Histopathological, ultrastructural and biochemical alterations in the kidney of male albino rats intoxicated with cypermethrin and the protective effects of propolis and curcumin

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ABSTRACT

Cypermethrin (CYP), which belongs to the type II synthetic pyrethroid insecticide, is mainly used as pesticides for domestic and industrial applications. Thus evaluation of their toxic effects is of major concern to public health. The purpose of this study was to investigate the adverse effects of CYP on rat kidney and to elucidate the possible protective effects of propolis (PRO) (100 mg/kg/day) and curcumin (CUR) (100 mg/kg/day) against CYP. The effects of oral administration of CYP at a dose of 30 mg/kg/day for 28 days on the biochemical, histopathological and ultrastructural changes of the kidney in male rats were studied. In the present study, rats were divided into six groups: the 1st group was used as a control, the 2nd group was treated with PRO, the 3rd group was treated with CUR, the 4th group with CYP, the 5th group was treated with PRO and then received a daily dose of CYP, and the 6th group was treated with CUR followed by CYP. Samples from kidney serum were collected from all groups at the end of the experimental period. CYP-intoxication elicited significant elevations in kidney function indicators (urea and creatinine) and lipid peroxidation (LPO), as well as marked reduction in the antioxidant enzymes [superoxide dismutase (SOD) and glutathione peroxidase (GPx)] levels in kidney. CYP-intoxication also showed marked histopathological, histochemical and ultrastructural alterations in the kidney. On the other hand, treatment with PRO and CUR led to an obvious improvement of the injured kidney tissues and ameliorate the damage effects of CYP. The toxic effects on kidney tissues were greatly ablated with a significant reduction in urea, creatinine and LPO level and elevation in SOD and GPx after treatment with PRO and CUR. Thus, PRO and CUR may be used with CYP to improve the biochemical, histopathological, histochemical and ultrastructural changes of renal toxicity induced by CYP due to their antioxidant effects. In conclusion, PRO is markedly effective than CUR in protecting rats against CYP-induced histopathological, ultrastructural and biochemical parameters in kidney and enhances the antioxidant defense mechanism in male albino rats.

Key words: Cypermethrin, biochemical, oxidative stress, histopathology, ultrastructure, propolis and curcumin.

INTRODUCTION

Numerous pollution problems of the environment have occurred as a consequence of industrial pollution and high residues of insecticides. Cypermethrin (CYP) is a member of the family of synthetic pyrethroids, which belongs to type II class of pyrethroids and is widely used in agricultural and other household applications (Lukowicz-
Ratajczak and Krechniak, 1991). CYP is a universally used pesticide, and therefore has a maximum chance of accumulation in various food chains and thus causing related toxicity (Bhushan et al., 2013; Sangha et al., 2013). It has a hydrophobic nature which can easily pass through the cell membrane and disturb its structure and cause leakage of cytoplasmic enzymes (Hussien et al., 2013). CYP produced ROS and damaged DNA directly proportional to the dose (Huang et al., 2016). There is a direct reaction between ROS and cellular biomolecules. It damage lipids, proteins and DNA in cells, inducing cell death (Ferrari, 2000).

Natural antioxidants strengthen the endogenous antioxidants defenses and restore the optimal balance by neutralizing ROS (Heeba and Abd-Elghany, 2010). Propolis (PRO) (bee glue) is a sticky resinous substance applied by honey bees, Apis mellifera L., as a building material in their hives and as a defensive substance against infections (Bankova et al., 2016). It has more than 180 compounds including flavonoids, phenolic acids and its esters (Li et al., 2005; Marquele et al., 2005). The antioxidant activity of PRO extract is mainly attributed to its flavonoid content, that is capable of scavenging free radicals and thereby protection against LPO (Yousef and Salama, 2009).

Curcumin (CUR) is a constituent (up to ~5%) of the traditional medicine known as turmeric (Nelson et al., 2017). CUR is considered as a source of food additive or dietary pigment and in traditional medicine (Perrone et al., 2015; Pattanayak et al., 2016). Moran et al. (2016) indicated that CUR is a potent scavenger of a variety of ROS and reactive nitrogen species (RNS), including O$_2^·$, hydrogen peroxide (H$_2$O$_2$) and nitric oxide (NO$^·$) and also, has enhanced activities on different antioxidant systems, such as catalase (CAT), SOD, GPx, glutathione, reduced glutathione (GSH) and heme oxygenase -1.

