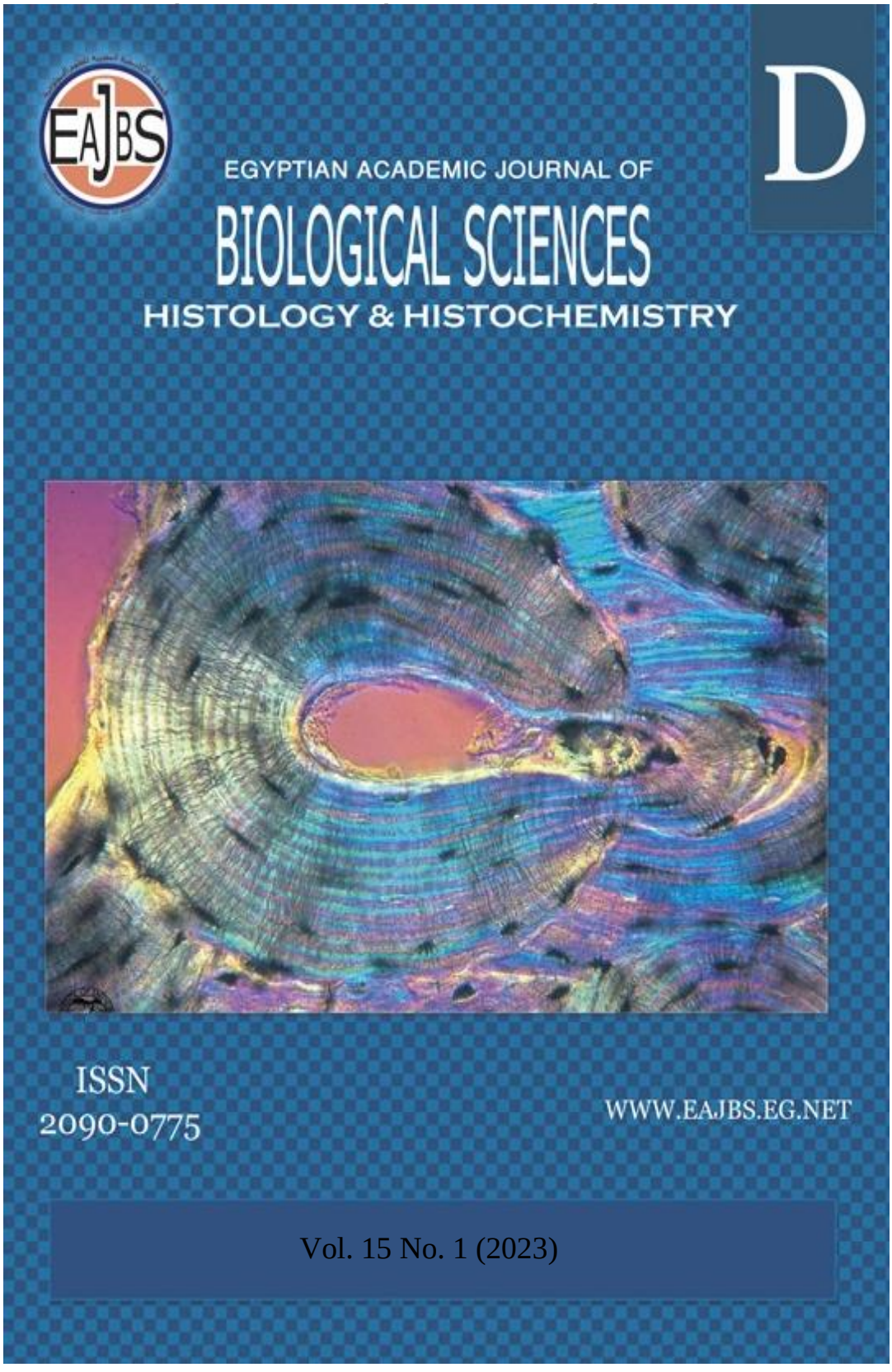

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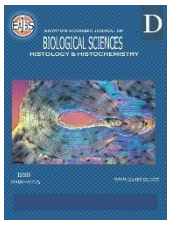

Histopathological Effects of Curcumin Versus Combined Captopril and Losartan Therapy in The Liver of Type I Diabetic Albino Rats

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Diabetes mellitus, curcumin, captopril and losartan.

ABSTRACT

Introduction: Hyperglycemia is a major complication of diabetes mellitus (DM), a disease of glucose metabolism that associated with major organ damage. **Aim of the work:** The goal was to compare the preventative effects of curcumin versus combination of losartan and captopril medication on rats' liver damage from type I diabetic. **Material and methods:** Fresh streptozotocin (STZ) solution, dissolved in sodium citrate buffer, was utilized by a single intraperitoneal injection (50 mg/kg body weight) to induce diabetes mellitus in rats within 15 minutes of formulation. Study groups were control group, diabetic group, diabetic group treated with captopril/losartan (C/L+D), diabetic group treated with curcumin (Cur+D), each group contains 6 randomly distributed rats. Blood samples were used to detect liver enzymes and assess markers of oxidative stress, also, paraffin liver sections were taken. **Results:** STZ-induced diabetic inflammation and apoptotic effects were better ameliorated by curcumin, while the combination of captopril and losartan was better in healing STZ-induced diabetic fibrosis. **Recommendation:** To improve biochemical and histopathological results, three drug combinations (captopril, losartan and curcumin) should be tested.

INTRODUCTION

Insulin synthesis, production and utilization were significantly altered in diabetes mellitus, resulting in persistent hyperglycemia, and fluctuating the metabolism of macromolecules; carbohydrates, lipids, and proteins (Roglic, 2016). This causes serious illnesses that impact numerous organs, including the brain system, retina, kidney, heart, and liver. (Adiga & Malawadi, 2016; Bril, 2014; Nentwich & Ulbig, 2015; Samir *et al.*, 2019). Diabetes mellitus led to hepatocellular damage, sinusoidal enlargement, and altered liver's histology in rat models of streptozotocin-induced DM. (Carnovale & Garay, 1984; Kohl *et al.*, 2013). Oxidative stress is thought to be the main trigger of liver damage in diabetic patients, resulting from hyperglycemia and the consequent disruption of carbohydrate, protein and lipid metabolism (Mohamed *et al.*, 2016).

