoplasmic reticulum (Hui, et al., 2017).

### **Results and Discussion**

The aqueous extract of Cinnamon (*Cinnamum selenium*) bark (200 mg/kg) have shown significant reduction in blood glucose levels in normal, alloxan-induced diabetic.

Table (1) showed that the daily treatment with *Cinnamomum zeylanicum* extract of 200 mg/kg b.w. led to a significant reduction in the blood glucose levels after 3, 6, 9 days of the treatment which recorded 186, 155, 131 mg/dl respectively. The effect seems to reach maximum on 12<sup>th</sup> day of the treatment period (122 mg/dl) with *Cinnamomum zeylanicum* extract and then became stable as control treatment. Significant reduction in the blood glucose level was observed as compared to the normal group (118 mg/dl) and diabetic group (321 mg/dl) after 12 days with gradually reduction till reached 121 mg/dl at the end of the test period (12 days). Diabetes can change the number of MCs in the liver tissue. The possible effects of diabetes on the histochemical and morphological differences of MCs in liver (Tugrul et al., 2022).

The results showed that cinnamon (*Cinnamomum zeylanicum*) extract significantly lowered blood glucose levels, with the effective dose (200 mg/kg). These results showed that *Cinnamomum zeylanicum* extract have hyperglycemic activity as compared to the control treatment. The effect of the insulin-like protein and the active compounds such as flavonoids that found in *C. zeylanicum* on glucose levels in the serum of diabetic mice caused a significant decrease in blood glucose levels in diabetic mice similar

to that reported on the hypoglycemic activity of *C. zeylanicum* by Meysam et al.(2023) who reported that it also possesses cytoprotective, antigenotoxic, and antioxidant effects and can be used as an anti-diabetic agent and added to control glycemic indices in patients.

The hypoglycemic activity in this study may be related to the presence of flavonoids compounds which had very pronounced effect in the bark extract of this plant. Flavonoids may preserve  $\beta$ -cell function by reducing oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes.

**Table (1): Effect of Cinnamon (*Cinnamomum zeylanicum*) extract on reducing blood glucose levels of white albino mice after different periods of time**

Group/treat	Dose	0 days	3 days	6 days	9 days	12 days
Normal mice	0.1 ml distilled H <sub>2</sub> O	121	122	118	120	116
Induce diabetic mice (Alloxan)	0.1 ml (150 mg/kg)	311	316	309	314	321
Diabetic mice cinnamon extract	0.1 ml (200 mg/kg)	287	186	155	131	122

Pairwise Comparisons				
Dependent Variable: Data				
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Sig.	C.S.
Normal	Induced (Alloxan)	-192.2	0.00	HS
	Cinnamon extract	-52.2	0.041	S
Induced (Alloxan)	Cinnamon extract	142.0	0.00	HS

Based on estimated marginal means

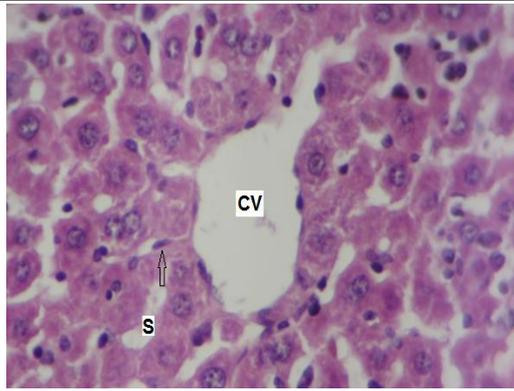
\*The mean difference is significant at the .05 level.

Adjustment for multiple comparisons: Least Significant Difference

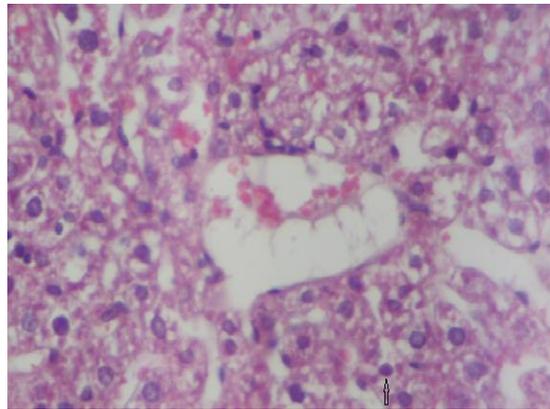
Hematoxylin and Eosin (H and E) stained sections examination of control group revealed the normal architecture of the classic hepatic lobules. The hepatocytes form branching and anastomosing cords radiating from the central vein. Vesicular nuclei appeared and some of them seen binucleated. The cells appeared to be separated by the blood sinusoids that were seen to be lined by flat endothelial cells (Fig. H and E-stained sections of the untreated diabetic group revealed degenerative changes in the hepatocytes. Cells all over the hepatic lobules were observed to have many vacuoles as foamy appearance and some of them showed pyknotic nuclei (fig. 2). While liver sections of this group revealed sinusoidal dilations and hyperemia in sinusoids and central veins (Fig. 3).

