Comparing The Protective Effects of L-carnitine and Erdosteine against Malathion-induced Nephrotoxicity: A Biochemical and Histopathological Experimental Study

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KEYWORDS
Malathion,
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ABSTRACT

Malathion-induced renal impairment and the protective agents against it; are still points of research. The current study aimed at investigating and comparing between L-carnitine (LC) (100 mg/kg/day) and Erdosteine (Er) (10 mg/kg/day) regarding their protective effects against subacute nephrotoxicity of low dose Malathion (M27) (27 mg/kg/day) as well as high dose Malathion (M54) (54 mg/kg/day). Ten equal groups of adult male Sprague Dawley rats (n=6) were used as the following: Control, Corn oil, (M27), (M54), (LC), (LC+M27), (LC+M54), (Er), (Er+M27) and (Er+M54). Rats received treatments by oral gavage for 28 days. Malathion showed significant dose dependent elevation of serum creatinine, urea and renal malondialdehyde, in line with decreasing serum albumin, renal superoxide dismutase activity and renal reduced Glutathione concentration. Histologically, Malathion induced marked degenerative and inflammatory changes as well as significant dose dependent increase in Caspase-3 expression in both renal cortex and medulla compared to control. Co-administration of either LC or Er with Malathion; was associated with significant improvement of renal biochemical functions, renal oxidative stress markers, histological degenerative as well as inflammatory changes and Caspase-3 expression in both low dose and high dose Malathion groups. Moreover, LC nephroprotective effects appeared to be better than those of Er, although these differences were statistically insignificant. To conclude, it was the first time to demonstrate the nephroprotective effects of LC and Er against Malathion-induced renal dysfunction; yet the upper hand was to LC.

Introduction

Malathion (O, O-dimethyl-S-1,2-bis ethoxy carbonyl ethyl phosphorodithionate) is one of the most widely used organophosphate pesticides (Selmi et al., 2018). Inhibiting the cholinesterase enzyme and subsequent accumulation of acetylcholine at the nerve ending are considered the main toxic actions of Malathion-induced acute neurotoxicity (Ahmed, 2014). Chronic and subacute Malathion exposure are reported to have other toxic mechanisms as oxidative stress, inflammation and apoptosis which resulted in neurotoxicity, cardiotoxicity, hepatotoxicity, nephrotoxicity, reproductive toxicity, immunotoxicity and carcinogenic effects (Badr, 2020).

There is growing evidence of an association between the exposure to pesticides and renal impairment in both human and experimental data (Sobolev et al., 2022). Persons exposed to organophosphates had a higher risk of developing acute renal failure by 6.17 folds compared to non-exposed individuals (Cavari et al., 2013). Malathion

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was associated with increased risk of chronic kidney disease by more than one-fold in a human survey study (Wan et al., 2021). In addition, experimental studies showed renal function perturbation followed Malathion administration alongside histological degenerative and inflammatory changes in the kidney (Khalifa and Alkhalaf, 2020; El Okle et al., 2022; Ghamry et al., 2022; Gur and Kandemir 2023). Several mechanisms were considered in Malathion-induced renal injury e.g., oxidative stress, inflammation, autophagy, and apoptosis (Sobolev et al., 2022).

L-carnitine (LC) (3-hydroxytrimethyl-amino butyric acid) is a naturally occurring nutrient (Jafari et al., 2013). The LC has two sources, dietary sources and endogenous biosynthesis, which represent (75%) and (25%) of LC requirements, respectively. Dietary sources of LC are mainly animal sources and to a lesser extent plant sources. Endogenous biosynthesis of LC occurs in the liver, kidneys and brain from lysine and methionine essential amino acids (Elkomy et al., 2020). Physiologically, LC is essential for long-chain fatty acids transportation across mitochondrial membrane to be used in mitochondrial adenosine triphosphate (ATP) production (Kunak et al., 2016). Moreover, LC has antioxidant effects as it acts as scavenger for free oxygen radicals as well as products of fatty acids metabolism (Morid et al., 2023). In addition, LC has a membrane stabilizing effect on the lipid bilayer cellular membrane (Salama et al.,2022). Several studies concerned with the nephroprotective effects of LC against different nephrotoxic agent e.g., diazinon (Shokrzade et al., 2013), cisplatin (Elkomy et al., 2020), gentamycin (Sakr and Kamel 2023); combined doxorubicin and cyclophosphamide toxicity (Morid et al., 2023) and combined glycerol and contrast-induced nephropathy (Kunak et al., 2016).

Erdosteine (Er) [*N*-(caboxy-methyl-thioacetyl)-homocysteine thioacetone], is a widely used as mucolytic agent in the treatment of pulmonary diseases (Yildirim et al., 2003). The Er is considered as a thiol derivate containing two blocked sulfhydryl groups which are activated and become free after hepatic metabolism. The free sulfhydryl groups have reducing capacity that can scavenge the free oxygen radicals (Tutanc et al., 2012). In addition, Erdosteine has an active metabolite, (N - thio-diglycolyl-homocysteine). The active metabolite and the free sulfhydryl groups are responsible for the antioxidant, anti - inflammatory and antiapoptotic protective effects of Er (Mutneja et al., 2020). Previous studies investigated the nephroprotective role of Er against different nephrotoxic agents in animal models, e.g., cisplatin (Yildirim et al., 2003); gentamycin (Cabuk et al., 2008) and cyclosporine (Tutanc et al., 2012).

Hence, the aim of the present study was to investigate and compare between the biochemical, antioxidant as well as antiapoptotic protective effects of LC and Er against subacute malathion-induced nephrotoxicity of both low and high dose of Malathion in adult Sprague Dawley male rats.

Material and Methods:

Chemicals

Malathion (commercial grade 95%): in the form of white liquid was purchased from (El Helb for Pesticides & Chemicals Factory, Damietta, Egypt). Both LC and Er were obtained from (Global Napi for Pharmaceutical company, Egypt). All other chemicals used in the study were analytic grade and purchased from (Bio-diagnostic company, Giza, Egypt).

Animals

Sixty healthy adult male Sprague-Dawley rats weighing (200-220 g) were purchased from the animal house Faculty of Veterinary medicine, Mansoura University, Egypt. Animals were maintained in stainless steel cages and kept on a photoperiod of 12 h light-dark cycle with a controlled temperature (20 ± 2^{o} C) and relative humidity (55 ± 5%). All rats had unrestricted access to commercial rodent feed pellets (Al Wadi Company, Giza, Egypt) and water throughout the experiment. Rats were handled following the National Institutes of Health (NIH) general guidelines for the care and use of laboratory animals. The present study was authorized by Mansoura university Animal care and use committee code number (VM.R.23.01.46)

Experimental Design

After two weeks of acclimatization, rats were randomly divided into ten groups each group comprised six rats as the following:

Group 1 (Control): rats received distilled water (vehicle of LC and Er) (1ml/day) by oral gavage for 28 days.

Group 2 (Oil): rats received corn oil (vehicle of Malathion) (0.5 ml/day) by oral gavage for 28 days.

Group 3 (M27): rats received low dose of Malathion (27 mg/ kg/day) (equivalent to 1/50 of the LD50 for an oral dose) (Jalili et al., 2018) dissolved in (0.5 ml corn oil) by oral gavage to the for 28 days.

