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Renoprotective potential of olive leaves extract against cadmium-induced chronic kidney damage

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ABSTRACT

Environmental pollution remains a major global health concern, particularly when it comes to heavy metals, which are associated with various health hazards. Among the prevalent heavy metals in the environment is cadmium (Cd). As a result, the current study aimed to investigate the nephroprotective potential of olive leaves extract (OLE) against Cd-induced chronic renal injury. Forty-eight albino rats were divided into four equal groups. Rats in the control group were received distilled water orally, while those in the OLE group were orally administered OLE 200 (mg/kg b.wt.) daily. The cadmium-intoxicated group rats were orally gavage 5 mg Cd/kg b.wt. twice a week and the OLE+Cd group received the same doses of both OLE and CdC_{l2}. After 4 and 8 weeks of the experiment, blood and kidney samples were collected for analysis of kidney function test and histopathological examination. Oral administration of cadmium chloride ($CdCl_2$) for 4 and 8 weeks resulted in nephrotoxicity, evidenced by a significant increase ($P<0.05$) in urea, uric acid, and creatinine levels. These biochemical changes were accompanied by distinct histopathological alterations, including renal hemorrhage, edema and vasculitis with severe glomerular and tubular necrosis as well as periglomerular fibrosis and marked interstitial fibrosis. Treatment with OLE exhibited a remarkable reduction in these histopathological changes and restored the serum parameters toward normal levels. In conclusion, OLE has a time-dependent mitigating effect against Cd-induced nephrotoxicity by reducing inflammation, glomerulotubular injury, and renal fibrosis, indicating that it is a potential natural product in counteracting chronic nephrotoxicity.

Introduction

Environmental pollution is one of the major issues facing modern society (Ali and Khan, 2017), which categorized as volatile organic compounds, dyes, pharmaceuticals, pesticides, industrial wastes and heavy metals that pose direct adverse effects on human and animal health (Rasheed *et al*., 2020). Heavy metals are particularly concerning due to their toxicity, persistence in the environment, contamination of food chains and bioaccumulative nature (Ali *et al*., 2019). One such hazardous heavy metal that has become a serious public health concern is cadmium (Hernández-Cruz *et al*., 2022). Despite its rarity, over 90% of environmental Cd originates from industrial and agricultural activities, which have significantly increased in the past century (Pan *et al*., 2010; Elinder and Traub, 2013). Consequently, both humans and animals face substantial health risks as a result of environmental Cd exposure, leading to renal dysfunction, osteoporosis, cardiovascular disease, and liver damage (Nawrot *et al*., 2010; Chargui *et al*., 2011; Rani *et al*., 2014). Notably, chronic Cd exposures are associated with reduced glomerular filtration rate and increased risk of chronic kidney disease (Zang *et al*., 2019). Although numerous studies have explored the cellular and molecular aspects of Cd-induced kidney damage, the specific pathways involved in Cd-induced renal injury remain unknown. One of the primary mechanisms underlying Cd-induced nephrotoxicity is oxidative stress (Wang *et al*., 2013; El-Boshy *et al*., 2015; Nimse and Pal, 2015) and the key feature of Cd-induced nephrotoxicity is apoptosis triggered by this oxidative stress, primarily affecting proximal tubular cells, leading to kidney dysfunction (Nemmiche *et al*., 2012; Yuan *et al*., 2014).