The Cinnamon-treated diabetic group showed normal hepatic architecture which was almost similar to that of the control group (Fig. 4). PAS-stained sections of the control group showed PAS positive granules in most of the hepatocytes (Fig. 5). While those of untreated diabetic group showed few PAS positive granules as compared to that of the control group (Fig. 6). The cinnamon-treated diabetic group showed that most of the hepatocytes were studded with PAS positive granules (Fig. 7).

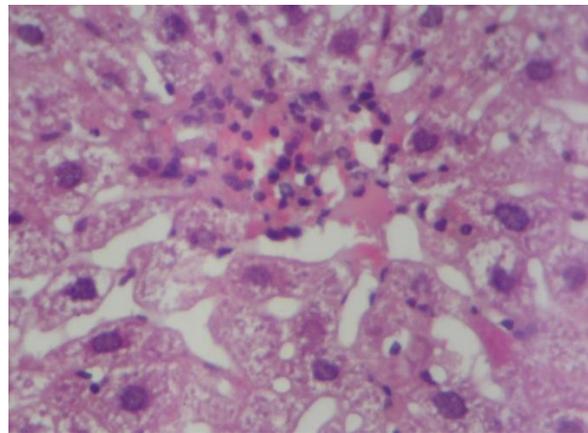
Liver sections of control group stained with methylene blue and eosin showed that hepatocytes contained bluish granules in their cytoplasm (Fig. 8) while those of untreated diabetic group showed fewer bluish granules in their cytoplasm (Fig.9). The Cinnamon-treated diabetic group showed that hepatocytes were studded with bluish granules (Fig.10).



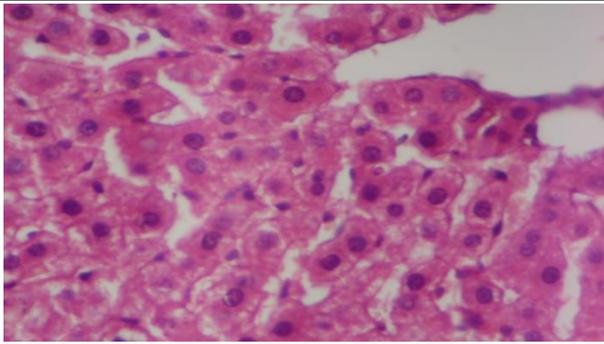
**Figure (1):**H and E-stained sections of control group normal architecture



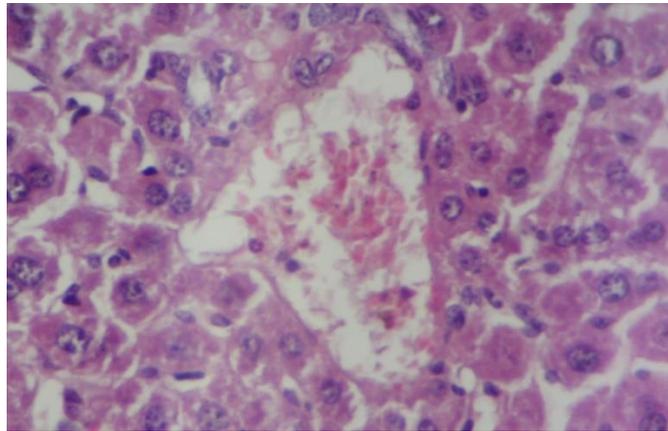
**Figure (2):** H and e-stained sections of untreated diabetic group degenerative changes in the hepatocytes



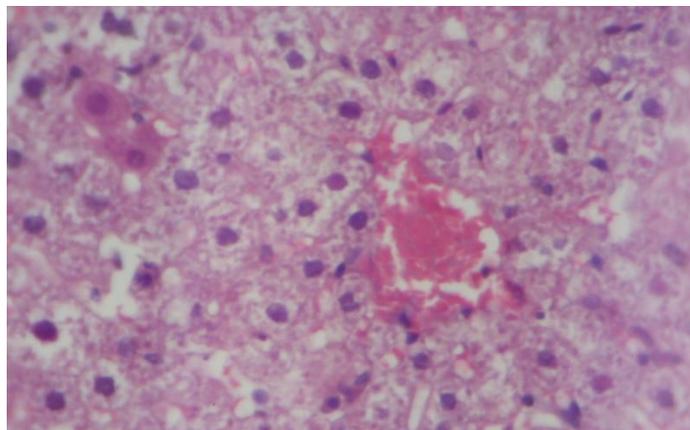
**Figure (3):** H and E stained of liver sections of untreated group