This study aims to investigate the protective effect of PRO and CUR against histopathological, ultrastructural and biochemical changes of kidney induced by CYP in the various control and tested groups.

MATERIALS AND METHODS

Chemicals

Cypermethrin (CYP) was purchased from the pesticides store in Beni-Suef, Egypt. Propolis (PRO) was purchased from Sigma Pharmaceutical Industries (Cairo, Egypt). It was found in the form of Biopropolis capsules, each one containing 400 mg. Curcumin (CUR) was obtained from Merk Company, Germany.

Animals and experimental design

A total of 42 adult laboratory males albino rats (Rattus norvegicus) of almost the same age, weighing 120-150 g, were obtained from the Ophthalmology Research Institute, Giza, Egypt. The rats were kept under standard management conditions of temperature (25°C). The animals were fed a standard commercials diet (ATMID Company, Giza, Egypt) and tap water ad libitum. All the rats were acclimatized for at least 15 days before performing the experiment. In this study, animal care was carried out following the European Community Directive (86/609/EEC) and national rules, this is in accordance with the NIH Guidelines for care and use of Laboratory Animals, 8th editions. This was administrated by the committee of the Zoology Department, Beni-Suef University, Egypt. Rats were separated to six groups containing seven rats each as follow:

**Group 1:** Served as an untreated control group under the same laboratory conditions.

**Group 2:** Treated with PRO at dose of 100 mg/kg/day, dissolved in distilled water and given gastric gavage for 28 days as described by Saleh (2012).

**Group 3:** Treated with CUR, dissolved in distilled water and given via oral rout at dose of 100 mg/kg/day for 28 days as described by Sankar et al. (2012).

**Group 4:** Received CYP, dissolved in distilled water and given orally at dose of 30 mg/kg/day for 28 days as described by Inayet et al. (2007).

**Group 5:** Administered with PRO at 100 mg/kg/day and then treated daily with oral dose of CYP (30 mg/kg/day).

**Group 6:** Received CUR at 100 mg/kg/day and then treated with oral dose of CYP (30 mg/kg/day).

At the end of experiment, the rats were starved for 12 h and then sacrificed by anesthesia under light diethyl ether and blood samples were collected and allowed to coagulate at room temperature then centrifuged at 3000 r.p.m. for 30 min. The clear non-haemolyzed supernatant sera was quickly removed and kept at -20°C for subsequent biochemical analysis. Liver homogenate was obtained by grinding a small piece of freshly excised tissue in 10 volumes of 0.9% NaCl (10% w/v) using Teflon homogenizer (Glas-Col, Terre Haute, USA). The supernatants were kept at -20°C till use in the determination of LPO, SOD and GPx.

The symptoms of CYP toxicity in the laboratory animals included burrowing, salivation, tremors, writhing and seizures. Also, there were mortality; out of the eight rats in the CYP-treated group, two died.

The assay of serum biochemical

Serum urea concentration was determined according to the method of Fawcett and Scott (1960) and Tabacco et al. (1979) using kits purchased from Diamond Diagnostic (Egypt). Serum creatinine concentration was determined according to the method of Murray (1984) using reagent kits obtained from Diamond Diagnostics (Egypt).
Assessment of oxidative stress and antioxidant enzyme activity

The activity of lipid peroxidation (LPO) in liver homogenate was determined according to the method of Yagi (1987). The activity of superoxide dismutase (SOD) in liver homogenate was assayed according to the method of Marklund and Marklund (1974). Glutathione peroxidase (GPx) activity in liver homogenate was assayed according to the method suggested by Matkovics et al. (1998).

Statistical analysis

The data were expressed as mean ± SEM. Package for the Social Sciences (SPSS for WINDOWS, version 20.0; SPSS Inc, Chicago) (IBM crop, 2011) was used for the statistical analysis of data. Data were analyzed by one way ANOVA test followed by Duncan’s multiple range tests post hoc analysis values. A value of P < 0.05 was considered statistically significant.