Cadmium induces severe functional and morphological changes in the kidney, necessitating the use of natural antioxidants to combat the free radical generation induced by Cd. Further research is needed to elucidate the potential therapeutic targets to mitigate the adverse effects of Cd exposure on kidney health. Natural antioxidants are widely used by the majority of people as they have few side effects (Unsal *et al*., 2020). In this context, Olive leaves extracts (OLE) have gained special interest due to their therapeutic properties, containing various biophenols such as phenolic acids, phenolic alcohols, flavonoids, and secoiridoids (oleuropein) (Sabry, 2014). These constituents possess antioxidative, antimicrobial, antiviral, antiatherogenic, antihypertensive, and anti-inflammatory properties (Şahin and Bilgin, 2018; Clodoveo *et al*., 2022). Thus, OLE could be an effective approach for preventing nephrotoxicity. OLE's beneficial effects extend not only to tubular cells but also to glomerular cells, resulting in a significant reduction in renal damage (Geyikoglu *et al*., 2017). Furthermore, OLE significantly prevented oxidative stress, inflammation and apoptosis (ALHaithloul *et al*., 2019). In the meantime, OLE significantly ameliorated renal dysfunction, and morphological alterations besides relieving oxidative stress against chronic cadmium exposure (Ranieri *et al*., 2019; Abugomaa and Elbadawy, 2020). Studies have shown OLE's protective effects against various renal injuries induced by carbon tetrachloride (Al-Sowayan and Mousa, 2014), diazinon (Al-Attar and Abu Zeid, 2013), doxorubicin (Kumral *et al*., 2015), cyclosporine (Mostafa-Hedeab *et al*., 2015), deltamethrin (Maalej *et al*., 2017) and streptozotocin (Al-Attar and Alsalmi, 2019).

Consequently, this research used rats as an experimental animal model to further verify the protective effects of OLE in alleviating the adverse effects of cadmium on kidney function and structure.

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Materials and methods

Chemicals

Cadmium chloride ($CdCl_2$) monohydrate 98% was obtained from LOBA CHEME PVT LTD, India. All chemicals used in this study were of high analytical grade and high quality.

Preparation of olive leaves extract

Fresh olive leaves (Olea europaea) were collected from Faculty of Agriculture, Benha University, Egypt. Botanical identification and authentication were performed by the Department of Horticulture, Faculty of Agriculture, Benha University.

The collected leaves were washed to remove any foreign matters, dried and grinded into fine powder. The resulting 600 g of dry powder was macerated with 10 L of 70% ethyl alcohol (B.P 78.37°C) twice for three days, six hours every day with a Heidolph stirrer. The alcoholic extract that resulted was decanted, filtered, and evaporated under vacuum using a Heidolph rotavapor until dryness, yielding the hydroalcoholic extract. The dried hydroalcoholic extract was then dissolved in 2% Tween 80 in distilled water with a sonicator at 50-55°C before being refrigerated at 4°C until use (Tavafi *et al*., 2012).

Animals

Forty-eight apparently healthy adult male albino rats weighing 200±10 g were obtained from the Nile Pharmaceutical Company. Al-Sawah Street, Al-Amiriya, Cairo, Egypt. These animals were housed in stainless steel wire cages with a controlled environment at 21±2°C and relative humidity at 50±5 under a 12 h dark and light cycle for one week of acclimatization. Rats were fed standard rat's ration and provided water ad libitum. The Ethics Committee of the Faculty of Veterinary Medicine, Benha University approved the protocol of the study (Ethical Approval Number BUFVTM 16-10-22).

Experimental design

Rats (n= 48) were randomly divided into four groups, (n= 12 in each group): In the first group (control), rats were daily orally received 1 ml of distilled water as a vehicle. In the second group (OLE) rats were orally administered OLE at a daily dose of 200 mg/kg bw (Liu *et al*., 2014). In the third group ($CdCl_2$), the rats received oral doses of $CdCl_2$ twice a week at a dose of 5 mg/kg bw (Sadek *et al*., 2017). In the fourth group (OLE + $CdCl_2$), rats were first given oral supplementation of OLE at a dose of 200 mg/kg bw day, followed by intragastric gavage of $CdCl_2$ at a dose of 5 mg/kg bw twice a week, one hour later. After the experiment period, blood samples and kidney specimens were collected for further analysis.

Body and kidney weights

All animals' body and kidney weights were recorded in this study. The body weight of each animal was recorded before and after treatment by placing it in a closed plastic container and weighing it with a digital balance. At the end of the experiment, the kidney weight and kidney weight ratio (kidney weight per body weight × 100) of each animal were calculated (Bharathiraja *et al*., 2013).