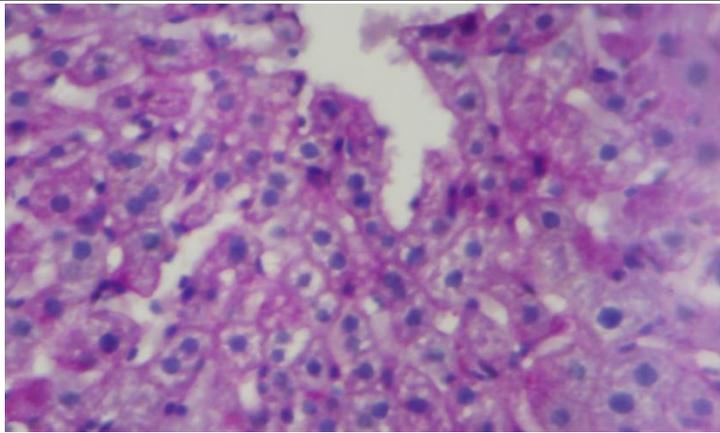
**Figure (4): H and E cinnamon treated diabetic group normal hepatic architecture similar to the control group**



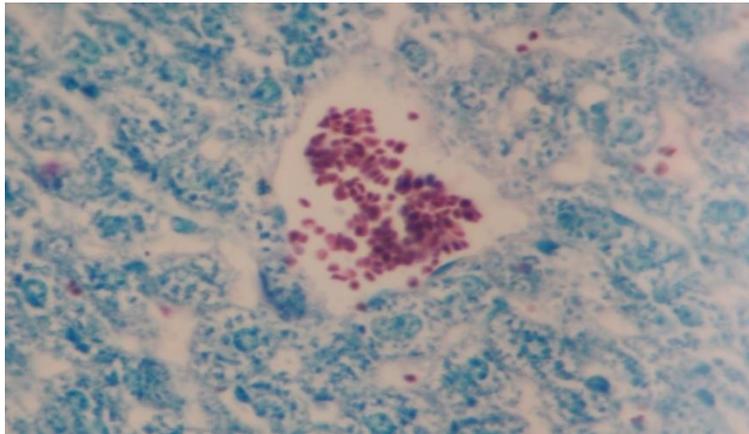
**Figure (5): PAS-stained sections of the control group, PAS positive Granules**



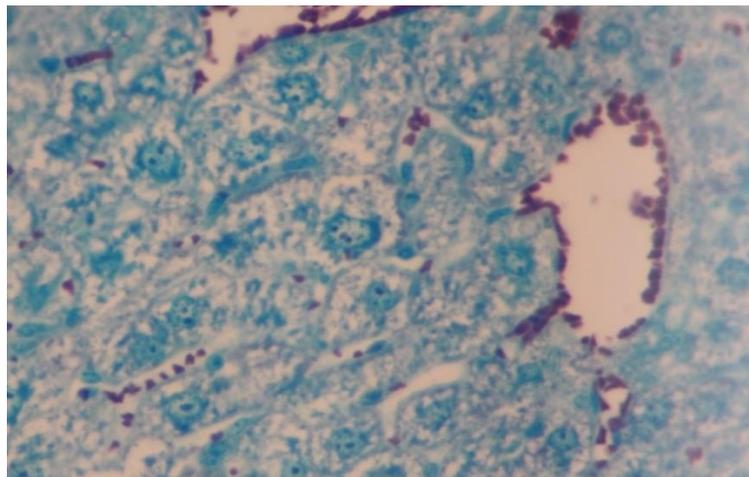
**Figure (6): Untreated diabetic group, few PAS positive granules**



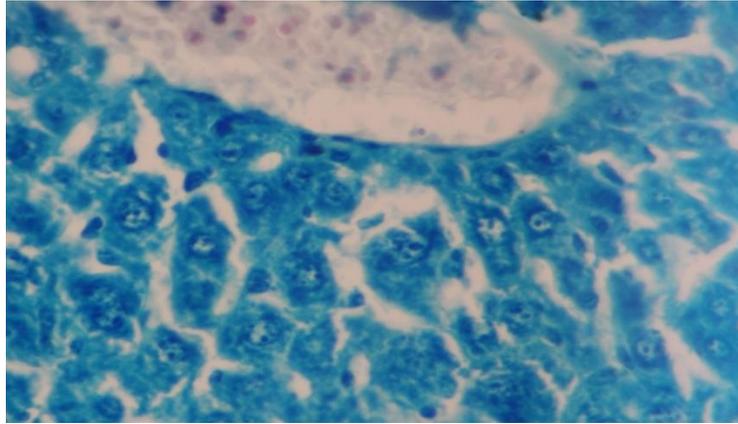
**Figure (7): Cinnamon treated diabetic group, hepatocytes studded with PAS positive granules**