Preparations of histology

In the final of 28 days of treatment, we used light diethyl ether for rats anesthesia and dissected to detach the kidney. After fixation in 10% neutral buffered formalin for 24 h, they were dehydrated through ascending series of ethyl alcohol, cleared in xylene and fixed in paraffin wax. 5 µm thick sections were stained with haemotoxylin and eosin for histopathological studies (Bancroft and Gamble, 2002).

Histochemical preparations

We determined polysaccharides, proteins and DNA by staining the liver sections of all the groups with periodic acid schiff (PAS) reaction (Hotchkiss, 1948), mercuric bromophenol blue method (Mazia et al., 1953) and Feulgen method (Feulgen and Rossenbeck, 1924), respectively.

Ultrastructural preparations

Small pieces of liver of all groups were immediately fixed in 3% gluteraldehyde-formaldehyde at 4°C for 18-24 h, rinsed in phosphate buffer, followed by post fixation in 1% osmium tetroxide. We dehydrated specimens in a series of alcohols, cleared in propylene oxide and finally embedded in Epon epoxy resin. Thereafter, we trimmed the blocks and sectioned them with glass knives by an ultramicrotome. Semithin sections (1 µm) were stained with toluidine blue and examined on light microscope to select the suitable area for the ultrathin sections. Ultrathin sections (70-90 nm) were cut on the same ultramicrotome and stained with uranyl acetate and lead citrate (Bozzola and Russell, 1999). Examination of the stained sections was done using Joel CX 100 transmission electron microscope operated at an accelerating voltage of 60 kV.

RESULTS

Effects of cypermethrin, propolis and curcumin on serum biomarkers

CYP-intoxicated rats showed a significant increase in serum urea concentration when compared with the control, PRO, and CUR groups. However, administration of PRO or CUR in concomitant with CYP, produces observable amelioration when compared with CYP group (Figure 1a).

Also, CYP-treated rats significantly (P < 0.05) increased creatinine concentration as compared with control, PRO, CUR groups values. Administration of PRO or CUR in concomitant with CYP decreased creatinine concentration (Figure 1b).

Effects of cypermethrin, propolis and curcumin on lipid peroxidation

Figure 2 indicated that lipid peroxidation in the form of malonaldehyde (MDA) significantly (P<0.05) increased in CYP treated group as compared with the control, PRO, CUR, PRO+CYP and CUR+CYP groups.

Effects of cypermethrin, propolis and curcumin on enzymatic antioxidant enzyme

CYP administration induced a significant (P<0.05) decrease in SOD levels and GPx when compared with the control, PRO, CUR groups. However, administration of PRO or CUR to CYP-treated rats revealed significant amelioration in the altered level of SOD and GPx activities when compared with CYP-treated group (Figures 3a and b).

The most biochemical parameters revealed that PRO is significant effective than CUR in ameliorating the biochemical alterations.

Histopathological results

Histological examination of control, PRO and CUR treated rats revealed normal organization of healthy kidney with their characteristic; an outer grainy-appearing cortex (Figure 4a, b, c, d, and f). The administration of CYP showed some tubules cells displayed pyknotic nuclei,
Figure 1: Concentration of serum (a): Urea (b): Creatinine (mg/dl) of CYP, PRO, CUR, CYP plus PRO and CYP plus CUR in serum of male rats.

Values were expressed as mean ± standard error. Means which share the same superscript symbol(s) are not significantly different. No. of samples in each group is six.

(Propolis: (PRO), curcumin: (CUR), Cypermethrin: (CYP), Cypermethrin plus Propolis (CYP+PRO) and Cypermethrin plus Curcumin (CYP+CUR).

partial degenerated glomerulus and interstitial hemorrhage (Figure 5a). The cortex region displayed dissolution in some tubules, other tubules cells showed vacuolar degeneration of the cells and disorganization (Figure 5b). In addition, widening of urinary space, atrophic glomerulus, vacuolization, intratubular casts and detachment of cells in the lumen of some tubules were observed (Figure 5c). Hyperplasia of blood vessels, oedema and inflammatory cells infiltrations were also detected (Figure 5d). Also, completely demolished glomeruli, vacuolization, dilated tubules and interstitial hemorrhages were also distinguished (Figure 5e). On the other hand, kidney of CYP plus PRO group and CYP plus CUR group showed a relatively normal architecture, however some renal tubules still have cells with vacuolated cytoplasm. Also, there was a less degree of dilatation in the tubules and no intratubular casts were detected (Figures 6a and b).

