Original Article

The activity of curcumin combined with $ZnCl_2$ on streptozotocin-induced diabetic rats: An anti-diabetic, anti-hyperlipidemic study

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ABSTRACT

Diabetes plays a direct role in the development of oxidative stress. Numerous molecules have been evaluated to see whether they can improve diabetes. Curcumin (CUR) and Zinc Chloride ($ZnCl_2$) have various health-beneficial properties. The present study aims to assess the efficacy of CUR combined with $ZnCl_2$ on oxidative changes in Streptozotocin(STZ)-induced diabetes in rats. Forty-eight Albino rats were treated with STZ (60 mg/kg b.w. intraperitoneally) and divided into six groups (G1-G6). G1 was the negative control, while G2 was the positive control. G3,G4 received STZ+CUR (100, 200 mg/kg b.w, respectively). G5 and G6 received (15 mg/kg b.w STZ+$ZnCl_2$ +100 mg/kg b.w CUR) and (15 mg/kg b.w STZ+$ZnCl_2$ +200 mg/kg b.w CUR), respectively. Serum levels of glucose, insulin, lipid profile, liver transaminases (ALT, AST), and lipid peroxidation (GSH, SOD, TBA, and CAT) were determined. Furthermore, morphological changes of the liver were studied. Results: The results showed that glucose, TC, TG, LDL-C, ALT, AST, SOD, TBA, and CAT levels increased, while insulin, HDL-C, and GSH decreased in the STZ group. Treatment with (200 CUR +15 mg/kg b.w $ZnCl_2$) ameliorated these changes with superior results than other groups. The study confirmed that CUR+$ZnCl_2$ improved the oxidative stress condition in rats' serum and tissues; therefore, they could work effectively as preventive agents against STZ- induced diabetes.

Keywords: Curcumin, Streptozotocin, Oxidative stress, $ZnCl_2$, Diabetic rats

Introduction

Diabetes mellitus (DM) is a metabolic disorder with a variety of etiologies [1, 2]; it is characterized by chronic hyperglycemia and disturbance in protein, fat, and carbohydrate, metabolism due to a lack of insulin excretion/or insulin action. DM has become a public health threat around the globe [3-5]; it is also a major health issue in the Kingdom of Saudi Arabia. The progression of diabetic hepatosclerosis is triggered by oxidative stress. It is caused by an excess of reactive oxygen/nitrogen radicals, as well as a reduction in endogenous antioxidant systems, which leads to the development of degenerative diseases [6, 7]. Although there are several anti-diabetes drugs that regulate hyperglycemia, treatment options that target DM sequelae, including oxidative stress and dyslipidemia, are limited. Therefore, the focus of the current research is to explore new factors and strategies that would curb the spread of the diabetes epidemic and its fatal consequences. A combination of anti-diabetic drugs supplied with other chemicals has emerged as an interesting strategy for managing hyperglycemia and other DM disorders. Curcumin (CUR) is a South Asian spice and is a highly pleiotropic molecule that is a widely-

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known antioxidant [7] and has been demonstrated to have anti-bacterial, [8] anti-inflammatory, anticancer [9], antiatherosclerotic, antimicrobial [10], wound healing [11], and other pharmacological actions. Moreover, it has the ability to scavenge reactive oxygen species (ROS), including alkoxy radicals, hydroxyl radicals, and superoxide radicals, directly in the living cells [12], completely degrades superoxide through mimicking superoxide dismutase [13, 14], and acts as a protective barrier in conditions when high levels of peroxynitrite and hydrogen peroxide molecular oxidants are produced inside the cells [15].

Zinc, an essential trace element, has been found to improve glucose absorption and transport in numerous tissues while suppressing pancreatic insulin release and raising pancreatic insulin content [16]. Evans' research [17] has also revealed that zinc supplementation has a beneficial influence on the progression of age-related macular degeneration; individuals who took antioxidants and zinc supplements were less likely to lose visual acuity. Furthermore, studies have found promising results when curcumin is used in combination with anti-diabetic drugs [18] or other phytochemicals [19] to regulate glycemia and reduce other diabetic problems.

The aim of this research is to study how CUR combined with ZnCl2 affects the levels of oxidative stress biomarkers and serum lipid profile; and how the combination exerts defensive actions against morphological changes in liver tissue in STZ-induced diabetic rats. **(Figure 1)** This would benefit in determining the role of the combination, as well as alleviating the deteriorating effects of diabetes worldwide.

