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Renoprotective Potential of Coenzyme Q10 and Quercetin in an Experimental Model of Carbon Tetrachloride–Induced Kidney Injury

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Abstract

Carbon tetrachloride (CCl_4)-induced nephrotoxicity is a long-established model of oxidative stress-induced renal injury. This paper compared the protective properties of quercetin and coenzyme Q10, singly and in combination with CCl_4 to produce renal dysfunction in rats, using silymamarin as a control. The study was done on 42 male rats that were distributed into six groups and treated over a period of 60 days. Renal condition was determined through serum urea, creatinine and cystatin C whereas oxidative stress and inflammation was measured using malondialdehyde (MDA), reduced glutathione (GSH), HMGB1 and TNF-alpha. To determine the correlations between biochemical changes and structural damage, histopathological analysis was conducted. Administration of CCl_4 caused significant renal dysfunction, oxidative imbalance, and inflammatory activation, with glomerular and tubular damage. Quercetin or coenzyme Q10 pretreatment had a great effect in ameliorating these changes. It is worth noting that combined therapy had a better protective effect, normalizing renal biomarkers and tissue architecture to near-normal levels, similar to or better than silymarin. The results underscore the potential of combined antioxidant therapy as synergistic nephroprotectant.

Keywords: Carbon tetrachloride, Nephrotoxicity, HMGB1, Cystatin C, Oxidative stress.

القدرة الوقائية للكلى للإنزيم المساعد *Q10* والكيرسيتين في نموذج تجريبي لإصابة الكلى الناجمة عن رابع كلوريد الكربون

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الخلاصة

يُعدّ رباعي كلوريد الكربون (CCl_4) نموذجًا تجريبيًا معروفًا لإحداث السمية الكلوية المرتبطة بالإجهاد التأكسدي. هدفت هذه الدراسة إلى مقارنة التأثير الوقائي لكلٍ من الكويرسيتين (Quercetin) والإنزيم المساعد Q10 (Coenzyme Q10)، منفردين ومجتمعين، ضد الخلل الكلوي المستحث بـ CCl_4 في الجرذان، مع اعتماد السليمارين (Silymarin) كمرجع وقائي. شملت الدراسة 42 جرذًا ذكرا وُزّعت إلى ست مجموعات وعولجت لمدة 60 يومًا. تم تقييم وظائف الكلى عبر قياس اليوريا والكرياتينين والسيستاتين C في المصل، بينما قُيّم الإجهاد التأكسدي والالتهاب من خلال المالونداياليديهايد (MDA) والكلوتاثيون المختزل (GSH) وHMGB1 وTNF-α)، كما أُجري فحص نسجي لتحديد الارتباط بين التغيرات الكيميائية والبنيوية. أدى إعطاء CCl_4 إلى خلل كلوي واضح، واختلال تأكسدي، وتنشيط التفاعل الالتهابي مع أضرار في الكبيبات والنبيبات. في المقابل، حسّن الكويرسيتين وQ10 هذه التغيرات بشكل ملحوظ، بينما أظهرت المعالجة المشتركة تأثيرًا وقائيًا أعلى، إذ أعادت المؤشرات الحيوية والبنية النسيجية إلى مستويات قريبة من الطبيعي، وبكفاءة مماثلة أو أفضل من السليمارين. تشير النتائج إلى فعالية المعالجة المشتركة كمقاربة تآزرية واعدة للحماية الكلوية.

1. Introduction

Kidney is a crucial organ which helps to maintain homeostasis in the internal condition and balance of fluids and electrolytes, metabolic waste products and acid-base equilibrium[1]. Kidney is highly susceptible to toxic damage due to its high blood perfusion rate and active involvement in the filtration of xenobiotics and their metabolites in the circulation[2]. Nephrotoxicity that is produced experimentally by using chemical agents is thus vital in the study of the pathophysiology of damage to kidneys and in assessing nephroprotective drugs[3]. Carbon tetrachloride (CCl_4) is a famous toxic substance that has been widely used in laboratory experiments to cause oxidative damage to different organs[4]. Even though its hepatotoxicity has been well-documented, growing evidence suggests that CCl_4 also causes serious renal damage[5]. After metabolic activation, CCl_4 forms highly reactive levels of free radical intermediates that reduce oxidative stress, damage to cell membranes, mitochondrial dysfunction, and induction of inflammatory reactions in kidney tissue. All these occurrences culminate to both functional and structural changes in the kidney[6, 7].

The oxidative stress is a key factor in the nephrotoxicity caused by CCl_4, Overproduction of reactive oxygen species saturates the endogenous antioxidant defenses, which leads to lipid peroxidation, damage to mitochondria, and cell damage in the kidney[8]. Simultaneously, oxidative damage triggers inflammatory processes, which only enhance tissue damage propagation and cause development of renal dysfunction[9]. The tight interrelation between inflammation and oxidative stress is one of the mechanisms involved in toxic kidney injury. Since oxidative and inflammatory processes play a central role in nephrotoxicity, anti-oxidant and anti-inflammatory agents have gained much interest as potentially protective agents[10].

Quercetin is the naturally occurring flavonoid that is found in great quantities in fruits and vegetables and is known to possess strong antioxidant and anti-inflammatory effects[11]. Quercetin is revealed to be capable of scavenging of ROS, mitochondrial protection, and regulating of inflammatory signaling, and thus offer protection against oxidative damage in kidney[12]. Coenzyme Q10 (CoQ10) is an endogenous lipid soluble molecule that is found majorly in the mitochondrial membrane, which is critical in the generation of energy in the cell[13]. CoQ10 has a strong antioxidant effect on top of its bioenergetic activity, where it is good to stabilize mitochondrial membranes and reduce the extent of oxidative damage. They have been shown to have an experimental effect in reducing renal damage in a variety of models of oxidative and toxic stress when CoQ10 is supplemented[14].

Silymarin is a flavonolignan complex that is extracted in Silybum marianum, which is commonly used as a reference antioxidant and cytoprotective agent[15]. In addition to its well-known hepatoprotective properties, silymarin has been described to have nephroprotective effects owing to its antioxidant, anti-inflammatory, and membrane-stabilizing effects and therefore serves well as a standard comparator in nephrotoxicity research[16]. Although there has been increasing evidence in support of the protective effects of quercetin, Coenzyme Q10 and silymarin, there are still limited comparative studies assessing the effects of these agents in comparison with the effects of CCl_4 -induced renal injury under identical experimental conditions. Thus the current research was aimed at exploring the CCl_4 induced nephrotoxicity in rats, and the protective properties of quercetin and Coenzyme Q10 individually and in combination with silymarin as a reference drug.