Captopril used widely as a therapy for renal complications of diabetes, heart failure, acute myocardial infarction, and hypertension with minimal side effects through inhibition of angiotensin-converting enzyme (ACE) (Herman *et al.,* 2017). Losartan is a selective type 1 angiotensin II receptor blocker (ARBs). Previous studies found that administration of losartan improved liver histopathology and prevented the progression of non-alcoholic steatohepatitis (NASH) to liver fibrosis (Taha *et al.,* 2020). In addition, losartan has been shown to reduce the number of activated hepatic stellate cells (HSCs), which are important contributors to the progression of liver fibrosis (Yokohama *et al.,* 2006).

There has been a recent increase in the use of traditional and herbal medicine. especially in light of the link between environmental contamination, organ disorders, and cancer in developed countries (Elmetwaly *et al.,* 2019). The Middle Eastern countries frequently use curcumin, a natural product of turmeric, in their cuisine. Several studies have described its contribution to altering many of the biological mechanisms responsible for liver damage. Adipogenesis-related genes including SREBP-1c, PPARc, and C/EBPa are induced by curcumin, which suppresses hepatic stellate cells (HSC) activation by raising intracellular lipid content (Tang & Chen, 2010). This study compared the structural alterations in the rat liver treated with curcumin to those treated with a combination of losartan and captopril in order to emphasize the preventative role of curcumin against diabetic rat liver problems.

MATERIALS AND METHODS

Pharmaceuticals and Chemicals:

Streptozotocin (Sigma Aldrich, St. Louis, MO, USA) was dissolved freshly in sodium citrate buffer. Captopril (ACEI) (AMRIA Pharm. Ind) was dissolved in 0.9% saline. Losartan (ARB) was obtained from (Alexandria Company for Pharmaceuticals). A freshly made curcumin suspension was made from curcumin (Sigma) suspended in in 0.5 g of carboxymethyl cellulose (Zhu *et al.,* 2014)

Study Design:

Twenty-four male albino adult rats were used in this study, weighing between 150 to 200 g. Rats were kept under controlled housing conditions, 4°C temperature, and twelve hours cycles of dark and light. Rats get free access to water and tainted laboratory feed. Study groups were control group, diabetic group, diabetic group treated with captopril/losartan (C/L+D), diabetic group treated with curcumin (Cur+D), each group contains six randomly distributed rats. Untreated diabetic rats were sacrificed fourteen weeks after DM induction. Treatment by captopril (50 mg/kg/day) (Boonla *et al.,* 2014), losartan (10 mg/kg/day) (Ibañez *et al.,* 2007), and curcumin (100 mg/kg/day) (Qi *et al.,* 2022) were done eight weeks after DM induction with oral gavage. All animal care and experiments were done under the rules and regulations of the Animal Care and Use Committee of Mansoura University.

Induction of Diabetes:

Streptozotocin solution was used to induce type 1 diabetes by a single intraperitoneal injection (50 mg/kg body weight). Animals were kept without food overnight for twelve hours before STZ injection. To avoid hypoglycemia after streptozotocin injection, the rats were kept on an oral 10% glucose solution for the following two days in addition to a regular meal. If the fasting blood glucose level (FBG) was 250 mg/dl or greater for two consecutive days, the diabetic state was confirmed (Wen *et al.,* 2008).

Rats body weight were monitored at the beginning of the experiment and throughout the study. At the scarification time, rats were anesthetized (after eight hours of fasting) and blood samples were taken to measure liver enzymes, blood glucose and markers of oxidative stress (MDA

and GSH). Rat scarification was then performed. Livers were excised, fixed for histopathology, and processed for light microscopy.

Biochemical Studies:

Serum levels of liver enzyme glutamate pyruvate transaminase (SGPT), liver enzyme glutamate oxaloacetate transaminase (SGOT), glutathione (GSH), and malondialdehyde (MDA) were monitored by spectrophotometry using clinical test kits (Elitech, UK) (Ramakrishnan & Sulochana, 2012).

Histopathological Studies:

Paraffin tissues sections of 5-6 μm thickness were stained with hematoxylin and eosin stain (H&E). For immunohistochemical study, the following primary antibodies were used; Anti-NF-KB p52 (ABclonal, China, rabbit polyclonal IgG, 1:200 dilution, A3108) to assess inflammation (Fattori *et al.,* 2017), Anti-caspase 3 antibody (Servicebio, China, rabbit polyclonal IgG, 1:500 dilution, GB11532) to assess apoptosis (Abdel-Salam *et al.,* 2014), Anti-alpha SMA antibody (Servicebio, China, rabbit polyclonal IgG, 1:500 dilution, GB111364) to assess fibrosis (Yoshiji *et al.,* 2001). Histological slides were analyzed under a light microscope and a brownish color was taken as evidence of positive expression (Li *et al.,* 2015).

Morphometric Analysis:

An Olympus® optical microscope (X400) and Olympus® digital camera were used to inspect and take pictures of ten non-overlapping fields from each slide. Following the program's instructions, morphometric analysis was carried out with NIH Image J program (National Institutes of Health, Bethesda, MD, USA). Areas of NF-kb, α-SMA, and caspase-3 proteins expressions were calculated using a slightly altered protocol of Schipke *et al.* (2017). Hepatic stellate cells (α-SMA stained sections) and apoptotic cells (caspase-3-stained sections) were manually counted (Ascher *et al.,* 2001).

Statistical Analysis:

Data analysis for different parameters (body weight, blood glucose, serum MDA and GSH, liver enzymes, NF-kb, caspase 3 and α-SMA areas percentage, caspase 3 stained nuclei and α-SMA-stained nuclei) were done by SPSS program (Statistical Package for Social Sciences) version 22.0. Analysis of variance (ANOVA) was used for comparing two or more groups of numerical (parametric) data, followed by post hoc Tukey for multiple comparisons.

