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Original Research

Effect of curcumin and angiotensin II receptor antagonist on insulin sensitivity and on inflammation in induced diabetic rats

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ABSTRACT

Objective: In this study, we aimed to discuss the effect of curcumin and/or irbesartan on diabetic complications in induced diabetic rats. **Materials and Methods:** Diabetic rats induced by intraperitoneal injection of 45 mg/kg dose of streptozotocin (STZ) in adult rats. Rats were divided into six groups of 10 rats on each. Serum glucose, creatinine, and urea were estimated using a colorimetric method. Serum insulin and serum and kidney homogenate interleukin 6 (IL-6) were measured using enzyme-linked immunosorbent assay technique. While paraffin-embedded kidney and pancreas specimens were performed for histopathological examination. While homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. **Results:** Curcumin and/or irbesartan reduce the degradation of kidneys and pancreatic tissues as indicated in both histopathology and tissue IL-6. They improve the weight loss and IR. In addition, they lower circulating level of IL-6, serum creatinine, and urea. Diabetic rats received the medications show significantly better insulin and glucose levels as compared to STZ group. **Conclusion:** Chronic administration of curcumin and/or irbesartan for diabetic rats decreased serum glucose and HOMA-IR and lowered the increased serum levels of pro-inflammatory cytokines. Using such combination could be beneficial in diabetic managements, especially if diabetes accompanied with hypertension.

KEY WORDS: Diabetes mellitus, inflammation, insulin resistance, nephropathy

INTRODUCTION

Diabetes is the most common chronic metabolic disease that is characterized by hyperglycemia. Diabetes is related to either low insulin level due to beta cell destruction or the body's inability to respond to and use the insulin produced known as insulin resistance (IR) [1]. The increased level of glucose triggers inflammation for different organs, especially beta cells of the pancreas which produce pro-inflammatory mediators such as interleukin (IL-1ß) which leads to ß-cell dysfunction [2]. High glucose levels alter the mitochondrial electron transfer chain, causing the formation of reactive oxygen species (mROS) induce oxidative stress on ß-cell causing ß-cell dysfunction and producing more pro-inflammatory mediators such as IL-6, tumor necrosis factor-α (TNF-α), and IL-1ß [3,4]. These cytokines develop IR by disturbing the flow of blood into ß-cell and reduce the expression of glucose transporter-4 and insulin receptor substrate-1 [5,6].

Diabetic patients suffer from abnormal lipid and protein metabolism, along with specific long-term complications including nephropathy, retinopathy, and nervous system disorders [7,8]. Diabetic nephropathy is defined as persistent clinically detectable proteinuria in association with hypertension and reduced glomerular filtration rate [9]. Diabetic nephropathy results from a structural and functional change of collagen [10], interaction with the renin-angiotensin system, and oxidative stress with the induction of cytokines and growth factors [11,12]. Water and sodium depletion results from polyuria and glycosuria stimulate renin secretion which converts angiotensin I to angiotensin II leading to hypertension [13,14].

Irbesartan is one of the angiotensin II receptor antagonist used as an antihypertension drug. Irbesartan activates peroxisome proliferator-activated receptor gamma which regulates lipid uptake, IR, and inflammatory cytokines levels [15].

Curcumin is extracted yellow powder of *Curcuma longa* [16] shows anti-inflammatory effect through inhibition of some inflammatory mediators such as IL, TNF, cyclooxygenase-2, lipoxygenase, and inducible nitric oxide synthase, so curcumin

reduces oxidative stress and plays a protective role against inflammation [17].

This study was aimed to assess the effect of administration of curcumin and/or irbesartan on insulin sensitivity through evaluation of insulin and glucose levels and on pro-inflammatory cytokines through investigation of serum and tissue levels of IL-6 in experimentally–induced diabetic rats.

MATERIALS AND METHODS

Materials

Streptozotocin (STZ) was purchased from Sigma-Aldrich Corporation (Cat. no. S0130, Sigma, USA) for induction of diabetes. Curcumin, which is the yellow extract of *C. longa*, from the family *Zingiberaceae*, is imported from India by Elgomhoria Chemical Company, Egypt. Irbesartan was donated from Medical Union Pharmaceuticals Company (MUP), Egypt. The vehicle consists of dimethylsulfoxide (DMSO) and ethanol provided by Elgomhoria Chemical Company, Egypt. Curcumin extract was prepared by dissolving 5 g curcumin in 12.5 mL of 5% DMSO in phosphate-buffer saline (pH 6.1) [18]. Hence, each 0.1 mL of solution contains 40 mg curcumin, and the dose of curcumin was 400 mg/kg/day dissolved in 1 mL 5% DMSO solution.