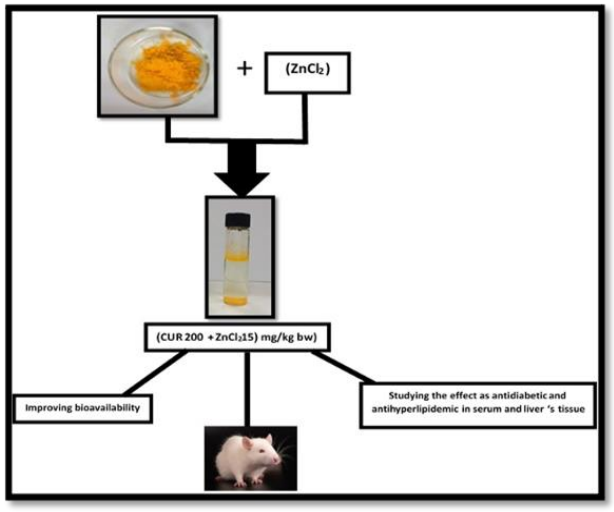

Figure 1. Enhancing curcumin's bioavailability by combining it with $ZnCl_2$ - application on rats

Materials and Methods

Chemicals and experimental procedures

High purity analytical grade chemical reagents were procured from Sigma, Merck, Aldrich. All kits were manufactured by Biosystems (Alcobendas, Madrid, Spain), Sigma (St. Louis, MO, USA), and Biodiagnostic (Cairo, Egypt). Seventy-two Albino Wister male rats, in the weight range of 200g to 205g, were purchased from Animal House, King Faisal University. Rats were kept in an individual cage in a room at a controlled temperature of 25°C, 50% humidity, 12 h of light and dark cycle with screened bottoms. These rats were fed for 10 days with the basal diet containing corn starch (70%), casein (10%), corn seed oil (10%), cellulose (5%), a mixture of salt (4%), and vitamins (1%). Then, rats were weighed and separated into six groups, with 12 rats in every group. The assigned 6 diet groups were as follows: G1 signified the negative control (NC), G2 designated the positive control (PC) in which rats were injected by STZ (60 mg/kg BW), G3 enclosed the rats that received STZ (60 mg/kg BW) + CUR (100 mg/kg BW), in G4 rats were treated with STZ (60 mg/kg BW) + CUR (200 mg/kg BW), rats in G5 were treated with [STZ (60 mg/kg BW) + CUR (100 mg/kg BW)+ ZnCl2 (15 mg/kg BW)] and those in G6 were treated with [STZ (60 mg/kg BW) + CUR (200 mg/kg BW) + ZnCl2 (15 mg/kg BW)]. Decapitation was used to kill all the rats at the end of the experiment (21 days). Following Schermer [20], blood samples were obtained from the orbital plexus using heparinized capillary glass tubes. First, a dry clean centrifuge tube was utilized to place each blood sample and then centrifuged at 4°C with the revolution of 1500g for 30 min to acquire the blood serum. An approval **(IRB-2018-10-216)** from the University was obtained to perform the study.

Biochemical analysis

ALT, AST, glucose, insulin, and lipid profile assay

ALT, AST activities [21], Glucose [22], Insulin [23], Total cholesterol [24], Triglycerides [25] and HDL-C and LDL-C [26] concentrations were estimated by using standard kits.

Thiobarbituric acid (TBA) assay

Lipid peroxidation was estimated according to Hommouda *et al.*'s method [27] and upon the TBA reactivity principle. Briefly, 2.5mls of 10% trichloracetic acid (TCA) and 0.5ml of plasma were mixed in test tubes. After 15 minutes of incubation at 90°C and then cooling via a cold water bath, the mixture was centrifuged for 10 minutes at 3000 rpm. Then 2mls of the supernatant was added to 1ml of 0.675% TBA. The tubes were capped and incubated for 15 minutes at 90°C and then allowed to cool down at room temperature. The optical density was measured at 532nm by a spectrophotometer (Schimadzu, AA6800, Tokyo, Japan).

Superoxide dismutase (SOD) assay

The SOD activity of erythrocytes was determined in the hemolysates via commercially available kits from Randox Laboratory, Crumlin, Ireland. Hemolysis of erythrocytes was done by using ice-cold deionized water followed by strong

vortexing. SOD activity was determined through the superoxide radicals that are generated from xanthine and xanthine oxidase, which in turn react with 2-(4- iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to produce a red formazan dye. The degree of inhibition of such a reaction is correlated to SOD activity [28].

Catalase (CAT) assay

CAT activity was determined via Aebi method [29], which employs a UV spectrophotometer at 240nm. To determine the ceruloplasmin activity in serum, 5ml of phenylenediamine substrate was added to 2 tubes (test and curve tubes). Then, 1ml sodium azide (NaN3) solution was added only into the curve tube while 100µls of serum was added to both tubes. Both tubes were mixed and kept for 15 minutes at 37°C. Finally, 1ml of NaN3 was added to the test tube only, and a spectrophotometer was used to measure the OD at 546nm.