Histochemical studies

The renal corpuscles of the control, PRO and CUR groups showed strong PAS-positive reaction in basal lamina, while the visceral epithelial showed moderate reaction. Concerning the proximal tubules, the basal membrane and the brush borders showed strongly PAS-positive, while the cytoplasm reacted moderately (Figures 7a, b and c). However, marked reduction of PAS reaction was observed in CYP treated group (Figure 7d). On the other hand, PAS-positive reaction in PRO plus CYP and in CUR plus CYP groups were nearly similar to that of the control group.
Figure 2: Concentration of renal malondialdehyde (MDA) of CYP, PRO, CUR, CYP plus PRO and CYP plus CUR in liver homogenate of male rats. Values were expressed as mean ± standard error. Means which share the same superscript symbol(s) are not significantly different. No. of samples in each group is six. Propolis: (PRO), curcumin: (CUR), Cypermethrin: (CYP), Cypermethrin plus Propolis (CYP+PRO) and Cypermethrin plus Curcumin (CYP+CUR).

Figure 3: Concentrations of (a): renal superoxide dismutase (SOD) and (b): Glutathione peroxidase (GPx) of CYP, PRO, CUR, CYP plus PRO and CYP plus CUR in liver homogenate of male rats. Values were expressed as mean ± standard error. Means which share the same superscript symbol(s) are not significantly different. No. of samples in each group is six. Propolis: (PRO), curcumin: (CUR), Cypermethrin: (CYP), Cypermethrin plus Propolis (CYP+PRO) and Cypermethrin plus Curcumin (CYP+CUR).
Figure 4: Photomicrographs of sections of kidney of (a and b): control rats, (c and d): PRO and (e and f): CUR groups for 28 days stained with HandE showing Control kidney showing renal corpuscles, glomerulus (G), proximal tubule (P), distal tubule (D) and collecting tubules (C) (Scale bar=100 µm, 50 µm respectively).

especially in the glomeruli, as well as the brush border of the proximal tubules cells and their basement membranes (Figures 7e and f).

Kidney sections of the control, PRO and CUR groups revealed intense blue colour with bromophenol blue stain. Fine blue granules were shown in the glomerular tufts, as well as in the cytoplasm of tubular cells (Figures 8a, b and c). After treatment with CYP, obvious depletion of proteins content was seen in most of the renal tissue (Figure 8d). Treatment of CYP-treated rats with PRO and CUR induced great improvement and restoration of normal distribution of protein content in the renal tissues (Figures 8e and f).

Using Feulgen reaction to detect DNA, kidney sections of untreated control group, PRO and CUR groups showed normal content in the nuclei of the glomerulus and renal tubules cells (Figures 9a, b and c). The kidney section of CYP-treated group showed decrease amount of DNA in the nuclei of the glomerulus and renal tubules cells (Figure 9d). On the other hand, administration of PRO and CUR to CYP caused marked increase in DNA content in the nuclei.
Figure 5: Photomicrographs of sections of kidney of CYP group for 28 days stained with HandE. (a): showing partial degenerated glomerulus (G), interstitial hemorrhage (H) and some tubule cells appeared with pyknotic nuclei (arrows) (Scale bar= 50 µm). (b): showing cortex region with dissolution of some tubules (arrows), vacuolar degeneration of cells (arrow heads) (Scale bar= 50 µm). (c): showing widening of urinary space (US), atrophid glomerulus (G), vaculization (arrow heads), intratubular casts (thick arrows) and detachmentment of cells in lumen of some tubules (thin arrows) (Scale bar= 50 µm). (d): showing hyperplasia of blood vessels (HB), oedema (O) and inflammatory cells infiltrations (IF) (Scale bar= 100 µm). (e): showing completely demolished glomeruli (G) and dilated tubules (arrows), vacuolization (arrow heads) and interstitial hemorrhages (H) (Scale bar= 50 µm).

