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Protective Effects of Quercetin against Lambda Cyhalothrin Induced Hepatotoxicity and Nephrotoxicity Disorders in Mice

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Research Article

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Abstract

Although the antioxidant, anti-inflammatory actions of quercetin (QE) are well established, no study has measured its protective actions against the toxicity of cyhalothrin lambda (LCT). This study was designed to determine the acute toxicity of LCT on male mice and investigate the effect of repeated sub lethal dose (1/10 LD_{50}) for 14 days on body and organ weights, some biomarkers and histopathological changes in liver and kidney. In addition, the regulatory effect of QE on the hepatotoxicity and nephrotoxicity induced by LCT. The results showed that oral administration of LCT significantly reduced the body weight gain while increased the relative weights of liver and kidney. In addition, LCT significantly increased the activity of the liver function parameters; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) and level of kidney indices; creatinine and urea as well as oxidative stress biomarkers; lipid peroxidation (LPO) level and glutathione peroxidase (GPx) activity, while, declined the glutathione content (GSH). The above findings were confirmed by histopathological examination of liver and kidney. The administration of QE can effectively prevent LCT-induced hepatotoxicity and nephrotoxicity by attenuation oxidative stress, inflammation and tissue injury. QE enhanced antioxidant defenses, suggesting that it could be used as a potential therapeutic intervention to minimize LCT hepatotoxicity and nephrotoxicity.

Keywords: Lambda Cyhalothrin; Biomarkers; Quercetin; Oxidative Stress; Histopathology

Introduction

The greatest challenge of today is to feed the growing population and restore the natural resources. The increase of crop production requires the increase use of fertilizers and pesticides. Pesticides have played a vital role in controlling crop pests during growth, post-harvest and storage to minimize losses of agricultural products and control insect vectors to prevent outbreak of human and animal epidemics [1]. LCT is an insecticide that represents a good compromise between efficacy and toxicity. It has extensively been used in public and animal health applications where it effectively controls a broad spectrum of insects and ectoparasites [2].

Thousands of pollutants enter the environment every day and put diverse pressures on organisms and ecosystems. These pollutants can decrease the environmental quality, loss of living resources and affect many non-target organisms, including human beings [3]. Due the increasing awareness of the potential ecotoxicological hazards posed by increasing levels of contaminants in the environment, scientists directed their efforts toward biomontoring studies. Bio-monitoring is an effort to screen environmental quality by using biological systems (Biomarkers) to assess the ecological risks posed by toxic substances [4]. The biomarker is any measurable biochemical, physiological, behavioral, molecular or cellular change in an organism or population that indicates exposure to contamination stress by focusing on the sub-lethal concentrations of a toxin. This developing field aims to reveal environmental threats before a lethal effect becomes apparent. An ideal biomarker has been used as an accurate, quick, simple, inexpensive and useful tool for risk assessment of chemical compounds and provides ecotoxicological diagnoses, which can represent an early-warning system [5].

The liver is often the main target for the detoxification of toxic substances that enter the body [6]. The Kidney is a highly specialized organ that regulates metabolism and participates in endocrine system functions. It can also discharge body metabolites and harmful exogenous substances [7,8]. Furthermore, the liver and kidney are the organs most susceptible to pesticide damage and can be used as an index for the toxicity of xenobiotics. In toxicity studies, a variety of biomarkers are measured to assess a wide range of physiological and metabolic functions that influence the identification of target organs and the evaluation of tissue lesions. A combination of some common biochemical parameters provides better information for pattern recognition, e.g. enzymes like ALP, ALT and AST for hepatotoxicity, creatinine and urea as nephrotoxicity [9,10].

Histopathology is a critical part of the toxicological and risk assessment of foods, drugs and chemicals. Investigation of histopathological changes in animal tissues is a sensitive and rapid method, commonly used to detect effects of pesticides on various tissues and organs and to evaluate the toxic potential of chemicals in the environment [11].

The pesticide-induced toxic manifestation may be accompanied by the increased production of reactive oxygen species (ROS), which provides an explanation for the different types of toxic responses. Oxidative stress refers to the enhanced generation of ROS and/or depletion of antioxidant defense system, causing an imbalance between pro-oxidants and antioxidants [10,12].