2. Materials and Methods

2.1 Ethical Approval

The Ethical Approval Committee of the University of Anbar gave its consent to this study No. 98, dated 9/10/2025). Any experimental procedure was carried out in compliance with the available ethical guidelines of the treatment and use of laboratory animals. There was an attempt to reduce the suffering of the animals and treat them humanely during the study. The study was conducted in an academic supervision and in accordance to the institutional standards.

2.2 Experimental Animals

Forty-two (42) adult male rats were utilized in this study, and their weight was 245-280g. Animals were kept in the animal house in the Department of Biology, College of Education for Pure Sciences, University of Anbar, Iraq. Rats were kept under the usual laboratory conditions (22 o C, 50-60 percent humidity), a light/dark cycle or 12 hours light/ 12 hours dark, and given standard pellet diet and water *ad libitum*. One week before the experiment, the animals were acclimatized.

2.3 Chemicals

Coenzyme Q10 (CoQ10), carbon tetrachloride (CCl_4), quercetin, and silymarin were of analytical grade and were purchased commercially. CCl_4 was prepared as a 50% (v/v) solution in olive oil and used fresh before the administration[17]. Both quercetin and coenzyme Q10 and silymarin were completely dissolved in the lipid based carrier, olive oil, which was used to enhance the absorption of the two. Quercetin 50 mg/kg has been extensively studied with regard to its ability to effectively reduce oxidative stress and hepatic damage without causing toxicity[18]. Likewise, CoQ10 at a dosage of 30 mg/kg has been proven to enhance the function of mitochondria and minimize oxidative liver damage in experimental models [19]. The doses were thus chosen as the biologically effective and safe concentrations for testing the hepatoprotective activities of the individual and combined doses**.**

2.4 Experimental Design and Treatment Protocol

Following the acclimatization, the rats were randomly chosen into six groups (n = 7) and treated over 60 days.

G1 (Control): Rats were given drinking water and olive oil (3 mL/kg, orally).

G2 (CCl_4): CCl_4 (1 mL/kg) was intraperitoneally injected twice a week into the rats that had been diluted 1:1 (v/v) with olive oil.

G3 (Quercetin + CCl_4): In G3, rats were given quercetin (50 mg/kg, orally) daily, but 1 h after oral administration, CCl_4 was injected as it was in G2.

G4 (CoQ10 + CCl_4): Coenzyme Q10 (30 mg/kg) was orally administered to the rats once daily followed by CCl_4 injection after 1 h as in G2.

G5 (Quercetin + CoQ10 + CCl_4): Rats were administered to oral quercetin (50mg/kg) combined with Coenzyme Q10 (30mg/kg) once daily after 1 h of treatment with CCl_4. G6 (Silymarin + CCl_4): Rats were given silymarin (100 mg/kg) orally once a day, and then 1 h later, CCl_4 was injected as in G2.

2.5 Sample preparation

Blood samples were taken at the expiration of the experimental time, and left to clot at room temperature, then centrifuged (3000 rpm 10 to 15 min) to get serum. In the case of oxidative stress, kidney tissues were dissected, rinsed in ice-cold normal saline, blotted on a towel and homogenized (10% w/v) in cold phosphate-buffered saline (PBS, pH 7.4). Homogenates were centrifuged (10,000g per 10-15 min with 4 o C) and the supernants employed in biochemical analysis.

2.6 Renal Function Biomarkers

2.7 Urea and Creatinine

Serum urea and creatinine were measured by enzymatic colorimetric kits (Randox Laboratories, UK) as previously described. The absorbance was read at the specified wavelengths and concentrations determined from the equations of the calibration curves[20].

2.8 Cystatin C

The findings were measured by ELISA kit (Elabscience Biotechnology, China) before the manufacturer protocol, using serum cystatin C. In short, antibody-coated wells were added with standards and samples, incubated and washed and then enzyme conjugate and substrate were added. The optical density was obtained at 450 nm and the concentrations calculated using the standard curve.

2.9 Inflammatory Biomarkers(HMGB1, TNF-α)

The level of serum high mobility group box 1 (HMGB1) was measured with the aid of a rat HMGB1 ELISA kit (ELK Biotechnology, China). Following the incubation of standards/samples in pre-coated plates, washing procedures and substrate development, a 450 nm absorbance was taken and concentrations were determined by use of standard curve.

The concentration of the serum tumor necrosis factor-alpha (TNF-α) was measured according to the instructions of the rat TNF-α ELISA kit (ELK Biotechnology, China). The optical density was read at 450 nm and the concentrations were determined from the standard curve.

2.10 Malondialdehyde (MDA)

The amounts of renal malondialdehyde (MDA) were obtained as per the procedure reported by[21] .The principle on which this assay was founded is the interaction of MDA with thiobarbituric acid (TBA) to form a colored complex that is detected spectrophotometrically.

2.11 Reduced glutathione (GSH)

The concentration of reduced glutathione (GSH) in kidney homogenates was measured using a colorimetric kit based on the DTNB (Ellman's reagent) reaction (Cayman Chemical, USA) according to the manufacturer's protocol. The absorbance was read at the appropriate wavelength (405-412 nm) and the GSH concentration was determined from the standard curve provided. In some cases, the data were expressed as per mg of protein[22].

2.12 Histopathological Examination

The rats were sacrificed and the kidneys were thoroughly removed and they were flushed with normal saline to eliminate blood traces. This was followed by fixation of the tissues in 10 percent neutral buffered formalin to ensure the tissue was well preserved. After fixation, the samples were then dehydrated in a series of ethanol and cleared in xylene and embedded into paraffin wax. With a microtome, paraffin blocks were sliced into thin sections about approximately 4-5 µm. The sections were then placed on glass slides, deparaffinized, rehydrated and stained with hematoxylin and eosin (H&E) and examined under a light microscope under the histopathology[23].

2.13 Statistical Analysis

The results were represented as mean standard deviation (SD). Statistical package for the social sciences (SPSS) version 26.0 software was utilized in the statistical analysis. One-way analysis of variance (ANOVA) was used to evaluate the differences between experimental groups, and then Tukey used the post hoc test to compare the multiple results. The value p of 0.05 was taken as statistically significant.

3. Results

3.1 Effect of treatments on serum urea and creatinine levels

Administration of carbon tetrachloride (CCl_4) led to substantial rise in serum urea and creatinine levels relative to the control (Figure 1 and 2) indicating that there was a marked impairment of the renal function. This increase indicates decreased renal clearance and impaired glomerular filtration that develops after CCl_4-induced nephrotoxicity. As a result, pretreatment with quercetin or Coenzyme Q10 resulted in a substantial drop of serum urea and creatinine levels in comparison to the CCl_4 -treated group, indicating a partial recovery of renal functional capacity. The quercetin and Coenzyme Q10 combination showed a stronger reduction in the two parameters with the values being close to the control group. Silymarin-treated group also significantly improved the level of serum urea and creatinine in comparison to the CCl_4 group.