Blood sample collection and storage

Blood samples were taken from each rat after 4 and 8 weeks of treatment through retro-orbital venous plexus puncture under diethyl ether anesthesia of each rat. These blood samples were collected into gel and clot activator tubes, allowed to coagulate at room temperature. Following collection, the blood samples underwent centrifugation at 3000 g for 15 minutes, and the resulting serum was carefully transferred to clean and dry Eppendorf tubes. To ensure optimal preservation, the serum samples were stored at -20°C until further analysis. Quantitative analysis of serum uric acid, urea and creatinine was carried out using a semi-auto chemistry analyzer according to Caraway (1955); Jing *et al*. (2018) and Larsen (1972) respectively.

Histopathological analysis

During necropsy, kidney specimens were collected from rats and rapidly preserved in 10% neutral buffered formalin. The fixed specimens were dehydrated in ethyl alcohol, cleared in xylol, and embedded in paraffin wax. After that, 5 µm tissue paraffin sections were prepared and stained with H&E stain (Bancroft and Layton, 2019a). Histopathological changes were examined with a Nikon Eclipse E800 microscope and images were captured with an Olympus digital camera.

Van Gieson staining technique

Van Gieson staining technique was used to assess collagen deposition. Tissue sections were incubated for 2 minutes in 1% acid fuchsin in aqueous saturated picric acid. After staining, collagen fibers appear pink to red, whereas remaining tissue appears yellow (Bancroft and Layton, 2019b).

Statistical analysis

Statistical analysis was conducted using Prism GraphPad software version 9.0 (San Diego, California, USA). Statistical comparisons between the groups were performed using a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer post hoc test. The results were expressed as the mean ± SD. P-values ≤ 0.05 were considered statistically significant.

Results

Body and kidney weight changes

Data in Table 1 revealed that there was no significant decrease in body weight and kidney weight between $CdCl_2$-challenged rats and the control one after 4 weeks of treatment. While after 8 weeks of treatment, oral administration of $CdCl_2$ caused a significant ($P<0.05$) decline in body

Table 1. The effect of OLE and $CdCl_2$ on the final body and kidney weights after 4 and 8 weeks of treatment.

Groups	Body weight (g)		Kidney weight (g)	
	4 weeks	8 weeks	4 weeks	8 weeks
Control	274.8± 31.78	332.25± 14.67 [a]	1.683± 0.06504	2.0012± 0.1044 [a]
OLE	276.0± 27.06	335.01± 9.933 [a]	1.6913± 0.06893	2.0165± 0.2112 [a]
$CdCl_2$	265.3± 6.455	268.3± 23.26 [c]	1.541± 0.01396	1.5952± 0.1469 [c]
OLE+$CdCl_2$	272.2± 7.550	305.7± 4.933 [b]	1.648± 0.02170	1.903± 0.04233 [b]

Values (means ± SD) within the same column carrying different superscripts are significantly different ($p \leq 0.05$).

weight and kidney weight in intoxicated rats, when compared to control group. Treatment with OLE after 8 weeks significantly (P<0.05) increased the body weight and kidney weight toward normal levels. However, after 4 weeks treatment with OLE in Cd intoxicated group has no effect in body or kidney weights.

Serum renal biomarkers

After 4 and 8 weeks of $CdCl_2$-intoxication serum urea, uric acid and creatinine levels were significantly (P<0.05) compared with control group. Interestingly, after 8 weeks, the levels of these serum biochemical parameters were higher in cadmium intoxicated rats than after 4 weeks. However, serum levels of urea, uric acid and creatinine in rats in OLE + $CdCl_2$ group were significantly restored to near normal as compared to Cd intoxicated groups after 4- and 8 weeks (Fig. 1).