Group 4 (M54): rats received high dose of Malathion (54 mg/kg/day) (equivalent to 1/25 of the LD50 for an oral dose) (Geng et al., 2015) dissolved in (0.5 ml corn oil) by oral gavage for 28 days.

Group 5 (LC): rats received LC (100 mg/kg/day) (Salama et al., 2022) dissolved in (1ml distilled water) by oral gavage for 28 days.

Group 6 (LC+M27): rats received LC (100 mg/kg/day) dissolved in (1ml distilled water) by oral gavage followed by low dose of Malathion (27 mg/kg/day) dissolved in (0.5 ml corn oil) by oral gavage after 2 hours for 28 days.

Group 7 (LC+M54): rats received LC dose (100 mg/kg/day) dissolved in (1ml distilled water) by oral gavage followed by high dose of Malathion (54 mg/kg/day) dissolved in (0.5 ml corn oil) by oral gavage after 2 hours for 28 days.

Group 8 (Er): rats received Er (10 mg/kg/day) (Uz et al., 2011) dissolved in (1ml distilled water) by oral gavage for 28 days.

Group 9 (Er+M27): rats received Er (10 mg/kg/ day) dissolved in (1ml distilled water) by oral gavage followed by low dose of Malathion (27 mg/kg/day) dissolved in (0.5 ml corn oil) by oral gavage after 2 hours for 28 days.

Group 10 (Er+M54): rats received Er (10 mg/kg/ day) dissolved in (1ml distilled water) by oral gavage followed by high dose of Malathion (54 mg/kg/day) dissolved in (0.5 ml corn oil) by oral gavage after 2 hours for 28 days.

Samples collection and preparation:

After 28 days, animals were anesthetized by thiopental sodium (40 mg/ kg intraperitoneal) and blood samples were collected by cardiac puncture (Abdelrahman and Abdelmageed, 2020). Blood samples centrifuged at (4000 r.p.m / 4 ^{0}C) for 15 min to obtain serum. The serum was preserved at -

20°C until used for biochemical investigations of renal functions. After that, animals were sacrificed by cervical dislocation, where both kidneys were harvested and washed with ice-cold NaCl isotonic solution (0.9%). For the biochemical measurements of oxidative stress markers, the right kidneys homogenates were centrifuged at (4000 r.p.m/4 °C) for 15 min, and the supernatant layer stored at $^{-}80^{o}$C until used. Left kidneys were kept for histopathological and immunohistochemical analyses by immersion in 10% neutral buffered formalin for 48 h preparing for staining.

Biochemical evaluation of renal function in the serum:

Serum creatinine and urea were measured by absorption spectrophotometry using commercial kits supplied by (Spinreact®, Santa Coloma, Spain) according to the methods described by (Bartels et al., 1972) and (Fawcett and Scott,1960) respectively. Quantitative assay of serum albumin was done by using SPIN 800 Autoanalyzer and commercial kits supplied by (Spinreact® Santa Coloma, Spain) according to the method of (Doumas et al., 1971). The results were expressed as milligram per deciliter (mg/dL) for serum creatinine and urea, while as results were expressed as gram per deciliter(g/dL) for serum albumin.

Biochemical evaluation of oxidative stress markers in the renal tissue homogenate:

Oxidative stress markers in renal tissue were assessed by colorimetric methods using commercial kits purchased from (Bio-diagnostic®, Giza, Egypt). The activity of super oxide dismutase (SOD) was determined according to the method described by (Nishikimi et al., 1972) and the results were expressed as unit per gram of tissue homogenate (U/g. tissue). Reduced Glutathione (GSH) renal concentration was measured according to the method of (Beutler et al., 1963) and the concentrations of GSH were expressed as milligram per gram of tissue homogenate (mg/g. tissue). The malondialdehyde (MDA) was determined according to the method of (Ohkawa et al., 1979) and MDA concentrations were expressed as nanomole per gram of tissue homogenate (nmol/g. tissue).

Histopathological Examination:

Paraffin sections of five microns thickness were cut and stained by hematoxylin and eosin (H&E) for histological examination according to the methods of (Bancroft and Layton, 2013) by using a light microscope (Olympus Bx51 model, Tokyo, Japan) equipped with a digital camera (Olympus DP20, Tokyo, Japan).

Immunohistochemical investigation

For immuno-histochemical examination, renal tissue sections of four microns thickness were obtained (section / animal). Renal sections were stained with polyclonal anti-cleaved-Caspase-3 rabbit antibodies obtained from (Service biotechnology Co., Wuhan, China), according to the avidin-biotin-peroxidase complex (ABC) technique (Hsu et al., 1981). Stained slides were analyzed under 400× magnification using digital camera (Olympus DP20, Tokyo, Japan) installed in (Olympus Bx51 model, Tokyo, Japan) light microscope. In the obtained digitalized images, the areas of positive (brown) staining were analyzed using image analysis (http://fiji.sc). For quantitative analysis of Caspase-3 expression; the area of positive

Caspase-3 expression was calculated as the average of positive areas in four non overlapped fields per each slide then data from each group were presented as the means ± standard error of mean (SEM).

Statistical analysis

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 26) and data normality was approved by using One-sample Kolmogorov-Smirnov test. Continuous variables were presented as means ± standard deviation (SD) or means ± SEM. One-way analysis of variance (ANOVA) test followed by Tukey-Kramer multiple comparisons post hoc test were used for comparing the mean values between the studied groups. The threshold of significance was fixed at 5% level (p-value). The results were considered significant when the probability of error is less than 5% ($p < 0.05$).

Results

No toxic manifestations as well as no mortality were reported among animals of all studied groups during the period of the study.

Biochemical evaluation of renal function in serum

As shown in (Table 1) significant elevation of serum creatinine and urea in line with significant decrease in serum albumin were noticed in both (M27) and (M54) groups compared to control group with significant difference between both groups. There were significant lowering of both serum creatinine and urea as well as significant elevation of serum albumin in both (LC+M27) group and (LC+M54) compared to (M27) group and (M54) group, respectively. Only serum urea and albumin in (LC+M27) achieved normal values as they did not show difference with the control group. On the other hand, serum creatinine in (LC+M27) as well as all renal functions in (LC+M54) were still significantly higher than control group. Moreover, (Er+M27) group and (Er+M54) group showed significant lowering of serum creatinine and urea as well as significant elevation of serum albumin compared to (M27) and (M54), respectively. In both (Er+M27) group and (Er+M54) group, all the renal functions were still significantly different from control group except serum urea in (Er+M27) which was the only renal function parameter that return normal values. Notably; (LC+M27) group and (LC+M54) group had lower serum creatinine and urea as well as higher serum albumin values compared to (Er+M27) group and (Er+M54) group respectively, although these differences were statistically insignificant. Although both LC and Er showed amelioration in renal function abnormalities induced by Malathion; LC was slightly better than Er. Oil, LC and Er groups had normal renal functions and no significant differences compared to the control group.

Table (1): Comparing the effects of Malathion administration alone and with L-carnitine or Erdosteine on the renal functions in the serum of adult spurge dewily rats (n= 60).

