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Using Pomegranate Peel Extract to Change the Adverse Effect of Ethephon by Enhancing its Antioxidant, Anti-inflammatory, and Anti-apoptotic Effects in Rats

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Abstract:

Organophosphorus insecticide and growth regulator namely Ethephon (2-chloroethylphosphonic acid) are widely used as a ripening process accelerator and a cultivation duration inhibitor. Pomegranate extract (PPE) has recently been taken into consideration due to its pharmacological effects especially those associated with renal diseases. Thus, this study aims to investigate the possible protective effect of PPE against ethephon-induced nephrotoxicity in rats. In this study four groups of adult male rats were divided into control group, PPE 400 mg/kg group, Ethephon 250 mg/kg group, and finally, PPE + Ethephon group (treated with the same dose of PPE group and Ethephon group). In the current study, kidney function parameters (KIM-1, creatinine, and urea) along with oxidative stress markers, heme oxygenase-1 (HO-1) and nuclear factor erythroid 2–related factor 2 (Nrf2), glutathione (GSH) and its correlated enzymes, nitric oxide (NO), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT) were estimated. Additionally, mediators of renal inflammation: interleukin 1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NF-κB) were measured. Apoptotic biomarkers (Bax, caspase 3, and Bcl2) in addition to renal histopathological data were also investigated. Results revealed that Ethephon elicited a significant increase in oxidation markers and reduced antioxidant levels, accompanied by oxidative renal tissue injury. Consequently, administration of Ethephon was reported to provoke secretion of the pro-inflammatory mediators. Moreover, histopathological results showed that deformities in the renal tissues were noticed which is attributed to Ethephon exposure. Interestingly, co-administration of PPE and Ethephon resulted in significantly ameliorated the biochemical and histopathological alterations produced by Ethephon. Current results propose the potential effect of PPE in the protection of renal tissue from Ethephon induced nephrotoxicity in rats.

Keywords: Antioxidants markers, Ethephon, inflammation, Kidney, Oxidative stress, Pomegranate peel extract.

Introduction:

Despite the beneficial effects of agrochemical compounds, the extreme application of plant growth regulators is related to many depraved effects on health [1]. Organophosphorus insecticide namely Ethephon (2-chloroethyl phosphonic acid) is widely used as a ripening process accelerator and a cultivation duration inhibitor. Upon Ethephon metabolism by the plant; ethylene oxide, ethanediol, hydroxyethyl-glutathione, and mercapturic acid are respectively formed [2]. It has been reported that the consumption of artificially ripened crops treated with Ethephon may be regarded as the main reason for Ethephon toxicity [3]. A recent study implicated in embryonic fibroblasts has shown that Ethephon is capable to enhance lipid peroxidation and induce excessive production of reactive oxygen species (ROS) at low doses [1].

Furthermore, administration of Ethephon at the sub-chronic level persuades renal tissues histological changes and oxidative homeostasis [4]. Likewise, Bhadoria et al. have found that intoxication of Ethephon leads to degeneration and infiltration in hepatocytes [2]. Moreover, exposure of mice to Ethephon could suppress the development of the immune system in their offspring [5].

Additionally, it has been reported that Ethephon also can cause reproductive impairment through decreasing levels of sex hormones, disturbing spermatogenesis and sperm counts [6].

Renal injury is one of health problem which results from exposure to harmful toxins or even from some medications [7]. Organophosphorus compounds induce nephrotoxicity and lead to acute and/or chronic renal failure due to pathophysiological disturbance [8]. ROS formation as subsequent oxidative stress regarding as one of the main causes of acute renal injury. The catastrophic effect of toxicity of organophosphorus on kidney tissue represented by damage of cellular phospholipid layers, dysfunction of mitochondria, and disturbance in intracellular calcium level, which lead to accumulation of ROS and development of oxidative stress and toxicity of renal tubules [9]. Furthermore, it has been reported that ROS enhances the progression of fibrosis and inflammation, then stimulates cytokines production and growth factors [10]. Metabolites of ROS are assumed to trigger organophosphorus insecticide induced nephrotoxicity [11]. Consequently, intracellular accumulation of free radicals may underlie lipid peroxidation results disorder in the permeability and viscosity of cellular membrane [12].

Several natural compounds and their constituents have recently been taken into consideration due to their pharmacological effects especially those associated with the amelioration of renal diseases [13-14]. Administration of medicinal plants that contain nephroprotective effects together with different nephrotoxic agents showed attenuation of toxicity [15]. Pomegranate belongs to the family Punica granatum L. and is commonly cultivated in South-east Asia, tropical Africa, Malaya, India, the East Indies, and China [16].

Pomegranate peel was broadly used a long time ago due to its therapeutic advantages as an antidiarrheal agent and anthelmintic. Lately, pomegranate peel extract (PPE) has shown to have bioactive compounds which are associated with many pharmacological properties including anti-inflammatory and anti-oxidant, henceforward becoming a target of many researchers because of its protective role against severe diseases [17].

A recent broad study applied on more than 1000 extracts of different plants has found that extracts of pomegranate peel were more influential than all others [18]. Furthermore, it has been established that natural polyphenols content in the pomegranate peels precisely ellagic acid, Punicalagin, punicallin, ferulic acids, ellagitannins, quercetins, catechins, anthocyanins, and gallotannins [19], have potential anticancer [20], antibacterial, as well as a protective effect against hepatotoxicity [21] and nephrotoxicity [22]. PPE exhibits marked antioxidant properties, which shows noticeable antioxidant activity through attenuate oxidative mediators which attributed to its content of polyphenols compounds [23].

It has been reported that presence of miscellaneous phenolic compounds in the PPE namely; gallic acids, ellagic acids, ellagitannins, and ferulic acids, play a crucial role in inhibiting lipid peroxidation, suppress oxidative stress precursors, and scavenging free radicals, which exert their antioxidative activity in cells [24]. Furthermore, studies on animals have shown that PPE does not exhibit any toxic effects [20].

However, although several hypotheses have been established to explain the precise mechanism of organophosphorus intoxication-induced kidney failure, still the knowledge of this matter doubtable due to the lack of sufficient experimental data [25]. Therefore, in order to support further experimental studies, the current study has been established to investigate whether PPE can attenuate or eliminate the harmful effects of organophosphorus insecticide namely Ethephon on renal tissue through assessing histopathological changes, kidney function markers, oxidative stress parameters, apoptotic and inflammatory mediators.

