BIOLOGICAL SCIENCES

Nephroprotective effect of *Pleurotus ostreatus* extract against cadmium chloride toxicity in rats

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Abstract: Cadmium, present in the environment, accumulates in different organs of animals and humans, and has deleterious effects on the kidney. In this study, we investigated the protective effects of the methanolic extract of *Pleurotus ostreatus* in comparison with silymarin on renal function in cadmium-intoxicated rats for five days. Rats intraperitoneally injected with cadmium chloride (1 mg/kg). These rats were treated with either *P. ostreatus* extract (200 mg/kg) or silymarin to investigate the protective effects of the extract. Cadmium treatment induced significant histopathological impairments and increased cadmium levels, DNA fragmentation, and renal oxidative stress. However, treatment with *P. ostreatus* extract or silymarin improved the pathology, reduced the level of cadmium in renal tissue, and restored DNA fragmentation. In addition, a significant reduction in lipid peroxidation and reactive oxygen species levels, and a significant increase in the levels of glutathione and catalase activity were observed. Thus, protective effects of *P. ostreatus* extract to its components. Chromatographic analysis of the *P. ostreatus* confirmed the presence of five phenolics (gallic acid, chlorogenic acid, catechin, propyl gallate, and cinnamic acid) that exhibit strong antioxidant properties as free radical scavengers. Therefore, our findings demonstrate that treatment with *P. ostreatus* extract protects against cadmium-induced nephrotoxicity in female rats.

Key words: cadmium, P*leurotus ostreatus*, Kidney, Rats.

INTRODUCTION

Cadmium (Cd) is a highly toxic heavy metal (Yang & Shu 2015), with unknown beneficial biological function in humans or animals. Cd is not normally found in body fluids or tissues; therefore, the presence of Cd in animals or humans is suggestive of environmental exposure (Alkushi et al. 2018). Cd is capable of entering the food chain; thus, it is considered a deleterious environmental pollutant, because it threatens both animal and human health (Katole et al. 2013) and has a very long biological half-life (about 30 years) in humans, especially in the

kidneys. Cd bound to proteins, like sulfhydryl-containing molecules, may cause nephropathy due to heavy metal accumulation. In addition, Cd toxicity stimulates the induction of oxidative stress in different organs (Morales **et al. 2006**), and chronic toxicity due to Cd causes renal proximal tubular dysfunction (Koçak & Akçil 2006).

Silymarin (SIL) is an extract of the seeds of *Silybum marianum* (also known as milk thistle), a plant belonging to the family Asteraceae and has been used for its medicinal properties for several centuries (Basiglio et al. 2009). SIL has antioxidant, anti-inflammatory, anti-allergic,

and anti-hyperglycemic properties (Pradhan & Girish 2006). According to Rafieian-Kopaie & Nasri (2012), SIL may be important for the maintenance of a healthy kidney and liver, and Ghosh et al. (2016) reported that SIL has a beneficial role in the pathophysiology of the mouse kidney.

Pleurotus ostreatus is a widely cultivated edible mushroom that exerts a wide range of pharmacological properties, including immunomodulatory (Jesenak et al. 2013), antitumor (Kong et al. 2014), and antioxidant (Ogbomida et al. 2018) properties. According to Zhu et al. (2019), *P. ostreatus* revealed hepatoprotective effects on CCl_4-induced toxicity in rats. Moreover, Bindhu & Das (2018) showed that *P. ostreatus* extract ameliorated the cognitive impairment and oxidative stress in Diabetic mellitus.

Therefore, the main goal of this study was to investigate the beneficial effects of *P. ostreatus* in comparison with SIL on renal function in response to Cd-intoxication.

MATERIALS AND METHODS

Chemicals and chromatographic analysis of POE

Anhydrous cadmium chloride ($CdCl_2$) of analytical grade, CAS number 10108-64-2, was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Silymarin (SIL) tablets (South Egypt Drug Industries"SEDICO", 6th of October City, Egypt) were purchased from local pharmacies in Cairo, Egypt. *P. ostreatus* methanolic extract (POE) was prepared using the dried and homogenized fruiting bodies of *P. ostreatus* (50 g) that were purchased from the Agriculture Research Center, Cairo, Egypt. The metabolites were extracted using a constant ratio of 2:2:1 of methanol: chloroform: distilled water (Kim et al. 2010). The

hydrophilic upper layer was removed from the extract and dried under vacuum for 24 h.