Cells have different mechanisms to mitigate oxidative stress and repair injured macromolecules. The primary defense is obtainable by enzymatic and non-enzymatic antioxidants that have been shown to clean, free radicals and ROS. Antioxidants belonging to the second line of defense (exogenous compounds) including vitamins and flavonoids can prevent the uncontrolled formation of free radicals or inhibit their reaction with biological sites [13,14]. QE is one of the most frequently studied bioflavonoid that has been shown to be a very potent antioxidant. It exerts beneficial effects including anti-carcinogenic, anti-inflammatory and antiviral that contribute to human health. QE can act by scavenging free radicals, chelating of divalent cations, inhibiting of some enzymes, protecting against DNA damage and may consider as an effective attenuating factor for preventing various disorders [15,16].

Despite the known adverse effects of lambda cyhalothrin insecticide on mammals, to our knowledge this study represents the first investigation on the role of quercetin as an antioxidant against lambda-cyhalothrin-induced hepatotoxicity and nephrotoxicity to non-target organism. Therefore, the present study planned to assess the potential risks of consecutive repeated sub lethal dose of LCT for 14 days on some successful biomarkers such as body and organ weights, oxidative stress parameters; LPO, GPx and GSH, liver function parameters; AST, ALT and ALP, kidney function parameters; creatinine and urea and histopathological changes of male mice. In addition, the role of QE in overcoming the damage induced by LCT was investigated.

Materials and Methods

Animal

Male Swiss albino mice (*Mus musculus*) weighting, 23 ± 2 g were obtained from the Faculty of Science, Alexandria University, Alexandria, Egypt. Animals were housed in stainless steel cages under the laboratory conditions of 25 ± 5 ºC and 70 ± 10 % humidity with 12:12 h (dark: light) provided by diet and water *ad libitum*. The animals were allowed to acclimatize for two weeks prior to the start of the study. The protocol of the present study was approved by the Institutional Animal Care and Use Committee of Alexandria University (Approval Code AU08181231306).

Chemicals

Lambda cyhalothrin (96 % purity) was kindly supplied from the National Company for Agrochemicals (AGROCHEM), Egypt. Quercetin (98 % purity) and all of the other reagents of analytical grades used in this study were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA).

Toxicity Studies

Different concentrations of LCT were dissolved in corn oil and administered to mice orally in a single dose via gavage (3 mice for each dose). Mice serving as control had received corn oil only. The medium fatal dose (LD_{50} value) and its confidence limits of LCT were calculated [17].

Experimental Design

Twenty male mice were divided into four experimental groups (five/each) and were orally treated for 14 consecutive days as follows:

Group 1: Mice treated with corn oil and kept as a control.

Group 2: Mice treated with QE alone (50 mg/kg body weight).

Group 3: Mice treated with LCT alone in a dose equivalent to 1/10 LD50.

Group 4: Mice treated with LCT (1/10 LD50) plus QE (50 mg / kg body weight).

The animals were examined daily and their body weight was recorded.

Sample Preparation

At the end of the experimental period the animals in each group were sacrificed by decapitation, then liver, kidney and spleen were quickly removed, weighed individually and homogenized in ice cold saline solution (10% w/v) using polystyrene Homogenizer (Tekmar tissumizer), Germany for 30 Sec. The homogenates were centrifuged at 10,000 x*g* for 30 min at 4°C using Janetzki cooling centrifuge, type K23, Berlin, Germany. The resultant supernatants were used for all different parameters assays except lipid peroxidation and glutathione content were measured in the liver homogenate.

Biochemical Estimation

Oxidative stress parameters: LPO level was determined by the thiobarbituric acid (TBA) method [18], which estimates the malondialdehyde formation (MDA). LPO level was expressed as n moles MDA/mg tissue. GSH content as a non-enzymatic antioxidant was measured [19] and the level of reduced glutathione present was calculated as µg/mg tissue. GPx activity was carried out [20], and the specific activity was expressed as µmol / mg protein min.

Liver function parameters: AST and ALT activities were measured [21], using a commercially available kit from ERBA Diagnostic Mannheim GmbH, Germany. ALP activity was estimated using sodium *p*-nitrophenyl phosphate as a substrate in the alkaline medium at 405 nm [22].

Kidney function parameters: Creatinine and urea were analyzed, using commercial kits from BioScope Diagnostics and ERBA Diagnostics, Mannheim GmbH, Germany [23].