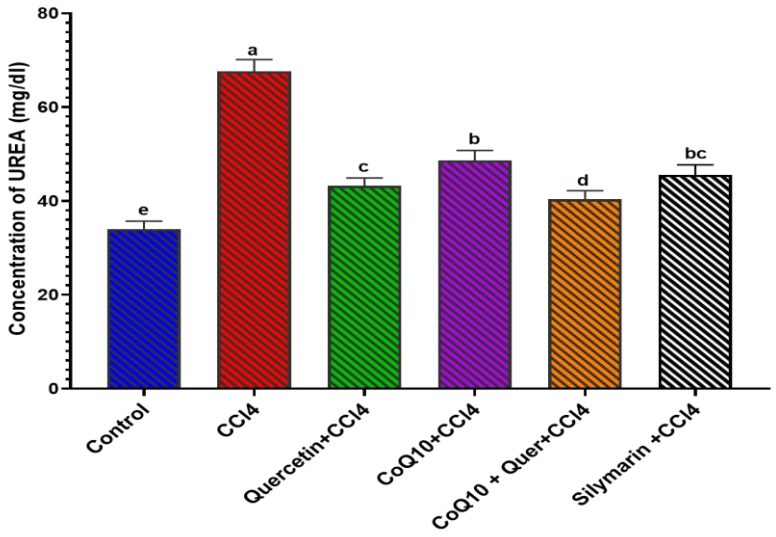

Figure -1 illustrates the effect of different treatments on serum urea concentration in male rats treated with CCl_4. The values are expressed as (Mean ± SD) for a sample size of (n = 7), and different letters indicate significant differences at a significance level of ($P < 0.05$).

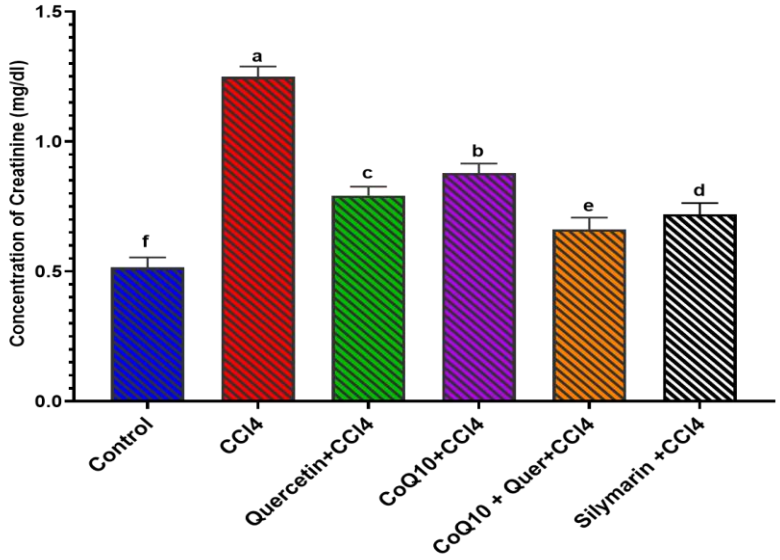

Figure -2 illustrates the effect of different treatments on serum Creatinine concentration in male rats treated with CCl_4. The values are expressed as (Mean ± SD) for a sample size of (n = 7), and different letters indicate significant differences at a significance level of ($P < 0.05$).

3.2 Effect of treatments on serum cystatin C levels

Serum cystatin C level was much higher in the treated group with CCl_4 than in the control group depicting the fact that there was a sensitive and early hindrance of the renal filtration function. Quercetin or Coenzyme Q10 treatment also caused a significant reduction in cystatin C levels when compared with the CCl_4 group indicating effective protection of renal dysfunction induced by CCl_4. It was interesting to note that the highest drop in the levels of cystatin C was recorded in the combination treatment group which was almost similar to the

control group. On the same note, high doses of silymarin had a significant effect in reducing the level of cystatin C that was instigated by CCl_4.

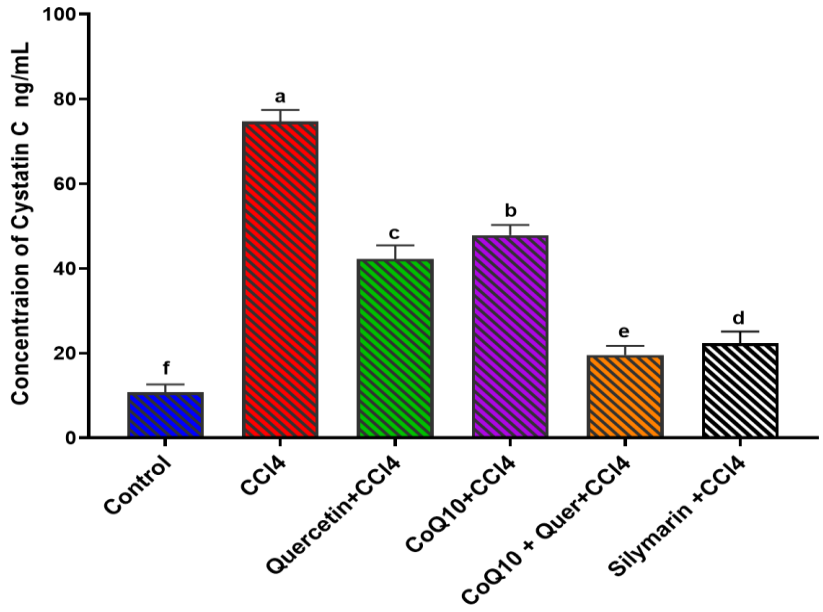

Figure-3 illustrates the effect of different treatments on Cystatin C concentration in male rats treated with CCl_4. The values are expressed as (Mean ± SD) for a sample size of (n = 7), and different letters indicate significant differences at a significance level of ($P < 0.05$).

3.3 Effect of treatments on renal oxidative stress markers

As shown in Figure 4 and 5, carbon tetrachloride (CCl_4) administration found a high increase in renal malondialdehyde (MDA) level than the control group, suggesting greater lipid peroxidation and oxidative stress. At the same time, the depletion of endogenous antioxidant defenses was substantially reduced in the CCl_4-treated group, which was characterized by the minimized levels of glutathione (GSH). Pretreatment using quercetin or Coenzyme Q10 resulted in a significant decrease in MDA and an enormous replenishment of GSH content in comparison to the CCl_4 group. The Coenzyme Q10 presence with quercetin gave the strongest effect on the oxidative stress parameters as both MDA and GSH levels were close to the levels found in the control group. On the same note, the level of oxidative stress was considerably reduced by administration of silymarin, as indicated by reduced levels of MDA and increased levels of GSH in comparison to those of CCl_4-treated group.