Variables	Control (n=6)	Oil (n=6)	M27 (n=6)	M54 (n=6)	LC (n=6)	LC + M27 (n=6)	LC+ M54 (n=6)	Er (n=6)	Er+ M27 (n=6)	Er+ M54 (n=6)	One-Way ANOVA test
Serum Creatinine (mg/dL)	1.12± 0.19	1.05 ± 0.24	4.62 ± 0.52 [a]	5.35 ± 0.71[a,b]	1.03 ±0.28	2.17 ± 0.30[a,b,c]	3.08 ± 0.60 [a,b,c,]	1.13 ± 0.29	2.80 ± 0.38 [a,b,c]	3.63± 1.18 [a,c]	F= 49.413 p < 0.001*
Serum Urea (mg/dL)	27.50± 8.44	28.50 ± 8.64	58± 15.47 [a]	68.5 ± 12.05[a,b]	31 ± 9.12	38 ± 5.97 [b,c]	49.33 ± 4.97 [a,c]	31.83 ±10.11	40.83 ± 7.99 [b,c]	54.5 ± 9.39 [a,c]	F= 12.522 p < 0.001*
Serum Albumin (g/dL)	4.25± 0.51	4.28 ± 0.45	2.32 ± 0.32 [a]	2.05± 0.29 [a,b]	4.27± 0.62	3.63 ± 0.32[b,c]	2.98 ±0.31[a,c]	4.20 ± 0.51	3.45 ± 0.25[a,b,c]	2.62± 0.15 [a,c]	F= 28.467 p < 0.001*

Data were expressed as mean ± (SD).
F: One-Way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test.
Values shown with different letters (a, b, and c) are statistically different at $P < 0.05$*
a: statistically significant difference in relation to control group
b: Statistically significant difference in relation to M27 group
c: Statistically significant difference in relation to M54 group

(Control) : group received distilled water, (Oil): group received corn oil, (M27) group received Malathion low dose (27 mg/kg/day) dissolved in corn oil, (M54) group received Malathion high dose (54mg/kg/day) dissolved in corn oil, (LC) group received L-carnitine (100 mg/kg/day) in distilled water, (LC+ M27) group received L-carnitine (100 m/kg/day) dissolved in distilled water + Malathion low dose (27 mg/kg/day) dissolved in corn oil, (LC+M54) group received L-carnitine (100m/kg/day) dissolved in distilled water + Malathion high dose (54 mg/kg/day) dissolved in corn oil , (Er) group receive Erdosteine (10 mg/kg/day) dissolved in distilled water, (Er+M27) group received Erdosteine (10 mg/kg/day) dissolved in distilled water + Malathion low dose (27 mg/kg/day) dissolved in corn oil, (Er+ M54) group received Erdosteine (10 mg/kg/day) dissolved in distilled water+ Malathion high dose (54 mg/kg/day) dissolved in corn oil, n: number of rats in group, mg: milligram, dL: deciliter, g:gram, p: significance, SD: standard deviation.

Biochemical evaluation of oxidative stress markers in the renal tissue homogenate

As illustrated in (Table 2) Malathion groups showed significant deficiency of SOD and GSH as well as significant elevation of MDA compared to control group. Malathion induced renal oxidative stress was in dose dependent manner as significant differences were observed between (M27) and (M54) groups. There was significant elevation of SOD and GSH as well as significant lowering of MDA in (LC+M27) and (LC+M54) groups compared to (M27) and (M54) groups, respectively. In addition, (Er+M27) and (Er+M54) groups had significantly higher SOD and GSH as well as lower MDA compared to (M27) and (M54) groups, respectively. The (LC+M27), (LC+M54), (Er+M27) and (Er+M54) groups still had significantly lower SOD and GSH in line with significantly higher MDA compared to the control group. The antioxidant effect of LC was slightly better than that of Er; as (LC+M27) and (LC+M54) groups had higher SOD and GSH as well as lower MDA compared to (Er+M27) and (Er+M54) groups, respectively, although these differences were statistically insignificant. The Oil, LC and Er groups had no significant differences with each other or with the control group as regard SOD, GSH and MDA.

Table (2): Comparing the Malathion oxidative stress effect and the antioxidant effect of co-administration of L-carnitine or Erdosteine with Malathion in renal tissues of adult spurge dewily rats (n= 60).

Variables	Control (n=6)	Oil (n=6)	M27 (n=6)	M54 (n=6)	LC (n=6)	LC + M27 (n=6)	LC+ M54 (n=6)	Er (n=6)	Er+ M27 (n=6)	Er+ M54 (n=6)	One-Way ANOVA test
Renal SOD (U/g. tissue)	198.83 ± 8.70	206.17 ± 6.79	98.67 ± 12.89[a]	84.17 ± 10.30[a,b]	200.5 ± 8.87	171.83 ±17.75[a,b,c]	127.17 ± 10.67[a,b,c]	204.50 ± 9.99	160.67 ±16.61[a,b,c]	111.83 ± 11.84[a,b,c]	F= 94.911 p < 0.001*
Renal GSH (mg/g. tissue)	224.67 ± 7.20	224.17 ± 8.16	96.50 ± 4.51[a]	76.67 ± 8.09[a,b]	218.67± 7.53	174 ± 14.13[a,b,c]	112 ± 9.38[a,b,c]	222.83 ± 6.24	162.33 ±20.46[a,b,c]	96.83 ± 10.34[a,b,c]	F= 198.572 p < 0.001*
Renal MDA (nmol/g. tissue)	0.89 ± 0.05	0.86 ± 0.05	8.67 ± 1.43 [a]	10.74 ± 1.54[a,b]	0.87 ± 0.05	3.70 ± 0.65 [a,b,c]	6.41 ± 1.32[a,b,c],	0.89 ± 0.04	4.29 ± 0.80[a,b,c,]	7.10 ± 0.79[a,b,c]	F= 101.898 p < 0.001*

Data were expressed as mean ± SD.

F: One-Way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test.

Values shown with different letters (a, b, and c) are statistically different at $P < 0.05$*

a: statistically significant difference in relation to control group

b: Statistically significant difference in relation to M27 group

c: Statistically significant difference in relation to M54 group

(Control) : group received distilled water, (Oil): group received corn oil, (M27) group received Malathion low dose(27 mg/kg/day) dissolved in corn oil, (M54) group received Malathion high dose (54mg/kg/day) dissolved in corn oil, (LC) group received L-carnitine (100 mg/kg/day) in distilled water, (LC+ M27) group received L-carnitine (100 m/kg/day) dissolved in distilled water + Malathion low dose (27mg/kg/day) dissolved in corn oil, (LC+M54) group received L-carnitine (100 m/kg/day) dissolved in distilled water + Malathion high dose (54 mg/kg/day) dissolved in corn oil , (Er) group receive Erdosteine (10 mg/kg/day) dissolved in distilled water, (Er+M27)group received Erdosteine (10 mg/kg/day) dissolved in distilled water + Malathion low dose (27 mg/kg/day) dissolved in corn oil, (Er+ M54) group received Erdosteine (10 mg/kg/day) dissolved in distilled water+ Malathion high dose (54 mg/kg/day) dissolved in corn oil, (SOD) Superoxide dismutase, (GSH) Reduced glutathione, (MDA) malondialdehyde, (U/g tissue) unit per gram of tissue homogenate, (mg/g. tissue) milligram per gram of tissue homogenate, (nmol/g. tissue), nanomole per gram of tissue homogenate, n: number of rats in group, p: significance, SD: standard deviation .