Total Protein Content

It was determined, using bovine serum albumin as the standard [24].

Histopathological changes: Liver and kidney of different groups were removed, fixed in 10 % formalin solution, dehydrated by standard procedures and embedded in paraffin. Five microns thick paraffin sections were stained with hematoxylin - eosin (H&E) and examined by light microscope.

Statistical Analysis

All data were expressed as mean ± standard error. Data were subjected to analysis of variance (ANOVA) and analyzed using a randomized complete design with Student-Newman Keuls test. The statistical analysis was performed using the computer software CoStat program, Version 6.311, 2005 [25]. Comparison between means was made at $p \leq 0.05$.

Results

Toxicity study

The acute oral LD_{50} value of LCT against male mice was 51.38 mg/kg body weight, with a confidence limits ± 0.145 .The *in vivo* effects of the repeated dose of one-tenth LD_{50} of LCT (5.1 mg/kg BW), QE (50 mg/kg) and their combination for 14 days on the body and organ weights, biochemical parameters and histopathological changes were studied. Treatment with QE alone has no significant effect on all the tested parameters when compared with control oil.

Body and organ weights

No mortality was occurred in any group throughout the experimental period. The physiological status of control and treated animals was noticed as the change in body weight gain and relative organ weights (organ

weight/body weight). Results in Table 1 showed that there was a marked decrease in the body weight of mice treated with LCT when compared with those of the control group ($p \leq 0.05$). The liver and kidney weights of LCT treated mice were significantly increased than untreated ones, on the contrary, the spleen weight was decreased. The co-administration group of QE plus LCT showed an increase in the body weight gain compared with pesticide alone, reaching for the mouse's weight in control. The liver weight was significantly decreased, while spleen weight was increased than that in pesticide group, reaching approximately to the weight in the control group. On the other hand, kidney weight was non-significantly decreased than that in pesticide alone (Table 2).

Table 1: Body and relative organ weights of male mice after 14 days of exposure to LCT, QE and their combination.

Treatments	Body weight gain (%)	Relative organ (%)		
		Liver	Kidney	Spleen
Control	14.11 ± 0.19^{b}	5.94 ± 0.19^{a}	1.01 ± 0.05^{a}	1.06 ± 0.06^{b}
QE	13.53 ± 0.23^{b}	6.24 ± 0.28^{a}	1.07 ± 0.06^{a}	1.06 ± 0.08^{b}
LCT	7.14 ± 1.40^{a}	7.35 ± 0.33^{b}	1.53 ± 0.08^{b}	0.72 ± 0.02^{a}
Ouer + LCT	12.09 ± 1.09^{b}	6.16 ± 0.09^{a}	1.35 ± 0.05^{b}	1.05 ± 0.08^{b}

Values are expressed as means (5 mice) ± standard errors.
Values in column with different letters are significantly different at P ≤ 0.05.

Table 2: Effect of LCT, QE or their combination on oxidative stress parameters of male mice after 14 days.

Treatments	Oxidative stress parameters		
	Lipid peroxidation nmoles MDA/mg tissue	Glutathione GSH/mg tissue	Glutathione peroxidase µmol/mg prot. min
Corn oil	82.03 ± 0.45^{a}	6.12 ± 0.045^{b}	24.83 ± 0.54^{a}
QE	82.42 ± 1.13^{a}	6.13 ± 0.06^{b}	26.16 ± 0.34^{a}
LCT	132.33 ± 4.22^{b}	5.06 ± 0.11^{a}	39.23 ± 0.46^{c}
QE+ LCT	92.59 ± 3.26^{a}	5.99 ± 0.06^{b}	33.57 ± 0.89^{b}

LCT: lambda cyhalothrin; QE: quercetin
Values are expressed as means (5 mice) ± standard errors.
Values with non identical superscripts are significantly different at P ≤ 0.05.

Oxidative stress parameters

LCT caused a significant increase of hepatic LPO level and GPx activity, while QE minimized the toxic effects of LCT in treated mice.

Liver function parameters: Results in Table 3 showed that hepatic AST, ALT and ALP activities were significantly increased ($p \leq 0.05$) by LCT treatment compared with untreated mice. QE supplementation has been shown to counteract the toxic effects of LCT.