**Figure (8): Liver section of control group M, E, contain bluish granules in their cytoplasm**



**Figure (9): Untreated diabetic group, few bluish granules in the cytoplasm**



**Figure (10): Cinnamon treated diabetic group hepatocytes studded with bluish granules**

It was observed that accumulation of lipid droplets in the cytoplasm of hepatocytes of diabetic group. This change was reminiscent to the formation of fatty liver. It could be due to the increased influx of fatty acids into the liver induced by hypo-insulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apolipoprotein B synthesis. Hyperlipidemia could be another factor for fatty liver formation. Our findings of fatty liver formation are in agreement with the findings of (Sazan, M. and Talaat., 2023).

The present study showed few PAS positive granules in the untreated diabetic group indicating a decrease in the glycogen content in the liver. These results were similar to a previous study which showed a marked decrease of glycogen granules in the diabetic mice (Elizabeth, H. et al.2019).

In diabetes as the activities of glycogen synthase and hexokinase were diminished as a result of insulin deficiency, glucose cannot be transformed into glycogen and glycogenesis was reduced and thus the amount of glucose increased (Soonham, S. et al., 2010).

Insulin is considered as an anabolic hormone with a wide range of effects on metabolisms including stimulation of protein synthesis (Mohammed, M. and Mutassina, M. 2016).

It was demonstrated that hepatocytes of diabetic mice exhibited well defined rough endoplasmic reticulum with dilatation of their cisternae and swollen mitochondria with loss of its cristae (Fang, F. et al., 2019).

Therefore, in this study, hepatocytes of diabetic group demonstrated fewer bluish granules than in normal group which indicated that ribosomes were decreased in hepatocytes of this group and consequently, protein synthesis was diminished. In diabetic group treated with Cinnamon extract, hepatocytes were studded with bluish granules which indicated that Cinnamon extract stimulated protein synthesis by increasing the number of ribosomes.

In conclusion, the present results showed that cinnamon extract consumption reversed most of the histological changes in the liver of the diabetic mice. This effect was due to the hypoglycemic effect of the Cinnamon and improving the insulin resistance. In addition, in diabetes there was an increase in the oxidative stress which was significantly reduced by Cinnamon consumption owing to its antioxidant effect. So, can say that Cinnamon had a significant hepatoprotective role in diabetic mice and offers promising perspectives deserve further investigation.