Animals

60 albino rats of both sexes, weighing 180-200 g and aged 10-12 weeks were used in the present study. Animals were obtained from and housed at Faculty of Pharmacy (Boys), AL-Azhar University, Cairo, Egypt. A high-fat diet was obtained from the Animal House of Faculty of Medicine, Benha University, Qaliubyia, Egypt.

Rats were kept at temperature 25 ± 1°C and a relative humidity of 55% with a regular 12 h light/12 h dark cycles. The animals were fed standard chow with free access to water. The experimental animal protocol was approved and carried out according to guidance from Medical Research Ethics Committee, Al-Azhar University, Egypt.

Induction of diabetes

Diabetes mellitus was induced by intraperitoneal injection of rats with a single dose of STZ (Sigma, USA) in a dose of 45 mg/kg body weight dissolved in 0.2 ml of citrate buffer (pH 4.5) [19].

Experimental design

After the period of acclimatization which was 1 week, the rats were randomly divided into six experimental groups (n = 10; 5 males and 5 females in separate cages) as the following:

- Group I (Control group): Rats received no medications and kept under the same conditions as before start of the study
- Group II (Vehicle group): Rats received 0.2 mL vehicle only/rat for 6 weeks
- Group III (STZ group): Induced diabetic rats received no treatment for the period of 6 weeks
- Group IV (curcumin group): Induced diabetic rats were treated with curcumin in a dose of 400 mg/kg/day in 5% DMSO by oral gavage technique for the period of 6 weeks
- Group V (irbesartan group): Induced diabetic rats were treated with Irbesartan in a dose of 5 mg/kg/day in 0.33 ml 70% aqueous ethanol by oral gavage method for the period of 6 weeks
- Group VI (combination group): Induced diabetic rats treated with a combination of curcumin in a dose of 400 mg/kg/day and irbesartan in a dose of 5 mg/kg/day by oral gavage method for the period of 6 weeks.

Animals were closely monitored for any complication at daily basis. At the end of the study, rats were anesthetized with diethyl ether inhalation and fasting blood samples were withdrawn from their ocular vein, and sera were separated by centrifugation at 4000 rpm for 20 min and then, stored at −80°C until the measurement of biochemical parameters. Then, animals were euthanized, kidneys, and pancreatic tissue samples were harvested and prepared and stored at −80°C until histological examinations.

Methods

All biochemical measurements were performed in the Central Laboratory Unit of the Biochemistry Department, Faculty of Pharmacy (Males), Al-Azhar University.

Assessment of glucose and insulin

Serum glucose was measured using colorimetric diagnostic kit according to the manufacturer's instructions [20]. Serum insulin was determined by enzyme-linked immunosorbent assay (ELISA) kit (Calbiotec, USA) [21]. Homeostatic model assessment of IR (HOMA-IR) was calculated according to an equation of Gutch *et al*. [22].

Determination of renal functions

As a marker of renal function and potential nephrotoxicity, Serum creatinine and urea were determined using colorimetric diagnostic kits (Spinreact, Santa Coloma, Spain and Vitro Scientific, Hanover, Germany, respectively) according to the manufacturer's instructions.

Evaluation of IL-6

IL-6 of serum and kidney's homogenate was determined using ELISA kit (Boster Biological Technology Co., Ltd., Pleasanton, California, USA) according to the manufacturer's instructions.

Histopathological examination

Paraffin-embedded kidney and pancreas specimens were cut into the sections and stained with hematoxylin and eosin stain according to the method of Bancroft and Gamble [23].

Statistical Analysis

Data were expressed as a mean ± standard error of the mean. Statistical differences were analyzed using unpaired Student's *t*-test. Data analysis was performed using GraphPad Prism software (GraphPad Software, LaJolla, CA, USA). Statistical significance was accepted at $P < 0.05$.