Kidney function parameters: LCT caused a significant ($p \leq 0.05$) increase of renal creatinine and urea concentrations compared with control animals (Table 4). Meanwhile, they were significantly decreased in the LCT plus QE group but could not reach to control value.

Histopathological examination: No histopathological alteration was observed in liver (Figures 1 a & b) and kidney (Figures 2a & b) of untreated animals and those receiving QE, respectively. LCT induced histopathological changes in the liver as follows: Moderate lymphocyte infiltration, dilated sinusoids, moderate sinusoidal enlargement, vacuoles in hepatocytes, congestion in a central vein, slight hemorrhage in hepatic tissue interstitium, and moderate necrosis (Figure 1c). QE administration plus LCT for 14 days revealed portal areas containing elements of the hepatic triads that are one or more small branches of the portal vein, a branch of the hepatic artery, a small bill duct along with lymphatic vessels and very small amount of connective tissue. The presentations of portal areas along with central venues provided evidence of a globular structure. The plates or cords of liver cells are separated by sinusoidal capillaries; also, sections reveal mild lymphocytic infiltration, dilated sinusoids and minimal necrosis (Figure 1d).

Concerning the kidney of animals treated with LCT alone appeared severe tubule necrosis, congestion of the renal glomerulus along with hemorrhage, tubules inflammation, marked interstitial and cellular infiltration (Figure 2c). In addition, examination of mouse kidney

sections treated with LCT plus QE showed that glomerulus, proximal and distal convoluted tubules were nearly to control group with mild necrosis and cellular infiltration (Figure 2d).

Table 3: Effect of LCT, QE or their combination on liver function parameters of male mice after 14 days.

Treatments	ALP µmol p-nitrophenol/ mg prot. min	ALT U/mg prot.	AST U/mg prot.
Corn oil	32.19 ± 0.86[a]	706.47 ± 33.95 [a]	690.39 ± 17.08 [a]
QE	31.39 ± 0.05[a]	684.55 ± 17.19[a]	694.44 ± 11.48[a]
LCT	53.52 ± 3.43[b]	1084.48 ± 17.67[c]	1092.95 ± 12.19[c]
QE + LCT	38.93 ± 1.38[a]	866.44 ± 29.06 [b]	902.43 ± 29.96 [b]

ALP: alkaline phosphatase
ALT: alanine aminotransferase
AST: aspartate aminotransferase
Values are expressed as means (5 mice) ± standard errors.
Values with non identical superscripts are significantly different at P ≤ 0.05

Table 4: Effect of LCT, QE or their combination on kidney function parameters of male mice after 14 days.

Treatments	Kidney function parameters (mg / mg prot min)	
	Urea	Creatinine
Corn oil	333.72 ± 22.69[a]	0.12 ± 0.01[a]
QE	305.21 ± 2.98[a]	0.12 ± 0.01[a]
LCT	882.47 ± 46.31[c]	0.01 ± 0.36[c]
QE + LCT	476.43 ± 45.84[b]	0.19 ± 0.01[b]

Values are expressed as means (5 mice) ± standard errors.
Values with non identical superscripts are significantly different at P ≤ 0.05.