Materials and Methods:

Experimental Design

Sixty male rats were divided into four groups (each group contained 15 animals), all groups were treated for 28 repeated days as follows: control group was gavage administrated daily with physiological saline (0.9% NaCl), PPE group; was daily gavage administrated with PPE 400 mg/kg [26], Ethephon group; was orally administered with Ethephon 250 mg/kg, and PPE+Ethephon group; was co-administered with Ethephon and PPE orally with the same doses. The chosen dose of Ethephon was according to results recorded by Tudor et al. [27] which found that Ethephon 250 mg/kg induced hepatotoxicity, histopathological, and oxidative status as well as biochemical alterations.

Compounds and Reagents

Ethephon (2-chloroethyl phosphonic acid) compound was obtained from Chema Industries, Cairo, Egypt. Pomegranate peel extract (PPE) was obtained from Tizan (XI'AN 710119, China).

Experimental Animals

Sixty male adult rats (Wistar albino) weighing about 200-250g, were brought from the University of Salahalddin, Erbil-Iraq. Before starting the experiment, the experimental animals were reserved in the animal house cages and

supplied with laboratory food and water for one week for adaptation.

Collection of Blood and Tissue Samples

After 24 hrs of the last dose, animals were euthanized using sodium pentobarbital (300 mg/kg; Sigma-Aldrich, St Louis, Missouri, USA). Blood samples were obtained from the plexus veins of retro-orbital and left-hand for a half-hour, the serum was separated after centrifugation of the blood samples at 3000 rpm by refrigerated centrifuge for 15 min, and the serum was stored at -20°C to perform the biochemical investigations. The right kidney of rats was collected, weighed and by using 50 mM Tris-HCl (pH 7.4), 10% (w/v) homogenized specimens of renal tissue were obtained, the homogenate was centrifuged by refrigerated centrifuge for 10 min at 5000 xg. The harvested supernatant was stored at -80°C for later biochemical investigations. The left kidney of rats was used for histological measurements.

Renal Homogenates Preparation

The homogenate of kidney specimens was gained by using 0.05 mM Tris-HCl pH 7.4 the obtained homogenate was centrifuged by refrigerated centrifuge for 10 min at 5000×g. The harvesting supernatants were kept at -80°C for consequent biochemical investigations. Content of renal protein was measured according to Lowry et al. [28] method.

Estimation of Relative Kidney Weight

The relative kidney weight was calculated according to Abdel-Daim et. al. [13] method as following formula:

$$\text{Relative Kidney weight} = \frac{\text{Right kidney weight}}{\text{Total body weight}} \times 100$$

Estimation of Kidney Function

Creatinine level in serum was calculated by spectrophotometer using commercial kits (Sigma-Aldrich, St. Louis, Missouri, United States), while the renal KIM-1 contents were analyzed using ELISA kits (Elabscience, Houston, Texas, United States) according to Abdel-Daim et. al. [13] method.

Estimation of Oxidant Levels in the Renal Tissue

Malondialdehyde (MDA) assayed was performed as an index of lipid peroxidation which is carried out according to Ohkawa et al. [29] protocol. The reduced level of glutathione (GSH) in the renal tissue homogenates was measured according to Ellman's [30] technique. The level of nitric oxide (NO) was examined using the Griess reagent according to Green et al. [31] method.

Estimation of Antioxidant Status

Enzyme activity of glutathione reductase and peroxidase in the renal tissue were assessed according to methods of Paglia and Valentine [32] and De Vega et al. [33]. The activity of superoxide dismutase (SOD) in the renal tissue was examined using Nishikimi et al. [34] method. Catalase (CAT) activity was assessed using procedures described by Aebi [35]. Renal protein contents were measured depending on Lowry et al. [28] method which referenced Bovine serum albumin.

Measurement of Heme Oxygenase-1 (HO-1) and Nuclear Factor Erythroid 2–related Factor 2 (Nrf2) in Renal Tissue

The levels of HO-1 and Nrf2 in the renal tissue were measured depending on the manufacturer's instructions (MyBioSource).

Measurement of Inflammatory Markers

Estimation of nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β) tumor necrosis factor-α (TNF-α), were measured using ELISA kits according to the manufacturer's instructions (Cusabio Biotech) according to Abdel-Daim et. al. [13] method.

Measurement of Apoptotic Markers

Bax, caspase-3, and Bcl2 contents in the renal tissue were measured using an ELISA kit depending on the manufacturer's instructions (MyBioSource).

Histology Investigation

The left kidney tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and then cut into 4-μm sections, stained with hematoxylin and eosin (HE) staining, and examined with an optical microscope Nikon Eclipse E200-LED (Tokyo, Japan) microscope at 400× magnification [36].

Histomorphometric Investigation of the Renal Tissue

Image J software (Version,1.8.0-112) was used to perform the histomorphometric analysis of the Bowman's capsule and the proximal convoluted tubules diameters according to Stojiljkovic and Mihailovic [37] procedure.

Statistical Analyses

Statistical analysis was performed using one-way analysis of variance (ANOVA), the significance between groups was calculated by Newman-Keuls post-test using StatsDirect computer software. $p < 0.05$ was considered

statistically significant. All data were expressed as the mean ± 2SD.

Results:

In order to investigate whether PPE can attenuate or eliminate the harmful effects of Ethephon on renal tissue, assessing histopathological changes, kidney function markers, oxidative stress parameters, apoptotic and inflammatory mediators were performed. The results of the main findings of treatment of the rats with Ethephon, PPE, or a combination of both are stated below.

PPE Ameliorates the Effect of Ethephon Intoxication on the Kidney's Weight and Functions

The administration of Ethephon induced a significate increase ($p < 0.05$) in the kidney weight comparing with the control group. Whereases combination of PPE and Ethephon was significantly ($p < 0.05$) able to reduce the increment in the weight of the kidney comparing with the Ethephon group (Fig. 1).

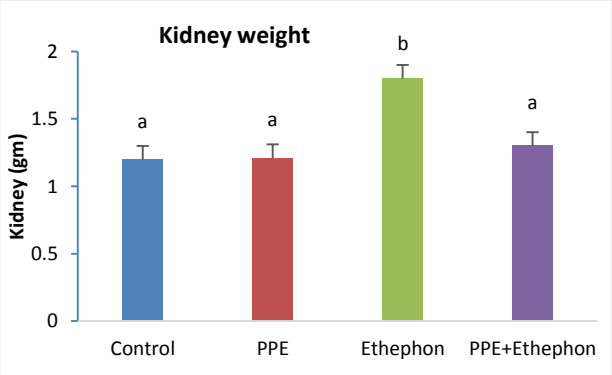

Figure 1. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on the relative weight of the kidney. Different letter values refer to the significant difference P < 0.05. (n= 15).

