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Physiological and Histological Effects of Aqueous Extract of Cinnamon and Frankincense on Albino Rats with Induced Diabetes

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Abstract | One of the major causes of inability in human body to properly use or produce insulin is diabetes mellitus. Cinnamon is a food flavoring produced from the bark of the *Cinnamon cassia* tree. It contains many compounds including cinnamic acid, cinnamon anhydride, tannin, and methyl-hydroxychalcone polymer (MHCP) etc. Frankincense (FRN) is an aromatic resin extracted from tree species belonging the genus Boswellia within the family Burseraceae family. This study was initiated to evaluate the physiological activities of Cinnamon and Frankincense aqueous extracts, which included blood sugar level, B. urea, lipid profiles, TNF-α, LDH, creatinine, ALT, AST, MDA, CAT, and SOD. Thirty albino rats were used in this experiment, divided into three groups(10/group). The first was considered a negative control group, the second was considered a diabetic group without treatment, and the third was a diabetic group treated with the aqueous extract of the of aqueous extract of Cinnamon and Frankincense (250g/kg). A significant increase in both blood sugar levels, cholesterol, triglyceride, LDL, VLDL, Urea, creatinine, ALT, AST, MDA.CAT, TNF-α, LDH and SOD was shown in the diabetic group without treatment. The diabetic group treated with aqueous extract of Cinnamon and Frankincense showed a clear significant decrease in the above values at the end of experiment. Based on these finding, it can be concluded that using the aqueous extract of cinnamon and frankincense can lower the blood sugar level and reduce the negative side effects of diabetes and offer animals as good model to study impact of compounds.

Keywords | Cinnamon, Frankincense, TNF-α, Creatinine

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INTRODUCTION

Humans use plant extracts for various purposes to protect their food and health. They serve to protect plants from insect and other pest infestation and to protect food sources from infection (Nawar et. al., 2024; Jameel, 2023). The failure of pancreatic glands, to produce or proper use insulin by human body is termed to as diabetes mellitus (DM). Characterization of this chronic endocrine disorder can be through a high concentration of blood glucose resulted from an absolute or relative insulin deficiency, associated with insulin resistance (Jimoh *et al.*, 2013; Deepti *et al.*, 2017). DM can be a silent killer affecting millions of people worldwide (Chaudhary and Tyagi, 2018). DM as a common metabolic disease referred to as hyperglycemia as well, may occur from one or both

the insufficient insulin sensitivity and failure of insulin secretion, causing the loss of glucose homeostasis. DM can impact almost every organ system of human body, but the damage level can be mainly related to the disease severity and duration (Pari and Saravanan, 2004; Kareem and Galeel, 2022).

Diabetes mellitus can be grouped into four categories: Diabetes Type-1: Insulin dependent diabetes mellitus or also termed hyperplasia because it is characterized by an absolute insufficiency of insulin. Beta cells are destroyed because of invasion by virus, action of chemical toxins or due to action of autoimmune antibodies (Obaid *et al*., 2020). This beta cell necrosis is causing insulin deficiency and caused Type-1 diabetes (Wang *et al*., 2011).

Diabetes Type-2: Non-insulin dependent diabetes mellitus occurs when target organ insulin resists the boundary responsiveness to both exogenous and endogenous insulin (Bacha *et al*., 2010). Diabetes Type-3: This type is caused by chronic drug treatment with glucocorticoids, growth hormone thiazids and diuretics (Tripathi and Verma, 2014). Diabetes Type- 4: This type of diabetes presented in about 4-5% of total pregnancies, can caused by placental hormones that develop insulin resistance (Tripathi and Verma, 2014).