Glutathione (GSH) assay

Concentrations of serum GSH were measured according to Beutler *et al*. method [30].

Statistical analysis

Data were analyzed by comparing the results of various treatment groups to the results of control groups. All data were presented as mean±SD. ANOVA with posthoc least significant difference test (LSD) at (p 0.05) were integrated and used to examine significant differences between values.

Histopathology

Liver specimens were fixed in 10% formalin, paraffinized, sectioned (4µm thickness), and stained using hematoxylin and eosin dyes. Light microscopy was used to demonstrate hepatic pathological alterations such as degeneration, atrophy, cell distortion, and necrosis, as well as the effectiveness of micronutrients in alleviating these pathological aspects [31].

Results and Discussion

Table 1 shows the effect of CUR+$ZnCl_2$ on serum glucose during 21 days in STZ-induced diabetes in rats. The level of serum glucose increased significantly (p≤0.05) in PC group (STZ-treated); and reduced significantly (p≤0.05) in CUR (100 & 200 mg/kg b.w) and CUR coupled with $ZnCl_2$ (15 mg/kg b.w) treated groups, concerning the PC.

Table 1. Effect of curcumin combined with $ZnCl_2$ on serum glucose level in diabetic rats

Treatments \ Time	Glucose (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
G1 (NC)	99.907a ±0.100	100.513f±0.320	99.955e±0.027	100.297f±0.258
G2 (PC)	99.752a±0.187	391.685a±0.624	435.090a±0.535	502.443a±0.258
G3 (STZ+ CUR 100mg/kg b.w)	99.756a±0.182	222.407b±0.300	178.485b±0.310	163.107b±0.368
G4 (STZ+ CUR 200mg/kg b.w)	99.982a±0.035	218.027c±0.341	177.987b±0.328	153.540c±0.262
G5 (STZ+ $ZnCl_2$ 15 mg/kg b.w + CUR 100 mg/kg b.w)	99.912a±0.073	202.167d±0.364	163.450c±0.217	111.118d±0.303
G6 (STZ+ $ZnCl_2$ 15 mg/kg b.w + CUR 200 mg/kg b.w)	99.742a±0.208	193.217e±0.274	151.457d±0.408	101.993e±0.140
LSD	**0.437**	**1.122**	**0.990**	**0.790**

Results for 12 rats in every group are shown as mean±SD; statistical significance at p≤0.05 than NC (ANOVA was obtained via Fischer's LSD test). The values with distinct superscript letters (a, b, c, and d) differ significantly from each other at p ≤0.05.

The levels of insulin were given in **Table 2**. The results demonstrated that the level of serum insulin was reduced significantly (p≤0.05) in PC when compared with NC. Administration of CUR (100 & 200 mg/kg b.w) and CUR combined with $ZnCl_2$ (15 mg/kg b.w) led to a marked increase (p≤0.05) in insulin level when compared to PC. The treatment of 200 mg/kg b.w CUR + $ZnCl_2$ 15mg/kg b.w gave results near to the NC after 21 days.

The results of TC, TG, HDL-C, and LDL-C were given in **Table 3**. The data indicated that serum levels of TC, TG, and LDL-C were significantly (p≤0.05) elevated in PC; however, HDL-C was significantly (p≤0.05) reduced compared to NC and other treated groups. A significant (p≤0.05) inhibition in TC, TG, and LDL-C levels was observed in CUR (100 & 200 mg/kg b.w) and CUR combined with $ZnCl_2$ treated groups while HDL-C levels were elevated, compared to PC. Moreover, the group that was treated with CUR 200 + $ZnCl_2$ 15mg/kg b.w, gave the best results.

Table 2. Effect of curcumin+$ZnCl_2$ on serum insulin level in diabetic rats

Treatments \ Time	Insulin (pg/ml)			
	Day 0	Day 7	Day 14	Day 21
G1 (NC)	45.852a±0.088	46.067a±0.394	46.128a±0.147	45.850a±0.086
G2 (PC)	45.882a±0.094	11.205e±0.176	8.347f±0.112	6.255f±0.121
G3 (STZ+ CUR 100mg/kg b.w)	45.967a±0.037	18.865d±0.169	27.002e±0.160	31.977e±0.031
G4 (STZ+ CUR 200mg/kg b.w)	45.985a±0.035	22.298c±0.278	28.935d±0.223	36.218d±0.139
G5 (STZ+ $ZnCl_2$ 15 mg/kg b.w + CUR 100 mg/kg b.w)	45.960a±0.041	22.710c±0.223	30.285c±0.208	38.730c±0.186
G6 (STZ+ $ZnCl_2$ 15 mg/kg b.w + CUR 200 mg/kg b.w)	45.930a±0.058	28.280b±0.260	32.843b±0.275	42.140b±0.153
LSD	**0.184**	**0.754**	**0.564**	**0.374**

Results for 12 rats in every group are shown as mean ± SD with statistical significance at $p \leq 0.05$ than NC (ANOVA was obtained via Fischer's LSD test). The values with distinct superscript letters (a, b, c, and d) differ significantly from each other at $p \leq 0.05$.