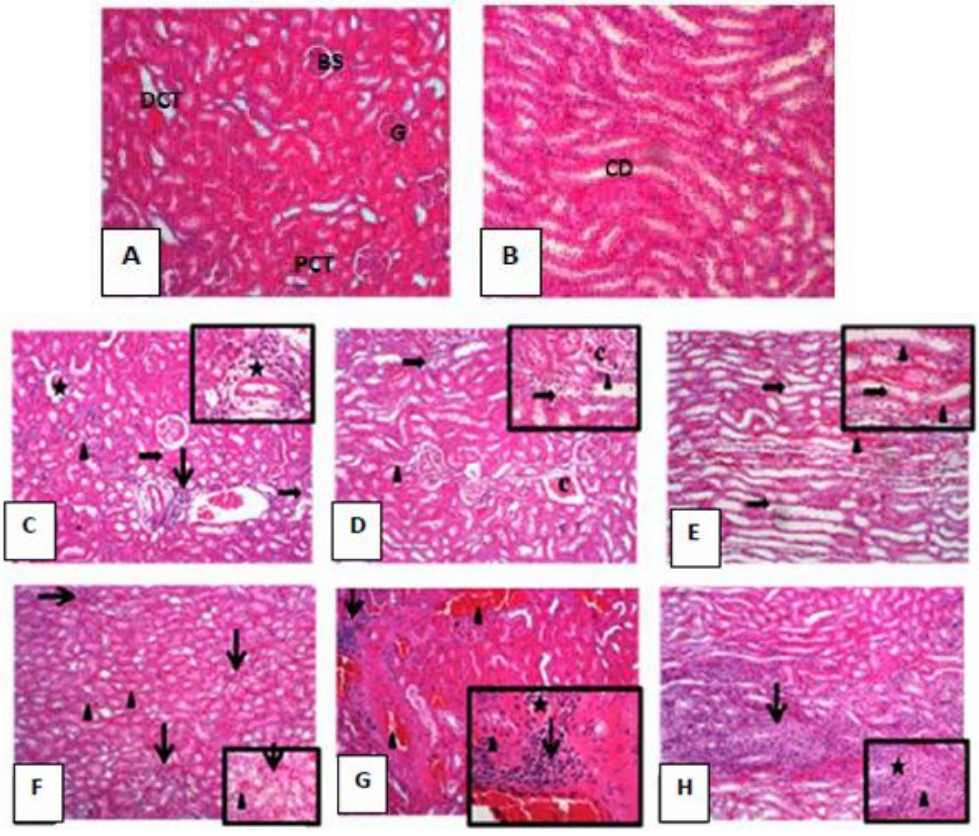
Histopathological Evaluation

The control group showed normal histological architecture in both renal cortex (Figure 1-A) and medulla (Figure 1-B). Oil group, LC group and Er group had normal histological architecture in both renal cortex and medulla like the control group.

As shown in (Figure 1-C& 1-D); (M27) group showed multifocal moderate cortical degenerative and inflammatory changes. Atrophied shrunken glomeruli, congested glomeruli and periglomerular fibrosis were observed. Proximal and distal convoluted tubules appeared dilated and degenerated with intraluminal hyaline cast and intratubular aggregation of sloughed epithelium. Cortical interstitial nephritis was evident in the form of mild perivascular aggregations of lymphocytes, plasma cells and macrophages admixed with perivascular edema. Renal medulla in (M27) group showed moderate changes in the form of degenerated ectatic collecting ducts with sloughed epithelium, interstitial congestion, moderate inflammatory infiltration, and peritubular fibroblast (Figure 1-E). Moreover, (M54) group showed all the above mentioned degenerative and inflammatory changes in (M27) but in more massive form in both renal cortex (Figure 1- F, 1-G) and medulla (Figure 1-H). In addition, in (M54) group, diffuse cortical tubular pyknosis and massive renal congestion were observed.

As shown in (Figure 1- I), renal cortex of (LC+M27) group showed marked improvement compared to (M27) group, as no abnormalities were detected in the glomeruli. Proximal convoluted tubules were markedly improved although minimal tubular degeneration and dilatation were observed. Only a few focal aggregations of inflammatory cells were reported. In addition, renal medulla of (LC+M27) group showed minimal tubular degeneration, dilatation, and inflammatory infiltration (Figure 1-J). Some improvement of cortical changes was observed in (LC+M54) compared to (M54) group as moderate changes were found as shrunken glomeruli, tubular degeneration, moderate focal tubulointerstitial inflammation and marked congestion (Figure 1-K). Renal medulla showed improvement in (LC+M54) compared to (M54) group as moderate medullary tubular dilatation, interstitial congestion and less inflammatory cells were detected (Figure 1-L).

Renal cortex and medulla in (Er+M27) and (Er+M54) groups showed less improvement of Malathion induced degenerative and inflammatory changes compared to (LC+M27) and (LC+M54), respectively. Widening of the glomerular Bowman's space, mild tubular changes and mild multifocal inflammatory infiltration were observed in (Er+M27) group (Figure 1-M). Mild medullary changes in the form of dilated degenerated renal tubules and focal inflammatory infiltrations were detected in (Er+M27) group (Figure 1-N). Group (Er+M54) showed marked cortical degenerative changes in the form of atrophic shrunken glomeruli, necrotic tubules, moderate inflammatory aggregations, congestion, and hemorrhage (Figure1-O). Severe medullary interstitial nephritis, degenerated collecting ducts, congestion, hemorrhage and numerous inflammatory and fibroblast were detected in (Er+M54) group (Figure1-P)