Most medicinal plants comprise potentially useful chemical compounds that can be utilized as basic materials for the synthesizing contemporary medications (Okigbo *et al*., 2009). Royal jelly also works as an antioxidant in addition to being an anti-diabetic (Salih *et al*., 2021). Cinnamon is a common spice produced from the bark of the *Cinnamon cassiae*, contains many compounds including cinnamic acid, cinnamon anhydride, methyl-hydroxychalcone polymer (MHCP) and tannins. Historically, it has been prescribed as an anti-diabetic spice but results from trials involving cinnamon supplementation were inconstant (Kirkham *et al*., 2009). Studies showed that cinnamon extract could cause a mild effect through minimizing the fasting plasma glucose concentrations in diabetic patients suffering poor control of glycaemia (Mang *et al*., 2006).

Frankincense (FRN) an aromatic resin can be extracted from tree species belonging to the genus Boswellia within the family Burseraceae. Traditionally, *Boswellia sacra* can be discovered and used in numerous geographical regions, possessing a range of pharmacological properties, including curing activities as anti-hyperglycemic, antioxidant and anti-inflammation (Mothana, 2011; Mothana *et al*., 2007). Formulations containing FRN extract can decrease blood sugar in STZ-induced DM (Azemi *et al*., 2012; Shehata *et al*., 2011) and alloxan-induced DM (Kavitha *et al*., 2007). FRN curing activity made it a potential candidate for many studies to investigate this resin as an antioxidant on DM. Thus, the current study was designated to investigate the antioxidant activities of FRN on RBC's against the initiation of DM on experimental rats.

This study was conducted to evaluate the physiological activities of Cinnamon and Frankincense aqueous extracts on blood sugar level and the negative side effects of diabetes.

Experimental design

Albino male rats (n=30) weighing 155-170 grams were selected for treatments and divided into three groups (10 rats/group). G1: negative control, G2: diabetic group without treatment (positive group), G3: diabetic group orally treated with 250 mg/kg of Cinnamon and Frankincense aqueous extracts as a single dose/day for 30 days. The rats were obtained from the Animal House of the Center for Biotechnology at the University of Nahrain (Al-Timimi, 2019; Al-Timimi and Taher, 2020).

Blood collection

Blood samples, collected under anaesthetized conditions, were immediately centrifuged for 10 min, at 3000 rpm (Obaid *et al*., 2020; Obaid, 2021). The resultant serum was used for further analyses including, ALT, AST, MDA, CAT, SOD, creatinine, blood urea, lipid profile, TNF-α, CRP and LDH (Raheem *et al*., 2024).

Lipid profile

Th concentration of serum cholesterol was estimated using a commercial diagnostic kit (Biolab, USA) following the manufacturer`s standard protocol. Spectrophotometry was applied to estimate the OD of the dyed complex. The absorbance values at a wavelength 500 nanometer were measured.

Triglyceride

Triglycerides concentration was estimated using a commercial diagnostic kit supplied by (Biolab, USA) of Fossati. The absorbance values were measured at a wavelength of 500 nm using a spectrophotometer.

High density lipoprotein (HDL)

This test was performed using a commercial diagnostic kit provided by (Biolab, USA). A colorimetric method was performed to measure the final reaction produced at a wavelength of 505 nm using a spectrophotometer (Obaid *et al*., 2025).

Low density lipoprotein (LDL)

It is measured according to the following equation:

Low-density lipoprotein = cholesterol concentration
- (high-density lipoprotein concentration +
high-density lipoprotein concentration)
Very Low-density lipoprotein (VLDL)

It was measured according to the following equation

Concentration of very low-density protein fats = concentration of triglycerides/5

ALT (Rndox/British), AST (Rndox/British), ALP (Biolabo/France). Enzymatic and colorimetric methods (Spinreact/Spain), (Cat) and Biovision-USA kits and protocols were used to measure urea and serum Creatinine in bloods of treated animals.

Diabetes induction

Diabetes in male rats was induced experimentally through a single intraperitoneal (IP) injection of alloxan (Sigma Chemical Co.) freshly dissolved in normal saline at a dose of 150 mg /kg body wt. To prevent hypoglycemia that might be resulted from alloxan week later after injection, experimental animals were let to feed overnight on glucose solution (5%) w/v. For blood glucose determination, Alloxan–treated rats were fasted for 12 hours, and blood samples were collected from the tail venous. Rats in diabetic group suffered blood sugar levels more than 250 milligram/dl were considered diabetic and chosen for additional studies (Salah *et al*., 2020).