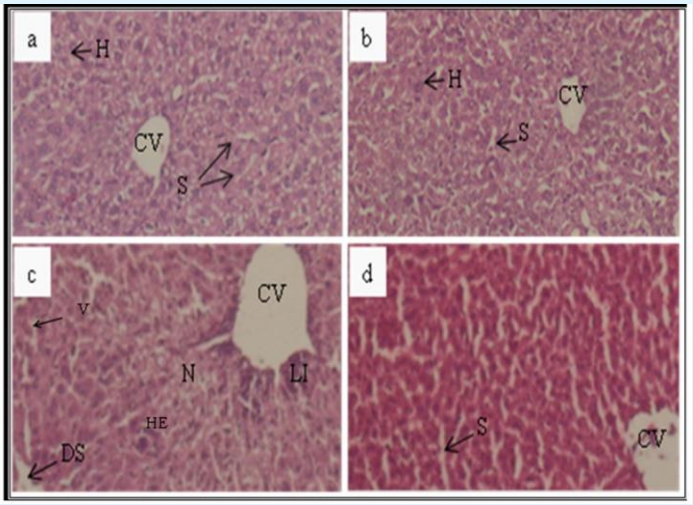

Figure 1: Photomicrograph of liver sections of male mice treated with LCT, QE and their combination for 14 days (H & E, 100 X): (a) Control and (b) QE treated mice show normal histological appearance of the liver including, hepatocytes (H), central vein (CV) and sinusoids (S). (c) Section of mice liver treated with LCT reveals considerable damaged in liver, with the appearance of lymphatic infiltration (LI), vacuole formation in hepatocytes (V), necrosis (N), congestion in central vein, hemorrhage in interstitue of hepatic tissues (HE) and sinusoids dilation (DS). (d) Mice treated with QE plus LCT demonstrates restoration of normal arrangement of hepatocytes, mild lymphocytic infiltration, dilated sinusoids and minimal necrosis.

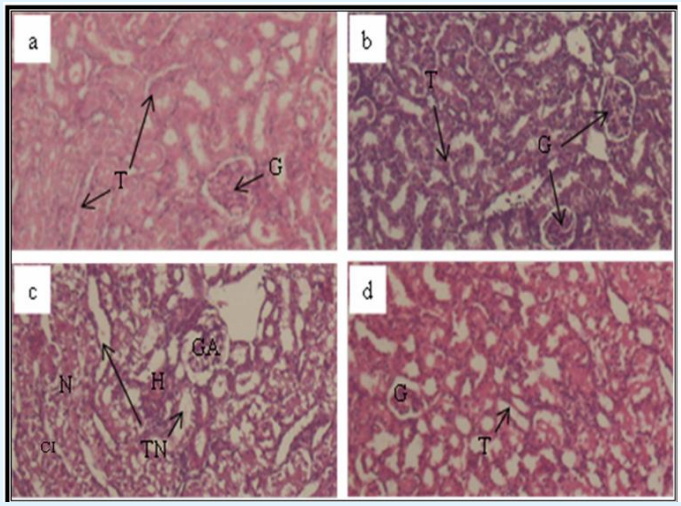

Figure 2: Photomicrograph of kidney sections of male mice treated with LCT, QE and their combination for 14 days (H & E, 100 X): (a) Control and (b) QE treated mice show normal renal histoarchitecture of the glomerular (G), surrounding normal tubules (T). (c) Mice kidney treated with LCT show severe tubular necrosis (TN), necrosis (N), marked interstitial and cellular infiltration (CI), hemorrhage (H) and glomerular atrophy (GA). (d) Mice treated with QE plus LCT demonstrates mild tubular necrosis and intact glomeruli.

Discussion

The widespread use of pesticides in public health and agricultural programs has caused serious environmental pollution and health hazards, including cases of severe acute and chronic human poisoning [3]. It is well known that the oral median lethal dose (LD_{50} value) of any pesticide is highly dependent on the pesticide and the organism. The obtained LD_{50} value of LCT (51.38 mg/kg BW) classified as moderately toxic; class III (LD_{50} 50-500 mg/kg BW) according to the ranking scheme for assessing the acute toxicity of chemicals [26]. The LD_{50} value of the tested compound greatly differs from those currently reported by other authors [27,28], because the LD_{50} of any compound depending on several factors such as animal species, strain, age, weight, sex, nutrition, season, time of day, place, temperature, route of toxicant administration, human error, and the toxicokinetic of the compound in the organism; absorption, distribution, metabolism and excretion [29]. So, the LD_{50} value is not an absolute biological constant and may be different from laboratory to another.

Body and organ weights of animals are important criteria for the evaluation of toxicity that provides information on the general health levels of animals [30]. The body weight of pesticide treated animals was significantly ($p \leq 0.05$) lower than that of the control group. This may attribute to a decreased food intake (anorexia, diarrhea or food avoidance), poor food palatability, increase the degradation of lipids and protein and oxidative stress effects [31,28]. Our data are in accordance with other researchers who observed reduction of body weight of mice exposed to ethoprophos and chlorpyriphos, respectively [10,32]. The increase of liver weight may be due to the increased circulation as a result of increased demands for the detoxification of toxic compounds, proliferation of hepatic cytochrome P 450 mono-oxygenase and the maintenance of the liver normal functional capacity [33]. Changes in kidney weight may be reflecting renal toxicity, tubular hypertrophy or chronic progressive nephropathy and the accumulation of interstitial connective tissue around the tubules [34]. The reduction of the relative weight of the spleen by LCT may be due to the toxic action of testing compounds on the red blood cells, the damage of the spleen cells and the suppressive effect on the immune response of mice [35]. A change in body and organ weight was used in toxicological experiments as a sensitive indicator of side effects [30,36].