RESULTS

Animal Weights

Animal's weights were measured pre and post 6 weeks treatment. After 72 h of STZ injection, there were no significant differences for all groups compared to control group as shown in Table 1. While the administration of curcumin and/or irbesartan for 6 weeks shows interesting results. Our results showed that a significant weight gain in both control and vehicle groups while significant weight loss was observed in the other groups that injected by STZ. Curcumin, irbesartan, and combination groups ameliorated weights significantly as compared to STZ group. However, combination group showed significant weight gain as compared to irbesartan group and non-significant weight gain as compared to curcumin group. Administration of curcumin ameliorated weights significantly as compared to irbesartan group as shown in Table 1.

Serum Glucose

Serum glucose levels were estimated either before and at the end of treatment. In control and vehicle groups, no statistically significant differences in serum glucose levels were observed before and after 6 weeks of treatment as illustrated in Table 1. The other four groups showed elevated serum glucose levels after 72 h of STZ injection. While after 6 weeks of treatment, irbesartan, curcumin, and combination groups showed a significant reduction in elevated glucose levels as compared to STZ group. In addition, combination group showed a significant reduction in serum glucose level as compared to curcumin group as shown in Table 1.

Serum Insulin

Serum insulin levels of each group were estimated after 6 weeks of treatment. Serum insulin levels of all STZ injected groups including STZ, curcumin, irbesartan, and combination groups were decreased significantly as compared to control group while no significant difference was observed between control and vehicle groups. Moreover, curcumin and combination groups showed a significant reduction in serum insulin levels as compared to STZ and irbesartan group while there were no significant differences in serum insulin levels between irbesartan and STZ group. Furthermore, combination group showed significant improvement in insulin level as compared to curcumin group as reported in Figure 1.

HOMA-IR

HOMA-IR was calculated at the end of treatment, and our results showed that HOMA-IR was significantly higher in all studied groups as compared to control or vehicle group. Moreover, STZ group was significantly higher in HOMA-IR as compared to curcumin, irbesartan, and combination groups. Combination group showed no significant difference in HOMA-IR as compared to curcumin group while a significant reduction in HOMA-IR was observed in curcumin and combination groups as compared to irbesartan group as recorded in Figure 2.

Serum IL-6

At the end of treatment, our results showed that IL-6 serum concentration of STZ, irbesartan, curcumin, and combination

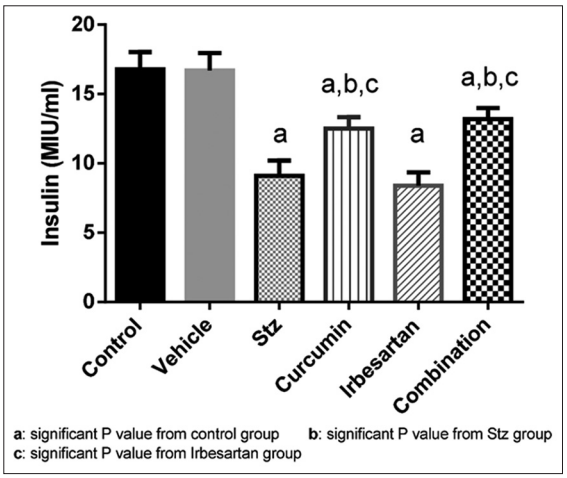

Figure 1: Serum insulin levels in all the studied groups

Table 1: Mean values of weight and serum glucose before and after treatments in all the studied groups

Group	Control	Vehicle	STZ	Curcumin	Irbesartan	Combination
Weight (g)						
M±SEM before	195±1.1	195±0.9	194.3±1.8	195.4±1.5	193.5±1.8	192±1.5
M±SEM after	204±1.0	206.4±0.9	153.9*,a±1.2	184.9#,a,b,c±1.6	156.5†,a,b±1.3	185‡,a,b,c±1.4
Serum glucose (mg/dL)						
M±SEM before	71±1.0	71.7±1.0	559.3±9.0	540±1.0	528.3±7.6	496.4±10.7
M±SEM after	72.7±0.7	72.9±0.7	649.7*,a±8.3	286.3#,a,b,c±6.3	517.2a,b±7.8	267.3‡,a,b,c,d±1.9