RESULTS

Data of the body weight showed a significant increase of the control animals on the last day of the experiment, when the final weight was 179.67 ±3.18 compared to the initial weight of the same group (160.30±3.09 g) (Table 1). In addition, a significant reduction was shown in the final weight of the diabetic categories (140.30±1.63)g compared with the initial weight of (168.22±2.09g) the same group. While a significant increase in the final body weight was shown in the diabetic group treated with the aqueous extract of Cinnamon and Frankincense scoring 167.32±2.35 g compared with the initial weight (157.10 ±3.64 g).

Blood test data

Testing cinnamon and frankincense extracts on Lipids profile

Cholesterol concentration data (Table 2) scored a significant increase at P≤0.05 value in in the group with diabetes and without treatment (209±5.82) mg/dl compared with the control group (100±3.56) mg/dL, while data revealed a significant decrease in glucose levels of the diabetic group exposed to Cinnamon and Frankincense (130±3.09b) mg/dl compared to the group with no treatment.

In contrast, there were significant differences in concentration of triglycerides between the group with diabetes (164±4.30)mg/dl compared to the control group scoring (89.76±3.5)mg/dl, while a significant decrease in triglycerides was noted in the group with diabetes mellitus treated with quercetin at a concentration of (102.32±2.85) mg/dl compared to the group with no treatment (Table 2).

Table 1: The activity of Cinnamon and Frankincense aqueous extracts on blood body weight in rats with induced diabetes.

Group name	Mean ± SE		
	Initial weight/g	Final weight/g	LSD
Control	160.30 ±3.09b	179.27±3.06A	16.32 *
Diabetes	168.22 ±2.09a	140.30±1.63 b	18.52*
Diabetes + moringa aqueous extract	157.10 ±3.64b	167.32±2.35a	6.237 *

Means with the different letters at the same row were differed significantly. * (P≤0.05).

As for high-density lipoprotein (HDL) (the beneficial ones), a significant decrease in the level of these fats was noticed in the group with diabetes (22.43±4.21) mg/dl compared to the group in negative control (30.79±3.65). While data showed a significant decrease in the concentration of good fats in the group treated with Cinnamon and Frankincense extracts (38.42±3.90).

A significant increase in the concentration of low-density lipoprotein (LDL) (bad) was noticed in the diabetic group (151.29 ±5.70) mg/dl compared to the negative control group (88.00±5.36) mg/dl, while the level of these fats reduced in the group that having diabetes and treated by Cinnamon and Frankincense extracts (109.59 ±6.85) mg/dl.

About very low-density lipoprotein (VLDL), data showed a significant variance in the positive control group (35.20 ±3.52) mg/dl compared to the control group (22.43±2.73) mg/dl. and the diabetic group treated with Cinnamon and Frankincense extracts (30.45±2.90)

Table 2: The activity of Cinnamon and Frankincense extracts on the lipid profiles in different treated diabetic rat groups.

Group	Mean ± SE (mg/dl)				
	Cholesterol	Triglyceride	HDL	LDL	VLDL
Negative Control	100±3.56c	89.76±3.54c	30.79±3.65a	53.00±5.36 c	17.54±2.73b
Positive diabetic	209±5.82a	164±4.30a	22.43±4.21b	155.29±5.70a	32.20 ±3.52a
Diabetic +quarectine	130±3.09b	102.32±2.85b	38.42±3.90 a	72.59 ±6.85b	20.45±2.90a
LSD value	14.65**	21.43**	6.21**	12.40**	5.42**

Means with the different letters at the same row were differed significantly. * (P≤0.05).