RESULTS

Assessment of the Body Weight (Table 1):

At week 10, weight loss in treatment groups was significant to control group ($p \leq 0.001$), however, compared to the diabetic group, there were no appreciable differences between any of the treatment groups.

At week 12, (Cur+D) group showed significantly increased their body weight in contrast to the diabetic group ($p=0.03$), meanwhile (C/L+D) group showed no significant differences. Both groups (Cur+D) and (C/L+D) gained significantly, ($p<0.001$) and ($p = 0.04$), bodyweights compared to the diabetic group at week 14.

Throughout the 16^{th}, 18^{th} and 20^{th} weeks, both groups express a significant increase in body weight in comparison to diabetic group, (Cur+D) group express a very high significance, while (C/L+D) group express gradual significance increase, ($p = 0.02$), ($p = 0.004$), and ($p>0.001$) at 16th, 18th and 20th weeks, respectively

By week 20, all groups showed highly significant weight loss compared to controls ($p<0.001$).

Table 1: The body weight in experimental groups throughout 20 weeks.

		Control	Diabetic	(Cur+D)	(C/L+D)
10th week		257±12.06	193±6.78*	210±5.09*	184±44.85*
	P1		0.006	0.054	0.002
	P2			0.847	0.983
	P3				0.543
12th week		266±11.66	185.75±5.7*	212.5±5.2*$	204±11.6*
	P1		0.000	0.000	0.000
	P2			0.026	0.196
	P3				0.825
14th week		274.20±13.68	177.5±6.13*	215.25±4.64*$	203.75±9.9*$
	P1		0.000	0.000	0.000
	P2			0.003	0.043
	P3				0.662
16th week		284±15.9	171.67±4.9*	217.75±5.56*$	204.33±6.02*$
	P1		0.000	0.000	0.000
	P2			0.001	0.022
	P3				0.555
18th week		294.8±15.8	160.3±1.5*	221.3±3.2*$	202.67±8.02*$
	P1		0.000	0.000	0.000
	P2			0.000	0.004
	P3				0.326
20th week		311.4±15.07	150.67±2.08*	220±3*$	199±1*$
	P1		0.000	0.000	0.000
	P2			0.000	0.000
	P3				0.155

The data was presented as mean ± SD, A one-way analysis of variance test (ANOVA) and Tukey's multiple comparison test were used to conduct the statistical analysis. P: Probability, P_1: significance in comparison to the control group, P_2: significance in comparison to the diabetic group, P_3: significance in comparison to the (Cur+D) group, $^{*,\$,\#}$: Significance in comparison to the Control, Diabetic, and (Cur+D) groups, respectively. (C/L+D): diabetics treated with captopril and losartan; (Cur+D): diabetics treated with curcumin.

Biochemical Results (Table 2):

a) Blood Glucose Level:

At the 20th week, in comparison to the control group, the diabetic and (C/L+D) groups had a highly significantly increase in blood glucose levels ($P < 0.001$). While the (Cur+D) group showed a substantial increase ($P > 0.05$).

b) Malondialdehyde (MDA) level:

When compared to the control group, the level of MDA in the diabetic and (C/L+D) groups increased significantly ($p> 0.01$), and (Cur+D) group showed no significant difference ($p>.05$). While the (Cur+D) group exhibited a substantial decline in comparison to the diabetes group ($p> 0.05$).

c) Glutathione (GSH) level:

When compared to the control group, there were high significant decreases in the level of GSH in the diabetic group and (C/L+D) groups ($p< 0.01$), while there was no significant difference between (Cur+D) group ($p>0.05$).

d) Liver enzymes (SGPT and SGOT):

In comparison to control group, there were highly significant increases in the level of both SGPT and SGOT in the diabetic group ($p< 0.01$). In comparison to diabetic group, both (Cur+D) and (C/L+D) groups showed a significant decrease in their levels. However, the decline in (Cur+D) group was more than in (C/L+D) group ($p< 0.05$).

Table 2: The biochemical results in experimental groups**.**

		Control	Diabetic	(Cur+D)	(C/L+D)
Blood glucose (mg/dl)		88.5 ± 2.5	392±36*	169±33.5$^{*\$}$	217±16.8$^{*\$}$
	P_1		0	0.002	0
	P_2			0	0
	P_3				0.809
Serum MDA (nmol/mg protein)		2.35±0.15	3.91±0.80*	2.99±0.55$^{\$}$	3.98±0.20$^{*\#}$
	P_1		0.003	0.137	0.002
	P_2			0.044	0.871
	P_3				0.033
Serum GSH (nmol/mg protein)		0.15±0.04	0.05±0.02*	0.09±0.04	0.04±0.006*
	P_1		0.005	0.062	0.003
	P_2			0.158	0.768
	P_3				0.097
Serum SGPT(U/L)		23.95±5.89	65.48±8.97*	31.43±9.18$^{\$}$	46.88±7.69$^{*\$\#}$
	P_1		0	0.395	0
	P_2			0	0.004
	P_3				0.016
Serum SGOT(U/L)		79.05±6.61	219.8±16.91*	127.33±12.67$^{*\$}$	155.93±24.29$^{*\$\#}$
	P_1		0	0	0
	P_2			0	0
	P_3				0.03

The data was presented as mean ± SD, A one-way analysis of variance test (ANOVA) and Tukey's multiple comparison test were used to conduct the statistical analysis. P: Probability, P_1: significance in comparison to the control group, P_2: significance in comparison to the diabetic group, P_3: significance in comparison to the (Cur+D) group, $^{*,\$,\#}$: Significance in comparison to the Control, Diabetic, and (Cur+D) groups, respectively. (C/L+D): diabetics treated with captopril and losartan; (Cur+D): diabetics treated with curcumin.