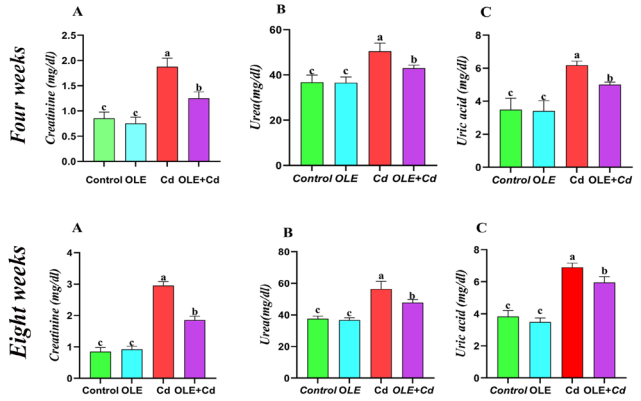

Fig. 1. Levels of serum (A) urea, (B) uric acid and (C) creatinine from Control, OLE, and OLE plus Cd groups after 4 and 8 weeks from the experiment. Data are expressed as the mean ± SD. Differences were considered statistically significant at P values < 0.05.

Histopathological changes

Almost all examined kidney sections of rats in control group after 4 and 8 weeks from experiment revealed normal histological structure of cortex and medulla. The cortex consists of renal corpuscles, proximal and distal convoluted tubules. The renal corpuscle containing glomerulus enclosed by Bowman's capsule which formed from outer parietal layer and inner visceral layer. While the renal tubules were lined with a single layer of cuboidal cells with eosinophilic cytoplasm and rounded nuclei (Fig. 2A, 2H). The examined kidneys of rats intoxicated with $CdCl_2$ for 4 weeks showed vascular damage in the form of mild endothelial cell proliferation in association with vacuolation of the blood vessel wall with perivascular edema (Fig. 2B) admixed with mononuclear leucocytic infiltration (Fig. 2C). Cadmium glomerulopathy was prevalent and represented by vacuolation of the lining endothelial cells of the glomerular tuft with glomerular hypersegmentation, while other glomeruli showed necrosis and shrinkage of their capillary tuft with thickening of Bowman's capsule and focal epithelial proliferation (Fig. 2D). Additionally, Cd toxicity was associated with severe tubular damage where vacuolation and even necrosis of the lining epithelial cell of renal tubules with the presence of eosinophilic cellular debris in the lumen of some renal tubules were frequently observed. Multifocally, peritubular and interstitial leucocytic cellular infiltration was detected with diffuse proliferation of interstitial fibrous connective tissue (Fig. 2E).

On the other side, OLE treatment of $CdCl_2$ intoxicated rats for 4 weeks resulted in attenuation of the Cd-renal damage where mild congestion of renal blood vessels and intertubular capillaries with mild perivascular hemorrhage were the only detectable microscopic renal lesions in the most of the examined kidney sections. However, mild proliferation and vesiculation of the glomerular tuft with mild thickening of the Bowman's capsule were infrequently seen. Accidentally, the renal tubules displayed mild vacuolation of their lining epithelium with the presence of eosinophilic debris intermixed with desquamated epithelial cells in the lumens (Fig. 2F,2G).