According to Roberts et al. (2018), high performance liquid chromatography (HPLC) analysis for POE was performed using an Agilent 1260 series (Agilent, Santa Clara, CA, USA).

Animals and experimental design

Thirty female albino rats weighing 200-250 g (10-12 weeks old) were purchased from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt).

To investigate the protective effects of POE on Cd-induced renal toxicity, all rats were divided into five groups (nine rats per group). Group 1 (control) was intraperitoneally (i.p.) injected with saline and served as the control group. Group 2 (POE) was treated with POE only; rats received a dose of POE at 200 mg/kg body weight (b.wt.). Group 3 (untreated Cd-intoxicated) was orally administered saline, and only $CdCl_2$ (1 mg/kg b.wt.) was injected i.p. after 1 h (Kataranovski et al. 2009). This dose did not cause any mortality in Cd-inoculated rats. Group 4 (POE-treated Cd-intoxicated) was orally administered POE (200 mg /kg b.wt.), and $CdCl_2$ was i.p. injected after 1 h. Group 5 (SIL-treated Cd-intoxicated) was orally treated with SIL (100 mg/kg b.wt.), and $CdCl_2$ was i.p. injected after 1 h. All treatments were performed for five consecutive days. All rats were sacrificed by decapitation on day 6 post treatment. This study was approved by state authorities in accordance with the ethical committee for animal protection of National Organization for Drug Control and Research, Egypt (approval no: NODCAR/ II/18/19).

Histopathology

Formalin-fixed kidneys were embedded in paraffin, and 6 μm sections were stained with hematoxylin and eosin. Histological damage was scored as follows: 0: absent; +: mild; ++:

moderate; and +++: severe (Drury & Wallington 1980).

Cd level in renal homogenate

The Cd ions in renal tissue homogenates were estimated using the protocol published by Murphy (Murphy 1987).

DNA fragmentation assay

According to Aljanabi & Martinez (1997), DNA was extracted using the methods and data published by Wlodek et al. (1991) where a DNA ladder was used to determine the size of the apoptotic DNA fragments.

Oxidative/anti-oxidative biomarkers

The kidneys were homogenized in ice-cold Tris-HCl (50 mM) and 300 mM sucrose, (pH 7.4) to produce a 50% (w/v) homogenate (Tsakiris et al. 2004). The renal homogenate was centrifuged at 600×g for 10 min and the supernatant was separated.

Glutathione (GSH) level in renal homogenates was measured according to Ellman (1959). The catalase (CAT) activity was measured according to Aebi (1984). Using previously published methods by Ohkawa et al. (1979), malondialdehyde (MDA) level was determined. To measure ROS production, nitro blue tetrazolium (NBT) was converted into formazan by superoxide anion (Vrablic et al. 2001).

Statistical analysis

The data are expressed as means ± standard error. In addition, the statistical (SPSS version 17.0) package program was used to perform one-way ANOVA. P≤0.05 is considered statistically significant.
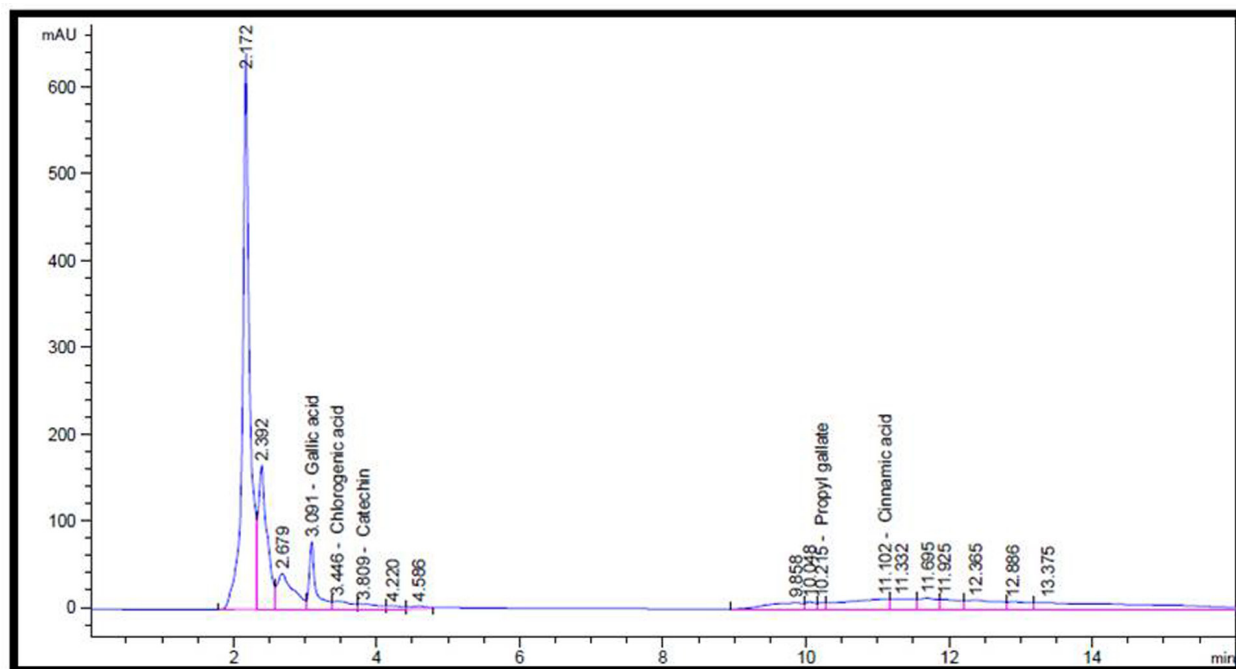

