Journal of Pharmacology Research
ISSN: 0976-7134 & E-ISSN: 0976-7142, Volume 3, Issue 1, 2013, pp.-64-69.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000279

CURCUMIN AMELIORATES NEPHROTOXICITY AND HISTOPATHOLOGICAL ALTERATIONS INDUCED BY CHLORPYRIFOS IN ALBINO RATS

AL-AMOUDI W.M.*

Biology Department, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, KSA.
*Corresponding Author: Email- wmamoudi@uqu.edu.sa

Received: September 04, 2013; Accepted: September 26, 2013

Abstract- In the present investigation an attempt was made to assess the possible ameliorative role of curcumin on nephrotoxicity and histopathological alterations caused by Chlorpyrifos in the kidney tissue of albino rats. Chlorpyrifos (0,0-diethyl-0-3,5,6-trichloro-2-pyridyl phosphorothioate; (CPF) is an insecticide used in agriculture against wide variety of insects. Rats were given daily doses of intragastric and sublethal concentrations of CPF ($1/_{20}$ LD_{50}, 6.7 mg/kg b.w. 5 days/ week for 6 weeks respectively). Several endpoints related to the histoarchitectural profile in the kidney tissues were evaluated. Histological examination showed alterations in the treated kidney such as degeneration of the renal tubules, atrophy and hypertrophy of glomeruli and intertubular leucocytic infiltrations. The histopathological changes were more pronounced with higher concentrations of CPF. The degree of degeneration of renal tubules found in higher concentration treatment of CPF was more pronounced. These alterations of the renal tubules and glomeruli affected the functioning of motor coordination of the rat body. Moreover, histochemical examination of the renal tubules showed depletion of glycogen and total proteins content and marked elevation in serum creatinine and blood urea nitrogen. However, treating animals with CPF and curcumin (150 mg/kg b. w. 5 days / week for 6 weeks) caused an improvement in histological and histochemical appearance of the kidney together with decrease of serum creatinine and blood urea nitrogen. It is concluded from the obtained results that CPF is highly toxic to albino rats by causing nephrotoxicity and may leads to oxidative stress and affect their population survival in environment. This effects appeared to be prevented and ameliorated by curcumin.

Keywords- Chlorpyrifos, Kidney, Glomeruli, Renal tubules, Curcumin, Histochemistry

Citation: Al-Amoudi W.M. (2013) Curcumin Ameliorates Nephrotoxicity and Histopathological Alterations Induced by Chlorpyrifos in Albino Rats. Journal of Pharmacology Research, ISSN: 0976-7134 & E-ISSN: 0976-7142, Volume 3, Issue 1, pp.-64-69.

Introduction

The wide use of chemical insecticides resulted in lethal effects on non-target organisms and has direct toxicity to users. Organophosphate insecticides are widely used in agriculture, home, gardens, and veterinary practice. They are accounting for about 50% global insecticide use. Exposure to the organophosphate by multiple routes can lead to serious toxicity in human and animal health. This chemical insecticides poison insects and mammals primarily by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. It has been reported that these insecticides can cause a syndrome called organophosphate-induced delayed neuropathy and is manifested chiefly by weakness or paralysis and paresthesia of the extremities [1].

Chlorpyrifos (CPF) (0,0-diethyl-0-3,5,6-trichloro-2-pyridyl phosphorothioate) are organophosphorus insecticides (OPs) widely used in the Kingdom of Saudi Arabia (KSA) to control agricultural pests. These chemical are one of the most used insecticides in agriculture worldwide and is significant because of its extensive use, persistence in the environment. Chlorpyrifos toxicity has led to lung and central nervous system damage, developmental, autoimmune disorders and vomiting [2]. Also, Wang, et al [3] has stated that chlorpyrifos is generally toxic to mammals and exposure to CPF induced histopathological alterations in liver [4] and kidney [5]. It has been recently reported that exposure to CPF leads to oxidative stress [6] and cause neurodegeneration in the fish brain by inhibiting acetylcholine in neuronal synapses [7]. The use of CPF was reported to have major health problems by inhibiting acetylcholinesterase (AChE) Endocannabinoids (eCBs, N-arachidonoyl-ethanolamine, AEA; 2-arachidonoylglycerol, 2AG) which modulate neurotransmission by inhibiting neurotransmitter release [8].