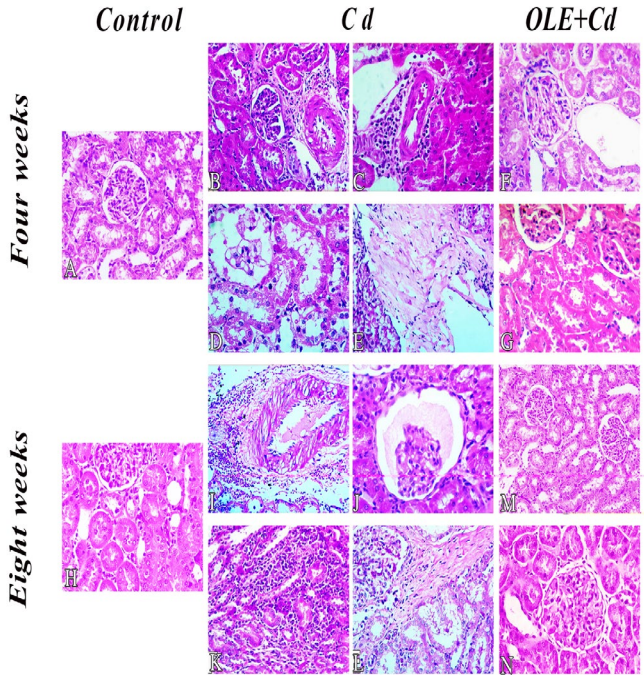

Fig. 2. Photomicrograph of renal tissue stained by H&E obtained after 4 weeks showing, (A) from control group showing normal renal glomeruli and renal tubules, from $CdCl_2$-intoxicated rats, (B) endothelial proliferation of renal blood vessels, vacuolation in tunica media with perivascular edema and hemorrhage×200, (C) perivascular mononuclear leucocytic infiltration×400, (D) degeneration, shrinkage and necrosis of glomerular tuft×400, (E) degeneration and necrosis of the lining epithelium of renal tubules with interstitial fibrous connective tissue proliferation×400, (F, G) $CdCl_2$ intoxicated rats treated with OLE revealing proliferation of glomerular tuft with mild degeneration in renal tubules×200, renal sections stained by H&E after 8 weeks showing, (H) normal renal tissue from control group, from $CdCl_2$ intoxicated rats, (I) severe endothelial cell proliferation, vesiculation in the blood vessel wall and recent thrombus formation with perivascular fibrosis, edema admixed with inflammatory cell and erythrocytes×400, (J) glomerular tuft shrinkage and necrosis with distension of Bowman's space by eosinophilic proteinaceous material with thickened Bowman's capsule×400, (K) glomerular synechiae with massive degeneration and necrosis of renal tubular epithelium and diffuse interstitial fibrous connective tissue proliferation with lymphocytic infiltration×400, (L) tubular atrophy with intertubular lymphocytic infiltration×200. (M, N) renal tissue obtained from rats treated with OLE and $CdCl_2$ showing proliferation of glomerular tuft with mild degeneration in the lining epithelium of renal tubules (×200, ×400).

Meanwhile, the histopathological examination of the renal tissues after 8 weeks of treatment with cadmium, revealed a marked increase in the severity of the pathological alterations in comparison to 4 weeks of treatment with cadmium as the renal blood vessels in all examined sections exhibited proliferation in the endothelial cell and vacuolation of the muscular layer accompanied by perivascular hemorrhage and edema was seen. Fibrin thrombi were detected in the lumen of degenerated blood vessels, attached to the injured intima in association with perivascular fibrosis was also prominent (Fig 2I). Thickening of the basement membrane of glomerular capillaries with hypersegmentation and hypercellularity of the glomerular tuft that characterized by the proliferation of their endothelial as well as proliferation of the epithelial cell lining of the Bowman's capsule. Glomerular tuft shrinkage with distension of the Bowman's space with proteinaceous material (Fig 2J), with narrowing of the uriniferous space in association with fibrosis of Bowman's capsule with synechiae between the glomerular tuft and the visceral layer and hypertrophy of partial epithelial cells were occasionally observed, along with tubulointerstitial fibrosis (Fig 2L). The tubular epithelium showed severe degeneration characterized by a large pale vacuolated cytoplasm. Focal areas of massive necrosis in the renal tubules manifested by pyknotic nuclei and hypereosinophilic cytoplasm were observed (Fig 2K). The remaining renal tubules in both the cortex and medulla showed tubular atrophy with diffuse lymphocytic infiltration (Fig 2K). The lumens of these tubules were packed with degenerated and desquamated epithelial cells. Conversely, in the kidney of rats treated with OLE and $CdCl_2$ for 8 weeks,

mild pathological changes were observed as mild congestion and vacuolation of endothelial cells and the muscular layer of renal blood vessels. The glomeruli showed mild proliferation of the glomerular tuft as well as the renal tubules exhibited nearly normal histological structure with only mild vacuolation and desquamation of their lining epithelium were observed in some treated rats (Fig 4M and 4N).