Table 3 shows the effect of CUR (100 & 200 mg/kg b.w) and CUR + $ZnCl_2$ 15mg/kg b.w on serum AST and ALT enzymes. ALT and AST activities significantly ($p \leq 0.05$) increased in PC group compared with NC and other treated groups. The CUR and CUR + $ZnCl_2$ treated groups showed a lower compared to PC, especially at CUR 200 + $ZnCl_2$ 15mg/kg b.w treated group.

STZ exposure significantly ($p \leq 0.05$) elevated the levels of SOD, TBA, and CAT, while GSH was significantly ($p \leq 0.05$) decreased as compared with the NC group. Administration of CUR (100 & 200 mg/kg b.w) and CUR combined with $ZnCl_2$ 15mg/kg b.w significantly ($p \leq 0.05$) lowered SOD, TBA and CAT levels **(Table 4)**. CUR 200 + $ZnCl_2$ 15mg/kg b.w treated group improved the levels of GSH and SOD close to NC.

Table 3. Effect of curcumin combined with $ZnCl_2$ on serum AST and ALT concentration in diabetic rats

Treatments	ALT (U/L)	AST (U/L)
G1 (NC)	63.395f±0.143	134.097f±0.080
G2 (PC)	153.623a±0.191	326.940a±0.041
G3 (STZ + CUR 100mg/kg b.w)	107.103b±0.135	218.202b±0.104
G4 (STZ + CUR 200mg/kg b.w)	92.367c±0.208	198.118c±0.063
G5 (STZ + $ZnCl_2$ 15mg/kg b.w + CUR 100mg/kg b.w)	86.407d±0.297	172.235d±0.186
G6 (STZ + $ZnCl_2$ 15mg/kg b.w + CUR 200mg/kg b.w)	67.025e±0.076	141.173e±0.123
LSD	**0.544**	**0.318**

Results for 12 rats in every group are shown as mean±SD with statistical significance at $p \leq 0.05$ than NC (ANOVA was obtained via Fischer's LSD test). The values with distinct superscript letters (a, b, c, and d) differ significantly from each other at $p \leq 0.05$.

Table 4. Effect of curcumin+$ZnCl_2$ on serum GSH, SOD, TBA, and CAT concentration in diabetic rats

Treatments	GSH Mmol/g	SOD U/ml	TBA (nmol/L)	CAT nmol/mg protein
G1 (NC)	23.847e±0.091	6.497e±0.138	31.473f±0.123	21.392f±0.220
G2 (PC)	15.482a±0.196	17.415a±0.137	67.492a±0.146	51.308a±0.162
G3 (STZ + CUR 100mg/kg b.w)	43.772b±0.129	11.433b±0.151	52.413b±0.203	39.970b±0.045
G4 (STZ + CUR 200mg/kg b.w)	38.723c±0.103	9.355c±0.176	43.445c±0.169	32.604c±0.107
G5 (STZ + $ZnCl_2$ 15mg/kg b.w + CUR 100mg/kg b.w)	30.775d±0.134	9.350c±0.118	41.243d±0.176	31.150d±0.052
G6 (STZ + $ZnCl_2$ 15mg/kg b.w + CUR 200mg/kg b.w)	23.868e±0.086	7.122d±0.061	33.295e±0.165	22.260e±0.159
LSD	**0.370**	**0.390**	**0.478**	**0.419**

Results for 12 rats in every group are shown as mean±SD with statistical significance at $p \leq 0.05$ than NC (ANOVA was obtained via Fischer's LSD test). The values with distinct superscript letters (a, b, c, and d) differ significantly from each other at $p \leq 0.05$.

Histopathological investigations revealed that the diabetic liver had a high degree of hydropic degeneration accompanied by coagulative necrosis **(Figure 2)**. Treatment with CUR (100 & 200 mg/kg b.w) and their combination with $ZnCl_2$ 15mg/kg b.w showing improvement appeared in the hepatocytes, Kupffer cells nuclei, and blood sinusoids **(Figure 2)**.