Numerous studies have reported the effectiveness of medicinal plant extracts and phytochemicals as a phytotherapeutic agent or as a preventive agent in different diseases. Curcumin (diferuloylmethane) is a traditional medicine obtained from the powdered rhizomes of *Curcuma longa* Linn. It is domestically used as a spice to give specific flavor and yellow color to Curry [9]. It has been used for treatment of a wide variety of tumors [2]. It has been found that curcumin exhibit a variety of biological activities including antitumor [10] and antioxidant, anti-inflammatory properties, and antiviral activities [11]. The antitumor effects of curcumin have attracted widespread attention worldwide. One of its major functions is to induce the apoptosis of tumor cells [2]. It also showed hepatopro-

tective and nephroprotective against chemicals and drugs [12,13]. It has been found that the beneficial effects of curcumin against liver oxidative damage were associated with prevention of mitochondrial dysfunction [14]. Some studies have indicated that long-term curcumin administration may reduce lung inflammation and fibrosis caused by radiation treatment [15]. The objective of this study was performed to investigate whether curcumin, which is known to have antitumor, anti-inflammatory and antioxidant properties, could ameliorate the nephrotoxicity and histopathological Alterations induced by Chlorpyrifos in irradiated renal tubules in kidney of albino rats.

Materials and Methods

Chlorpyrifos

Chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate], 48% purity, with commercial name Chlorzane was obtained from king Abdulaziz university for pesticide and chemicals Co. Before use, CPF was dissolved in corn oil and administered orally at a dose level of 1/20 LD_{50} (6.7 mg/kg b.w.) 5 days / week for 6 weeks [6].

Curcumin (*Curcuma longa*)

Dry turmeric rhizomes of the plant *Curcuma longa* were purchased from a local market at Makah, Saudi Arabia. They were crashed into powder, dissolved in distilled water and intragastrically given at a dose level of 150 mg/kg b. w. 5 days / week for 6 weeks [15,16].

Animals

Male albino Wistar rats weighting 140 ± 6 g were kept in the animal house under constant conditions of temperature (24 ± 2 ^{0}C) for at least one week before the experimental work, being maintained on a standard diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitamins. Water was available *ad-libitum*. All the experiments were done in compliance with the guide for the care and use of laboratory animals. Animals were divided into four groups of 10 rats each:

Group-1: Animals of this group served as a control group.

Group-2: Animals of this group were orally administrated with curcumin at a dose level of 150 mg/kg b. w. 5 days / week for 6 weeks.

Group-3: Animals of this group were orally given CPF at a dose level of 1/20 LD_{50} (6.7 mg/kg b.w.) 5 days / week for 6 weeks.

Group-4: Rats were given 1/20 LD_{50} CPF and curcumin (150 mg/kg b.w.) 5 days/ week for 6 weeks.

Biochemical Assays

For biochemical study sera were obtained by centrifugation of the blood samples and stored at -20°C until assayed for the biochemical parameters. Creatinine and urea were estimated using the methods of Henry [17] and Patton & Crouch [18] respectively.

Histological and Histochemical Study

Animals were dissected and their kidneys were removed. For histological preparations, the kidney was fixed in Bouin's fluid, dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 microns thickness were prepared and stained with Ehrlich's haematoxylin and eosin. Scoring of histopathological changes was done as follow: (-) absent; (+) mild; (++) moderate and (+++) severe [19]. For histochemical study, specimens were fixed in Carnoy's fluid. Periodic acid Schiff's reaction was used for demonstration of polysaccharides [20]. Total proteins were detected using the mercury bromophenol blue method [21].

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, P.A).

Results

Histological observations

The renal cortex of control rat kidney showed no changes in normal structure of renal tubules and the glomeruli. This observation appeared clearly in (Fig-1a). Also, the same observation was obtained in the rat Kidney sections treated with curcumin. The architecture of the renal cortex, renal tubules and glomeruli appeared normal. However, in the animal group treated with CPF variable degenerative changes were obtained in the structure of kidney section [Fig-1] and [Fig-2]. The semi-quantitative histological scoring of the effected kidney tissue is illustrated in [Table-1]. As appeared in [Fig-1b] after 3 weeks of treatment there was a congestion and dilatation in the renal blood vessels. In addition as illustrated in [Fig-1c] the renal tubules appeared with wide lumens and degenerated epithelial cells whereas the leucocytic infiltrations were abundant [Fig-1c]. Other histopathological changes were marked and noticed after 6 weeks of treatment, these changes were recorded when the renal tubules became necrotic with pyknoyic nuclei and the cytoplasmic content were noticeably degenerated [Fig-2a]. In the same section, the architecture of the glomeruli appeared hypertrophied with disrupted Bowman's capsules [Fig-2a]. Other histological changes were obtained from the same CPF treated animal these changes appeared as up normal shape of atrophied glomeruli, congested renal veins and degenerated renal tubules [Fig-2b]. However, in the group treated with CPF followed by treatment with curcumin, the results showed positive improvement in the histopathological alterations induced by CPF. The results have demonstrated that the kidney tissues were appeared normal and there was no histopathological changes were noticed [Fig-2c].

Table 1- Semi-quantitative histological scoring of kidney damage in different animal groups.