Figure 1. HPLC chromatogram recorded at 280 nm of a methanolic extract of P. ostreatus (POE). Only peaks corresponding to phenolic compounds or related compounds are indicated: gallic acid, chlorogenic acid, catechin, propyl gallate, and cinnamic acid.

RESULTS

As shown in Figure (1), a representative chromatogram of POE, five phenolics and related compounds (gallic acid, chlorogenic acid, catechin, propyl gallate, and cinnamic acid) were positively identified and quantified in POE (Figure 1, Table SI- Supplementary Material). This was determined by comparing the chromatographic characteristics and absorption spectra with that of standard compounds.

Cd-intoxication resulted in a significant decrease in the renal index of rats compared to that of the control group (ratio of kidney weight (mg/rat) to body weight (g/rat)) (Figure 2). However, POE/SIL treatment significantly increased the renal index in Cd-intoxicated rats relative to the Cd control group.

Cd intoxication resulted in significant histological impairments in renal tissue. The glomerular tuft shrunk or was damaged; and cytoplasmic degeneration of renal tubules and pyknotic nuclei were observed. In addition, some tubules were necrotic and exhibited multiple foci of hemorrhage as well as dilatation and congestion of **blood vessels** (Figure 3).
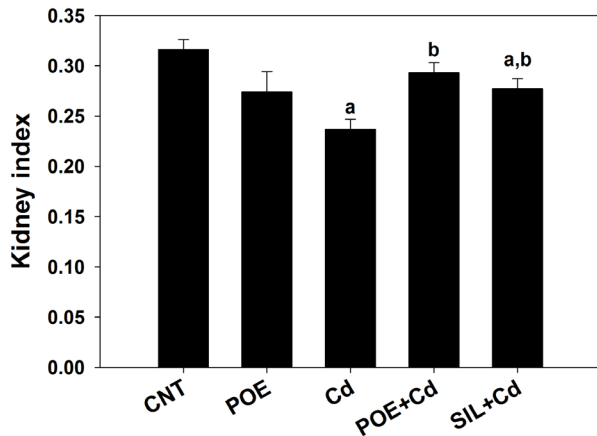

Figure 2. P. ostreatus methanolic extract (POE) and silymarin (SIL) treatment improved the kidney index of female albino rats in response to Cd-intoxication. Values are presented as mean ± SE.
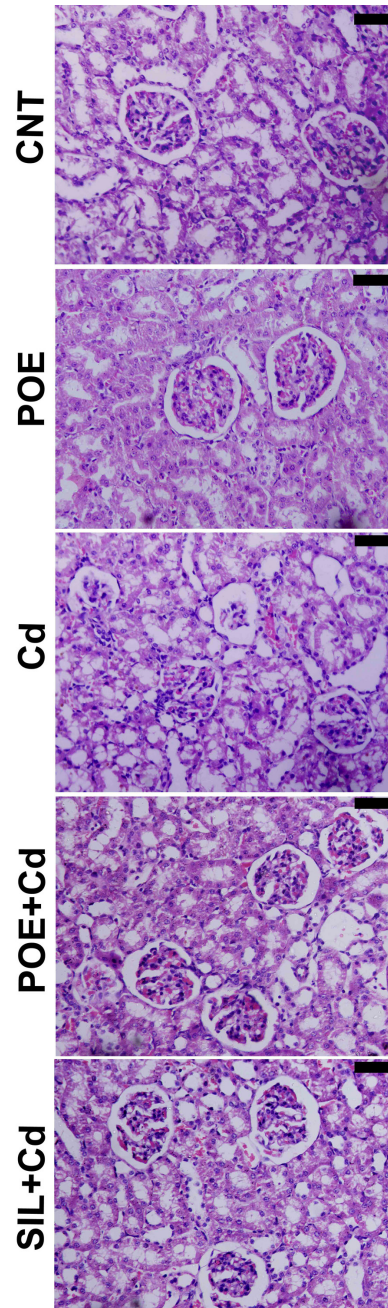