To assess the effect of the three treatments (Ethephon, PPE, and combination of both) on kidney functions, renal tissue content of KIM-1 along with levels of urea and creatinine were performed. Rats treated with Ethephon 250 mg/kg for four weeks showed a significant increase ($p < 0.05$) in KIM-1 content, urea and creatinine levels; compared with the control group. Whereas PPE administration 400 mg/kg for four weeks was able to reduce the KIM-1 content, urea, and creatinine levels to the normal values compared with the Ethephon-treated group (Fig. 2 and Tab. 1), respectively.

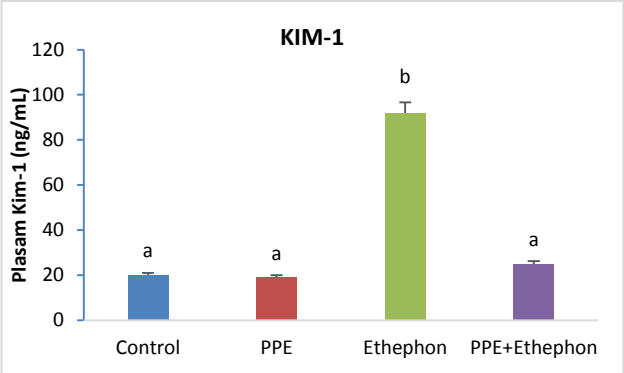

Figure 2. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on the KIM-1. Different letter values refer to the significant difference *P* < 0.05. (n= 15).

Table 1. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on urea and creatinine Levels

Parameters	Control	PPE	Ethephon	Ethephon+PPE
Urea mg/dl	25.18 ± 1.01 [a]	24.28 ± 1.06 [a]	41.30 ± 2.01 [b]	30.16 ± 2.38 [a]
Creatinine mg/dl	0.55 ± 0.05 [a]	0.56 ± 0.01 [a]	1.12 ± 0.07 [b]	0.66 ± 0.05 [a]

Different letter values refer to the significant difference $P < 0.05$. (n= 15).

PPE Suppresses Oxidative Stress Markers Resulting from Ethephon Intoxication in Renal Tissue

Ethephon administration persuaded significant elevation in NO and MDA ($p < 0.05$), and a reduction in the content of GSH compared with the control group. No significant effect of PPE on oxidative stress biomarkers in the PPE treated group. In contrast, significate reduction ($p < 0.05$) in levels of NO and MDA accompanied with significant raised ($p < 0.05$) in GSH content were shown in the co-administered of PPE and Ethephon group comparing to the Ethephon treated group Fig. 3.

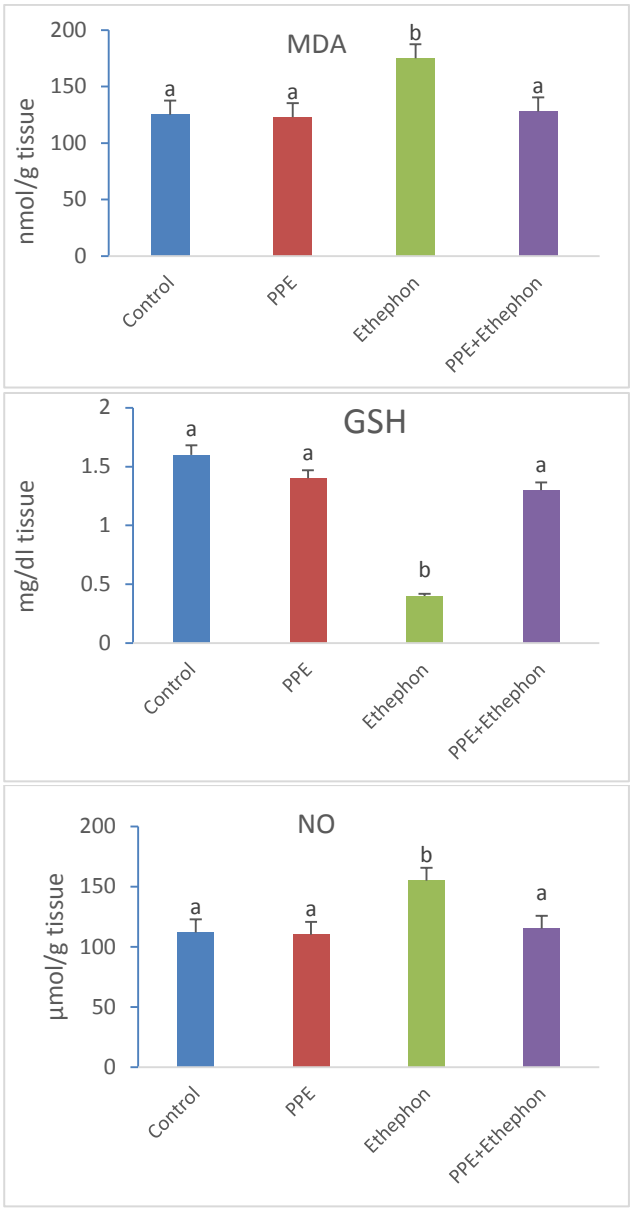

Figure 3. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on the malondialdehyde (MDA), glutathione (GSH), and nitric oxide (NO) in the kidney tissue. Different letter values refer to the significant difference $P < 0.05$. (n= 15).