Histopathological Study:

a) Haematoxylin and Eosin (H&E) Stain (Fig.1):

In the control group, there was normal liver architecture in the form of cords of hepatocytes separated by hepatic sinusoids, forming anastomosing plates radiating from the central vein. Hepatocytes were polyhedral with eosinophilic cytoplasm and open face vesicular nuclei with prominent nucleoli. Hepatic artery, portal vein, and bile duct branches could all be found in the portal tracts. Around the portal tracts, there was barely any fibrous tissue (Fig.1A, B). In the diabetic group, there was a distortion of the hepatic architecture in the form of oedema with the widening of the sinusoidal space. Some hepatocytes appeared with darker cytoplasm and dark nuclei. Other hepatocytes appeared shrunken and isolated from each other or with fading outlines. Other cells lost their nuclei. Central Veins were congested and Von Kupffer cells (hepatic stellate cells) appeared more numerous and prominent (Fig. 1C, D, E). Administration of captopril and losartan for 6 weeks partially preserved the hepatic architecture in the form of decreased congestion of the central vein, and decreased oedema with narrowing of

sinusoidal spaces, however, there is an apparent decrease in hepatic stellate cells (Fig. 1F). Administration of curcumin for 6 weeks partially preserved the hepatic architecture against hazards induced by STZ. Hepatocytes appeared normal with active euchromatic nuclei like control. Decreased congestion of central veins. Hepatic stellate cells were still numerous and prominent. No oedema with narrowing of sinusoidal spaces (Fig. 1G).

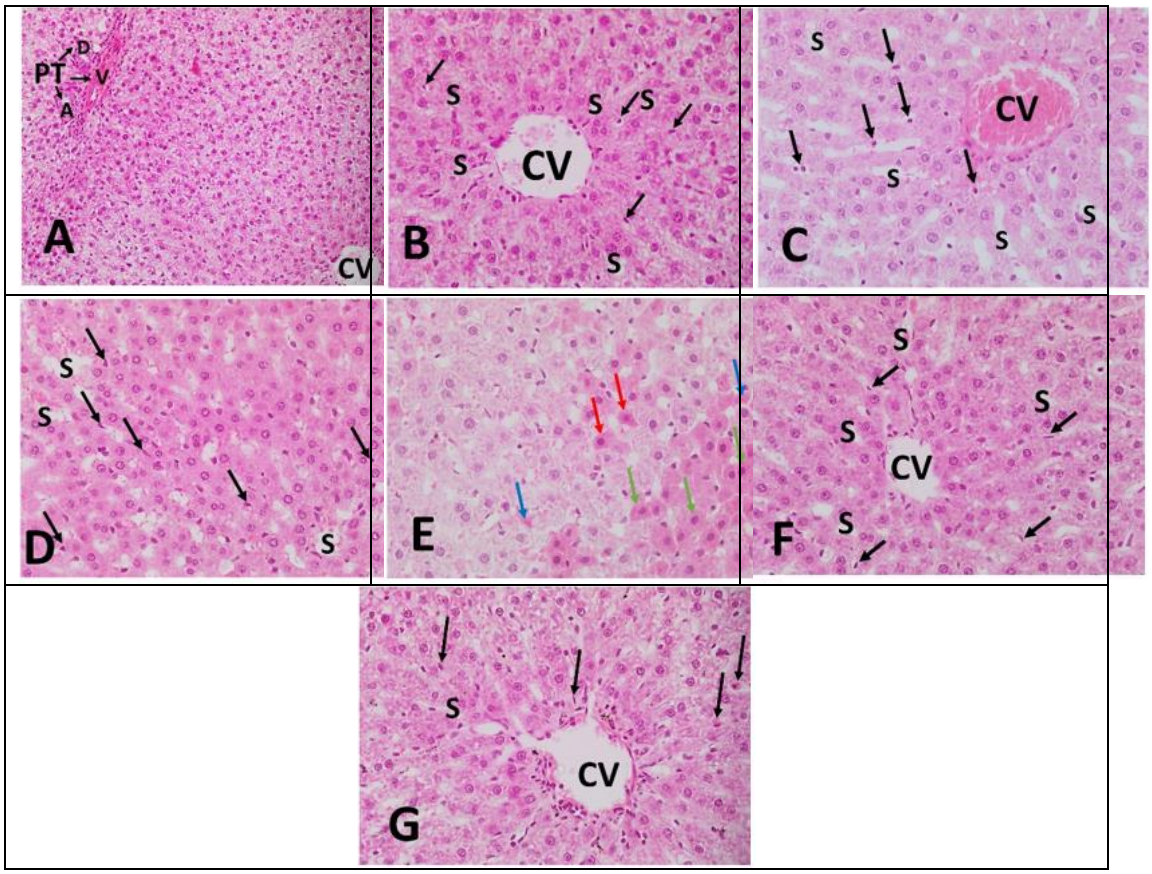

Fig.1: A, B: photomicrographs of a liver section of the control group, showing normal liver architecture in the form of cords of hepatocytes separated by hepatic sinusoids (S), forming anastomosing plates radiating from the central vein (CV). Hepatocytes were polyhedral with eosinophilic cytoplasm and open face vesicular nuclei with prominent nucleoli. Von Kupffer cells (hepatic stellate cells) appeared between the hepatocytes (black arrow). The portal tracts (PT) contained a branch of the hepatic artery (A), portal vein (V) and bile duct (D). A minimal amount of fibrous tissue was seen around the portal tracts., **C, D, E:** photomicrographs of a liver section from the diabetic group showing there was a distortion of the hepatic architecture in the form of oedema with the widening of sinusoidal space (S). Some hepatocytes appeared with darker cytoplasm and dark nuclei (green arrow). Other hepatocytes appeared shrunken and isolated from each other or with fading outlines (red arrow). Other cells lost their nuclei (blue arrow). Central Veins (CV) were congested and Von Kupffer cells (hepatic stellate cells) appeared more numerous and prominent (black arrow). **F:** a photomicrograph of liver section from (C/L+D) group showing partially preserved hepatic architecture in the form of decreased congestion of central vein (CV), decreased oedema with narrowing of sinusoidal spaces (S), however, there is an apparent decrease in hepatic stellate cells (black arrow). **G:** a photomicrograph of a liver section from (Cur+D) group showing partially preserved hepatic architecture. Hepatocytes looked normal with active euchromatic nuclei similar to control. Decreased congestion of central veins (CV). Hepatic stellate cells were still numerous and prominent (black arrow). No oedema with narrowing of sinusoidal spaces (S). A: *hematoxylin-eosin stain, original magnification: ×200. B, C, D, E, F and G: hematoxylin-eosin stain, original magnification: ×400*

a) Anti NF-Kb Immune-Stained Sections (Fig. 2, Table 3):