Figure 3. POE and SIL improved rat kidney damage induced by Cd. (CNT) Sections from control group with normal architecture. (POE) P. ostreatus methanolic extract inoculated group with normal structure. (Cd) Untreated Cd-intoxicated group with damaged glomeruli. (POE+Cd) Sections from P. ostreatus methanolic extract treated Cd-intoxicated female rats with improved histological structure. (SIL+Cd) Sections from silymarin-treatedCd-intoxicated rats with improved structure. Sections are stained with hematoxylin and eosin. Bar = 50 µm.

Moreover, treatments with POE or SIL in the Cd-intoxicated rats significantly improved the histological picture (**Figure 3**). This is confirmed by the histological score of both groups of mice, recorded in Table I.

Cd-intoxicated female rats had significantly elevated levels of renal Cd compared to that of the control group (Figure 4). Likewise, a significant increase in the Cd level was observed in groups 4 (POE-treated Cd-intoxicated rats) and 5 (SIL-treated Cd-intoxicated rats) relative to the control group. However, Cd levels significantly ($P < 0.05$) decreased in groups treated with POE before Cd injection. As with POE-treated rats, SIL treatment before Cd-intoxication resulted in a significant decrease in renal Cd compared to that of the untreated Cd-intoxicated group.

Cd-induced apoptotic DNA fragmentation in the kidneys of rats was determined by agarose gel electrophoresis and visualized by ethidium bromide fluorescence (Figure 5). No ladder was observed on the agarose gel in the DNA of normal kidney tissue (Figure 5), and no DNA laddering was detected in response to POE-only treatment, similar to the control group. Genomic DNA ladder formation was observed in the DNA of rats treated with $CdCl_2$ (1 mg/kg b.wt.) (Figure 5). Treatment of Cd-intoxicated rats with POE (Figure 5) restored the DNA laddering profile induced by the toxic effect of $CdCl_2$ treatment, to normal. The degradation of DNA into oligonucleotide fragments was also observed in the $CdCl_2$ group treated with SIL (Figure 5). These results suggest that POE was more effective than SIL in protecting against the nephrotoxic effect of Cd-intoxication in female rats.

In addition, Cd induced a significant increase in MDA and ROS levels ($P \leq 0.05$) in renal homogenates, whereas the level of non-enzymatic and enzymatic activity of antioxidants decreased significantly compared to those of the control group. Moreover, treatment of

Table I. P. ostreatus extract (POE) ameliorates histopathological kidney damages induced by cadmium.

Group	Microscopic observation			
	Tubular vacuolization	Hydropic degeneration change	Glomerular damage	Inflammatory cellular infiltration
CNT	+	0	0	0
POE	+	0	0	0
Cd	+++	++	+++	+++
POE+Cd	+	+	+	+
SIL+Cd	+	+	+	+

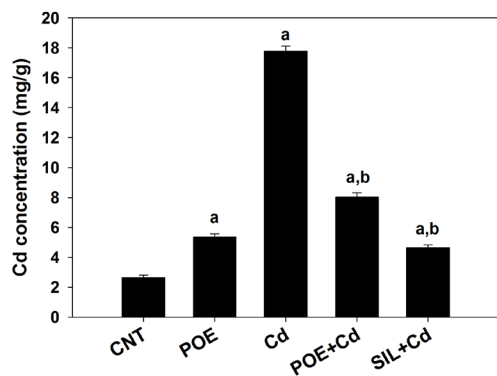**0: absent; +: mild; ++: moderate; +++; severe.**