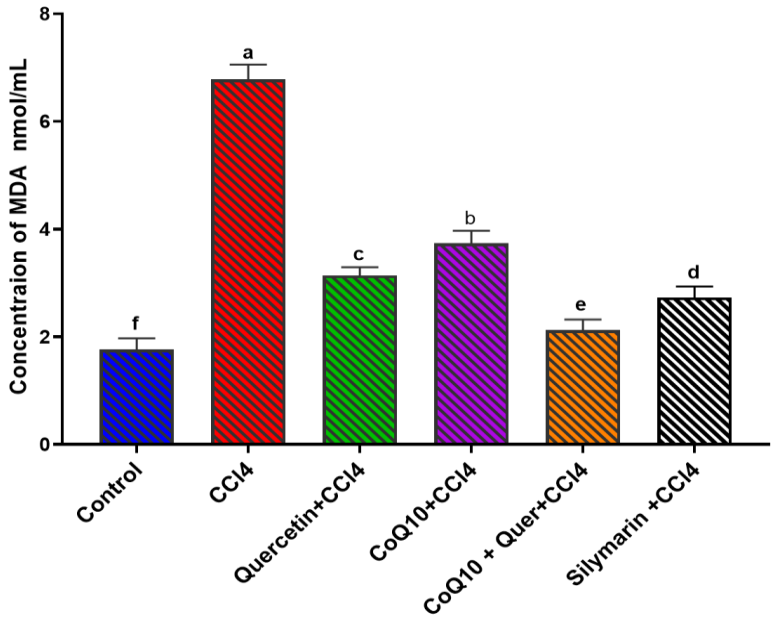

Figure -4 illustrates the effect of different treatments on MDA level in male rats treated with CCl_4. The values are expressed as (Mean ± SD) for a sample size of (n = 7), and different letters indicate significant differences at a significance level of ($P < 0.05$).

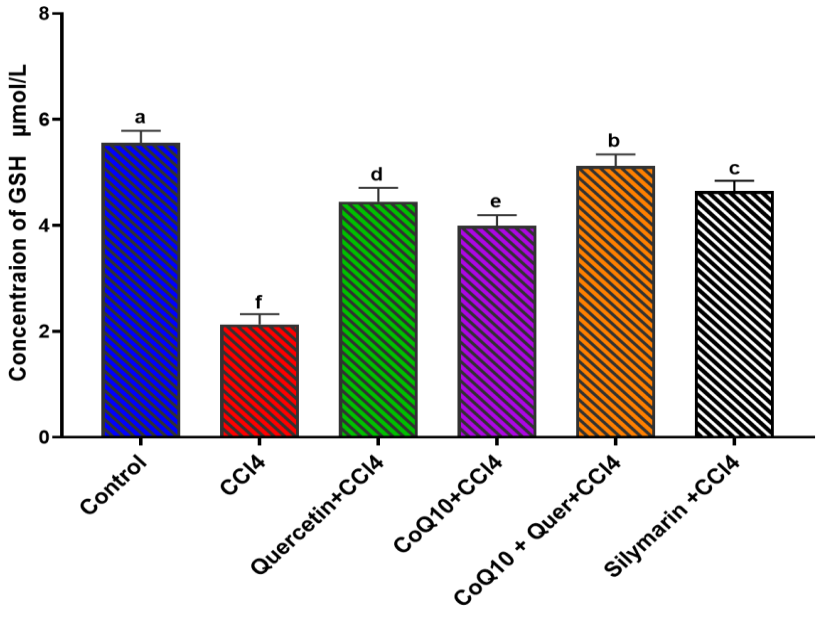

Figure -5 illustrates the effect of different treatments on GSH level in male rats treated with CCl_4. The values are expressed as (Mean ± SD) for a sample size of (n = 7), and different letters indicate significant differences at a significance level of ($P < 0.05$).

3.4 Effect of treatments on inflammatory biomarkers

The results presented in Figure 6 and 7 reveal that administration of carbon tetrachloride (CCl_4) led to a significant rise in serum high mobility group box 1 (HMGB1) and (TNF-α) levels relative to the control group, suggesting that inflammatory processes were triggered by carbon tetrachloride (CCl_4). Quercetin or Coenzyme Q10 pretreatment had a significant effect on CCl_4-induced increment of TNF-α and HMGB1. The quercetin and Coenzyme Q10 combination treatment was the most effective in reducing both inflammatory markers with few levels that could be compared to control group. On the same note, the administration of silymarin drastically decreased the HMGB1 and TNF-α levels in comparison to the CCl_4-treated group.

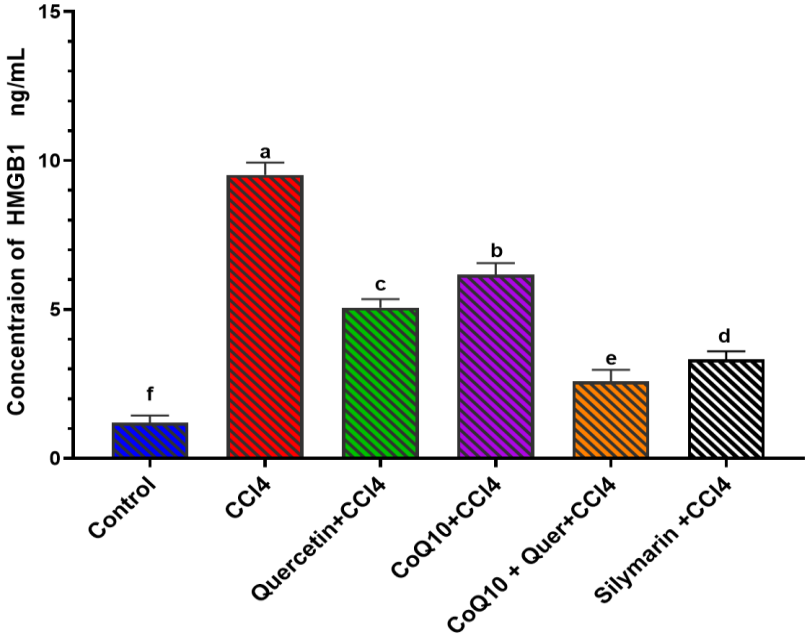

Figure -6 illustrates the effect of different treatments on HMGB1 level in male rats treated with CCl_4. The values are expressed as (Mean ± SD) for a sample size of (n = 7), and different letters indicate significant differences at a significance level of ($P < 0.05$).