Sections of control group showed NF-kb positive reaction in the lining of the hepatic sinusoids (Fig. 2A). Diabetic sections showed an increase in the intensity of NF-kb positive reaction in the hepatocytes and in the hepatic sinusoids lining compared to the control group (Fig. 2B). The area % of positive NF-kb reaction revealed a significant increase (43.86 ± 5.63) compared to the control group. In (C/L+D) treated liver sections, the intensity of NF-kb positive reaction decreased in the hepatocytes and in the hepatic sinusoids lining compared to the diabetic group (Fig. 2C). In (Cur+D) treated liver sections, NF-kb positive reaction was observed in the hepatocytes and in the hepatic sinusoids lining (Fig. 2D). By image analysis, administration of captopril and losartan (C+L group) insignificantly decreased the area % of NF-kb positive reaction (38.60 ± 2.01) compared with the diabetic group. On the other hand, curcumin succeeded in significantly decreasing the area occupied by NF-kb positivity (36.93 ± 5.32) in comparison to the diabetic group. However, both failed to normalize it.

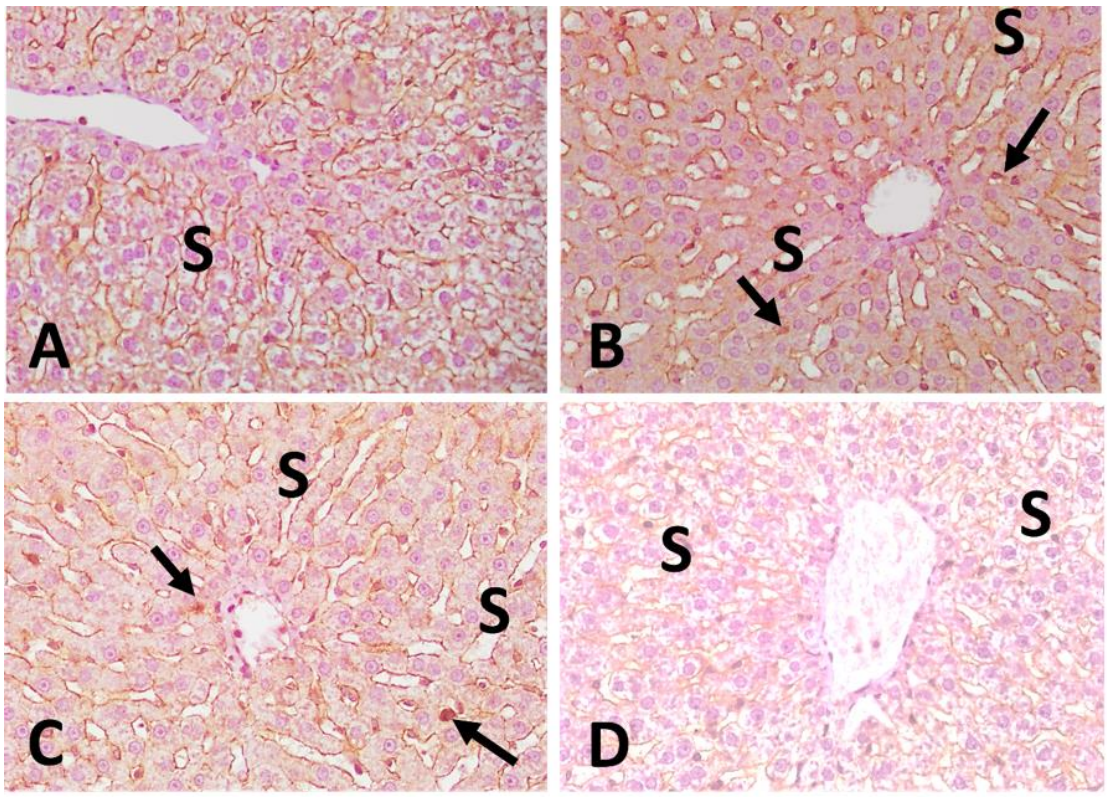

Fig.2: A: a photomicrograph of a liver section of the control group, showing NF-kb positive reaction in the lining of the hepatic sinusoids (**S**). **B**: a photomicrograph of a liver section of the Diabetic group showed an increase in the intensity of NF-kb positive reaction in the hepatocytes (**black arrow**) and in the lining of the hepatic sinusoids (**S**). **C:** a photomicrograph of a liver section of the (C/L+D) treated liver sections, the intensity of NF-kb positive reaction decreased in the hepatocytes (**black arrow**) and in the lining of the hepatic sinusoids (**S**). **D**: a photomicrograph of a liver section of (Cur+D) treated liver sections, NF-kb positive reaction was observed in the hepatocytes (**black arrow**) and in the lining of the hepatic sinusoids (**S**). *A, B, C and D: NF-Kb original magnification: ×400*

Table 3: NF-Kb, Caspase 3 and α- SMA protein area percentages (%) by image analysis in the different groups.