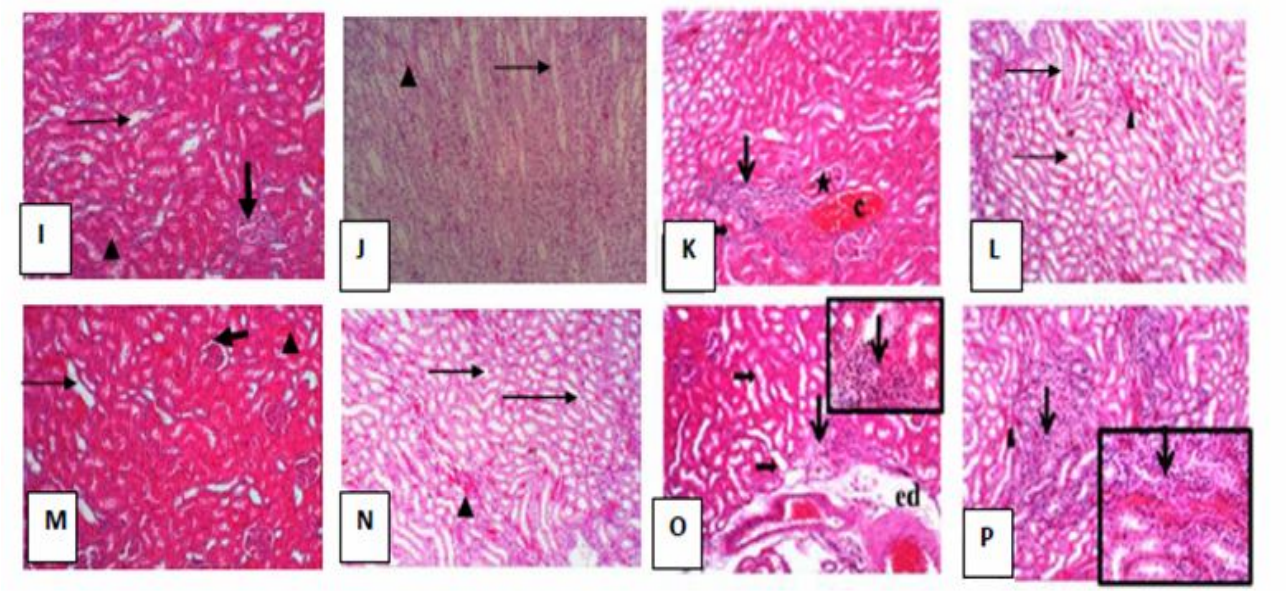


Fig. (1): **Representative photomicrographs of hematoxylin and eosin (H&E) stained renal sections from different experimental groups**: **(A) and (B)** showed normal renal cortex and medulla, respectively in the control group. G: glomeruli, BS: bowman's space, PCT: proximal convoluted tubules, DCT: distal convoluted tubules and CD: collecting ducts. **(C) and (D)** showed moderate cortical affection in (M27) group. **(C)** cortical interstitial nephritis (long arrow), degenerated tubules (short arrows), intraluminal hyaline casts (arrowhead) and atrophied glomerulus (star). The insert in image: perivascular inflammatory cells infiltrations (star). **(D)** showed mild fibrosis (arrowhead), tubular damage (arrow) and congested glomeruli (c). The insert in image: intratubular sloughed epithelium (arrow), congested glomeruli (c) and periglomerular fibroblasts (arrowhead). **(E)** showed diffuse ectatic renal medullary tubules in (M27) (arrows) and hemorrhage (arrowhead). The insert in image: sloughed medullary tubular epithelium (arrow) and hemorrhage mixed with few fibroblasts (arrowheads). **(F) and (G)** showed massive cortical degenerative and inflammatory effects in (M54) group. **(F)** showed diffuse cortical tubular pyknosis (arrowheads), extensive inflammatory cells (arrows). The insert in image: inflammatory infiltrations (arrow) and tubular pyknosis (arrowhead). **(G)** showed necrotic cortical tubules with extensive congestion (arrowheads) and interstitial nephritis (arrow). The insert in image: aggregations of lymphocytes (arrow), atrophic glomerulus (arrowhead) and intraluminal hyaline cast (star). **(H)** showed renal medullary tubules in (M54) were massively dilatated (arrow). The inset in image: extensive inflammatory infiltrations and fibroblast (arrowhead), necrotizing tubules (star) **(I)** showed cortical improvement in (LC+M27) group as glomeruli appeared normal (Thick arrow), few dilated tubules (Thin arrow) and minimal focal inflammatory infiltration (arrowhead). (**J**) showed medullary improvement in (LC+M27) group as some dilated medullary tubules (arrow) and focal inflammatory infiltration (arrowhead). **(k)** (LC+M54) showed moderate cortical changes as focal coalescing inflammation (arrowhead), necrotic tubules (arrow), extensive congestion (c) and shrunken glomeruli (star). **(L)** (LC+M54) showed some improvement as low interstitial congestion (arrowhead) and few inflammatory cells and some dilated medullary tubules (thin arrows). (**M**) (Er+M27) showed mild cortical affection as wide Bowman's space (thick arrow), tubular dilatation and epithelial sloughing (thin arrow) with inflammatory infiltrations (arrowhead). (**N**) (Er+M27) showed mild medullary changes as dilated renal tubules (arrows) and focal inflammatory infiltrations (arrowhead). (**O**) (Er+M54) showed less improvement of the renal cortex as necrotic tubular (short arrows) with focal, perivascular edema (ed) and inflammatory aggregations (long arrow). The insert image: focal inflammatory aggregations and fibroblast (arrow). **(P)** (Er+M54) showed obvious, severe medullary interstitial nephritis (arrow) with congestion (arrowhead). The insert in image: hemorrhage, inflammatory infiltrations, and fibroblasts (arrow). All images at 100x and the inset in images= 400x.

Immunohistochemical Evaluation

The control group showed weak positive Caspase-3 reaction in both renal cortex and medulla (Figure 2-A & 2-B) respectively. Oil, LC and Er groups; showed week positive reaction to Caspase-3 in both cortex and medulla with no statistically significant difference on comparison with the control group (Table 3).

Caspase-3 reaction was stronger in both renal cortex (Figure 2-C) and medulla (Figure 2-D) of (M27) group, in the form of brownish staining of mainly tubular cells and to less extent the glomerular cells and the interstitial tissue. The strongest reaction to Caspase-3 was observed in renal sections of (M54) group as massive brownish staining involved both cortex and medulla with preference to tubular cells was detected (Figure 2-E & 2-F). Both (M27) and (M54) groups had significantly increasing in Caspase-3 expression compared to the control group in both renal cortex and medulla. In addition, (M54) group had significantly higher Caspase-3 expression compared to (M27) group in both renal cortex and medulla (Table 3).

As shown in (Figure 2-G,2-H,2-I &2-J) and (Table 3); administration of LC with Malathion was associated with marked improvement of apoptosis in both renal cortex and medulla. A significant decrease in Caspase-3 expression was observed in both (LC+M27) and (LC+M54) groups compared to (M27) and (M54) respectively. In addition, Er combination with Malathion was associated with significant decrease in Caspase-3 reaction. Both renal cortex and medulla of (Er+M27) and (Er+M54) groups showed significantly weaker Caspase-3 reaction compared to (M27) and (M54) groups respectively (Figure 2-K,2-L,2-M & 2-N) (Table 3). As shown in (Table 3), (LC+M27) and (LC+M54) groups had weaker Caspase-3 reaction compared to (Er+M27) and (Er+M54) groups respectively, although this difference was statistically insignificant. Hence, the antiapoptotic protective effect of LC against Malathion-induced nephrotoxicity seemed to be slightly stronger than Er although both of them did not recover the Caspase-3 expression to control levels.