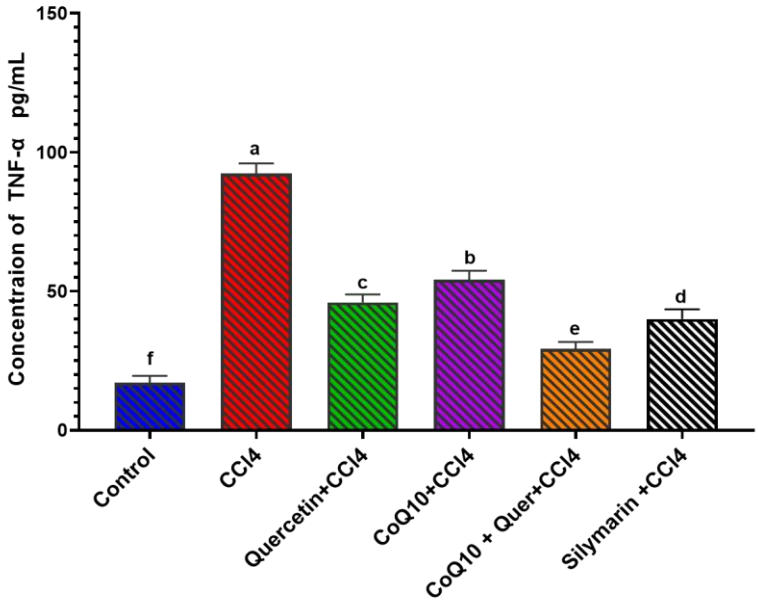

Figure -7 illustrates the effect of different treatments on TNF-α level in male rats treated with CCl_4. The values are expressed as (Mean ± SD) for a sample size of (n = 7), and different letters indicate significant differences at a significance level of ($P < 0.05$).

3.5 Histopathological Findings:

Renal tissue samples of the control group (Figure 8) demonstrated the normal renal architecture, with the clearly defined glomeruli, intact Bowman's capsule and space, as well as normal convoluted tubules. Conversely, the CCl_4-treated group (Figures 9-12) had gross histopathological changes such as the glomerular congestion, distortion and shrinkage, and expansion of the Bowman space. Renal tubules had hydropic degeneration, significant dilatation, and cellular debris deposition in the lumen of the tubules. As well, there was a severe hemorrhage, inflammatory infiltration, and massive cellular necrosis that revealed the presence of major tissue damage in the renal tissues and a disturbance of normal histological organization. Quercetin pretreatment (Figure 13) did lead to a partial improvement in the renal histology with glomerular and tubular structures relatively intact, though glomerular congestion, hydrotrophy and tubular dilatation persisted. In the same way, CoQ10 treated group (Figure 14) showed enhanced renal architecture, where glomerular structure is almost normal, but the Bowman space and tubules are not destroyed, although there is a slight vascular congestion. Interestingly, the treatment of quercetin and CoQ10 (Figure 15) demonstrated a stronger protective effect, as kidney sections of the treatment appeared to have almost normal histological findings, such as intact glomeruli and tubules, with only mild congestion of the glomeruli and slight inflammatory infiltration in contrast to the CCl_4 treated group. The silymarin-treated group had an almost normal renal histological architecture demonstrating a significant protective effect. Nevertheless, there was still mild inflammatory infiltration with slight hemorrhage (Figure 16).

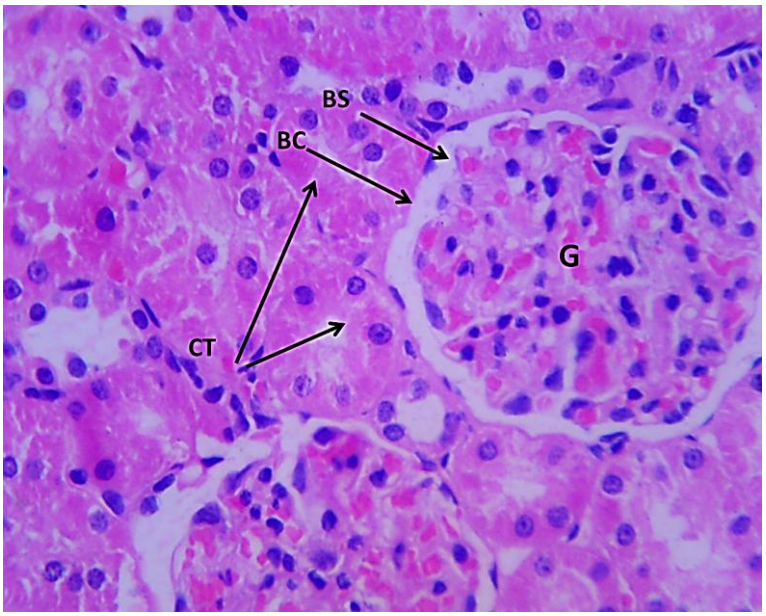

Figure-8 A cross-sectional view of the kidney from the control group, illustrating the glomerulus (G), Bowman's capsule (BC), Bowman's space (BS), and the convoluted tubules (CT). (Stained with hematoxylin and eosin; magnification ×400).

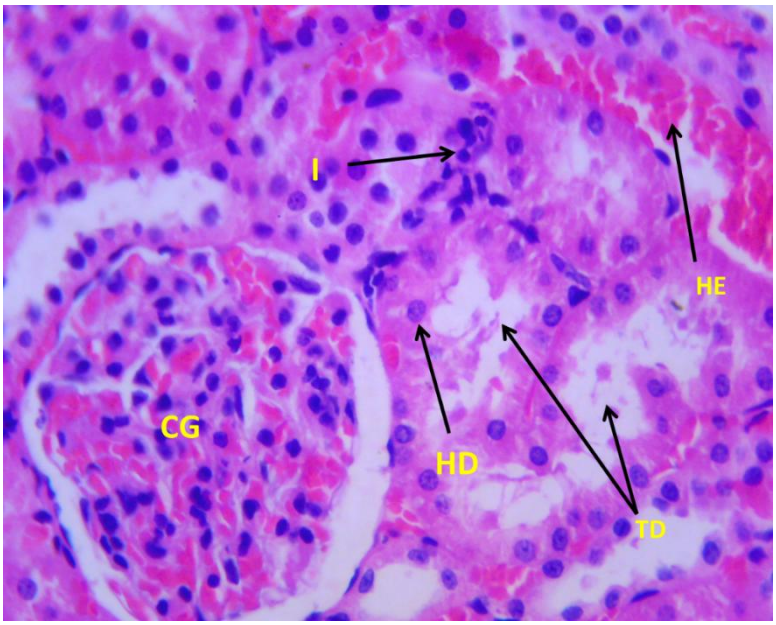

Figure -9 A cross-sectional view of the kidney from the CCl_4-treated group, showing glomerular congestion (CG), hydropic degeneration (HD), severe hemorrhage (HE), and tubular dilatation (TD). (Stained with hematoxylin and eosin; magnification ×400).