Assessment of the anti-fibrotic effects of OLE against Cd-induced renal fibrosis

Van Gieson stain was used to assess the anti-fibrotic effects of OLE against Cd-induced renal fibrosis. The examined kidney sections of rats in control and OLE groups showed only a thin layer of pink-stained collagen fibers in the tunica adventitia of renal blood vessels and wall of glomeruli and renal tubules (Fig. 3A and 4A). Contrary, there was moderate positive reaction of pink-stained collagen fibers was seen around blood vessels in the majority of kidney sections of $CdCl_2$-intoxicated rats after 4 weeks (Fig. 3B). Additionally, multifocal areas of pink-stained collagen fibers were seen in interstitial tissues, around glomeruli and in-between renal tubules were occasionally seen (Fig. 3C). Compared to $CdCl_2$ group, the examined kidneys of rats in the OLE+ $CdCl_2$ group showed weak positive reaction of pink-stained collagen fibers around blood vessels and glomeruli (Fig. 3D).

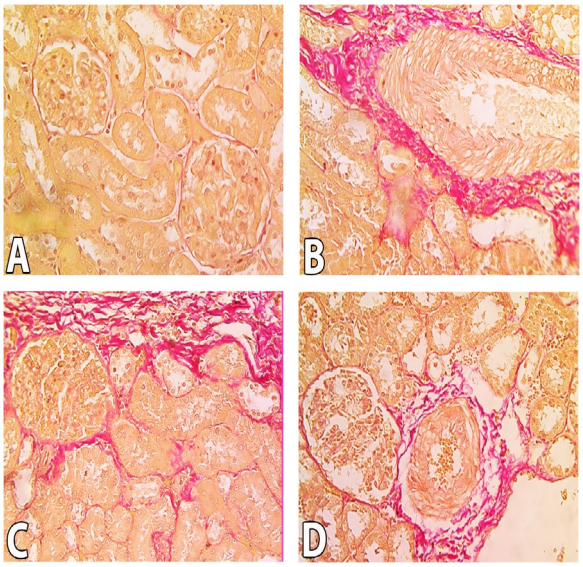

Fig. 3. Representative photomicrographs of Van Gieson-stained kidney sections after 4 weeks in control group showing thin strands of pink-stained collagen fibres around glomeruli and renal tubules, (A) in Cd group showing severe positive reaction of Van Gieson-stained collagen fibres around a renal blood vessel, (B) glomeruli and in the interstitial tissue, (C) Cd+OLE group showing thin stands of pink-stained collagen fibers around glomeruli and renal blood vessel. x200.

On the other hand, after 8 weeks of $CdCl_2$-intoxicated severe positive reaction of Van Gieson stain was recorded indicating severe perivascular, periglomerular and interstitial fibrosis (Fig.4B). Moreover, there were multifocal areas of tightly packed, pink-stained collagen fibers in between renal tubules which replaced necrosed renal tissue (Fig. 4C). Remarkably, OLE treatment of $CdCl_2$-intoxicated rats revealed a marked reduction in in the severity of fibrous connective tissue proliferation and only weak positive reaction of pink-stained collagen fibers was seen in-between renal tubules (Fig. 4D). From the forementioned findings, it was cleared that the antifibrotic effect of OLE against cadmium toxicity-induced renal fibrosis is time-dependent.

Discussion

Chronic kidney disease associated with environmental heavy metal exposure is a major public health issue. Long-term occupational and environmental exposure to Cd causes kidney diseases (WHO, 2010). Thus, this study aimed to investigate the protective effect of OLE to attenuates chronic renal injury induced by Cadmium.