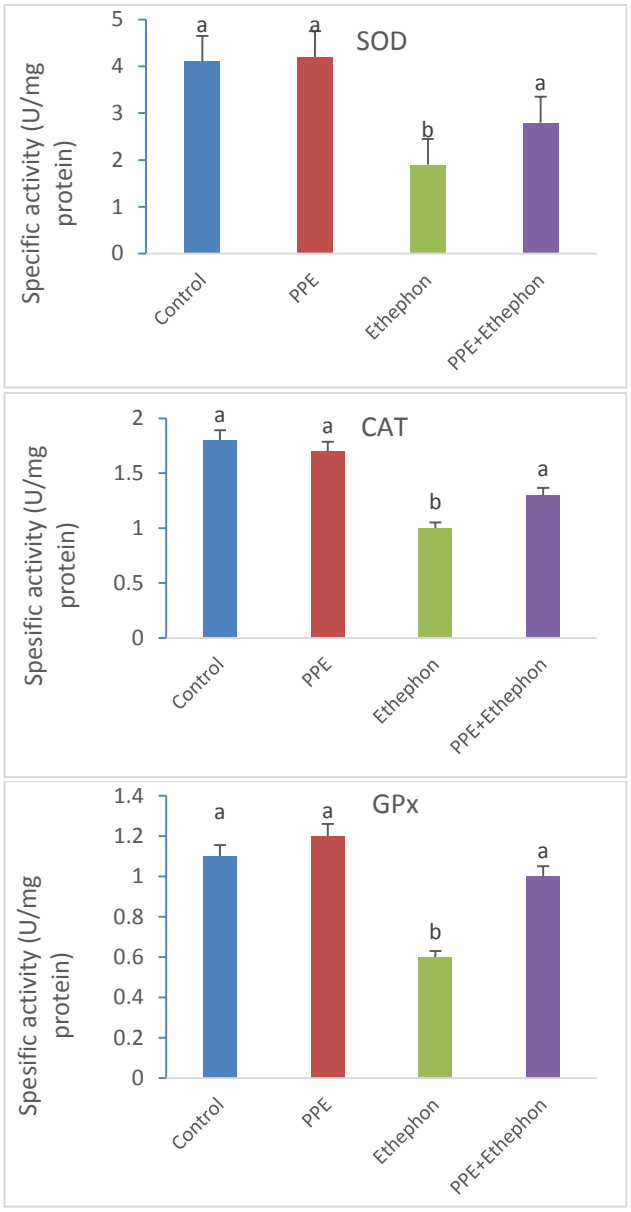

Figure 4. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the kidney tissue. Different letter values refer to the significant difference $P < 0.05$. (n= 15).

Furthermore, Ethephon treated group showed a significant reduction ($p < 0.05$) in the enzymatic activity of CAT, SOD, and GPx as compared with their enzymatic activity as antioxidants in the control group Fig. 4.

To explore the impact of Ethephon on the mechanism of antioxidation in the renal tissue, HO-1 and Nrf2 were investigated. Results from ELISA showed a significant decline ($p < 0.05$) in HO-1 and Nrf2 levels compared to the same parameters in the control group.

Whereas the PPE treated group showed no significant effect on the activity of the above-mentioned antioxidants parameter. Results collected from the PPE+Ethephon group revealed a significant increase ($p < 0.05$) in the HO-1 and Nrf2 levels in addition to the levels of CAT, SOD, and GPx comparison to the value of their activity in the group of Ethephon in the renal tissue Fig. 5.

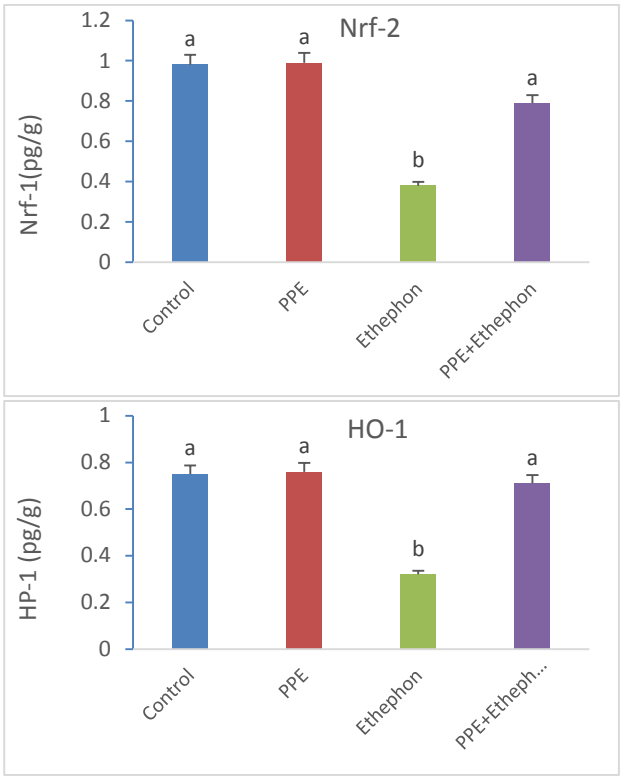

Figure 5. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on the nuclear factor erythroid 2–related factor 2 (Nrf2) and hemeoxygenase-1 (HO-1) in the kidney tissue. Different letter values refer to the significant difference $P < 0.05$. (n= 15).

PPE obstructs Ethephon induced- inflammatory signaling markers in the kidney tissue

The ethephon-treated group showed persuading inflammatory markers levels namely NF-κB, IL-1β, and TNF-α levels despite this elevation, was only statistically significant ($p < 0.05$) in NF-κB compared to their levels in the control group. However, the PPE group showed no significant alterations in the inflammatory status. In contrast, results from Fig.6, showed a significant inhibition ($p < 0.05$) of NF-κB, IL-1β, and TNF-α levels in the PPE+Ethephon group compared to their levels in the Ethephon group.