Ultrastructural studies

Electron microscopic examination of ultrathin sections of control kidneys showed that the renal corpuscles appear as dense round tuft of capillaries. The outer layer of Bowman’s capsule is the parietal layer and the inner is the visceral layer applies closely to the glomerular capillaries. Each capillary loop is lined with endothelial cells. Podocytes give rise to primary processes which in turn give numerous secondary foot processes (pedicels) that
rest on thin basal lamina. Each podocyte has a large nucleus and abundant cytoplasm. The pedicels are separated by split pores. The filtration barrier includes thin basal lamina (Figures 10a and b). The normal proximal tubular cells have a brush border of numerous microvilli. The nucleus is spherical in shape. Numerous mitochondria fill the cytoplasm, the basal lamina is thin and the basal infoldings run upwards among the mitochondria (Figures 10c and d).

The renal corpuscle of CYP-treated rats appeared with marked degeneration of almost structures of the glomerulus, degeneration of podocytes, marked thickening of the basal lamina and fusion of foot processes (Figures 11a, b and c). The proximal tubule cells of CYP-treated rats showed marked thickening of the basal lamina. Moreover, vacuolation and irregular basal infoldings, in addition to increased number of lysosomes which contain a variety of enzymes, enable the cell to break down various biomolecules it engulfs, including peptides, nucleic acids, carbohydrates, and lipids called phagosomes. The
most pronounced feature revealed dissolution and degeneration of some parts of the cytoplasm, degeneration of the microvilli on the apical parts of some cells and presence of electron dense mitochondria (Figures 11d, e and f).

After treatment with PRO and CUR, the renal corpuscle showed marked amelioration of almost structures including the podocytes, and the basal lamina. The basal lamina appeared thin as compared with CYP group (Figures 12a, b and 13a, b). The proximal tubule cell retained almost normal structures including the mitochondria and revealing thin basal lamina and basal

**Figure (7a, b and c):** Photomicrographs of sections of kidney of control, PRO, CUR groupsshowing strong PAS positive reaction in the basal lamina of renal corpuscle (thick arrow), luminal brush border (arrow heads) and basal lamina of the proximal tubules (arrows). **(d):** kidney of CYP treated rat showing faint PAS reaction in the basal lamina of renal corpuscle (thick arrow), luminal brush border (arrow head) and basal lamina of the proximal tubule (arrow). **(e and f):** kidney of CYP plus PRO and CYP plus CUR treated rat showing strong positive reaction nearly similar to control in the basal lamina of renal corpuscle (thick arrow), luminal brush border (arrow head) and basal lamina of the proximal tubules (arrow) (Scale bar = 50 µm for all Figures).
infolding after treatment with PRO and CUR as compared with CYP group (Figures 12c, d and 13c, d).

The most histopathological, histochemical and ultrastructural studies on kidney revealed that PRO is markedly effective than CUR in ameliorating the alterations caused by CYP.

**DISCUSSION**

CYP is expected to generate ROS that induce oxidative stress or accumulate in cell membrane and disturb membrane structure due to its hydrophobic nature (Saxana and Saxana, 2010). The renal biochemical results
of the present study indicated that treatment with CYP caused significant increase in serum urea and creatinine. This finding was in line with Ahmad et al. (2011), Sankar et al. (2011 and 2012) and Sakr and Albarakai (2014).

The present results showed that CYP induced many histopathological alterations in the kidney of rats, such as shrunken, atrophid glomerulus, dilated collecting tubules, oedema and inflammatory cells infiltration. Similarly, Abdou et al. (2012) and Soliman et al. (2014) showed that CYP caused histological and degenerative effects in kidney of rats. Inayet et al. (2007) reported that dermal exposure of CYP to rats caused congestion of vessels, diffuse and focal lymphocytic infiltration, oedema and necrosis of proximal tubules of the kidney. The renal cortex was more
affected than other parts in the kidney as the cortex received most of the blood nutrient flow to the organ. Thus, when a blood-borne toxicant is delivered to the kidney, a high percentage of toxins will reach the cortex (Oyama et al., 2006). The present ultrastructural study of the kidney showed that the renal corpuscle of CYP-treated rats appeared with marked degeneration of almost structures of the glomerulus, degeneration of podocytes, marked thickening of the basal lamina and fusion of foot processes. Also, the proximal tubule cells showed marked thickening of the basal lamina. Moreover, vacuolation and irregular basal infoldings were also observed in addition to increase in phagosomes. The most pronounced feature is represented by dissolution and degeneration of some parts of the cytoplasm, degeneration of the microvilli on the apical parts of some cells and electron-dense mitochondria. These results are in line with the findings of Luty et al. (1998).