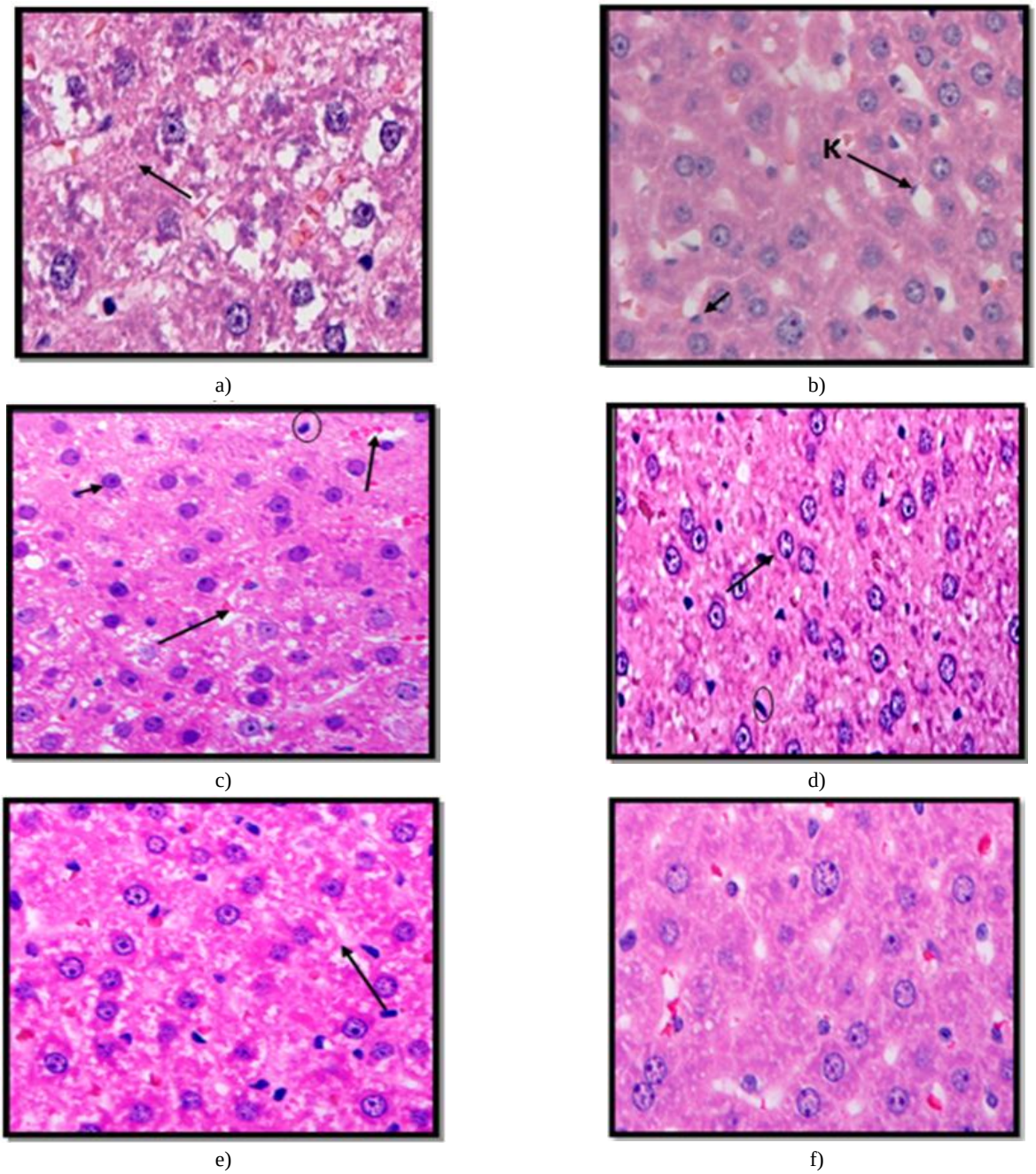

Figure 2. Light micrograph of liver (a) section of STZ (60mg/kg b.w) group showing high degree of hydropic degeneration (arrow) accompanied with coagulative. (b) section of negative control group showing the hepatocytes radial arrangement. The blood sinusoids were penetrated the hepatocytes strands and lined with endothelial cells (arrow). Also, Kupffer cells (K) were clearly noticed. (x40 H&E) necrosis. (x40 H&E) (c) section of CUR (100mg) +STZ(60mg) group showing some bleeding at the blood sinusoids (arrow), while proliferated Kupffer cells were appeared (circle). Improvement in the nuclei (arrow head) were observed. (x40 H&E) (d) section of CUR (200mg)+ STZ(60mg) group showing distinct marked improvement of the hepatocytes and nuclei (arrow). The Kupffer cells appeared diffuse proliferation (circle). (x40 H&E) (e) section of CUR (100mg)+ ZnCl2(15mg)+STZ (60mg) group showing narrowing of blood sinusoids (arrow) with slight coagulative necrosis of the hepatocytes, and normal nuclei were markedly noticed. (x40 H&E) (f) section of CUR (200mg)+ ZnCl2(15mg) +STZ (60mg) group showing mild improvement appeared in the hepatocytes, nuclei and blood sinusoids were clearly with normal architecture.