After: After the period of treatment, before: Before the period of treatment, *Significant difference versus STZ group before treatment, #Significant difference versus curcumin group before treatment, †Significant difference versus irbesartan group before treatment, ‡Significant difference versus combination group before treatment, [a]Significant difference versus control after period of treatment, [b]Significant difference versus STZ after period of treatment, [c]Significant difference versus irbesartan after period of treatment, [d]Significant difference versus curcumin after period of treatment, M±SEM: Mean±standard error of mean and significant $P<0.05$. STZ: Streptozotocin

groups were significantly higher as compared to control group. Irbesartan, curcumin, and combination groups showed a significant reduction in IL-6 serum concentration as compared to STZ group. Moreover, curcumin group showed a significant reduction in serum IL-6 than irbesartan group. Furthermore, IL-6 serum concentration was reduced significantly in combination group as compared to curcumin or irbesartan groups as shown in Table 2.

Tissue IL-6

IL-6 of kidney tissue homogenate showed that IL-6 tissue concentration of STZ, irbesartan, curcumin, and combination groups were significantly higher as compared to control group. Moreover, irbesartan, curcumin, and combination groups showed a significant reduction in IL-6 tissue concentration as compared to STZ group. Furthermore, combination group showed a significant reduction in IL-6 tissue concentration as compared to curcumin or irbesartan groups as reported in Table 2.

Serum Creatinine and Urea

Serum creatinine and urea were measured at the end of treatment. Significantly elevated levels of both serum creatinine and urea were observed in STZ, irbesartan, curcumin, and combination groups as compared to control group. Moreover, irbesartan, curcumin, and combination groups showed a significant reduction in both serum creatinine and urea as compared to STZ group. Furthermore, combination group showed a significant reduction in both serum creatinine and urea as compared to curcumin and irbesartan groups. On the other hand, the irbesartan group showed a significant reduction in serum creatinine and urea as compared to curcumin group as mentioned in Table 3.

Histopathological Findings

In vehicle group, no histopathological alteration was observed in both kidney and pancreatic tissues. In the kidney, it was observed that a normal histological structure of the glomeruli (g) and tubules (t) at the cortex as shown in Figure 3a. Moreover, a normal histological structure of acini (a) and islands of Langerhans cells (s) was observed in the pancreatic tissue as shown in Figure 3b.

In the STZ group, interesting results were observed. Figure 3c showed that periglomerular inflammatory cells infiltration (m), dilatation of the cortical blood vessels (v), and degradation in tubular lining epithelium (d) in the kidneys of STZ group. Furthermore, the pancreas of STZ group showed severe atrophy in islands of Langerhans with vacuolization in lining epithelium of the acini as illustrated in Figure 3d.

In curcumin group, congestion was noticed in cortical blood vessels with hypercellularity in lining endothelium of glomerular tuft of the kidney tissues as shown in Figure 3e. Moreover, moderate atrophy was observed in islands of Langerhans cells of pancreatic tissues as illustrated in Figure 3f.

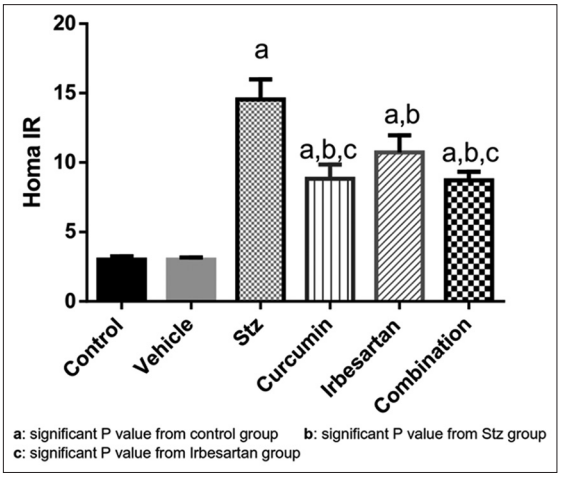

Figure 2: Homoeostasis model assessment of insulin resistance in all the studied groups. [a]Significant *P* value from control group, [b]Significant *P* value from streptozotocin group, [c]Significant P value from irbesartan group

Table 2: Mean values of serum and tissue IL6 after treatments in all the studied groups [43]