		Control	Diabetic	(C/L+D)	(Cur+D)
NF-Kb		32.31 ± 2.58	43.86 ± 5.63*	38.60 ± 2.01	36.93 ± 5.32$
	P_1		0.001	0.075	0.257
	P_2			0.167	0.045
	P_3				0.9
Caspase 3		9.03 ± 1.77	55.94 ± 6.82*	19.37 ± 4.33*$	16.34 ± 6.57$
	P_1		0.000	0.014	0.11
	P_2			0.000	0.000
	P_3				0.755
α- SMA		10.25 ± 1.51	27.83 ± 5.57*	8.74 ± 1.51$	19.92 ± 3.56*$#
	P_1		0.000	0.874	0.001
	P_2			0.000	0.004
	P_3				0.000

The data was presented as mean ± SD, A one-way analysis of variance test (ANOVA) and Tukey's multiple comparison test were used to conduct the statistical analysis. P: Probability, P_1: significance in comparison to the control group, P_2: significance in comparison to the diabetic group, P_3: significance in comparison to the (Cur+D) group, *,$,# : Significance in comparison to the Control, Diabetic, and (Cur+D) groups, respectively. (C/L+D): diabetics treated with captopril and losartan; (Cur+D): diabetics treated with curcumin.

a) Anti-Caspase 3 Immune-Stained Sections (Fig. 3, Table 3, 4):

Some hepatocytes' and von Kupffer cells' nuclei displayed positive caspase 3 responses in the control group sections (Fig. 3A). Diabetic sections showed an increase in the number of the positively stained nuclei (90.83 ± 14.29) compared to the control group (5.00 ± 1.79), in addition, there was a positive cytoplasmic reaction in some hepatocytes (Fig. 3B, C). This finding was confirmed by measuring the area % of positive caspase 3 reactions which revealed a significant increase (55.94 ± 6.82) compared to the control group (9.03 ± 1.77). In (C/L+D) treated liver sections, the number of positively stained nuclei decreased (10.83 ± 3.06), and there was a little cytoplasmic reaction in the hepatocytes compared to the diabetic group (Fig. 3D). In (Cur+D) treated liver sections, the number of positively stained nuclei decreased (7.33 ± 1.75), and there was a little cytoplasmic reaction in the hepatocytes compared to the diabetic group (Fig. 3E). By image analysis, administration of both (captopril and losartan) (C/L+D) (19.37 ± 4.33) and (Cur+D) (16.34 ± 6.57) significantly decreased the caspase 3 positive reaction compared to the diabetic group. However, the decline in the area stained by caspase 3 in the curcumin group was more than in the (C/L+D) group.

Table 4: Number of positive nuclei stained by Caspase 3 and α- SMA by image analysis in the different groups.

		Control	Diabetic	(C/L+D)	(Cur+D)
Caspase 3		5.00 ± 1.79	90.83 ± 14.29*	10.83 ± 3.06$	7.33 ± 1.75$
	P_1		0.000	0.54	0.95
	P_2			0.000	0.000
	P_3				0.85
α- SMA		11.83 ± 2.14	35.33 ± 7.12*	23.17 ± 3.82*$	27.33 ± 3.56*$
	P_1		0.000	0.000	0.002
	P_2			0.03	0.001
	P_3				0.41

The data was presented as mean ± SD, A one-way analysis of variance test (ANOVA) and Tukey's multiple comparison test were used to conduct the statistical analysis. P: Probability, P_1: significance in comparison to the control group, P_2: significance in comparison to the diabetic group, P_3: significance in comparison to the (Cur+D) group, *,$,# : Significance in comparison to the Control, Diabetic, and (Cur+D) groups, respectively. (C/L+D): diabetics treated with captopril and losartan; (Cur+D): diabetics treated with curcumin.

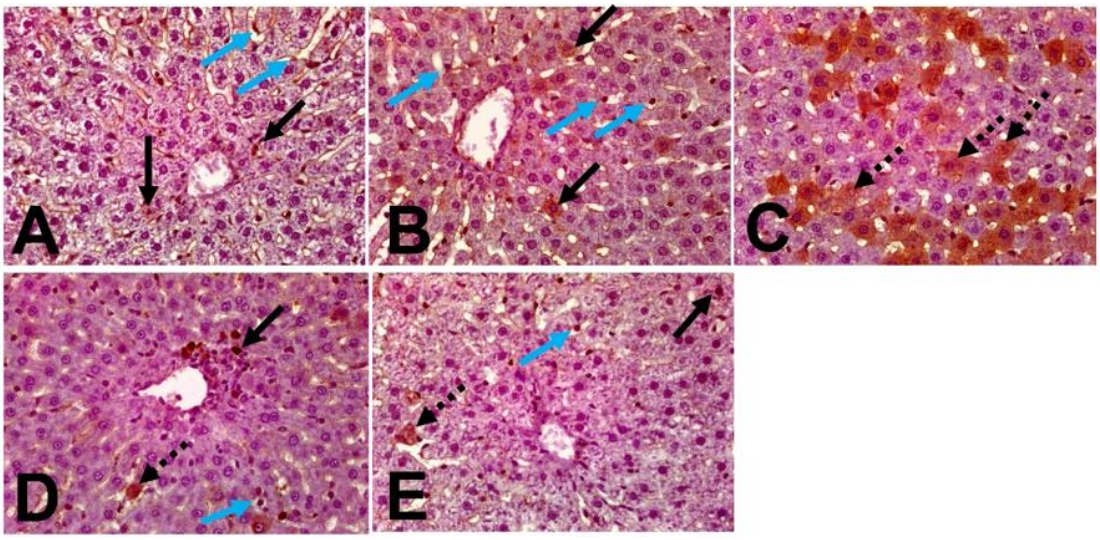

Fig.3: A: a photomicrograph of a liver section of the control group, showed a caspase 3 positive reaction in some nuclei of hepatocytes (black arrow) and von Kupffer cells (blue arrow). **B, C**: a photomicrograph of a liver section of the Diabetic group showed an increase in the number of the positively stained nuclei of hepatocytes (black arrow) and von Kupffer cells (blue arrow), in addition, there was a positive cytoplasmic reaction (dashed arrow) in some hepatocytes. **D:** a photomicrograph of a liver section of the (C/L+D) treated liver sections showed a decrease in the number of positively stained nuclei of hepatocytes (black arrow) and von Kupffer cells (blue arrow), and there was a little cytoplasmic reaction (dashed arrow) in the hepatocytes. **E**: a photomicrograph of a liver section of (Cur+D) treated liver sections showed a decrease in the number of positively stained nuclei of hepatocytes (black arrow) and von Kupffer cells (blue arrow), and there was a little cytoplasmic reaction (dashed arrow) in the hepatocytes. *A, B, C, D and E: caspase 3 original magnification: ×400*

a) Anti α- SMA Immune-Stained Sections (Fig. 4, Table 3, 4):