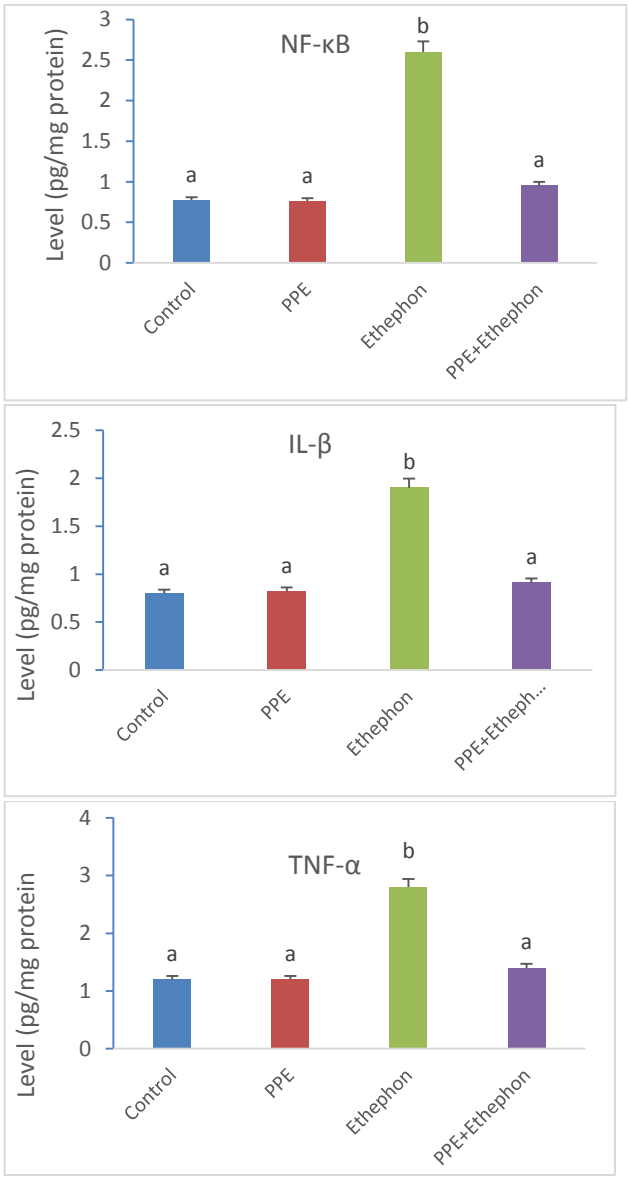

Figure 6. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on the nuclear factor kappa B (NF-κB), interlukin-1 β (IL-1β), and tumor necrosis factor-α (TNF-α) in the kidney tissue. Different letter values refer to the significant difference $P < 0.05$. (n= 15).

PPE Inhibits Ethephon Induced- apoptosis in the Renal Tissue

Increasing levels of Bax and caspase 3 as pro-apoptotic markers accompanied with decreasing Bcl2 level as an anti-apoptotic marker in the renal tissue of Ethephon exposure rats compared to the control group is an obvious indication of the ongoing apoptotic event. No significant alteration in the estimated apoptotic markers was found in the PPE-treated group. Remarkably, the co-administered of PPE+Ethephon has perceived to suppress cell apoptosis in renal tissue through reducing pro-apoptotic markers and inducing anti-

apoptotic markers compared to the Ethephon group Fig. 7.

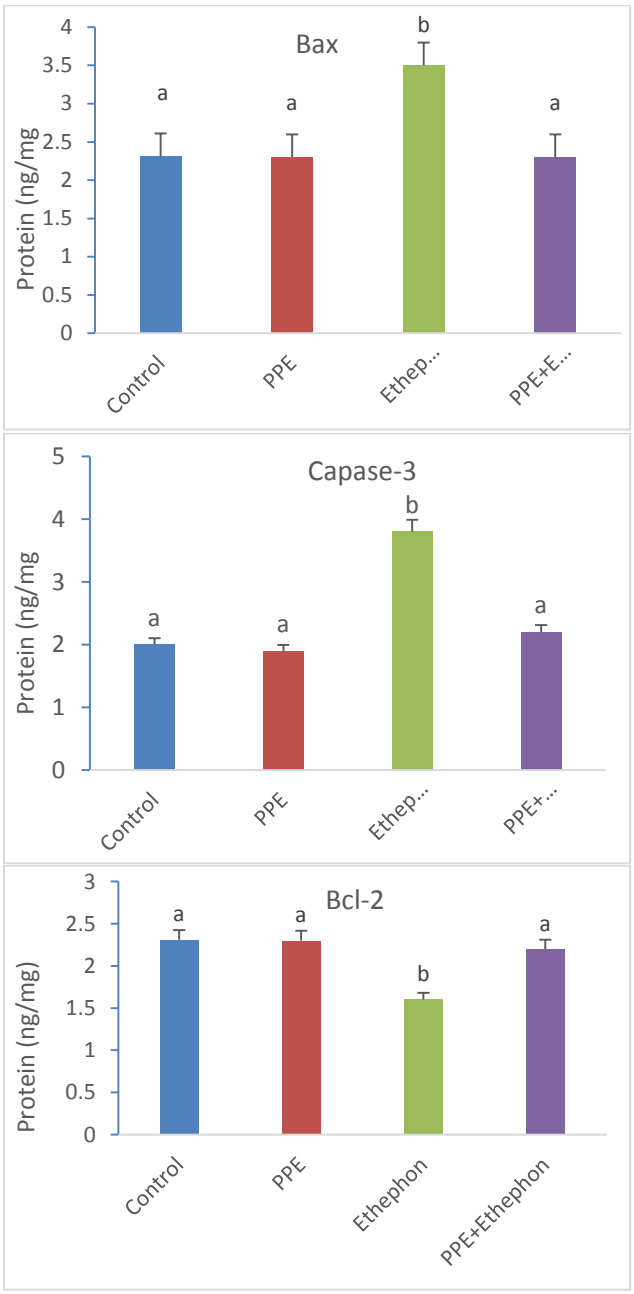

Figure 7. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on Bax, caspase-3, and Bcl2 in the kidney tissue. Different letter values refer to the significant difference $P < 0.05$. (n= 15).

Histopathological Impacts of PPE and/or Ethephon Treatments on Kidney Tissue

Microscopic examination of the section of the kidney showed normal mesangial cells with normal glomeruli; cuboidal epithelial cells having central rounded nuclei and eosinophilic cytoplasm in the adjacent renal tubules in the control and PPE groups as illustrated in Fig. 8A and 8B, correspondingly. Moreover, atrophying of glomeruli, reduction in mesangial cells, and congestion in the glomerular capillaries was noticed in the Ethephon group. Consequently, the Ethephon group also showed a scattering of apoptotic cells and disruption of the basement membrane in the adjacent renal tubules. Furthermore, inflammatory cell infiltration along with congesting of blood vessels was seen as well in the interstitial tissue of the Ethephon treated group (Fig. 8C). Interestingly, results from Fig.8D showed the ability of PPE to eradicate the catastrophic effects of Ethephon on the renal tissue through reducing the infiltration of inflammatory cells, improving glomeruli and the tubules, besides a decrease in the necrotic and apoptotic deformities in the histopathological section.