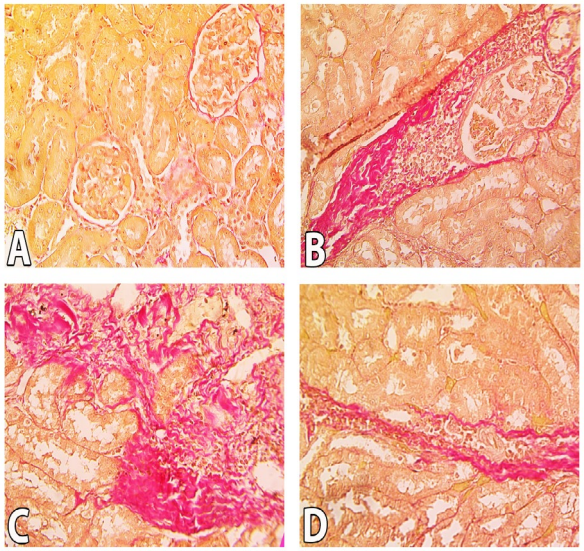

Fig. 4. Representative photomicrographs of Van Gieson-stained kidney sections after 8 weeks, (A) control group showing thin strands of pink-stained collagen fibres around glomeruli and renal tubules, (B) Cd group showing marked positive reaction of Van Gieson-stained of periglomerular, (C) and tubulointerstitial fibrosis, (D) Cd+OLE group showing positive reaction of pink-stained peritubular fibrosis. x200.

Cadmium toxicity for four weeks did not affect either body weight or kidney weight of intoxicated rats. This finding was in a line with previous report (Satarug, 2018). However, Cd exposure for eight weeks significantly induced renal damage, which was represented by reduced weights of the body and kidneys as previously reported by Fang *et al.* (2021). Interestingly, the treatment with OLE either for 4 or 8 weeks improves both body and kidney weights which may contribute to its ameliorative effects on the metabolic and oxidative stress parameters (Kumral *et al.*, 2015).

The kidney's waste-eliminating function is impaired due to hazardous substances, medications, heavy metals, or others, evidenced biochemically by elevating serum urea nitrogen and creatinine (Petejova *et al.*, 2019). In the present study, estimation of some serum biochemical parameters revealed that the Cd administration for 4 and 8 weeks was severely nephrotoxic and markedly increased the levels of serum urea, uric acid and creatinine which indicates this adverse effect of $CdCl_2$ on kidney function. These findings were supported by prior studies (Renugadevi and Prabu, 2010 and Nazima *et al.*, 2015). The same findings were also reported after 8 weeks (Sadek *et al.*, 2017). The increase in urea and creatinine levels could be attributed to the binding of cadmium with metallothionein in the liver, then released into plasma, filtered in the glomerular, and reabsorbed by the kidney's proximal tubules resulting in damage of the renal tissues (Johri *et al.*, 2010). Interestingly, these serum biochemical abnormalities were markedly attenuated in OLE-treated rats indicating that OLE relieved the Cd nephrotoxicity by reducing these parameters. These results were in concordance with Zari and Al-Attar (2011) who suggested that OLE exerts its ameliorative effects on these kidney biomarkers by preventing the decline of the antioxidant defense system and direct free radical scavenging activity. Furthermore, the decrease in blood serum urea and creatinine in the OLE-treated group may be related to an improvement in the kidney filtration mechanism (Mohammed *et al.*, 2018; Al-Hayaly *et al.*, 2020).

In the current study, serum kidney biomarker alterations are coupled with severe renal damage as revealed in histopathological examination. $CdCl_2$ administration for 4 and 8 weeks caused progressive tubular and glomerular necrosis with marked fibrosis and inflammation of the interstitial area, along with increased amounts of collagen-like material. Van Gieson staining was used to analyze collagen deposition. The severity of renal necrosis, inflammation and fibrosis markedly increased after 8 weeks of Cd toxicity compared to after 4 weeks. Our findings on renal morphology were supported by previous findings of renal fibrosis in cadmium-induced chronic kidney disease (Dong *et al.*, 2023). Cd exposure caused renal tissue damage including hyperemia of renal blood vessels and pyknotic nuclei in the renal tubular epithelial cells (Fang *et al.*, 2021). Besides, higher Cd doses and prolonged exposure resulted in extensive pathological changes to renal tissue which explained as Cd poisoning can partially alter intracellular ion transport in the renal tubules, leading to nuclei loss, massive necrosis of tubular epithelial cells and inflammatory cell infiltration, with numerous abnormalities in the glomeruli (Wan *et al.*, 2022). The causes of injury are due to direct tubular cell toxicity associated with ROS formation as well as by inducing regional ischemia (Tiong *et*

al., 2014). Oxidative stress has been widely demonstrated to be a pivotal molecular mechanism in nephrotoxicity caused by chronic Cd exposure (Nemmiche, 2017). Cadmium has been shown to enhance oxidative stress by triggering ROS production and inhibiting the antioxidant system in renal tubular epithelial cells (Aqeel *et al*., 2020).