Figure 4. Mitigating effects of POE and SIL treatment on levels of renal Cd in Cd-intoxicated rats.
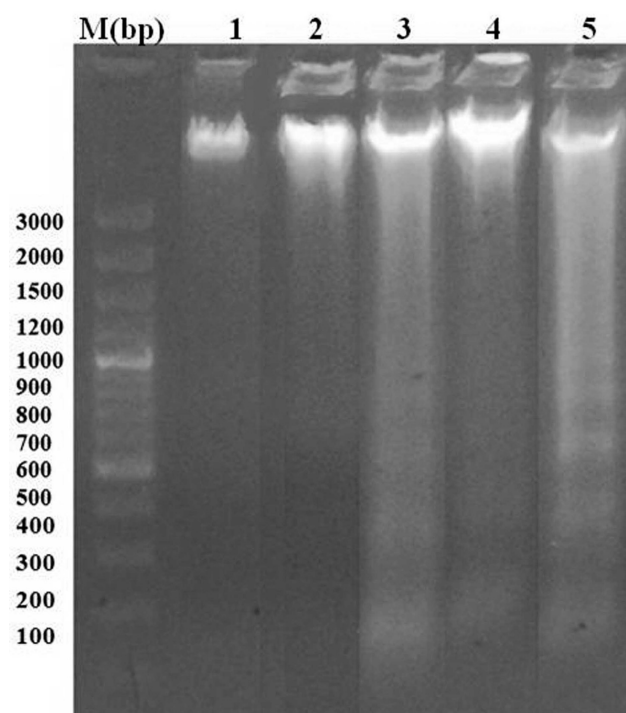
Figure 5. Effect of POE and SIL on DNA extracted from kidney tissue of normal and treated rats. Agarose gel electrophoresis photograph of M-Marker, lane 1: normal control group showed no DNA laddering, lane 2: POE-treated group showed no DNA laddering similar to control group, lane 3: CdCl$_2$ treated group showed DNA laddering band, lane 4: POE+CdCl$_2$ treated group restored DNA laddering, and lane 5: SIL+CdCl$_2$ treated group showed DNA laddering profile of the CdCl$_2$-induced nephrotoxicity.

Table II. Effect of *P. ostreatus* methanolic extract (POE) / SIL on glutathione (GSH) level, catalase (CAT) activity, malondialdehyde level (MDA) and reactive oxygen species (ROS) level in renal tissue of Cd-intoxicated female rats.

Groups	GSH (mg/g.tissue)	CAT (U/g)	MDA (nmol/g tissue)	ROS (µmol/g tissue)
CNT	67.63±2.75	1.47±0.06	59.07±2.57	1190±47.71
POE	71.04±1.71[a]	1.63±0.05[a]	47.64±2.00[a]	1063±45.51
Cd	52.54±2.02[a]	1.27±0.02[a]	77.91±1.43[a]	1525±45.51[a]
POE+Cd	62.49±1.59[b]	1.42±0.02[b]	71.88±1.76[ab]	1200±25.04[b]
SIL+Cd	58.67±2.38[a]	1.47±0.04[b]	71.35±1.77[ab]	1216±41.12[b]

Values are means ± SE. a: Significant against control group (CNT) at *P* ≤ 0.05, b: Significant against Cd group at *P* ≤ 0.05, n = 6.

Cd-intoxicated rats with SIL significantly reduced renal MDA and ROS levels; however, a significant increase was observed in CAT activity, and the increase observed in the level of GSH was not significant. Similarly, POE treatment significantly decreased renal MDA and ROS levels, *P* ≤ 0.05; and significantly increased CAT activity and GSH levels relative to those of the untreated Cd-intoxicated group (Table II).

DISCUSSION

The components of POE were identified as catechin, chlorogenic acid, gallic acid, propyl gallate, and cinnamic acid by HPLC. Burnett & Levy (2012) reported that catechin is a potent antioxidant that inhibits the production of MDA in response to Cd toxicity *in vitro* and *in vivo*. In addition, Suzuki et al. (2006) found

that chlorogenic acid is a strong polyphenolic antioxidant capable of scavenging free radicals. Chlorogenic acid inhibits Cd-mediated lipid peroxidation and reduces the utilization of non-enzymatic antioxidants, which improves the levels of GSH in brain tissue (Hao et al. 2015). Propyl gallate is extensively used as an antioxidant (Pop et al. 2013), and cinnamic acid exhibits antioxidant and free radical scavenging properties (Heim et al. 2002). SIL is also a strong antioxidant that may minimize the risks associated with free radical exposure (MDA, ROS) in the kidney (Tunca et al. 2009).