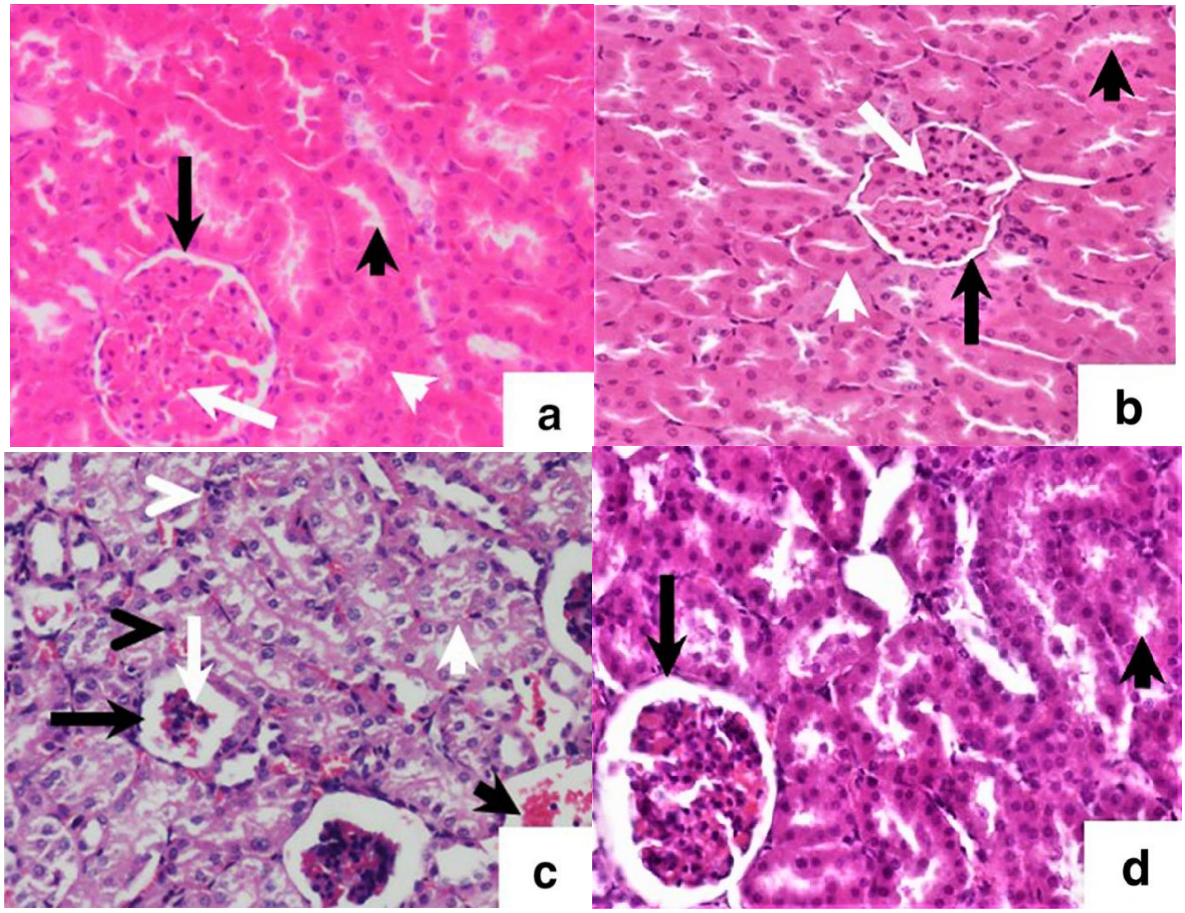

Figure 8. Images of a light photomicrograph of kidney tissues stained with (H&E, × 400).
(A)The control group and (B) PPE group showed normal mesangial cells (long black arrow), normal glomeruli (long white arrow), cuboidal epithelial cells having central rounded nuclei, and eosinophilic cytoplasm in the adjacent renal tubules (short white arrow). (C) The ethephon group is showing glomeruli atrophy (long black arrow), reduction in mesangial cells, in addition to congestion in the capillaries of glomeruli (long white arrow); also showed a scattering of apoptotic cells and disruption of the basement membrane were found in the adjacent renal tubules (short white arrow), along with infiltration of inflammatory cells (head white arrow) and congesting of blood vessels in the interstitial tissue (short black arrow). (D) The PPE-Ethephon group showed reduction in the infiltration of inflammatory cells, improving tubules (short black arrow) and glomeruli (long black arrow) in addition to reducing the necrotic and apoptotic deformities**.**

Analysis of Histoorphometric of Renal Tissue

Analysis data of histomorphometric of kidney tissue showed the effects of histopathological changes after PPE, Ethephon, or both. Results collected from Ethephon exposed rats, showed a significant reduction ($p < 0.05$) in diameters of glomeruli and proximal convoluted tubules compared to the control group. In contrast, results of PPE+Ethephon showed significant elevation ($p < 0.05$) in diameters of glomeruli and proximal convoluted tubules compared to the Ethephon-treated rats Fig. 9 which is another evidence of the ability of PPE to eliminate the injuries caused by Ethephon.

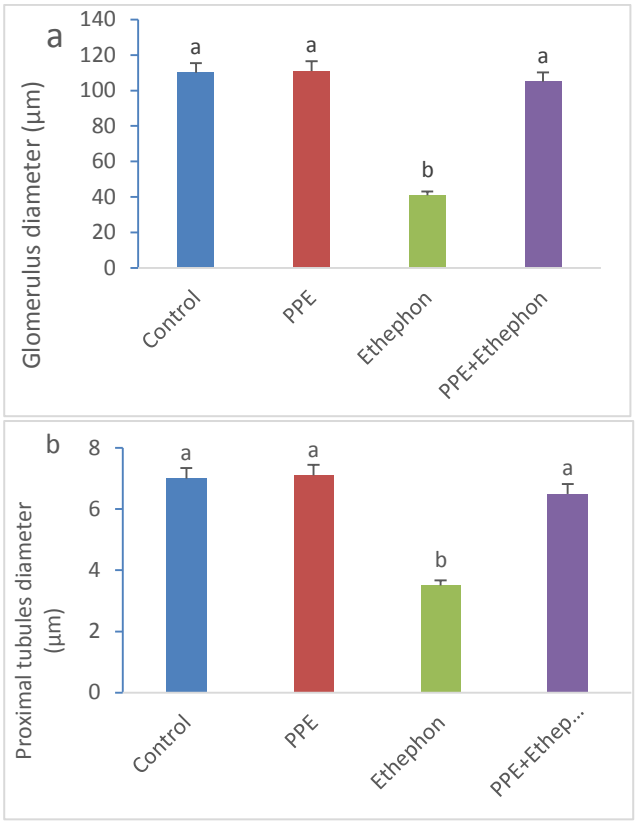

Figure 9. Effects of PPE 400 mg/kg, Ethephon and PPE-Ethephon 250 mg/kg on histomorphometry of glomerulus and renal proximal convoluted tubules. Different letter values refer to the significant difference $P < 0.05$. (n= 15).