However, the molecular mechanisms underlying CKD are complex, but include, at least, oxidative stress activation, inflammation and fibrosis (Gajjala *et al*., 2015), also, it is currently well-established that exposure to heavy metals is a major risk factor for the development of CKD (Gajjala *et al*., 2015). Chronic exposure to Cd results in its accumulation in proximal tubular cells of the kidney. This causes a variety of toxic effects that lead to renal cell death (Lee *et al*., 2019). The released cadmium from the cadmium-metallothionein complex within the tubular cells can bind to existing renal metallothionein in the proximal tubular region, leading to increased expression of metallothionein. However, when the renal metallothionein is depleted, Cd accumulates and causes nephrotoxicity, particularly in the proximal tubular region. Cadmium deposition in the kidneys can be significant, as up to 50% of the body's cadmium pool can accumulate there, and the half-life of cadmium in the kidneys is approximately 45 years. This poses a significant health risk, especially in regions with inadequate environmental regulation (Yan and Allen, 2021).

In this study, there were varying degrees of improvement in histopathological changes in the kidney due to OLE treatment after four and eight weeks. However, after 8 weeks OLE effect was stronger than after 4 weeks indicating that OLE has a time dependent effect. Olive leaves extract attenuated the vascular changes as degeneration of endothelial cells and vacuolation in blood vessel wall. which can be explained via the actions of absorbed phenolic metabolites of OLE are evidence that olive phenolics could be beneficial for vascular health (Lockyer *et al*., 2015). Additionally, OLE is effective in attenuating inflammation and oxidative stress in vascular tissue preventing endothelial dysfunction and improving vascular function (González-Hedström *et al*., 2021).

Moreover, in the current study, OLE improved the glomerular and tubular degeneration and necrosis in cadmium intoxicated groups after 4 and 8 weeks. This result was in a consistent with Al-Attar and Alsalmi (2019). In addition, Al-Hayaly *et al*. (2020) and Zari and Al-Attar (2011) proposed the probable therapeutic use of OLE as a new nephroprotective agent against severe renal failure as OLE-treated rats showed near normal histological structure of renal cortex and medulla. Interestingly, in the present research, treatment with OLE strongly decreased perivascular, periglomerular and interstitial inflammation, and fibrosis in the kidney. This result in agreement with Karanovic *et al*. (2021) who reported that OLE reduced renal inflammation and fibrosis with a reduction of fibronectin and collagen deposition. The improvement of renal structure could be related to multifunctional activities of phenolic constituents of OLE, including antioxidant, anti-inflammatory, and anti-fibrotic properties. Therefore, OLE was effective in preventing the infiltrations of fibroblasts and inflammatory mononuclear cells in the kidney (Abd El-Rahman, 2016; ALHaithloul *et al*., 2019; Karanovic *et al*., 2021). Geyikoglu *et al*. (2017) explained that OLE bioactive constituents such as oleuropein and hydroxy-tyrosol reversed fibrotic and collagen deposition. Moreover, OLE exhibits multiple protective actions that could prevent or attenuate pro-inflammatory activation, and influence vascular remodeling via modulation of MMP2 expression (Burja *et al*., 2019).

Conclusion

The current study demonstrated that the severity of cadmium nephrotoxicity was time dependent. OLE can counteract Cd-induced chronic renal toxicity via attenuating glomerulotubular damage, inflammation, and fibrosis with restoring the serum renal biomarkers. The protective effect of OLE was greater over time. This finding highlights the potential of OLE as a valuable therapeutic option to alleviate the harmful effects of Cd on the kidneys, providing hope for effective and natural treatment against Cd-induced nephrotoxicity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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