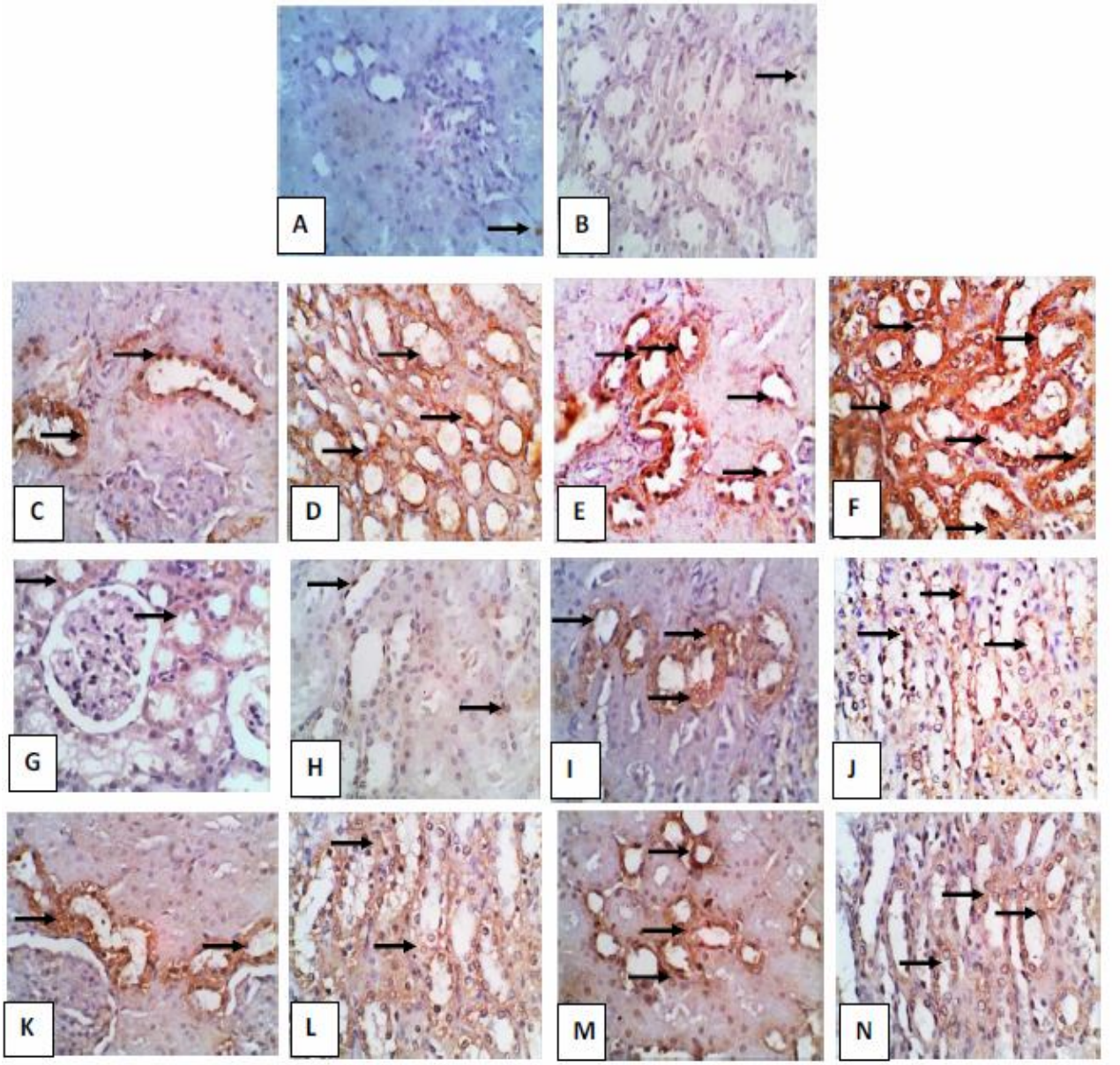


Fig. (2): Representative photomicrograph of immunohistochemical Caspase-3 expression in kidney from different experimental groups. **(A)** and **(B):** represented weak Caspase-3 expression in both renal cortex and medulla, respectively of the control group. **(C)** and **(D):** showed strong expression of Caspase-3 reaction in renal cortex and medulla of (M27) group, respectively. **(E)** and **(F):** showed the strongest expression of Caspase-3 in cortex and medulla, respectively of (M54) group with high preference of tubular cells in cortex and medulla**. (G**) and **(H)**: showed marked improvement in the form of decreased Caspase-3 expression of both cortex and medulla, respectively of (LC+M27) group compared to (M27) group. **(I)** and **(J):** showed improvement in Caspase-3 expression in both cortex and medulla respectively, of (LC+M54) group compared to (M54) group. **(K)** and **(L)**: represented decreased Caspase-3 expression in both cortex and medulla, respectively of (Er+M27) group compared to (M27). **(M)** and **(N)** showed a slight decrease in Caspase-3 expression in renal cortex and medulla respectively, of (Er+M54) group compared to (M54). The (black arrows) pointed to examples of positively stained cells with brownish stained cytoplasm (Positive Caspase-3 reaction). Image magnification= 400x.

Table (3): Comparison of Caspase-3 expression (area) in renal cortex and medulla between the studied groups.

Variables	Control (n=6)	Oil (n=6)	M27 (n=6)	M54 (n=6)	LC (n=6)	LC + M27 (n=6)	LC+ M54 (n=6)	Er (n=6)	Er+ M27 (n=6)	Er+ M54 (n=6)	One-Way ANOVA test
-Caspase-3 expression in renal cortex	0.11± 0.03	0.13± 0.03	9.47 ± 0.64 [a]	11.18 ± 0.67[a,b]	0.16 ± 0.04	3.46 ± 0.67[a,b,c]	6.53 ± 0.53[a,b,c]	0.14 ± 0.03	3.71 ± 0.52[a,b,c]	7.94 ± 1.29[a,b,c]	F= 307.376 P < 0.001*
-Caspase-3 expression in renal medulla	0.32 ± 0.03	0.32 ± 0.05	7.96 ± 0.39 [a]	11.17 ± 2.73[a,b]	0.31 ± 0.05	2.17 ± 0.31[a,b,c]	4.72 ± 0.56[a,b,c]	0.30 ± 0.04	2.48 ± 0.31[a,b,c]	6.24 ± 0.93[a,b,c]	F= 97.504 P < 0.001*

Data were expressed as mean ± (SEM)
F: One-Way ANOVA test followed by Tukey-Kramer multiple comparisons post hoc test.
Values shown with different letters (a, b, and c) are statistically different at P < 0.05*
a: statistically significant difference in relation to control group
b: Statistically significant difference in relation to M27 group
c: Statistically significant difference in relation to M54 group

(Control) : group received distilled water, (Oil): group received corn oil, (M27) group received Malathion low dose(27 mg/kg/day) dissolved in corn oil, (M54) group received Malathion high dose (54mg/kg/day) dissolved in corn oil, (LC) group received L-carnitine (100 mg/kg/day) in distilled water, (LC+ M27) group received L-carnitine (100 m/kg/day) dissolved in distilled water + Malathion low dose (27 mg/kg/day) dissolved in corn oil, (LC+M54) group received L-carnitine (100 m/kg/day) dissolved in distilled water + Malathion high dose (54 mg/kg/day) dissolved in corn oil , (Er) group receive Erdosteine (10 mg/kg/day) dissolved in distilled water, (Er+M27) group received Erdosteine (10 mg/kg/day) dissolved in distilled water + Malathion low dose (27mg/kg/day) dissolved in corn oil, (Er+ M54) group received Erdosteine (10 mg/kg/day) dissolved in distilled water+ Malathion high dose (54 mg/kg/day) dissolved in corn oil, n: number of rats in group, p: significance, SEM: standard error of means.

Discussion

Malathion is one of the most popular organophosphate pesticides that had been linked to renal impairment in both human and experimental data (Wan et al., 2021; Sobolev et al., 2022). Both LC and Er are well known antioxidant, anti-inflammatory and anti-apoptotic agents (Salama et al., 2022; Mutneja et al., 2020). The current study aimed at investigating as well as comparing the protective effects of LC and Er against high dose and low dose of subacute Malathion-induced nephrotoxicity.