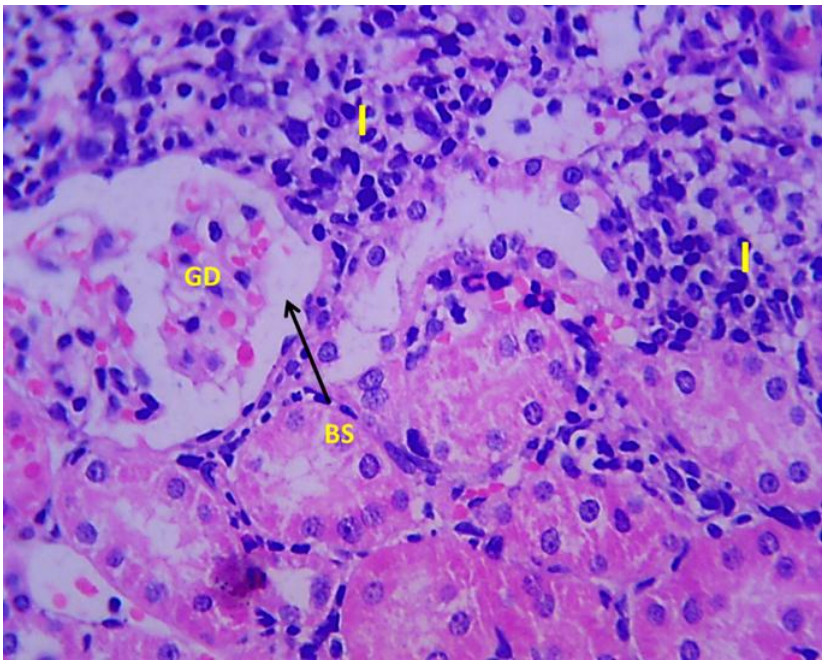

Figure-10 A cross-sectional view of the kidney from the CCl_4-treated group, showing glomerular distortion (GD), dilation of Bowman's space (BS), and severe inflammatory infiltration (I). (Stained with hematoxylin and eosin; magnification ×400).

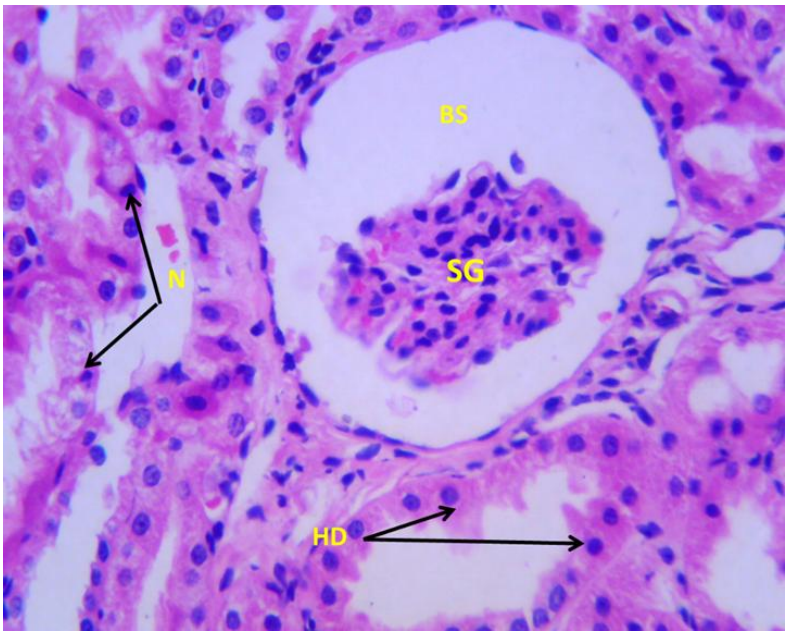

Figure-11 A cross-sectional view of the kidney from the CCl_4-treated group, showing glomerular shrinkage (SG), dilation of Bowman's space (BS), hydropic degeneration (HD), and cellular necrosis (N). (Stained with hematoxylin and eosin; magnification ×400).

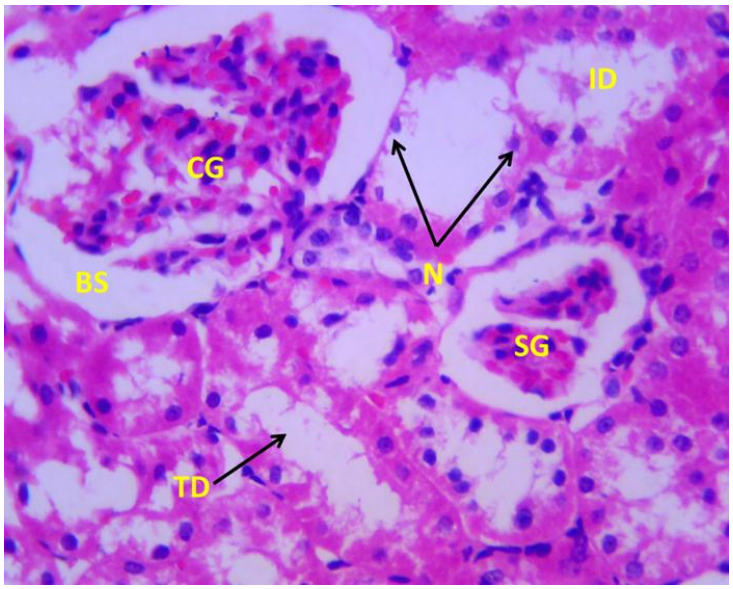

Figure-12 A cross-sectional view of the kidney from the CCl_4-treated group, showing glomerular shrinkage (SG) and congestion in other glomeruli (CG), dilation of Bowman's space (BS), cellular necrosis (N), tubular lumen dilatation (TD), and deposition of cellular debris within the tubules (ID). (Stained with hematoxylin and eosin; magnification ×400).