Sections of control group showed α- SMA positive reaction in the little number of von Kupffer cells and in relation to the central vein and portal triad (Fig. 4A). Diabetic sections showed an increase in the number of the positively stained cells (35.33 ± 7.12) compared to the control group, in addition, there was an excess positive reaction around the portal triad (Fig. 4B). This finding was confirmed by measuring the area % of positive α- SMA reaction which revealed a significant increase (27.83 ± 5.57) compared to the control group (11.83 ± 2.14). In (C/L+D) treated liver sections, the number of positively stained cells (23.17 ± 3.82) decreased, and there was little reaction in relation to the central vein and portal triad compared to the diabetic group (Fig. 4C). In (Cur+D) treated liver sections, the number of positively stained cells (27.33 ± 3.56) decreased, and there was little reaction in relation to the central vein and portal triad compared to the diabetic group (Fig. 4D). By image analysis, administration of both (captopril and losartan) (8.74 ± 1.51) and curcumin (19.92 ± 3.56) significantly decreased the α- SMA positive reaction compared to the diabetic group. However, the decline in the area stained by α- SMA in (C/L+D) group was more than in the (Cur+D) group.

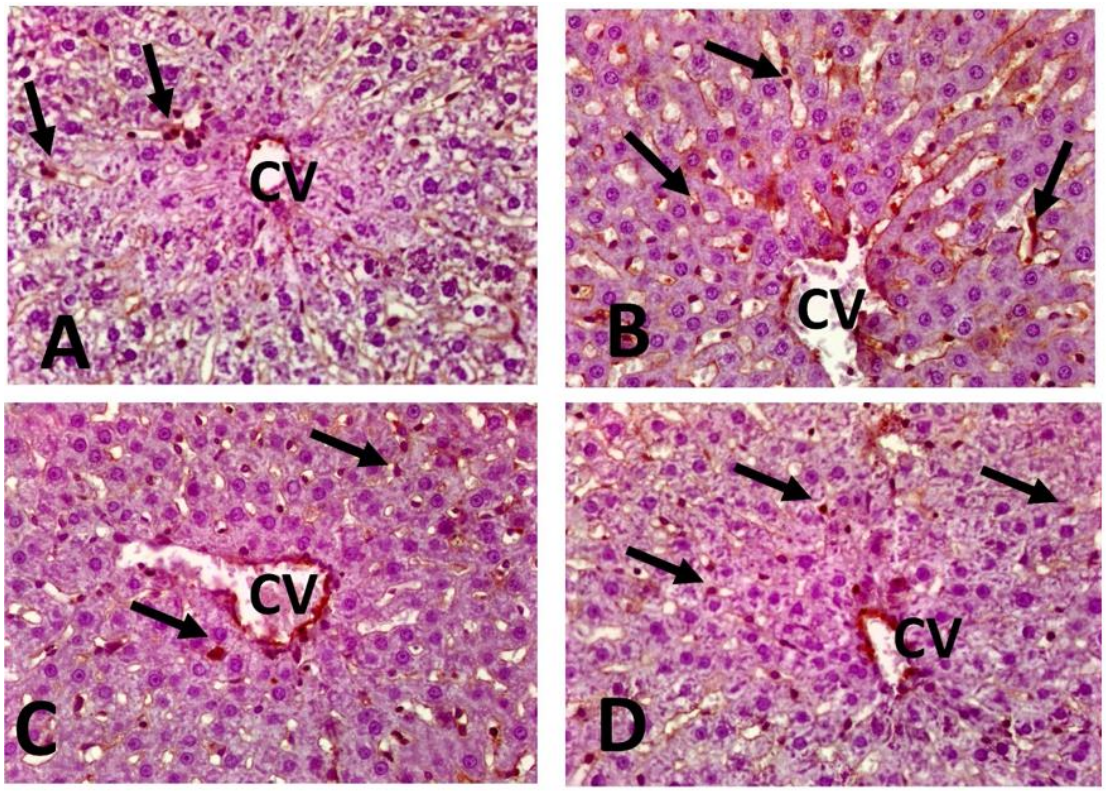

Fig.4: A: a photomicrograph of a liver section of the control group, showed α- SMA positive reaction in a little number of von Kupffer cells (black arrow) and in relation to a central vein (CV). **B**: a photomicrograph of a liver section of the Diabetic group showed an increase in the number of the positively stained cells (black arrow). **C:** a photomicrograph of a liver section of the (C/L+D) treated liver sections showed a decrease in the number of positively stained cells (black arrow), and there was little reaction in relation to the central vein (CV). **D**: a photomicrograph of a liver section of (Cur+D) treated liver sections showed a decrease in the number of positively stained cells (black arrow) and there was little reaction in relation to the central vein (CV). *A, B, C and D:* α- SMA *original magnification: ×400*

DISCUSSION

In this study, curcumin alone or combination of losartan and captopril treatment significantly improved changes in serum liver enzymes, blood glucose, and markers of oxidative stress resulting from STZ-induced diabetes. This study finding supports a study by Ghiamati Yazdi e*t al.* (2019). The most obvious sign of diabetes is hyperglycemia. The significant increase in serum glucose levels brought on by STZ proved that this trial's attempt to induce diabetes was successful. Diabetes was also associated with hepatic damage and significant changes in oxidative stress indicators. According to Giacco and Brownlee (2010), the primary reasons underlying diabetic complications are the production of free radicals and the corresponding decline in cellular antioxidant capacity. Diabetes creates significant injury to many organs, the liver was the most significant one (Ahmadieh & Azar, 2014). In comparison to untreated diabetic rats, blood glucose levels were considerably lowered by curcumin alone or with combined losartan and captopril treatment. On the same theme, giving curcumin to STZ-induced diabetic rats resulted in significantly lower blood glucose levels (El-Far *et al.,* 2017; Nishiyama e*t al.,* 2005). According to Bustanji *et al.* (2009), Kato *et al.* (2017) and Ye *et al.* (2017), curcumin's hypoglycemic effect may be a result of its ability to effectively block a number of important pathways involved in the pathophysiology of this diabetes. In the