The dose of 50 mg/kg body weight of QE was chosen based on the previous study which investigates the daily intake of QE by Chinese residents [7]. The obtained data showed that the tested parameters did not change significantly in the mice treated with EQ and in the control mice. The results of the present study concerning the role of QE to the male mice intoxicated with the tested

compound illustrated that the QE significantly ($p \leq 0.05$) ameliorated the effect of LCT on the body, liver, kidney and spleen weights compared to the mice treated with compound alone. The improvement in the body or organ weights caused by QE could be attributed to the presence of one or more aromatic hydroxyl group, which might provide protection against oxidative damage caused by pesticides [37].

In order to measure the effect produced by pollutants even at low concentrations, a successful battery of biomarkers or the integrated biomarkers response (IBR) methodology was applied to have a useful role for early-warning pollution [12,10]. Oxidative stress occurs due to imbalance between ROS and antioxidants, resulting in impairment in cellular functions and leading to potential tissue damage. In the present study, the animals treated with LCT showed an increase in the activity of GPx and the level of MDA. This increase in the antioxidant defense molecules leads to a concomitant decrease in the level of GSH.

Lipid peroxidation acts as an indicator of oxidative damage in cells and tissues, so it has been shown that LPO causes profound alterations in the structure and functions of the cell membrane, including decreased membrane fluidity, increased of membrane permeability, inactivation of membrane bound enzymes and loss of essential fatty acids, DNA damage and finally carcinogenic effects [13].

Reduced glutathione acts as a non-enzymatic antioxidant and is an important biomarker of oxidative stress due to its use for conjugation and/ or its role as an antioxidant in the neutralization of free radicals produced by pollutants and the maintenance of intracellular redox balance in mammalian cells [38]. The main function of GPx is to reduce soluble hydrogen peroxide and alkyl peroxides in tissues using glutathione as the essential substrate. The data obtained are consistent with the previously reported results [16,39]. The decrease in hepatic GSH level in the present study may be due to decreased GSH synthesis, the conversion of GSH to GSSG (glutathione oxide form) by GPx, or the most likely binding of GSH to the tested compound and/or its metabolites render them non-toxic, or interplay of all these suggestions [40]. QE is a well-known antioxidant that protects the body against radicals and pollutants as an electron donor and can directly capture free radicals and / or modulate the biochemical markers of oxidative stress and antioxidant enzymes [7,16,41].

The liver is the site of biotransformation where a toxic compound has been converted to a less harmful form to reduce toxicity; however, this will damage liver cells and the tissue membrane and produce hepatotoxicity. The most important outcomes of liver damage are the release of intracellular enzymes such as ALT and AST to the outside of the cell. Phosphatase is an important and sensitive indicator of change due to the toxicity of pesticides in biological processes [9]. Once LCT enters into the biological system, it is transformed into its metabolites, which can bind to cellular macromolecules and react with free amino groups of proteins. Therefore, the macromolecules may lose their physiological functions or stimulate hepatocytes to produce more toxic metabolites [42]. In the present study LCT induced significant elevation of the activity of AST, ALT and ALP in male mice. These findings are in coincidence with those reported in other articles [10,16,39]. The disruption of ALP and transaminases than the normal value denotes biochemical impairment, lesions of tissues, increase of cell membrane permeability and cellular functions because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions [32]. Administration of QE has a potent protective effect against liver damage in mice induced by the tested compound, as revealed by remarkable decrease of ALP, ALT and AST activities. The reduction of the activity of these enzymes is an indication of stabilization of cell membrane as well as repair of hepatic tissue damage caused by LCT. The liver of mice treated with the pesticide plus QE had a nearly normal appearance. The mechanism underlying hepatic protection of QE might be related to both its radical scavenging proprieties and indirect effect as a regular of anti-oxidative systems [16,43].