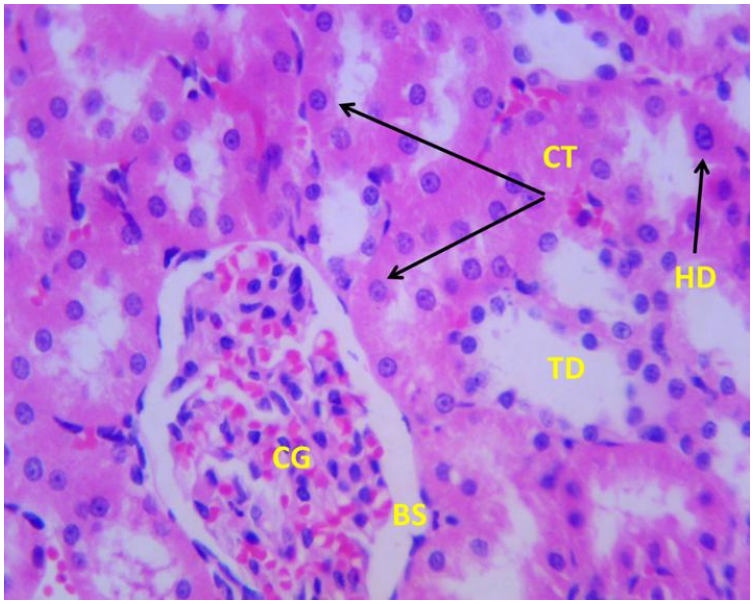

Figure -13 A cross-sectional view of the kidney from the (quercetin + CCl_4)-treated group, showing glomerular congestion (CG), Bowman's space (BS), convoluted tubules (CT), hydropic degeneration (HD), and dilatation of some tubular lumina (TD). (Stained with hematoxylin and eosin; magnification ×400)

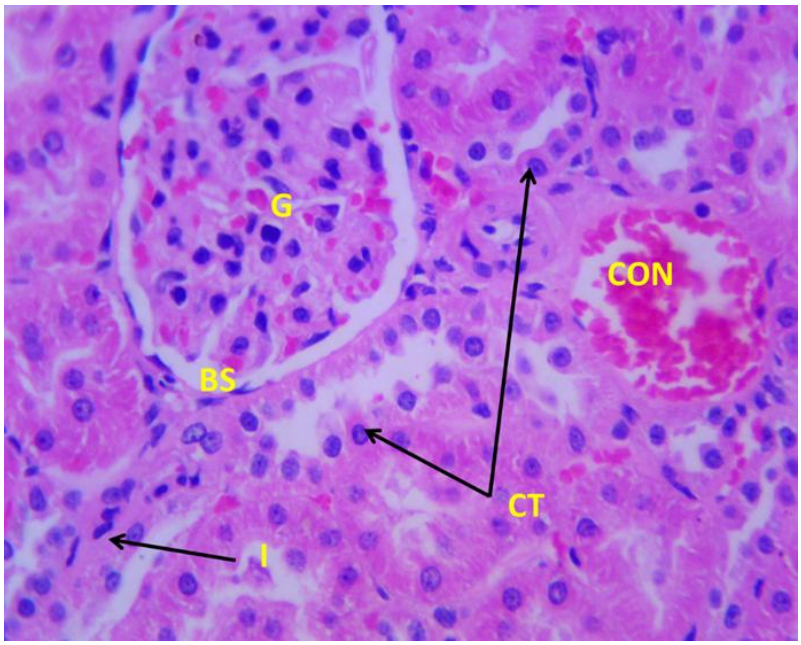

Figure -14 A cross-sectional view of the kidney from the (CoQ10 + CCl_4)-treated group, showing a normally structured glomerulus (G), Bowman's space (BS), convoluted tubules (CT), and vascular congestion (CON). (Stained with hematoxylin and eosin; magnification ×400).

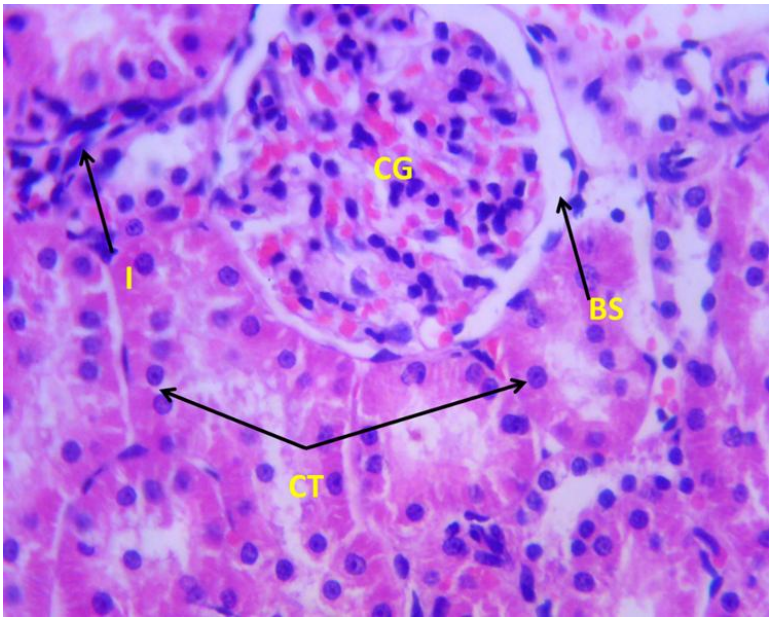

Figure -15 A cross-sectional view of the kidney from the (quercetin + CoQ10 + CCl_4)-treated group, showing mild glomerular congestion (CG), Bowman's space (BS), convoluted tubules (CT), and inflammatory infiltration (I). (Stained with hematoxylin and eosin; magnification ×400).

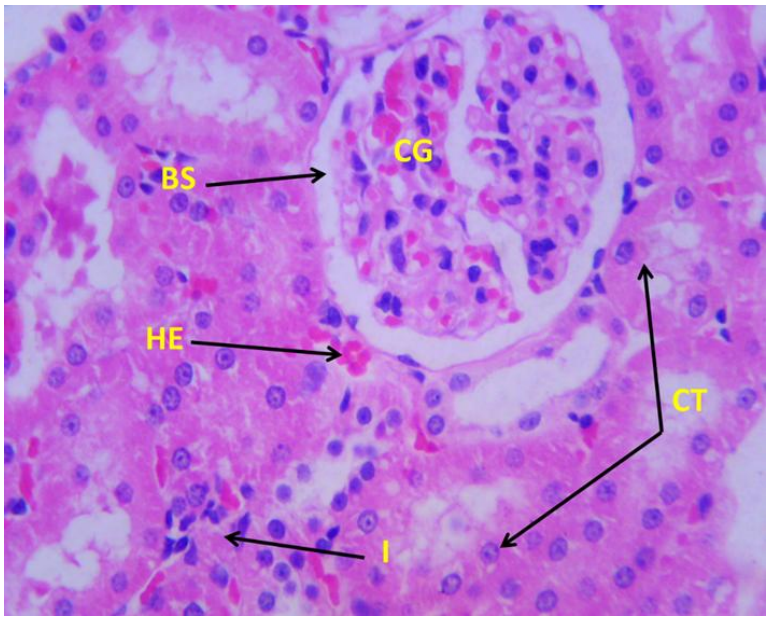

Figure -16 A cross-sectional view of the kidney from the (silymarin + CCl_4)-treated group, showing glomerular congestion (CG), Bowman's space (BS), convoluted tubules (CT), inflammatory infiltration (I), and hemorrhage (HE). (Stained with hematoxylin and eosin; magnification ×400).