Discussion:

The kidney is a vital organ that is responsible for several physiological processes including the removal of many toxic materials from the blood, therefore, it is more likely to be exposed to an injury. Consequently, impairment of kidney functions becomes one of the common diseases [38]. Renal impairment as a result of environmental pollutants has been reported to be related to dosage and duration period of exposure to xenobiotic materials [38].

The current study established the meliorative actions of PPE on the Ethephon induced kidney toxicity in the rats. The present study has shown that administration of Ethephon induced a significant increase in KIM-1 content, urea and creatinine (Fig. 2 and Table 1) in the renal tissue. These findings are similar to previous studies [4] and [39]. Increasing renal transmembrane protein (KIM-1 content) is evidence of kidney damage caused by the Ethephon effect since this protein is only over expressed during renal injury [40]. Furthermore, significant elevation of urea and creatinine reveals the impact of Ethephon-induced renal damage and impairment of renal functions.

Fascinatingly, the present study showed that PPE treatment suppressed the Ethephon-induced effects on functions and structure of the kidney (Fig. 3) through its high content of antioxidants and reno-protective compounds which could be particularly due to its high amount of phenolic ingredients [41-42]. Ethephon intoxication accompanied with free radical formation induce histopathologic deformities, thus, could lead to an increase in kidney function profile as a result of a disorder in glomerular filtration in addition to the changes in tubular reabsorption. Moreover, it has been reported that initiation of oxidative stress is regarded as a primary mechanism that is related to Ethephon-induced renal damage [43]. Thus, here, a significant reduction was reported in the enzymatic activity of CAT, SOD and also GPx (the enzyme of renal GSH) as antioxidants (Fig. 4), accompanied by a marked decline in the levels of Nrf2 and HO-1 (Fig. 5), along with rises in the release of NO and MDA (Fig. 3).

Exposure to organophosphorus compounds alters thiol-containing proteins which leads to renal dysfunction and tissue deformity [44]. The capability of GPx to conjugate with the metabolites of Ethephon and eradicate ROS formation and consequently suppress oxidative insults, that leads to depleting in the level of GSH with GPX and GR (GHS derived enzymes) since these enzymes are potent antioxidant factors against xenobiotics.

It has been reported that over production of ROS sways down regulation in the expression of CAT and SOD proteins following exposure to organophosphorus compounds [43]. Inactivation of Nrf2 in the renal tissue is suggested to be the reason for reduced antioxidants proteins since Nrf2 plays a crucial role in the regulation of the expression of several antioxidant genes. Overproduction of ROS was evidenced by elevation of MOD which is a marker of the development of oxidative damage [43, 45]. Most recent studies confirm that exposure to Ethephon stimulates ROS formation and depletes free radicle scavengers resultant in oxidative damage [4, 46].

Results of the present study revealed the potential effect of PPE in the kidney by conquering oxidative biomolecules formation through augmenting antioxidant biomolecules and attenuating synthesis of nitric oxide and lipid peroxidation (Fig. 3). The potential activity of PEE in the protection of kidney tissue against oxidative stress via enhancing antioxidant content and reducing lipid peroxidation has been established in previous studies [16, 42, 47].

In addition to oxidative insults, another renal damage has been found in this study which is

represented by the Ethephon-persuade inflammatory process through provoked proinflammatory mediators explicitly NF-κB, IL-1β, and TNF-α (Fig. 6). Ethephon increased inflammatory response in the experimental Japanese quail has been recorded in a recent study [48]. The same study has shown a cross-link between inflammation and oxidative stress [48]. Activation of proinflammatory mediators in response to ROS leads to an increase in the production of NF-κB [49]. The potency of PPE in reducing Ethephon- the induced inflammatory response was noticed in this study through its effect to decrease NF-κB, IL-1β, and TNF-α (Fig. 6). The previous study has found that polyphenolic phytochemicals compounds in the pomegranate suppress the signalling pathway of inflammation by inhibition of protein expression of TNF-α -induced COX-2, suppression p65 subunit phosphorylation, and NF-κB binding in colon cancer cells [50]. Also, a reduction in the protein expression of cyclooxygenase-2 and nitric oxide synthase as proinflammatory enzymes has been reported in rats treated with pomegranate juice [51].

Findings of the current study showed that administration of Ethephon induced apoptosis in kidney cells, which was demonstrated by increased caspase 3 and Bax as a pro-apoptotic marker and decreased by Bcl2 as an anti-apoptotic factor (Fig. 7), resulting in pathophysiological changes in renal tissue and further kidney dysfunction. During an acute renal injury, proapoptotic mediators distract the membrane of mitochondria and stimulate the production of apoptogenic proteins namely cytochrome c, which triggers caspases to provoke apoptosis [52]. Furthermore, oxidative stress and subsequential overproduction of ROS participate in renal apoptosis which induces damage of cellular macromolecules, for instance DNA, lipids, and lipoproteins [53-55]. Rats' exposure to Ethephon has shown programmed cell death in their thyroid gland [56]. The most recent study has recorded that exposure to Ethephon induces P53 tumor suppressor expression level, which is a key protein for the expression of many apoptosis-related genes, and inhibition of cell prefiltration consequently [6].

Results from the PPE+Ethephon group showed a reduction in caspase 3 and Bax levels and elevation in Bcl2 level (Fig. 7), which is a shred of further evidence on the potential effects of PPE to protect the kidney from phenobarbital and diethylnitrosamine arbitrated apoptosis in the kidney that induced by stress [53]. PPE-enhancing antiapoptotic protein and decreasing apoptotic markers suggest a potentially crucial role of PPE in the protection of kidneys from Ethephon induced apoptosis in renal tissue. Protection effects of PPE could be attributed to the activity of bioactive ingredients in pomegranate peel precisely Ellagic acid, Punicalagin and Punicallin.