Our study provided an advanced model and more information concerning using treatment mixtures and important measurements for the provided model of diabetes [32]. Oxidative stress plays an important role in the pathogenesis and etiology of diabetes [33]. The formation of ROS plays a role in the pathophysiology of diabetic retinopathy, possibly as a causal agent [34]. Many biochemical mechanisms strictly related to hyperglycemia, including glucose autoxidation and protein glycation, are enhanced under oxidative stress, which supports this theory. An increase in serum glucose was observed in the STZ-treated group; and an appreciable reduction in the groups treated with CUR and when combined with ZnCl2. Endothelial cells exposed to high glucose levels produce more superoxide anions, which may release nitric oxide, a potent endothelium-

derived vasodilator that promotes the vasculature's overall homeostasis. The discovery that several negative effects of excessive hyperglycemia on endothelial functions are overcome by antioxidants adds to the harmful role of oxidative stress [35]. A study showed that the oral digestion of CUR at the rate of 100 mg/kg/day for eight weeks could appreciably remove all STZ induced abnormalities and hyperglycemia, including blood glucose elevation [36]. Administration of CUR could prevent STZ-induced diabetes, which was supported by regular performance of glucose clearance and continued normoglycemia [37]. The capacity of CUR for improving the pancreatic islets was also reported [38]. Besides, it was shown that the death of isolated β-cells and dysfunction could be prevented by CUR in diabetic STZ-induced animals [39]. Balbaa *et al*. [40] showed a considerable boost in the plasma glucose level in animals after STZ vaccination. It has been found that zinc deficiency and altered zinc metabolism are linked to reduced glucose tolerance [41]. Oral administration of CUR and its combination with ZnCl2 may reveal a significant impact on the levels of blood glucose.

Table 2 summarized the influence of CUR+ZnCl2 on the serum insulin contents of diabetic rats. CUR of 100 and 200 mg/kg BW and combined with ZnCl2 could enhance the level of serum insulin in STZ-induced diabetes in rats. Primarily, the hyperglycemia in the β-cells and the deficiency of insulin were cropped up because of the direct effects from cytotoxic assisted by STZ. Furthermore, it has been postulated that the worsening in glucose tolerance in free radical initiating systems is due to reduced insulin activity [42]. It was supposed that inducing lipid peroxidation in the lipid moiety of the cell membrane by free radicals would change the functional and structural integrity of the cell membrane or the interior cellular components. This would impede insulin's ability to commence and spread its regular sequence of actions, which could partially explain STZ-induced hyperglycemia [43]. However, the inclusion of STZ that disrupted the Langerhans islet cells in the pancreas was slowly restored when treated with CUR, thus saving β cells from further damages. This indicated the defensive activity of CUR against pancreas cell damages caused by STZ induction in the rats [37]. Research on diabetic rats revealed a considerable reduction in the insulin level than control ($p<0.001$), wherein an insignificant alteration was observed after treating the rats with curcumin [44]. Diabetes has been related to zinc deficiency and altered zinc metabolism, which in turn might be attributed to impaired insulin sensitivity [41]. Similarly, Zn exerted insulin suppression activities by slowing down the insulin hypersecretion related to the pre-diabetic pancreas, thereby allowing the preservation of the remaining βcells' structure and functionality [45].

Table 3 exhibited that the serum LDL-C, TG, and TC were appreciably amplified in PC as well as other treated groups. In contrast, the HDL-C concentration was considerably reduced in diabetic rats. The observed significant decrease in the serum TC, TG, and LDL-C contents in all treated groups of diabetic rats indicated their efficacy against diabetes. Nevertheless, the serum HDL-C concentration was higher compared to PC. Moreover, the group treated with CUR 200 mg/kg b.w + ZnCl2 revealed the optimum outcome. Levels of serum TG and TC in diabetic rats were raised compared to the normal ones. However, such abnormalities were considerably attenuated when treated with CUR [39]. A similar study on diabetic rats disclosed a noteworthy amplification in the serum TC and TG levels compared to healthy (without diabetes) groups. A considerable reduction in the serum HDL-C level of diabetic rats was evidenced. Interestingly, after treating the rats with Zn, the serum HDL-C level was significantly augmented [42]. In short, CUR treatment in diabetic rats could appreciably lower the serum TC and TG concentrations [36, 40].