Antioxidants are the molecules that react with ROS to delay their function and to neutralize them and as such, they reduce oxidative stress and protect against diseases (Majeed et al., 2016). PRO, a mixture of phenolic acids,
flavonoids, ethers and other bioactive compounds, is known to have antioxidative effects (Funakoshi-Tago et al., 2015). PRO exhibits a wide spectrum of pharmacological properties such as antioxidant, anti-inflammatory, antibacterial, antiviral, anti-ulcerous, anticarcinogenic properties and immune system support (Araujo et al., 2012; Mahmoud and Mahmoud, 2013). The primary mechanism of the effect of PRO may involve the scavenging of free radicals that cause LPO. The other mechanism may comprise the inhibition of xanthine oxidase, which is known to cause free radicals to be generated (Kanbur et al., 2009).
In the present study, co-administration of PRO with CYP induced a significant decrease in the mean value of MDA and a significant increase in the mean values of antioxidant enzyme activities (SOD and GPx) as compared with CYP-treated group. Similarly, Eraslan et al. (2008) reported that PRO as an antioxidant substance decreased MDA levels, and increased SOD, CAT and GPx activities induced by CYP at 125 mg/kg/bw in plasma and tissues of rats. Ramadan et al. (2015) suggested that PRO has potent beneficial effects in LPO and oxidative stress.

In the present study, co-administration of PRO with CYP induced a relatively normal structure of renal corpuscle and the renal tubules appeared normal. Quita (2016) reported that administration of dacarbazine and PRO could effectively prevent adverse effects and protect the kidney, whereas the kidney restored the normal histological structure in both Malpighian corpuscles and Bowman’s capsules. The ultrastructural findings of this study revealed marked amelioration of almost glomerular structures including the podocytes and the basal lamina. While the proximal tubule cell retained almost normal structures including the mitochondria and revealed thin
basal lamina and basal infolding after treatment with PRO. Salem et al. (2015) indicated that PRO co-administration ameliorate the effects of Diazinon in the kidney of the rats, whereas podocytes appears normal with intact food processes, most area of basal lamina showed normal thickness, also the epithelial cells lining of the proximal tubules showed normal thickness of basement membrane.

CUR has protective effects against oxidative damage and has antioxidant and anticonvulsant properties exerting powerful ROS scavenging effects and increased intracellular glutathione concentration, thereby protecting against LPO (Aboul Ezz et al., 2011; Du et al., 2012). The free radical scavenging activity of CUR can arise by the resonance stabilization of its radicals from two phenolic

**Figure 13:** (a and b): Electron micrographs of renal corpuscle showing great recovery and restoration of the nearly normal structure of podocytes (P), basal lamina (BL) and pedicles (arrow) (Scale bar = 2 µm, 500 nm respectively). (c and d): Electron micrographs of proximal tubule showing restoration of almost structures including the nucleus (N), microvilli (MV) and the presence of few vesicles (V). The basement membrane (BM) is less than in CYP treated rats and basal infoldings (BI) (Scale bar = 2 µm, 500 nm respectively).
OH groups (mainly) or from the CH₂ group of the â-diketone moiety (Barzegar and Moosavi-Movahedi, 2011). Co-administration of CUR with CYP revealed a decrease in the concentrations of urea, creatinine and MDA as compared with CYP alone treated rats in this study. Earlier studies have also shown that CUR treatment decreased serum creatinine and urea concentrations in cyclosporine induced renal injury in rats in dose dependent (Tirkey et al., 2005). In the current study, the kidney of CUR plus CYP group displayed a relatively normal structure. In conclusion, the present study clarified that administration of PRO and CUR are beneficial to improve biochemical, histopathological and ultrastructural alterations in kidney of rats induced by CYP due to their antioxidants effects. The protective effects of PRO are markedly effective than CUR against CYP.

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