The results in Table 3 showed significant increase in the untreated diabetic group in the level of glucose (370.43±4.55) mg/dl and diabetic group treated with Cinnamon and Frankincense extracts (364±2.69) compared with the negative control group (90.56±3.05) mg/dl at the first day of experiment. Whilst a decreased level of glucose was noted in the diabetic group treated with Cinnamon and Frankincense extracts (154.43±1.96) mg/dl at the 30th, Compared with the same group on the first day, while the glucose level remained high throughout the treatment period in the group with diabetes without treatment (380±2.85).

Table 3: The glucose level of treated diabetic rats exposed to with Cinnamon and Frankincense extracts on

Group	Mean ± SE	
	Glucose(mg/dl) first day	Glucose(mg/dl) 20th day
Negative Control	90.56±3.05b	85.54±4.02 c
Positive diabetic	370.43±4.55 a	380±2.85a
Diabetic+ quercetin	364±2.69a	154.43±1.96 b
LSD value	9.32 *	7.16*

Means with the different letters at the same row were differed significantly. * (P≤0.05)

The results revealed an obvious significant increase in the untreated diabetic group in creatinine and blood urea levels (1.94 ±0.05, 42.54±3.53) mg/dl, respectively, compared with the negative control group (Table 4).When treated with Cinnamon and Frankincense extracts, the level of creatinine and blood urea decreased in the diabetic group treated scoring 0.95 ±0.06 and 29.50±2.43 mg/dl, respectively compared with the positive control group.

Table 4: Urea and creatinine concentrations in diabetic rats treated exposed to Cinnamon and Frankincense extracts.

Group	Mean ± SE	
	B. Urea (mg/dl)	Creatinine (mg/dl)
Negative Control	19.52±2.08c	0.53 ±0.02 c
Positive diabetic	42.54±3.53 a	1.94 ±0.05a
Diabetic+ quercetin	30.50±2.43b	0.80 ±0.06 b
LSD value	5.10 *	0.12 *

Means with different letters at the same column were differed significantly. * (P≤0.05).

The results shown in Table 5 indicate significant differences CRP level among tested groups. In contrast, a significant increase was observed in the enzymatic activity of the lactate dehydrogenase of rat group suffering diabetes scoring (176.54±3.32) in comparison with the -ve control group (120.1 8±4.3), while the activity of the enzyme decreased in the group of diabetic rats treated with Cinnamon and Frankincense extracts (137.54±3.65). Table 5 also showed a significant increase in the level of TNF-α in the group of diabetic rats (4.90±1.22) in comparison with the negative control group (1.53 ±0.05), while a no significant differences in level of TNF-α was observed between negative control and diabetic group treated with Cinnamon and Frankincense extracts (1.53 ±0.05, 2.05 ±0.39).

Table 5: Estimating the level of TNF-α, CRP, and LDH concentrations in in diabetic rats treated exposed to Cinnamon and Frankincense extracts.

Groups	Mean ± SE		
	TNF-α	CRP	LDH
Negative control	1.53 ±0.05b	2.78±0.89a	120.1 8±4.32c
Positive diabetic	4.90±1.22a	8.05±0.54a	176.54±3.32a
Diabetic + Cinnamon and Frankincense	2.05±0.39b	4.45±0.53a	137.54±3.65B
LSD value	1.45**	0.95**	10.50**

Means having with the different letters in same column differed significantly. * (P≤0.05).

Estimation of the concentrations of malondialdehyde, CAT and SOD enzymes in diabetic groups exposed to Cinnamon and Frankincense

Data revealed in the level of malondialdehyde level increased significantly, in the diabetic group scoring 4.94 ±0.48 mmol/ml compared to those in the control group and diabetic treated with Cinnamon and Frankincense groups, scoring (1.23±0.06) and (2.05±0.39 mmol/ml highest malondialdehyde levels, respectively.