In accordance with (Al-Attar, 2010; Khalifa and Alkhalaf, 2020; Ghamry et al., 2022; El Okle et al., 2022), the present study demonstrated that Malathion was associated with significant elevation of serum creatinine and urea in line with significant decrease in serum albumin compared to the control group. Like; (Kata, 2020; Massoud et al., 2022), the present renal biochemical changes were in a dose dependent manner. Physiologically, creatine and urea are byproducts of protein metabolism and their serum levels elevation indicating glomerular filtration deficiency (Kata, 2020). Hypoalbuminemia in Malathion groups could be assumed to decrease renal tubular reabsorption of albumin (Yokota et al., 2017), besides the decreased hepatic synthesis of albumin due to Malathion-induced hepatotoxicity (Selmi et al, 2015). Accordingly, histological findings of the current study and similar studies (Al-Attar, 2010; Zidan, 2015; Jalili et al., 2018; Selmi et al., 2018) reported extensive glomerular and proximal renal tubular degenerative changes in Malathion intoxicated rats. Like our results; Ahmed (2014) and Baiomy et al. (2015) reported marked degenerative and inflammatory changes in medullary collecting ducts in Malathion groups.

Kidney is a potential target of Malathion toxicity as kidney represents the main route of excretion of Malathion and its more toxic metabolite malaoxon (Akbel et al., 2018; Badr 2020). In addition, cytochrome P450 of renal proximal convoluted tubules share with the same enzyme system of

hepatocytes in production of the malaoxon (Knights et al., 2013). Moreover, Malathion affects the renal neural supply which regulates the renal microvascular supply (Ahmed, 2014). Hence, Malathion can induce renal hypoxia with all its sequences of oxidative stress, inflammation and apoptosis (Badr 2020).

The current findings came in harmony with (Jalili et al., 2018; Selmi et al., 2018; Abdel-Daim et al., 2020; Khalifa and Alkhalaf, 2020; Ghamry et al., 2022; El Okle et al., 2022) who reported significant alteration of renal oxidative stress biomarkers (SOD, GSH and MDA) in the Malathion groups compared to control group. Oxidative stress is the increasing of the free oxygen radicals (e.g., superoxide anion) to a degree that overcomes the neutralizing capacity of the enzymatic antioxidant (e.g., SOD) and non-enzymatic scavengers as (e.g., GSH) (Hosohata, 2016). Oxidative stress is a pivotal mechanism in renal impairment as well as in organophosphate-induced organs damage (Gyurászová et al., 2020; Sobolev et al., 2022). The SOD is highly expressed in renal tubules and catalyzes the transformation of superoxide into less toxic hydrogen peroxide and oxygen (Ghamry et al., 2022). The GSH is the major sulfhydryl donner in the renal tissue (Abdel-Daim et al., 2020). Free oxygen radicals react with macromolecules such as lipids, protein as well as nucleic acids inducing tissue damage. The MDA is a biomarker of lipid peroxidation which decreases the cell membrane fluidity and alters the functions of membrane receptors and linked enzymes (El Okle et al., 2022). Interestingly, the relationship between the renal oxidative stress and inflammation is bidirectional (Gyurászová et al., 2020). Free oxygen radicals stimulate the recruitment and activation of inflammatory cells through increasing of nuclear transcription factor κB (NF-κB). Moreover, inflammatory mediators as interleukin-6, tumor necrosis factor-α and myeloperoxidase are capable of induction of oxidative stress (Abdelrahman and Abdelmageed, 2020). The fact that explained marked inflammatory changes in the Malathion groups in the current work and in similar studies (Al-Attar, 2010; Ahmed, 2014; Zidan, 2015; Jalili et al., 2018; Selmi et al., 2018).

In accordance with Gur and Kandemir (2023); current findings demonstrated significant increase of renal Caspase-3 expression in Malathion groups compared to control group. Similarly, Atilgan et al. (2022) reported an increased number of apoptotic renal cells in Malathion intoxicated rats. Apoptosis occurs physiologically to get rid of abnormal cells, while as; massive apoptosis leads to organs dysfunction. Two pathways control apoptosis; the intrinsic mitochondrial pathway and the extrinsic pathway, which is initiated via death receptors on the cell membrane (Gur and Kandemir 2023). Caspases are cysteine - aspartic proteases which considered the initiators of both apoptotic pathways. Caspase-3 represents the common executioner of both pathways as its activation means that apoptosis will be irreversible (D'Arcy, 2019). Nuclear changes (pyknosis) due to Malathion-induced cell death were detected in (M54) group in current study as well as in the study of (Ahmed, 2014).

The LC antioxidant effect in the serum of acute organophosphate intoxicated patients was reported by Ghonem et al. (2018) in form of increased GSH and decreased MDA. Present results revealed for the first time the nephroprotective effects of LC against Malathion-induced renal injury in rats. Both (LC+M27) and (LC+M54) groups showed significant decrease in serum creatinine, urea, and renal MDA as well as significant increase in serum albumin, renal SOD and GSH

compared to Malathion groups. Similar LC antioxidant effects in line with improved renal functions, but without achievement of the control levels, were reported in toxic rat models of cisplatin (Elkomy et al.,2020), potassium dichromate (Salama et al., 2022) and gentamicin (Sakr and Kamel 2023). In present work, only serum urea and albumin achieved control levels in (LC+M27) group. In partial agreement, adjuvant LC treatment in methotrexate intoxicated rats returned the serum urea to control levels but not serum albumin (Tousson et al., 2014). Different from our results; Kunak et al., (2016) reported normalization of serum creatinine and urea with LC at doses (200 mg/kg/day and 400 mg/kg/day); while as renal SOD, GSH and MDA returned to control levels with LC at (400mg/kg/day) in rats with combined glycerol and contrast-induced kidney injury. These differences could be assumed to variations in LC dose as well as different mechanisms of nephrotoxic agents.

The LC cotreatment was associated with marked improvement of glomerular and tubular degenerative changes as well as renal tubulointerstitial inflammation and fibrosis, in the present Malathion toxic model as well as in rat models of diazinon toxicity (Shokrzade et al., 2013), tacrolimus toxicity (Zheng et al.,2021) and chronic kidney disease (Ahmad et al., 2016). The present histological improvements were more obvious in (LC+M27) group, although both (LC+M27) and (LC+M54) groups did not restore the normal renal architecture. Unlike our results, LC at dose (150 mg/kg/day) markedly ameliorated renal histological findings in rat model of combined doxorubicin and cyclophosphamide toxicity, while as LC at dose (300mg/kg/day) completely normalized renal tissue (Morid et al., 2023). Therefore, investigating higher doses of LC against Malathion-induced nephrotoxicity is highly recommended.

In the current work, LC adjuvant treatment ameliorated the Malathion-induced renal cells apoptosis via significant decrease in renal Caspase-3 expression compared to Malathion groups. Like our findings, LC led to decease in renal Caspase-3 expression in line with improved renal degenerative changes, inflammation and tubulointerstitial fibrosis in toxic animal models of cyclosporine (Xiang et al., 2013), combined glycerol and contrast-induced kidney injury (Kunak et al., 2016) and letrozole (Hassan et al., 2023).