Group	Control	Vehicle	STZ	Curcumin	Irbesartan	Combination
Serum IL6						
M±SEM after	79.8±2.8	80.8±2.8	264.6[a]±8.2	93.7[a,b,c]±1.8	138.1[a,b]±9.1	88.4[a,b,c,d]±1.7
Tissue IL6						
M±SEM	82.80±4.3	83.80±4.3	2105[a]±50.7	129.5[a,b]±7.3	127.1[a,b]±7.8	93.5[a,b,c,d]±5.0

[a]Significant difference versus control, [b]Significant difference versus STZ, [c]Significant difference versus irbesartan, [d]Significant difference versus curcumin. IL6: Interleukin 6, M±SEM: Mean±standard error of mean, STZ: Streptozotocin group and significant $P<0.05$

Table 3: Mean values of serum creatinine and urea after treatments in all the studied groups

Group	Control	Vehicle	STZ	Curcumin	Irbesartan	Combination
Serum creatinine						
M±SEM	0.42±0.03	0.42±0.04	1.96[a]±0.04	1.41[a,b,c]±0.10	1.01[a,b]±0.10	0.65[a,b,c,d]±0.04
Serum urea (g/dl)						
M±SEM	17.8±0.40	18.3±0.30	119.5[a]±3.50	57.7[a,b,c]±0.90	30.4[a,b]±0.90	25.4[a,b,c,d]±0.80

[a]Significant difference versus control, [b]Significant difference versus STZ, [c]Significant difference versus irbesartan, [d]Significant difference versus curcumin. M±SEM: Mean±standard error of mean, STZ: Streptozotocin group and significant $P<0.05$