Noticeably, the enzymatic level of catalase increased significantly in diabetic untreated rat group scoring 14.70±2.63. Whereas this enzyme scored (5.53±1.53) and (9.30 ±2.76) maximum levels in the negative control and diabetic group treated with Cinnamon and Frankincense, indicating a significant decrease in the enzyme level.

Table 6: Malondialdehyde, CAT, and SOD enzyme concentrations in experimental rat groups exposed to Cinnamon and Frankincense.

Groups	Mean ± SE		
	MDA	CAT	SOD
Negative control	1.23 ±0.06C	5.53±1.53b	2.48±0.34c
Diabetic	4.94 ±0.48 A	14.70±2.63a	9.86 ±2.94a
Diabetic+ quercetin	2.05 ±0.39B	9.30 ±2.76a	5.49 ±2.67b
LSD value	0.47 **	2.50**	288**

Means having with the different letters in same column differed significantly. * (P≤0.05).

Finally, the level of superoxide dismutase enzyme was significantly increased in the blood of diabetic rat group

scoring 9.86±2.94 IU/L. While those in the healthy control and treated with Cinnamon and Frankincense and the diabetic groups showed a significant decrease in the enzyme activity which was 2.48±0.34 and (5.49±2.67) IU/ liter, respectively.

Histological study

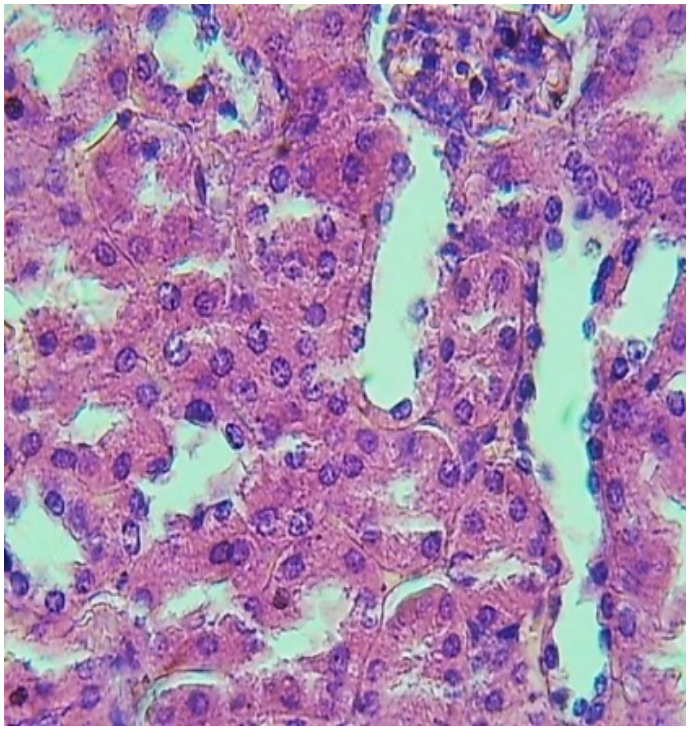

Figure 1: Section of renal cortex (control) shows: normal lining cells with and normal glomerulus. H and E stain. 400x.