## References:

- Adel**, Shalaby., Abd, El-Rahman, M., Abd, El-Mottaleb., Ahmad, Mohammad, Farag, Al-Kot. (2016). Comparative study to ginseng and cinnamon water extract on diabetic adult male albino rat. *al-azhar medical journal*, 45(3):527-544. doi: 10.12816/0033121
- Anas**, Sarwar, Qureshi., Junaid, Ghaffor., Muhammad, Usman., Nazia, Ehsan., Zaima, Umar., Adeel, Sarfraz. (2019). Effect of ethanolic preparations of cinnamon (*Cinnamomum zeylanicum*) extract on hematologic and histometric parameters of selected organs in Alloxan® induced diabetic female albino rats. *Journal of diabetes and metabolic disorders*, 18(2):505-512. doi: 10.1007/S40200-019-00457-4
- Elizabeth**, H, et al., (2019). Diabetes causes marked inhibition of mitochondrial metabolism in pancreatic B-cell. *Nature Communication*.
- Evi**, Lusiana., Nia, Savitri, Tamzil., Desi, Oktarina. (2020). The Efficacy of Cinnamon Extract (*Cinnamomum burmannii* ) on Reducing Staging Acute
- Gehad**, E., Elshopakey., Sara, T., Elazab. (2021). Cinnamon Aqueous Extract Attenuates Diclofenac Sodium and Oxytetracycline Mediated Hepato-Renal Toxicity and Modulates Oxidative Stress, Cell Apoptosis, and Inflammation in Male Albino Rats. *Veterinary Sciences*, 8(1):9-. doi: 10.3390/VETSCI8010009
- Fang**. Fang, Moi. et al. (2019). Protective mechanism of punicalagin against endoplasmic reticulum stress in the liver of mice with type 2 diabetes mellitus. *Journal of Functional foods*, Vol. 56, pp 57-64.
- Hatice**, Gül, Anlar., Merve, Bacanlı., Tuğbagül, Çal., Sevtap, Aydın., Nuray, Ari., Ülkü, Ündeğer, Bucurgat., A., Ahmet, Başaran., Ayşe, Nurşen, Başaran. (2018). Effects of cinnamic acid on complications of diabetes. *Turkish Journal of Medical Sciences*, 48(1):168-177. doi: 10.3906/SAG-1708-8
- Hui**, H. et al., (2017). Periodic acid Schiff staining method for function detection of liver cells. <http://doi.org/10.3892>.
- Laís, de, Souza, Gouveia, Moreira., Isabela, Brum., Drielly, C., M., de, Vargas, Reis., Liana, Trugilho., Tuany, R., Chermut., Marta, Esgalhado., Ludmila, F, M, F, Cardozo., Peter, Stenvinkel., Paul, G., Shiels., Denise, Mafra. (2023). Cinnamon: an aromatic condiment applicable to chronic kidney disease. *Kidney research and clinical practice*, doi: 10.23876/j.krcp.22.111
- Mahmoud**, E. et al., (2023). Curcumin and Cinnamon mitigate lead acetate-induced oxidative damage in the spleen of rats. *Phrontiers in Pharmacology*. 13 doi:10.3389/phar.2022-1072760.
- Meysam**, Zarezadeh., Vali, Musazadeh., Elaheh, Foroumandi., Majid, Keramati., Alireza, Ostadrahimi., Rania, A., Mekary. (2023). The effect of cinnamon supplementation on glycemic control in patients with type 2 diabetes or with polycystic ovary syndrome: an umbrella meta-analysis on interventional meta-analyses. *Diabetology & Metabolic Syndrome*, 15(1) doi: 10.1186/s13098-023-01057-2
- Moayad**, Mijbil, Ubaid., Shatha, Hussein, Kadhim., Zahraa, Abed, al-kareem. (2022). Protective effect of cinnamon oil against ciprofloxacin toxicity on liver and kidney of male Wistar rats. *Journal of Applied and Natural Science*, doi: 10.31018/jans. v14i4.3823
- Mohammed, M. and Mutassina, M. (2016). Role of insulin and other related hormones in energy metabolism. *Cogent food and Agriculture*. Vol. 2, issue 1.

**Navid**, Neyshaburinezhad., Mohammad-Reza, Rouini., Hoda, Lavasani., Yalda, H., Ardakani. (2021). Evaluation of Cinnamon (*Cinnamomum Verum*) Effects on Liver CYP450 2D1 Activity and Hepatic Clearance in Diabetic Rats. *Jundishapur Journal of Natural Pharmaceutical Products*, 16(1) doi: 10.5812/JJNPP.101797 Role of insuline and other related hormones in energy metabolism. *Cogent food and*

**Sanjay**, Kumar., Reshma, Kumari., SR, Mishra. (2019). Pharmacological properties and their medicinal uses of *Cinnamomum*: a review. *Journal of Pharmacy and Pharmacology*, 71(12):1735-1761. doi: 10.1111/JPHP.13173

**Sazan**, M., Talaat. (2023). Role of Cinnamon Supplementation on Glycemic Markers, Lipid Profile and Weight Status in Patients with Type II Diabetes. *ARO. The Scientific Journal of Koya University*, 11(1):1-9. doi: 10.14500/aro.11041

**Soonham**, S., Yaghmoor., Samir, M., Khoja. (2010). Effect of cinnamon on plasma glucose concentration and the regulation of 6-phosphofructo-1-kinase activity from the liver and small intestine of streptozotocin induced diabetic rats. *Journal of Biological Sciences*, 10(8):761-766. doi: 10.3923/JBS.2010.761.766

**Tugrul**, Y., Ertugrul., Nazife, ÜLKER, ERTUĞRUL. (2022). Effect of Cinnamon on Distribution and Amount of Mast Cells in Diabetic Rat Liver. *Turkiye Klinikleri Journal of Veterinary Sciences*, doi: 10.5336/vetsci.2022-92082

**Yusra, Hussain.**, Munawar, Ali., Faizan, Ghani., Muhammad, Imran., Aamira, Hashmi., Wajahat, Hussain., Muhammad, Hashim, Raza. (2018). Effects of Cinnamon on Diabetes. *Advances in Science, Technology and Engineering Systems Journal*, 3(3):57-60. doi: 10.25046/AJ030308