In the current study, co-administration of Er with Malathion led to a significant improvement of Malathion-induced renal biochemical and oxidative perturbations in line with some extent of reduction in renal inflammatory and degenerative effects particularly in (Er+M27). On reviewing literature, no previous studies concerned the Er protective effects against Malathion toxicity but Er had antioxidant and anti-inflammatory effects in the serum of diazinon intoxicated rats (Birdane et al., 2022). Similarly, Er had neuroprotective role in toxic rat models of cyclosporine (Yildirim et al., 2003; Uz et al., 2011) and cisplatin (Tutanc et al., 2012) without normalizing most of investigated renal parameters; while as; serum urea returned to control levels in (Er+M27) group in the current work. Unlike our results, Er maintained renal biochemical functions at normal control levels in vancomycin intoxicated rats via its antioxidant effect (Öktem et al., 2005). The present study and the above-mentioned studies used the least reported oral nephroprotective dose of Er (10 mg/kg/day). Higher antioxidant and anti-inflammatory nephroprotective doses of Er had been investigated against different agents in rat models. For example, Cabuk et al. (2008) used Er (50 mg/kg/day) in gentamycin toxicity. The Er (100 mg /kg/day) ameliorated radiation induced renal injury (Elkady and

Ibrahim.,2016). Unlike our results, Er (150 mg/kg/day and 300 mg/kg/day) normalized renal SOD, GSH and MDA despite sustained elevated serum creatinine in acetaminophen intoxicated rats (Isik et al., 2006). The difference could be assumed to Er doses variations as well as different mechanisms and durations of exposure to nephrotoxic agents. Hence, further investigation of higher nephroprotective of (Er) is highly recommended.

To the best of our knowledge, it was the first time to demonstrate the antiapoptotic effect of Er in renal tissues in vivo. The Er adjuvant treatment with Malathion significantly decreased renal Caspase-3 expression compared to Malathion groups. The antiapoptotic effect of Er via decreasing Caspase-3 expression had been demonstrated in testicular seminiferous tubules (Guven et al., 2014), pulmonary epithelial cells (Demiralay et al., 2013), auditory cell line (Kim et al., 2015), cardiac tissues (Mutneja et al., 2020) and hepatocytes (Hassan et al., 2020).

Interestingly, in the current study, LC has more potent antioxidant, anti-inflammatory and antiapoptotic nephroprotective effects compared to Er against both low and high Malathion doses although these differences were statistically insignificant. On reviewing literature, no previous studies concerned with comparing the neuroprotective effects of both agents. The current findings could be explained by the direct protecting effects of LC on renal glomeruli and tubules. For example, Martinez et al. (2009) demonstrated that LC maintained normal thickness of basement membrane and mesangial extracellular matrix of the glomeruli to prevent glomerular sclerosis and interstitial fibrosis. In addition, LC improved the apical plasma membrane of medullar collecting ducts via increasing their water channel aquaporin (Gao et al., 2017). Moreover, LC restored the normal organization of renal cells cytoskeleton particularly the intermediate filaments (vimentin and cytokeratin 18) (Elkomy et al., 2020). On the other hand, the decreased efficacy of Er nephroprotective effects compared to LC could be assumed to partial failure of hepatic transformation of Er to its active metabolite as well as unsuccessful releasing of the Er sulfhydryl groups due to Malathion-induced hepatotoxicity alongside the nephrotoxicity (Selmi et al, 2015; Mutneja et al., 2020).

In conclusion, the current study findings came in line with previously reported Malathion- induced nephrotoxicity via its oxidative, inflammatory, and apoptotic mechanisms which all were in dose dependent manner. Furthermore, it was the first time to evaluate as well as compare the nephroprotective effects of LC and Er against Malathion-induced nephrotoxicity. Both LC and Er proved to have antioxidant and antiapoptotic effects that reflected on improving renal degenerative and inflammatory changes as well as ameliorating renal biochemical perturbations in both low and high doses of Malathion. Notably, LC had more protective effects compared to Er, although no significant statistical differences were reported. In addition, the protective effects of LC were more evident with low dose of Malathion. Yet, the recorded improvements on co-administration of either LC or Er did not reach the control levels in most of the investigated parameters. From all the above, both agents and especially LC could be considered in ameliorating the Malathion-induced nephrotoxicity. Further investigating of the combined nephroprotective effects of both agents at higher doses in line with exploring other subcellular nephroprotective mechanisms

(e.g., autophagy and endoplasmic stress) are highly recommended in nephrotoxic models.

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مقارنة التأثير الوقائى للالكارنتين والاردوستين ضد السمية الكلوية الناتجة عن الملاثيون : دراسة مختبرية بيوكيماوية وهستوباثولوجية

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لا يزال القصور الكلوي الناجم عن الملاثيون والعوامل الوقائية ضده نقاط للبحث. هدفت الدراسة الحالية إلى استكشاف والمقارنة بين الإلكارنتين(LC) (١٠٠ مجم /كجم/يوم) والإردوستين (Er) (١٠ مجم/كجم/ يوم) فيما يتعلق بآثاركل منهما الوقائية ضد السمية الكلوية تحت الحادة لجرعة منخفضة من الملاثيون (M27) (27 مجم / كجم / يوم) وكذلك الجرعة عالية من الملاثيون (M54) (٥٤ مجم / كجم / يوم) .تم استخدام عشر مجموعات متساوية من ذكور جرذان سبرج داولى البالغة (عدد كل منها = ٦) على النحو التالي: المجموعة الظابطة ، زيت الذرة ، (M27)، (M54)، (LC)، (LC + M27)، (LC + M54)، (Er) و (Er + M27) و (Er + M54). تلقت الجرذان جميع العلاجات عن طريق الفم لمدة 28 يومًا.أظهر الملاثيون زيادة ذات اهمية احصائية تعتمد على زيادة الجرعة في كل من نسبة الكرياتينين واليوريا في البلازما والمالونديالدهايد الكلوي ، بالتوازي مع انخفاض كل من الألبومين فى البلازما ونشاط ديسموتاز الفائق الكلوي وانخفاض تركيز الجلوتاثيون الكلوي. من الناحية النسيجية ، تسبب الملاثيون في حدوث تغيرات تنكسية والتهابات ملحوظة بالإضافة إلى زيادة كبيرة تعتمد على الجرعة في تكون (Caspase -3) في كل من القشرة الكلوية ونخاعها مقارنة بالمجموعة الضابطة. اظهرت اضافة الإلكارنتين أو الإردوستين مع الملاثيون ؛ تحسن كبير في الوظائف البيوكيميائية الكلوية ، وعلامات الإجهاد التأكسدي الكلوي ، والتغيرات التنكسية النسيجية وكذلك التغيرات الالتهابية وتكون (Caspase- 3) في كل من المجموعات ذات الجرعة المنخفضة والجرعة العالية من الملاثيون. علاوة على ذلك؛ يبدو أن التأثيرات الوقائية للكلية الخاصة بالإلكارنتين أفضل من تلك الخاصة بالإردوستين ؛ على الرغم من أن هذه الاختلافات كانت غير ذات دلالة إحصائية. في الختام ، كانت هذه هي المرة الأولى التي يتم فيها دراسة التأثيرات الوقائية للكلى لكل من الإلكارنتين والإردوستين ضد الخلل الكلوي الناجم عن الملاثيون ؛ و كان الإلكارنتين الافضل .