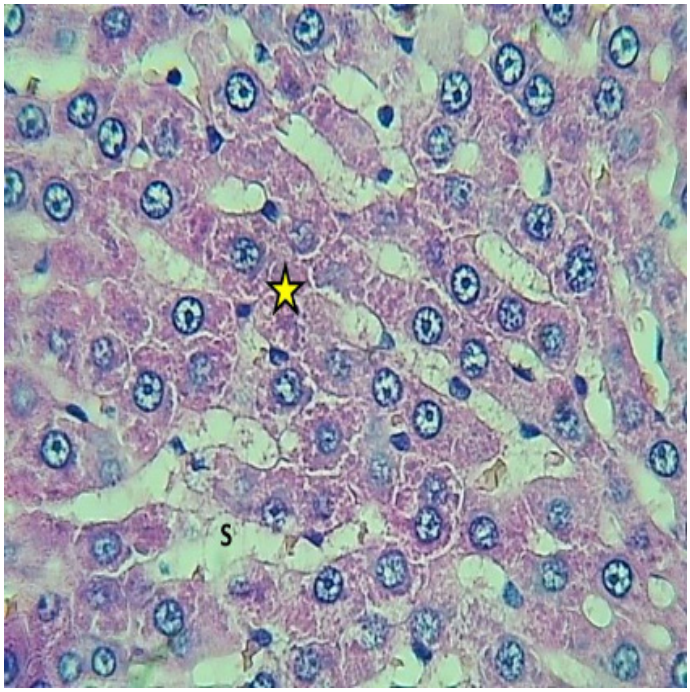

Figure 2: Section of hepatic lobule (5) shows normal hepatocytes (Asterisk), normal sinusoids (S) and kupffer cells. H and E. 400x.

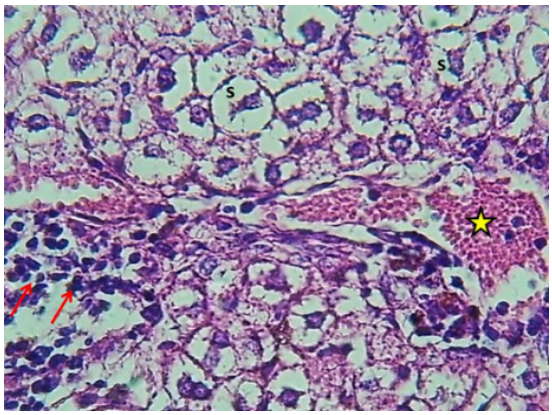

Figure 3: Hepatic lobule section (diabetic) showing mild congestion with dilation of portal vein (asterisk) with peri portal aggregation of MNCs (Arrow) and marked cellular swelling with nuclear pyknosis of hepatocytes (S). H and E stain. 400x.

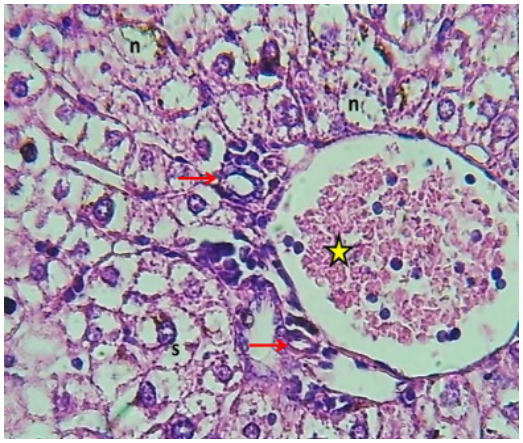

Figure 4: section of hepatic lobule (diabetic) showing mild congestion of central vein (Asterisk) with marked peri central histogenesis of newly formed hepatocytes (Arrow) and marked cellular swelling with nuclear pyknosis of hepatocytes (s). and necrosis (n) H and E stain. stain. 400x.

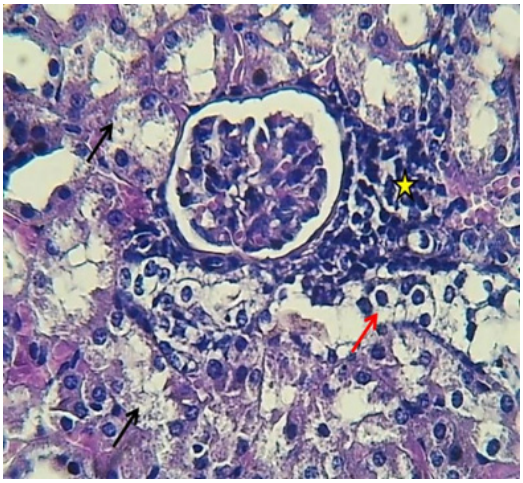

Figure 5: Section of renal cortex (diabetic) showing mild cellular swelling of lining cells (Red arrow) with necrosis (Black arrow) of the lining cells of renal tubules, with little peri glomerular aggregation of MNCs (Asterisk). H and E stain. 400x.