Current study results revealed that rats administrated with Ethephon for four weeks with 250 mg/kg induced a significant elevation in the relative kidney weight (Fig. 1). Increasing in kidney weight following the nephrotoxic effect of Ethephon treatment is resulted from decreasing in mesangial cells, glomeruli atrophy, accompanied by enlargement of epithelial and stromal cells [57, 58]. These findings are in agreement with a previous study [57], which mentioned that eleven weeks of injection of Ethephon (50 mg/kg i.p.) elevated the relative weight of kidneys in the experimental rats. Furthermore, other recent studies have stated that increment in the kidney weight after Ethephon administration is occurred due to renal tissue injury followed by renal edema and inflammation [2, 59].

Remarkably, Co-supplement of PPE and Ethephon showed a notable reduction in relative kidney weight (Fig. 1), which proposes the valuable role of PPE in the elimination of the nephrotoxic effect of Ethephon through reducing the infiltration of inflammatory cells, recovering tubular glomeruli in addition to decrease the necrotic and apoptotic deformities.

Histopathological investigation in this study (Fig. 8) showed there were several structural damages in the kidney tissue that happened, attributable to exposure to Ethephon, which are confirmed by results of biochemical parameters. Kidney tissue damages is characterized by congestion in the glomerular capillaries, atrophic glomeruli, and reduction in the mesangial cells. These histopathological changes in the renal tissue might be because of Ethephon accumulation in kidney tissue, thus, destructive renal tubules and filtration of the kidney, and consequently, epithelial cell inflammation.

These findings are in agreement with Mokhtari et. al. and Abou-Zeid et. al. [4, 54] who recorded histopathological deformation in the tubules particularly pyknotic cells in addition to hemorrhage and infiltration of inflammatory cells in renal tubules following an exposure to Ethephon.

Histomorphometry findings of glomerulus and renal proximal convoluted tubules of current study (Fig. 9) showed that administration of PPE with Ethephon perceived to abort Ethephon-induced histopathological changes in renal tissue, this magnificent PPE protection against Ethephon histopathological alteration could attribute the molecules of the active ingredients in the pomegranate extract as these ingredients could contribute to preventing oxidative stress,

degeneration, apoptosis and inflammatory mechanisms.

Conclusion:

Findings of the current study demonstrated that four weeks of administration of Ethephon induced disturbance in the kidney functions, provoked inflammation and oxidative stress in addition to histopathological changes in the renal tissue. Interestingly, co-administration of PPE and Ethephon showed to abort Ethephon-induced hepatotoxicity which attributed to the anti-inflammatory, anti-oxidant, and anti-apoptotic activity of PPE. The current study highlights the potential value of pomegranate peel as one of the renal-protectors against Ethephon toxicity in rats.

Abbreviations

PPE Pomegranate extract
KIM-1 Kidney Injury Molecule-1
HO-1 heme oxygenase-1
Nrf2 nuclear factor erythroid 2–related factor 2
GSH glutathione
NO nitric oxide
SOD superoxide dismutase
MDA malondialdehyde
CAT catalase
IL-1β interleukin 1 beta
TNF-α tumor necrosis factor-alpha
NF-κB nuclear factor kappa B
Bax Bcl-2 Associated X-protein
Bcl-2 B-cell lymphoma protein 2
ROS reactive oxygen species

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Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in Duhok Polytechnic University.

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استخدام مستخلص قشر الرمان لتغيير التأثير الضار الناتج عن الايثيفون وذلك من خلال تعزيز لمضادات الاكسدة ومضادات الالتهاب ومضادات موت الخلية في الجرذان

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الخلاصة:

المبيد الحشري الفسفوري العضوي ومنظم للنمو المسمى بالإيثيفون (2- كلورو إيثيل حامض الفوسفونيك) يستخدم على نطاق واسع كمسرع لعملية النضج ومثبط لمدة الزراعة. تم مؤخرًا أخذ مستخلص قشر الرمان (PPE) في الاعتبار نظرًا لتأثيراته الدوائية خاصة تلك المرتبطة بأمراض الكلى. وبالتالي ، كان الهدف من هذه الدراسة هو فحص في التأثير الوقائي المحتمل لمستخلص قشر الرمان ضد السمية الكلوية التي يسببها الإيثيفون في الجرذان. في هذه الدراسة، تم إنشاء أربع مجموعات من ذكور الجرذان البالغة (ن = 15) ، مجموعة التحكم ، مستخلص قشر الرمان 400 ملغم / كغم ، الإيثيفون 250 ملغم / كغم ، مستخلص قشر الرمان + الإيثيفون بنفس جرعة مجموعة مستخلص قشر الرمان ومجموعة الإيثيفون. معلمات وظائف الكلى: KLM-1 والكرياتينين واليوريا ؛ علامات الإجهاد التأكسدي: الهيم أوكسيجينيز -1 (HO-1) والعامل النووي 2 المرتبط بالعامل 2 (Nrf2) ؛ الجلوتاثيون (GSH) مع الإنزيمات المرتبطة به: أكسيد النيتريك (NO) ، ديسميوتيز الفائق (SOD)، malondialdehyde (MDA) و catalase (CAT). بالإضافة إلى ذلك ، تم فحص وسطاء الالتهاب الكلوي: إنترلوكين 1 بيتا (IL-1β) ، عامل نخر الورم ألفا (TNF-α) ، العامل النووي كابا ب (NF-κB). كما تم دراسة المؤشرات الحيوية لموت الخلايا (Bax، caspase 3، Bcl2) بالإضافة إلى فحص البيانات المرضية لأنسجة الكلى. أظهرت النتائج أن الإيثيفون أدى إلى زيادة معنوية في علامات الأكسدة ، وانخفاض في مستويات مضادات الأكسدة، مصحوبًا بإصابة الأنسجة الكلوية المؤكسدة. وبالتالي ، تم االاستنتاج أن إعطاء الإيثيفون يؤدي إلى إفراز العوامل المؤيدة للالتهابات. علاوة على ذلك ، أظهرت النتائج النسيجية المرضية وجود تشوهات في أنسجة الكلى تعزى إلى التعرض للإيثيفون. ومن المثير للاهتمام ، أن اعطاء مستخلص قشر الرمان مع الإيثيفون أدت إلى تحسين كبير في التغيرات الكيميائية الحيوية والنسيجية الناتجة عن الإيثيفون. تقترح النتائج الحالية التأثير المحتمل لمستخلص قشر الرمان في حماية الأنسجة الكلوية من السمية الكلوية التي يسببها الإيثيفون في الجرذان.

الكلمات المفتاحية: علامات مضادات الأكسدة، إيثيفون، الالتهاب، الكلية، الكرب التأكسدي، مستخلص قشر الرمان.