Table 4 described the influence of CUR+ZnCl2 on the serum AST and ALT contents in STZ induced diabetic rats. Results showed that serum ALT and AST levels notably increased in PC as well as other groups treated with CUR and CUR+ZnCl2. These treated groups disclosed a lower level of AST and ALT compared to PC, especially at 200 mg/kg BW of CUR+ZnCl2. Furthermore, hyperglycemia in hepatocytes could reflect the boost of blood serum aminotransferases as major effects of the diabetic problem. This enhancement in the serum markers can be attributed to the leaking of liver cytosol enzymes into the bloodstream because of hepatomegaly (fatty liver) [42]. Stimulation of diabetes by STZ in rats could produce hepatotoxicity and thus amplified the activity of serum marker enzymes, including ALP, ALT [46, 47]. Zinc can minimize the perturbations in serum AST, ALT, ALP in STZ-diabetic rats [48]. Necrosis or semipermeable membrane injury causes the discharge of those enzymes into the blood. However, the serum level of those enzymes is expounded with liver action. This is in agreement with the liver microscopic anatomy results of this study.

In **Table 4**, the exposure of rats to STZ could considerably increase the serum SOD, TBA, and CAT levels compared to NC, while it decreased the GSH levels. CUR and CUR+ZnCl2 could attenuate those levels. The serum GSH and SOD levels in 200 mg/kg BW CUR+ZnCl2 treated diabetic rats were almost close to NC. Peroxyl radicals and molecular oxidants are the main kinds of ROS rat cells. Upon reacting with the injected CUR, these free radicals led to the formation of H atom from the phenolic-OH that existed in CUR, which in turn released H. The released H further reacted to form phenoxy, which eventually became stable via resonance around the keto-enol structures. Such oxidants were less reactive compared to the peroxyl radicals that defend cells from ROS-induced oxidative stress [46]. The incorporation of STZ in the rats resulted in a decrease in the liver serum SOD contents. Therefore, weakening in the activities of SOD was ascribed to the excessive production of ROS. In this mechanism, the superoxides were neutralized by ROS due to their inability to cross the lipid membranes by generating H2O2 which can traverse biological membranes. The produced H2O2 being the catalase detoxifiers, played a vital role in damaging the tissues. Thus, such an effective decrease in the SOD level can be majorly ascribed to

the disruption of frontline enzymatic defense against H2O2 and superoxide anion [8, 47]. It was asserted that due to the exposure of STZ in the rats, the GSH was depleted and oxidized, which in turn created oxidative stress-mediated hyperglycemia when compared with normal (non-diabetic) rats [37]. It was arguing that hyperglycemic condition led to the formation of reactive species of oxygen [47], which in sequence inhibited antioxidant enzymes' activities, including SOD, CAT, and GSH-Px via the glycosylation mechanism in erythrocytes [49]. Gosh, *et al*. [46] revealed that exposure of animals to STZ could considerably enhance the hepatic lipid peroxidation than CUR treated ones. Furthermore, it was shown that CUR exposure to diabetic animals could appreciably inhibit hepatic lipid peroxidation. The exposure to STZ could cause fast depletion in the hepatic GSH, thus lowering the GSH to GSSG ratio in STZ induced animals than the normal ones. In brief, the levels of GSH, CUR treated animal groups remained unaltered (like normal animals) [47]. The exposure of STZ lowered the activities of antioxidant enzymes in the hepatic tissues, whereas the administration of CUR helped to restore these activities to make them normal. Thus, the protection of rat liver cells by CUR against the diabetic pathophysiology induced by the STZ exposure was primarily attributed to the antioxidant action of CUR [39, 44]. The presence of CUR in diabetic rats could normalize the activities of erythrocyte and hepatic antioxidant enzymes such as SOD, CAT, GSH-Px, and so forth, causing a considerable decrease in lipid peroxidation [42]. A recent study revealed that the presence of Zn is responsible for the efficient protection of free radicals from occasional injuries [45]. Such protective traits of Zn against enhanced lipid peroxidation and disintegration rates can be majorly ascribed to its capacity for effectively binding and stabilizing the cell membranes [45]. On top of that, the existence of Zn in the biological system is accountable for protecting the antioxidants against diabetic problems. Firstly, such protective action of Zn can be attributed to its excellent capacity to control hypoglycemic conditions, thereby inhibiting the occurrences of the injurious outcomes of hyperglycemia. Secondly, Zn can halt the development of the intrinsic alterations in the diabetic tissues, which gradually cause the disintegrations of cells [49]. The catalase activities revealed a considerable reduction in diabetic rats than the control group. Overall, the presence of Zn and CUR caused a considerable improvement in the specific activities in the diabetic rat's serum and hepatic catalase than those induced by STZ [41]. The observed noteworthy augmentation in the catalase by Zn in the diabetic cells can be ascribed to the competitive action of Zn to both Fe and Cu responsible for binding the cell membranes in diverse biological systems. Consequently, the formation of OH groups was hindered, and the generation of transition metal-free radicals was prevented [46-49]. The presence of Zn in the diabetic rats played an efficient and defensive role against diabetic syndrome that could produce injurious cell radicals to weaken the immune systems [44].