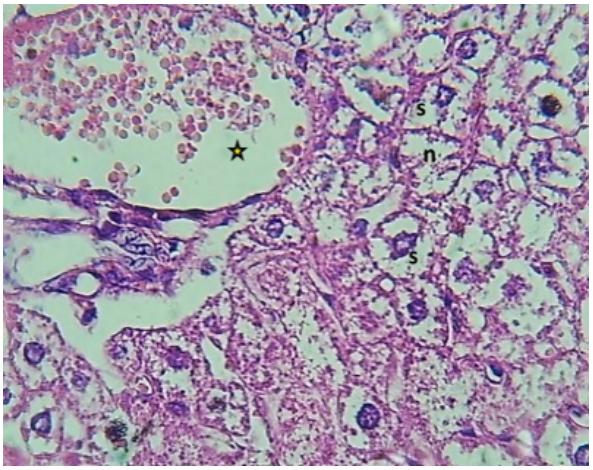

Figure 6: Central vein section (diabetic) showing dilation central vein with congestion with little cellular swelling (s) and necrosis (n) of hepatocytes. H and E stain. 400x.

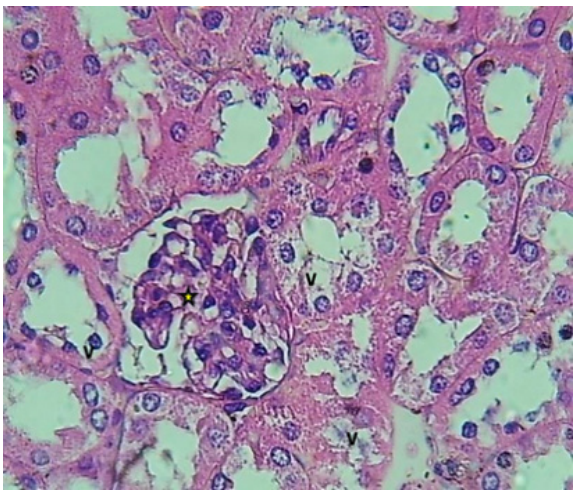

Figure 7: Renal cortex section (diabetic) showing little figures of vascular degeneration of lining cells of renal tubules (v) with normal glomerulus (Asterisk). H and E stain. 400x.

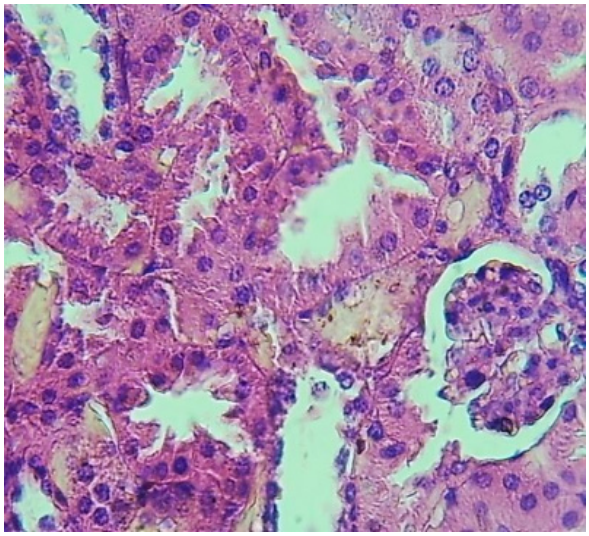

Figure 8: Renal cortex section (diabetic treated with extract) showing normal lining cells of renal tubules with normal cytoarchitecture of glomerular. H and E stain. 400x.