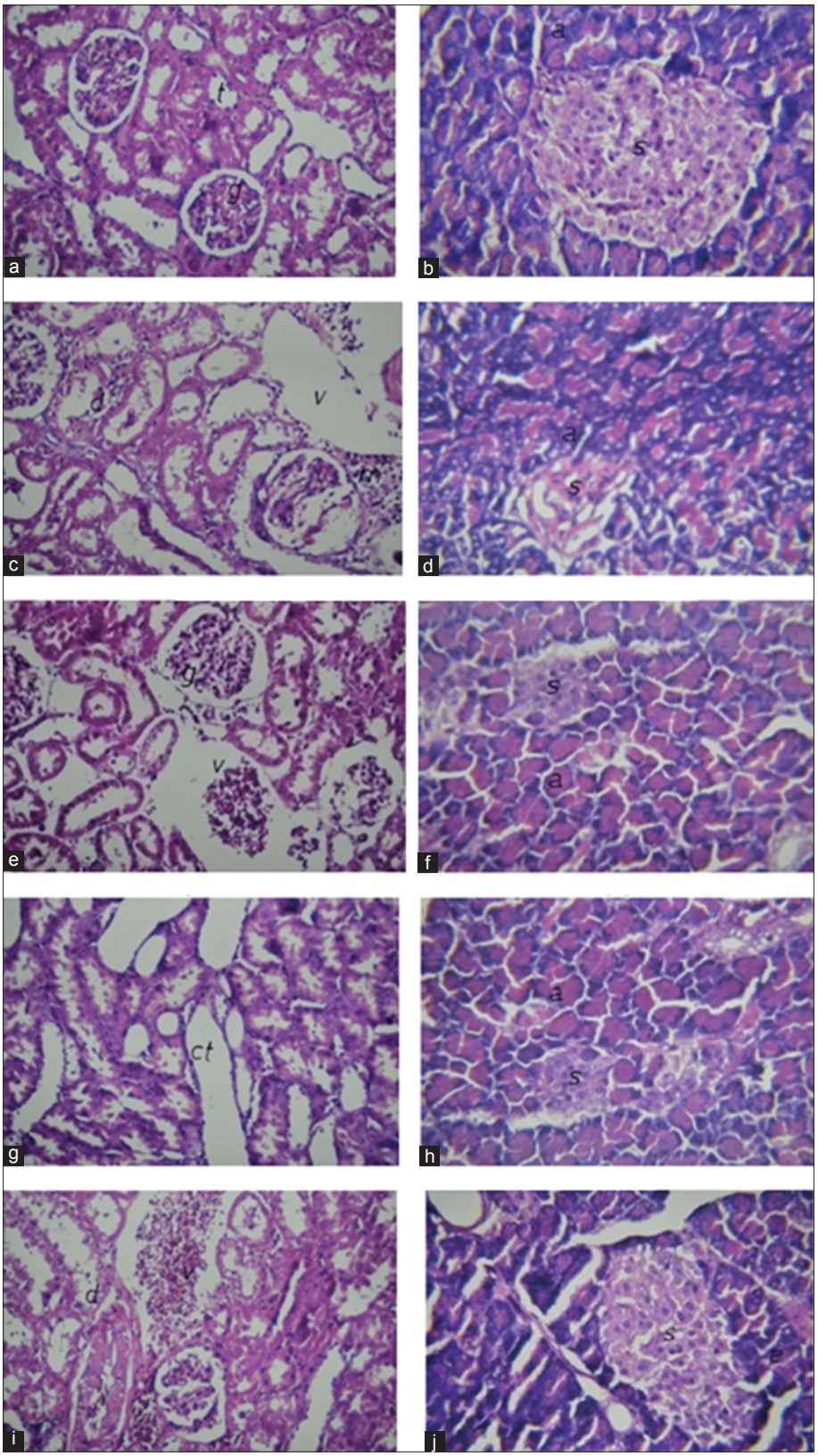

Figure 3: Kidney and pancreas sections collected from rats received vehicle, rats injected by streptozotocin (STZ) without treatment and rats treated by either curcumin, irbesartan or both. (a) Kidney section of vehicle group. (b) Pancreatic section of vehicle group. (c) Kidney section of STZ. (d) Pancreatic section of STZ group. (e) Kidney section of curcumin group. (f) Pancreatic sections of curcumin group. (g) Kidney sections of irbesartan group. (h) Pancreatic section of irbesartan group. (i) Kidney section of combination. (j) Pancreatic section of combination group

In irbesartan group, kidney tissues showed cystic dilatation with flattened lining epithelium of some tubules [24] at the corticomedullary portion. While the pancreatic tissues in irbesartan group showed moderate atrophy in the islands of Langerhans cells as illustrated in Figure 3g and h, respectively.

In the combination group, interesting findings were observed. Only congestion in the cortical blood vessels associated with degeneration in the lining tubular epithelium was observed in kidney tissue as shown in Figure 3i. While the normal histological structure of islands of Langerhans cells was observed in the pancreatic tissues of combination group as illustrated in Figure 3j.

DISCUSSION

This study evaluates the impact of chronic administration of curcumin and/or irbesartan in the management of diabetes mellitus through their effect on blood glucose level, pro-inflammatory cytokines as IL-6, IR, and through their expected ability to control the kidney function impairment in experimentally induced diabetic rats. In this study, irbesartan, curcumin, and combination groups failed to stop the weight loss resulted from dehydration and excessive loss of glucose in urine which was highly observed in STZ group more than other groups while the minimum weight loss was observed with the use of curcumin and irbesartan as a combination therapy.

These results were in agreement with Adibian *et al*. [25] and Yu *et al*., [26] who reported the same results. Curcumin therapy significantly reduces serum glucose in curcumin and combination groups as compared to STZ group which was in agreement with the results obtained by Miao *et al*. [27]. There is a discrepancy between our data and finding of Abdel Aziz *et al*. [28] that show no significant decrease in blood glucose level with the use of curcumin. However, in both studies, the treatment with curcumin was not enough to lower glucose level to normal levels. Irbesartan alone shows significant reduction of serum glucose as compared to STZ. This result was disagreed with Onishi *et al*. [29] and agreed to Mahfouz [19] who reported a significantly lower serum glucose with irbesartan treatment.

In this study, combination treatment could synergistically and significantly reduce serum glucose as compared to each drug alone which was corresponding to the findings obtained from Allah and El-Debakey [30]. In the present study, the serum insulin level of curcumin and combination group was significantly higher as compared to STZ group. This role of curcumin agreed with that reported by Pari and Murugan [31]. In support of the reported results, HOMA-IR indices were significantly improved in irbesartan, curcumin, and combination groups as compared to STZ group, with the effect was more significantly pronounced with combination therapy. A study by Huang *et al*. [32] explained irbesartan role as irbesartan increases the blood flow of pancreas and pancreatic islets leading to enhance late-phase insulin secretion and improve glucose tolerance.