The current study showed that the valuable results of the combined treatment of CUR with ZnCl2 on diabetes could be reached via 2 strategies: (i) the best results of the isolated treatments were kept, including the decrease in glucose and (AST, ALT) levels in (CUR and ZnCl2 effects), (ii) additive effects were achieved, mainly by a further reduction in blood serum TG, cholesterol, and TBA, in which levels were lower compared with those of isolated treatments. The highest benefits reached by combined CUR with ZnCl2 were the antioxidant potential of this treatment and the development of dyslipidemia.

As seen in **(Figure 2)**, in the negative control group, the liver tissue section had a hepatocytes arrangement. The blood sinusoids perforated the strands of hepatocytes and were bordered by endothelial cells. Likewise, Kupffer cells were observed. Histological investigation of the liver tissue exposed to STZ (60 mg/kg b.w) showed a high degree of hydropic degeneration accompanied by coagulative necrosis **(Figure 2)**. Treatment with CUR (100+200 mg/kg b.w) **(Figure 2)** showed distinct marked improvement of the hepatocytes and nuclei, while treatment with CUR (100+ 200 mg/kg b.w) + ZnCl2 15mg/kg b.w **(Figure 2)** showed more improvement appeared in hepatocytes and normal nuclei were markedly noticed. The results also indicated that treatment with (CUR 200 + ZnCl2 15 mg/kg BW) **(Figure 2)** showed the best results. Nuclei and blood sinusoids were clearly with normal architecture.

The present data reveal that CUR combined with ZnCl2 treatment to STZ-diabetic rats (especially at a high dose) produce a remarkable amelioration better than CUR treated groups. The combination of CUR with ZnCl2 exhibited a synergistic and powerful control over hyperglycemia; it was able to scavenge free radicals, normalize liver function as well as exerting a principal role in treating and ameliorating liver damage at the cellular level. Thus, the most promising therapeutic dose currently used in this study can be effective in the treatment and strengthening of liver tissue from damage caused by diabetes and may be used as a candidate anti-diabetes drug. According to Holemans *et al*. [41], beta cells are destroyed by necrosis as a result of streptozotocin activity. STZ causes degenerative changes and necrosis in pancreatic β-cells; in turn, this leads to insulin insufficiency and glucose oxidation impairment. Mitra *et al*. [49] found that after injecting STZ, diabetic livers became degenerated and congested. Hyperglycemia is characterized by a drop in blood insulin levels, followed by hypoglycemia that is caused by a decline in insulin levels. Experimental research indicated that nitric oxide (NO) could be the cause of the increased liver injury (42). Nitic oxide toxicity is increased when it reacts with superoxide radicals to produce powerful secondary toxic oxidizing species called peroxynitrite (ONOO). Such compound (ONOO) can oxidize cellular structure and cause lipid peroxidation [33], a process that causes membrane damage and is considered to be the fundamental cause of cell death.

This study proposes that CUR combined with ZnCl2 treatment acts in the liver as a potent free radical scavenger to prevent the hepatotoxicity effects of STZ-diabetic rats. I recommend

applying future studies on another natural mixture, recent synthetic organic materials, and provide continuous biomarkers related to antioxidants, inflammatory biomarkers, and its related gene expression to treat the risk of diabetes.

Conclusion

The results showed that CUR+$ZnCl_2$ is effective in protecting rats against STZ-induced diabetes in serum and tissue. The results showed the additive effects of this combination on decreasing oxidative stress and dyslipidemia in diabetic rats. They may be used as good therapeutic agents for a diabetic. The conclusion that CUR and $ZnCl_2$ as non-toxic and cheap sources of diabetes treatment can be applied on a large scale to protect the liver in diabetic rats. As far as we know, by targeting hyperglycemia, oxidative stress, and dyslipidemia, this study provides the first evidence about a promising therapeutic strategy by combined CUR with $ZnCl_2$ for managing diabetes complications, especially heart and blood vessels diseases. Further research is necessary to understand the mechanisms of actions of this combination therapy that result in helpful effects observed in diabetes.

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Ethics statement: The animal experiments were carried out by the National Institutes of Health guidelines for the care and use of laboratory animals. I certify that this work was performed following local ethical guidelines, with the approval of Imam Abdulrahman Bin Faisal University's Institutional Review Board (IRB). The IRB number for this study was IRB-2018-10-216.

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