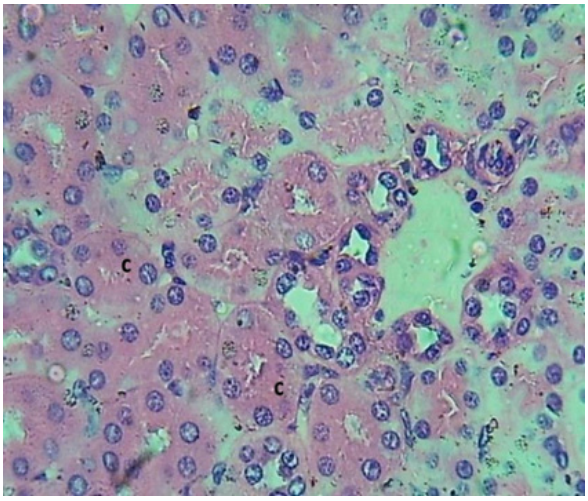

Figure 9: Renal cortex section (diabetic treated with extract) showing mild nephrosis little figures of cloudy swelling of lining cells of renal tubules (C). H and E stain. 400x.

DISCUSSION

Diabetes mellitus (DM) is amongst the most serious diseases which are expensive, reduce life expectancy and triggering disability, and causing life-threatening complications (Sun *et al.*, 2022). Long-term hyperglycemia can result in enormous and significant consequences, *e.g.*, increased glucose self-oxidation, oxidative stress and protein glycosylation resulting in reduced glucose absorption in muscle and fat cells decreasing insulin release from beta cells (Alrefaei *et al.*, 2023). Our results in Table 3 showed that, the level of glucose, Cholesterol, Triglyceride, HDL and VLDL decreased in the diabetic group treated with Cinnamon and Frankincense extracts, which were similar to those presented by Al-Muhammadi (2016) who found a significant elevation $p<0.05$ in Cholesterol, Triglyceride, HDL-C, VLDL-C concentration in rats with diabetes and after treatment with aqueous extract significant falling was occurred for the entirely parameters. LDH, and D-dimer considered as inflammatory markers (Abdul-Hussein and Fadhil, 2023). The body has a large amount of LDH, with high concentrations in the heart, liver, skeletal muscle, erythrocytes, and kidney and low concentrations in the lung, brain, and smooth muscle (Abdlkarem and Zainulabdeen, 2024). Our data did not indicate any significant differences in CRP level among the different groups. In contrast, a significant increase in the enzyme lactate dehydrogenase activity was noticed in the group of diabetic rats compared to those in -ve control group. Whereas this enzyme activity reduced in the group of diabetic rats treated with Cinnamon and Frankincense extracts, this result is in agreement with the result of Salih *et al.* (2014) who found that serum LDH was increased significantly in diabetic mice compared with controls. Our results showed that the level of TNF-a in diabetic mice

was higher than that of controls, this result is in agreement with the result of Lee *et al*. (2005) who concluded that TNF-α plays a crucial role in the initiation of T1DM by modulating DC–T cell interactions via modulation of DC development. On contemporary, a significant increase in the level of malondialdehyde was observed in the diabetic group compared to the control group and diabetic group treated with Cinnamon and Frankincense, this result is similar to the result of Gwarzo *et al*. (2014) who found a raising in the level of malondialdehyde among the diabetic rat prior to treatment and significantly decreased after the treatment. SOD and CAT considered as a protective enzyme that works as the first line of defense, converting superoxide radical to hydrogen peroxide (Oudah *et al*., 2017).

There was a significant increase in the level of the enzymes CAT and SOD in the diabetic group compared to the control group, this result is similar to the result of Ramanathan *et al*. (1999) who find that early diabetic rats exhibit increased SOD and CAT activities.

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NOVELTY STATEMENT

This study introduces a novel approach by the treatment of induced diabetes male albino rats with Cinnamon and Frankincense aqueous extracts and monitoring their effects on some physiological and histological parameters.

AUTHOR'S CONTRIBUTION

SMMA-C: Study conception and design
RMO: Literature search and manuscript preparation.
MHN: Data acquisition.
SMMA-C, MHN and RMO: Manuscript editing and review.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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