Curcumin significantly decreases lipid peroxidation, increases intracellular antioxidant as GSH, regulates antioxidant enzymes, and scavenges hyperglycemia-induced ROS [33]. Induction of diabetes using STZ leads to increase IL-6 due to inflammation, especially in the organs directly affected by beta cell destruction and high glucose levels such as kidney and pancreas. This explains the elevated level of IL-6 in STZ injected animals and the rule of IL-6 to estimate the drug efficacy [34]. In addition, curcumin is shown to inhibit the pro-inflammatory transcriptional factors, nuclear factor-κB, and activator protein1, responsible for cytokine transcription [16]. Cytokines such as IL-6 have an important role in cellular proliferation and survival. Overexpression of IL-6 has been observed in patients with inflammatory or degenerative diseases such as diabetes. IL-6 can be used as a marker of inflammatory diseases. Curcumin could produce their protective effect by inhibiting the production of IL-6 [12].

Our study demonstrated significant improvement of serum and tissue IL-6 in animals injected with STZ and then, treated by irbesartan and/or curcumin. Our study showed that strong reduction in serum and tissue IL-6, with the use of combination treatment, more than each medication alone while curcumin alone showed a significant reduction than irbesartan and STZ group which was in agreement with the data obtained from Aggarwal and Harikumar [35] and Jain *et al*., [36]. The highly significant reduction observed with combination group may be explained by the presence of a synergistic effect between curcumin and irbesartan. Diabetes leads to small blood vessels injury. When kidney blood vessels injured, the kidney cannot clean blood properly; the body will retain more water and salt than it should, the presence of protein in urine may occur [37]. High glucose level leads to produce excess ROS that damage the kidney glomeruli leading to albuminuria [38]. Irbesartan as antihypertensive drug classified as renin-angiotensin II receptor antagonist.

In our study, irbesartan could reduce renal inflammation through the reduction of tissue's IL-6. This data is in agreement with the data of Negro [39]. In our study, treating animals with both curcumin and irbesartan may have a synergistic effect and significantly improve IR and IL-6 levels which were matching with results obtained from Mahfouz [19]. The kidneys maintain the blood creatinine in the normal range. Creatinine and urea are reliable indicators of kidney functions. Impaired kidney's function is one of the most diabetic complications that lead to abnormal elevation of creatinine and urea levels [40].

In the present study, we assessed the effect curcumin/irbesartan combination on diabetic complications through assessing the kidney function tests and histopathological examination of kidneys in addition to pancreatic tissue samples. In this study, treating diabetic animals with irbesartan alone improved kidneys function tests significantly which was in agreement with De Rosa *et al*. [41]. The histopathological examination results of the present study showed pancreatic and kidney tissues damage due to STZ induction and diabetic progression. In our study, curcumin in both curcumin and combination group improve and maintain the structure of pancreatic tissues which was

agreed with another study of Bonner-Weir and O'Brien [42] who found a structural improvement in pancreatic tissue with curcumin. In the present work, irbesartan alone or in combination with curcumin also improve and maintain the structure of kidney tissues which was corresponding to the recent study of Yu *et al*. [26] who found an improvement of kidney tissues with irbesartan treatment.

Interestingly, it was observed that curcumin/irbesartan combination has a promising effect on diabetic complication through a strong reduction in the kidney function tests as compared to either irbesartan or curcumin alone. Moreover, less damage was observed through histopathological examination of kidneys and pancreatic tissue sample as compared to either irbesartan or curcumin alone. Hence, further studies should be recommended to confirm the present study's new findings.

CONCLUSION

Curcumin has an important role in improving insulin sensitivity, reducing diabetic inflammation, and decreasing diabetic complications. Irbesartan also as one of angiotensin II receptor antagonist is very useful in protecting kidney and delayed diabetic nephropathy as one of the most dangerous complication of diabetes, especially with diabetic hypertension patients. In conclusion, the administration of irbesartan/curcumin combination showed antidiabetic effect through reducing blood glucose levels with improving insulin sensitivity, decreasing the increased serum and tissue levels of pro-inflammatory cytokines IL-6 and protecting the islands of Langerhans cells of the pancreatic tissues. Hence, the use of this combination could be recommended for clinical trials to document its use for control of diabetes mellitus in human, especially if diabetes associated with hypertension.

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