The kidney is an important organ that performs many functions in the body, including hormone production, mineral absorption, blood filtration, and urine production. It is the critical target organ for xenobiotics, which produce various toxic effects on the kidneys involving tubular cells and glomeruli [8]. LCT inhibits the incorporation of amino acids into proteins causing alterations in creatinine and urea levels, which are the major nitrogen-containing metabolic products of protein metabolism [44]. Moreover, elevated urea level is known to be correlated with an increased protein catabolism in mammals and /or conversion of ammonia to urea due to increased synthesis of arginase enzyme involved in urea production [45]. The significant increase in the creatinine and urea concentrations with the sub-acute tested compound administration indicates a reduced ability of the kidney to filter waste products and excrete them in the urine. These findings were parallel with the data of

[39,46], who observed that LCT resulted in a marked increase in creatinine and urea levels in treated animals. An association between hyperuricemia and renal injury has also been reported where uric acid-mediated arteriolopathy and interstitial inflammation suggest mechanisms that would exacerbate the potential progressive renal functional decline after injury [45]. Co-administration with QE was decreased the elevation of creatinine and urea values induced by LCT due to the protective effect of QE on impaired tubular absorption capacity and glomerular filtration in mice [7,41].

The study of histological changes in animal tissues is a sensitive and rapid method, commonly used to detect effects of pesticides on various tissues and organs. Liver via the portal vein is the first organ exposed to internally absorb nutrients and other xenobiotic. It is composed of highly active metabolic tissue containing a considerable complement of the detoxification system. The kidney appears to be particularly sensitive to a variety of toxins due to several factors including; the high renal blood flow, the ability to concentrate substances and the biotransformation of the parent compound to a toxic metabolite [47]. The observed histopathological alterations in hepatic and renal tissues by LCT may be due to the ROS formation produced from LPO of liver and kidney, were probably responsible for the tissue damage, leading to necrosis of hepatocytes and tubular cells. These changes are entirely consistent with the changes in the various biomarkers that were previously observed. Several mechanisms have been identified on cell injury by three different ways: inhibition of fatty acid beta-oxidation, inhibition of respiratory enzymes or by a direct effect on mitochondrial DNA [48]. The result of the histopathological changes with the tested pesticide on the liver and kidney is in agreement with different scientists [39,46,49]. The QE co-treatment with LCT was mild renal- and hepato-toxicity effects, these protective effects may be due to their antioxidant capacity. Some authors explain this protection and beneficial effects of its chemical composition and its ability to stabilize membranes by decreasing membrane fluidity and exhibited regeneration of the liver and kidney tissue structures [31,50].

Previous studies have demonstrated that pesticides due to their hydrophobic nature, were largely accumulated in the biological membrane, especially in the phospholipid bilayers and in lipid rich internal tissues, including body fat, skin, liver, kidney, ovaries and elements of the central and peripheral nervous system [51]. The liver was the major site of pyrethroid metabolism, which accumulated a great concentration of its metabolites [36]. Their toxic effects occurred probably through the generation of ROS causing damage to various membranous components of the cell. Increase ROS may decrease the effectiveness antioxidant and increasing their harmful effects to liver and kidney tissues. Furthermore, IBR was shown to be a useful tool to predict the harmful effects of pollutants on animals, even at sublethal concentrations, and identify the efficiently antioxidant behavior of the QE. Flavonoids are plant phenolic compounds with strong antioxidant properties found in many dietary sources such as tea, onion, broccoli, fruits, green beans and many other sources [52]. QE is the most common flavonoid in the human diet and like other flavonoids can prevent oxidative damage as a result of their ability to scavenge free radicals directly, chelate metals, divalent cations, inhibit or strengthening antioxidant enzymes, protect against DNA damage, inhibit molecule oxidation and partition into the lipid bilayer [15,31].

In conclusion, the present study shows that repeated sublethal dose of LCT causes significant harmful effects in the different biochemical parameters; oxidative stress, liver and kidney functions, besides the histological changes of male mice. In addition, QE supplementation protected the adverse effects of LCT. It is a wise step to take care to avoid toxins such as pesticides as much as possible and to enrich our diets with life giving antioxidants such as QE especially in our increasingly toxic world. Thus, sufficient dietary intake of it by individuals who regularly come in contact with pesticides is beneficial in combating the adverse effects of pesticides.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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