present work, the serum MDA concentration in the (Cur+D) treated group was significantly lower and the serum GSH concentration was rather higher compared to the diabetic rats. Meanwhile, in comparison to the diabetic rats, neither captopril nor losartan were able to alter the levels of blood MDA or GSH. This is in line with earlier research showing how curcumin can reduce oxidative stress brought on by diabetes (Assis *et al.,* 2017; El-Azab *et al.,* 2011; Maithilikarpagaselvi *et al.,* 2016). In this investigation, the diabetic group showed a statistically significant rise in serum SGPT and SGOT activity. These liver enzymes are known to be increased due to hepatocyte damage. This is consistent with research conducted by Rodríguez *et al.* (2018) and Ghiamati Yazdi *et al.* (2019). In this study, the increased SGPT and SGOT activities were considerably reduced by either curcumin administration alone or by captopril/losartan administration together. Curcumin, however, outperformed the captopril/losartan combination. This can be explained by the antioxidant properties of curcumin in the treatment of diabetes-related liver damage. The histopathological image of a diabetic liver model used in this work demonstrated a distortion of the hepatic architecture in the form of oedema with enlargement of the sinusoidal space. Some hepatocytes have darker cytoplasm and nuclei than others. Other hepatocytes appeared smaller, separated from one another, or with vanishing borders. Central Veins were engorged and Von Kupffer cells (hepatic stellate cells) seemed more numerous and conspicuous. These results corroborated those of Rodríguez *et al*. (2018), and Mahata *et al*. (2021)

Morphometric analysis, which demonstrated a substantial increase in the area percentage of positive NF-kb, caspase 3, and α-SMA versus the control group. These data support that liver inflammation, fibrosis, and apoptosis were caused by STZ-induced diabetes. This could be explained by hyperglycemia, which causes inflammation and oxidative stress, aggravating the liver injury process by activating NF-KB, which in turn stimulates the genes that cause liver cells apoptosis, and releasing reactive oxygen species (ROS) (Ugwu *et al.,* 2013).

According to this study's findings, treating diabetic rats with curcumin alone or captopril/losartan in combination largely conserved the hepatic architecture by reducing central vein congestion, oedema, and sinusoidal spaces, as well as decreasing hepatic stellate cells, as seen by Hx & E. In comparison to the diabetes group, there was a decrease in both the percentage of positive NF-kb, caspase 3 and α-SMA stained nuclei and the overall number of positive nuclei in both treated diabetic groups. However, combined captopril and losartan treatment was superior in decreasing the area percentage of α-SMA while curcumin was superior in decreasing the area percentage of positive NF-kb and caspase 3.

According to Yekollu *et al.* (2011), the liver benefits from curcumin's suppression of the NF-kb signaling pathway during diabetes. In a rat model fed on fructose, curcumin blocked the (NF-kb) pathway activation, via preventing the breakdown of the inhibitor of kappa b and the subsequent release of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNFa).and C-reactive protein (Maithilikarpagaselvi *et al.,* 2016). Additionally, it was demonstrated by Qi *et al.* (2022) that daily curcumin administration reduces placental inflammation in rats with intrauterine growth retardation by blocking the NF-kB signaling pathway.

Another explanation by how curcumin reduces inflammation and apoptosis associated to diabetes is the suppression of endoplasmic reticulum stress (ERS). According to Brown and Naidoo (2012), diabetes-related causes can activate ERS and produce disturbances in glucose homeostasis and redox imbalance. In these

circumstances, the endoplasmic reticulum (ER) responds by surface membrane sensors, such as kinase/endoribonuclease 1a and serine/threonine-protein. These sensors trigger the activation of protein kinase R-like endoplasmic reticulum kinase and transcription factor 6, which in turn triggers the activation of cell death signaling pathways (NF-kb, caspases, and JNK, p38) (Rashid *et al.,* 2017).

In the non-alcoholic steatohepatitis rat model study by Yoshiji *et al.* (2001), the hepatoprotective impact of losartan was previously described. They linked the antifibrotic action of losartan to reduction of (HSC) activation. Losartan might also have antifibrotic effects via reducing oxidative stress, decreasing macrophages, down-regulating inflammatory cytokines, suppressing TIMP-1, and raising levels of circulating adiponectin (Paschos & Tziomalos, 2012). Curcumin's antifibrotic effects may be ascribed to its ability to reduce hepatic stellate cell activation by raising lipid levels in HSCs through the stimulation of genes associated with lipogenesis, including SREBP-1c, PPARc, and C/EBP a (Tang & Chen, 2010). The chemical structure of captopril, which contains a sulfhydryl (-SH) group and similar to cysteine in structure, an essential component of glutathione, can be used to explain why it has hepatoprotective effects (Habior, 1992). Sulfhydryl group is regarded as a hunter of oxygen free radicals (Kim *et al.,* 2013). In a variety of animal tissues, captopril was able to raise total glutathione levels as well as glutathione peroxidase and glutathione reductase activities (de Cavanagh *et al.,* 2000). Additionally, captopril was shown by Ackerman *et al.* (2008) to reduce the activity of glutathione reductase and peroxidase. These studies using the paracetamol-induced toxicity paradigm demonstrate that captopril has hepatoprotective properties (Al-Shaikh e*t al.,* 2016; Ali, 2012; Mahmood *et al.,* 2014). According to Mohamed *et al.* (2016), the main cause of liver damage is hyperglycemia-induced oxidative stress, hence a combination of losartan and captopril may be more effective in reducing it.

In summary, curcumin is superior at reducing the inflammatory and apoptotic effects that STZ causes in diabetics, while captopril and losartan together are superior at reducing the fibrosis that STZ causes in diabetics. Therefore, if all are merged, the outcomes might be superior. Finally, it is advised that further research to be continued.

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