4.Discussion

The current experiment has shown that the exposure to carbon tetrachlorine (CCl_4) led to severe renal impairment as the serum urea and creatinine levels were high. This effect may be explained by CCl_4-induced oxidative stress, in which formed free radicals result in the destruction of glomerular and tubular systems, which results in the lowering of the glomerular filtration rate and the inability of renal clearance. Moreover, lipid peroxidation and membrane damage are also to the development of nitrogenous waste products in the blood stream[17, 24].

Coenzyme Q10 pretreatment had a significant effect on the reduction of urea and creatinine levels and could be attributed to the fact that it enhanced the work of the mitochondrion and decreased the oxidative stress[25]. CoQ10 stabilizes the mitochondrial membranes, boosts energy production, and maintains renal cellular activity, as well as facilitating normal filtration[26]. On the other hand, quercetin treatment also resulted in a significant decrease in these renal biomarkers. This effect can be explained by the fact that it possesses strong antioxidant and anti-inflammatory effects, and it can help to eliminate free radicals, produce less oxidative damage, and preserve renal tissues against structural damage[27].

Urea and creatinine levels were the most reduced when quercetin and CoQ10 were used together, which shows that there was a synergistic effect. This mixture improves antioxidant defenses and gives the glomerular and tubular functions better protection against CCl_4-induced nephrotoxicity[5]. Moreover, the parameters of renal functioning were also significantly improved in the silymarin-treated group. This can be attributed to its high antioxidant effects, stabilizing membrane effects and capacity to stimulate cellular repair and regeneration thus enhancing the overall functioning of the kidney[28].

A sensitive early kidney dysfunction marker, cystatin C, was greatly increased after CCl_4 treatment, which means that there was early glomerular filtration dysfunction with slight structural destruction. This can be explained by the fact that the injury to the filtration capacity of the kidneys under the influence of oxidative stress is elevated[10]. Coenzyme Q10 therapy led to significant decrease in cystatin C levels, which is probably because of preservation of the mitochondrial activity and minimizing oxidative stress in the renal tissues[29]. Also, the administration of quercetin decreased the levels of cystatin C, which is also explained by the antioxidant and anti-inflammatory properties of the compound to preserve the integrity of the glomeruli[12]. It is worth noting that the synergy between quercetin and CoQ10 had the most remarkable effect on normalizing cystatin C, and thus it is possible that the combination of these two substances has a synergistic effect on early renal function and filtration. Moreover, cystatin C levels were also enhanced by silymarin therapy, which indicates its protective effect on renal structures and lessening the premature functional damage[30].

Oxidative stress is a key element of the pathogenesis of CCl_4-induced renal injury. Major increase in the levels of malondialdehyde (MDA) under the CCl_4-treated group indicates the increased lipid peroxidation and the serious oxidative injury to the membranes of the renal cells[31]. At the same time, endogenous antioxidant defenses are depleted as evidenced by depletion of reduced glutathione (GSH) as a result of excessive production of free radicals[9]. Coenzyme Q10 pretreatment significantly lowered the levels of MDA and the depleted GSH content, probably due to its ability to enhance the efficiency of the mitochondrion and reduce the extent of oxidative stress[32]. In the same way, the treatment with quercetin resulted in a marked decrease in lipid peroxidation and improvement of antioxidant ability that could be explained by the fact that quercetin possesses a strong free radical scavenging activity and regulates redox-sensitive pathways[33]. The quercetin and CoQ10 combination had the greatest effect on oxidative balance, which indicates that both quercetin and CoQ10 interact synergistically in strengthening the antioxidant defense mechanisms. Moreover, the same protective effect was also shown by silymarin through the reduction of the MDA level and an increase in the GSH content, which is not surprising in relation to its established antioxidant and membrane-stabilizing effects[34].

Activation of inflammatory pathways is closely linked to oxidative stress in CCl_4-induced renal injury. The marked increase in the levels of HMGB1 and TNF- α after exposure to CCl_4 shows the involvement of inflammatory cascades leading to renal cell damage and apoptosis[31]. Coenzyme Q10 treatment decreased these inflammatory indices, most probably by the power to counteract oxidative stress and consequently restrain inflammation[25]. Likewise, the use of quercetin led to a significant reduction in the level of HMGB1 and TNF-alpha and can be explained by its direct effect on pro-inflammatory signaling pathways[35]. The combination treatment group showed the most significant decrease in inflammatory mediators, which is indicative of synergy in regulating the inflammatory response and preserving renal tissues. Moreover, silymarin also reduced these markers significantly that indicates its strong anti-inflammatory and cytoprotective effects[36].

Histopathological observations were also used to support the biochemical results. Oxidative and inflammatory injury were observed because of severe renal damage in the CCl_4 treated group including congested glomeruli, tubular degeneration and inflammatory cell

infiltration[37]. Conversely, pre-treatment of rats with Coenzyme Q10 resulted in a visible improvement of renal structure with less tubular damage and partial remediation of glomerular integrity probable because of the preservation of mitochondrial function and decreased oxidative stress[38]. In the same vein, quercetin therapy led to an improvement in renal architecture and this could be explained by the fact that it is an antioxidant and anti-inflammatory agent and as such, it does not expose the renal tissues to structural damages[39]. The combined treatment group showed almost normal renal histology, which showed a high synergistic effect in terms of avoiding tissue damage. In addition, the silymarin-treated group showed significant histological improvement, which attests to the well-known nephroprotective activity of silymarin[40]. However, on the whole, these findings indicate that quercetin and CoQ10 have powerful protective properties in CCl_4-induced renal injury, especially when combined, as they can regulate oxidative stress, inflammation, and maintain renal structure and functionality.

5.Conclusion

This article has provided evidence of carbon tetrachloride's ability to cause severe renal injury through the initiation of persistent oxidative stress and inflammation. Quercetin and CoQ10 were significantly effective against the renal dysfunction and renal tissue damage caused by CCl_4. In particular, the optimal nephroprotective effect was obtained when Quercetin was administered in combination with Coenzyme Q10, demonstrating their synergistic protective activity, which was stronger than that of the standard compound Silymarin. The results of this study also validate this antioxidant combination as effective therapeutic approach to block toxic renal injury and retain renal function.

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