In our study, histological analysis revealed significant renal impairments in response to Cd intoxication. These results are in agreement with those of previous studies (Adi et al. 2016, Wongmekiat et al. 2018). In addition, Rafati et al. (2015) reported that Cd-induced glomeruli structural changes, increases in the mesangial matrix, and swelling of the glomeruli with wider urinary spaces were detected. Cd treatment resulted in tubular dysfunction and nuclear membrane damage in glomerular epithelial cells of rats (Adi et al. 2016). However, Gobe & Crane (2010) attributed the relationship between Cd-intoxication and renal cell injury to the sensitivity of the proximal tubular epithelium to oxidative stress. Similarly, Nazima et al. (2015) reported that increased nitric oxide and ROS generation is related to renal injury and induces the progression to renal failure.

Cinnamic, gallic, and chlorogenic acids maintain the normal architecture of the glomerulus and their antioxidant properties protect the kidneys of rats from nephrotoxicity (Yousuf & Vellaichamy 2015). Moreover, Wongmekiat et al. (2018) suggested that catechin effectively protects renal tissue from Cd nephrotoxicity, and propyl gallate in rats is therapeutic in response to diabetic glomerular endothelial proliferation (Tian et al. 2012).

Abouzeinab (2015) concluded that SIL antioxidant activity can directly protect cells by stabilizing the permeability of the cellular membrane.

Treatment with POE or SIL significantly decreased the levels of Cd compared to that of the untreated Cd-intoxicated rats. This result corresponds with the findings of Orr & Bridges (2017) who observed that after chronic Cd exposure, approximately 50% of the total Cd accumulates in renal tissue. Cd enters the epithelium of the kidney via several different mechanisms. As a result, transport of Cd into renal tissue may require different channels and transporters (Ca^{2+}, Fe^{2+}, and Zn^{2+}), or the formation of complexes with sulfhydryl (thiol)-containing biomolecules, such as GSH (Lee & Yu 2016). Moreover, these Cd-thiol complexes could penetrate the cells and regulate the transfer of some organic compounds (Ganger et al. 2016).

In this study, $CdCl_2$ induced apoptotic DNA fragmentation in the kidney while POE or SIL was able to reduce these changes. Rana et al. (2018) reported that $CdCl_2$ induces DNA fragmentation and apoptotic cell death in different types of cells due to its ability to induce the oxidative stress.

Treatment with chlorogenic, gallic, and cinnamic acids prevents oxidative DNA damage in experimental animals (Cheng et al. 2007). Moreover, Rafieian-Kopaie & Nasri (2012) showed that SIL accumulates in renal cells and aids in tissue repair and regeneration, by elevating the synthesis of proteins and nucleic acids.

Adi et al. (2016) suggested that exposure to Cd alters the metabolism of antioxidants and induces oxidative stress, generating free radicals and CAT activity and reducing other enzymatic antioxidants. In addition, ROS is involved in the deleterious effects of Cd-intoxication on human health. ROS accumulates in injured renal tissues and disturbs the balance of enzymatic and non-enzymatic antioxidant agents in cells.

After Cd absorption, ROS binds to metallothionein and enters the injured renal space via the glomerulus, forming the metallothionein-Cd complex. The formed complex is degraded by lysosomes of the renal proximal tubules, causing Cd release. Consequently, research related to recovery of the antioxidant levels after Cd toxicity is of major significance (Rana et al. 2018).

POE has potential antioxidant activity that inhibits GSH oxidation and protects related enzymes during aging (Jayakumar et al. 2010). Bindhu & Das (2018) also concluded that POE treatment inhibited MDA formation, raised GSH levels, and increased antioxidant enzyme activity (CAT).

Collectively, our findings provide evidence that POE protect against Cd nephrotoxicity in rats. The administration of POE to Cd-intoxicated rats improved the damaged renal architecture that resulted in $CdCl_2$ treatment. In addition, the level of Cd and DNA fragmentation were decreased. The POE treatment balanced the level of non-enzymatic and enzymatic antioxidants in Cd administered rats. We referred these improvements to the phenolic constituents of POE. However, further studies are required to understand the exact mechanisms responsible for protection.

Acknowledgments

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SUPPLEMENTARY MATERIAL

Table SI.

How to cite

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