Animal group	Renal tubules necrosis	Atrophy of glomeruli	Hypertrophy of glomeruli	Leucocytic infiltrations	Congestion of blood vessels
Control	-	-	-	+	-
Curcumin	-	-	-	+	-
CPF	+++	+++	++	+++	+++
CPF+Curcumin	+	+	-	+	-

(-) negative,(+) moderate, (++) and (+++) severe

Histochemical Observations

Histochemical examination of kidney sections of control rats stained by PAS method revealed that glycogen was found in the epithelial cells of the renal tubules in the form of magenta red granules distributed in the cytoplasm and the nuclei are negatively stained [Fig-3a]. Treatment with CPF for 3 weeks caused slight reduction in glycogen content; this reduction became marked after 6 weeks [Fig-3b]. Curcumin administration has prominently restored the normal content of glycogen [Fig-3c]. Examinations of kidney sections of control rats stained with bromophenol blue showed distribution of total proteins in the form of deeply stained diffuse granules in both the cytoplasm and nuclei of the tubular cells [Fig-4a]. CPF treatment led to depletion of total proteins in the tubular cells, especially after 6 weeks [Fig-4b]. Treatment with CPF and curcumin restored

protein content near to the normal levels [Fig-4c]. No change in glycogen or total proteins was observed in renal tubules of animals administered with curcumin alone.

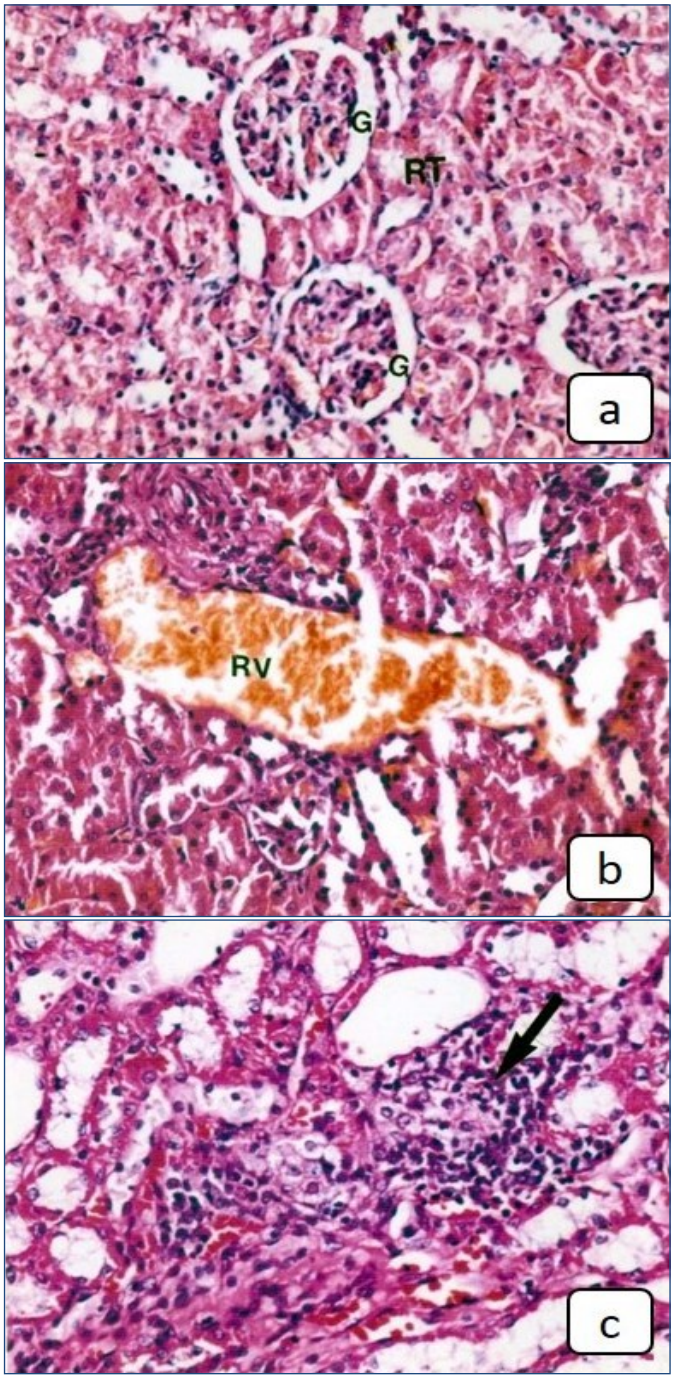

Fig. 1- Sections of rat kidney show **(1a):** Control section of normal glomeruli (G) and renal tubules (RT). **(1b):** Kidney treated with CPF shows enlarged and congested renal vein (RV). **(1c):** Intertubular leucocytic infiltrations, (X300).

Biochemical Results

Data in [Table-2] revealed that there is no remarkable change concerning urea and creatinine in sera of control rats and those treated with curcumin. In contrast, these parameters were elevated significantly ($p<0.05$) in animals treated with CPF for 6 weeks. Administration of curcumin resulted in a significant decrease in urea and creatinine levels compared to CPF group.

Table 2- Changes in urea and creatinine concentration in different animal groups.

Animal group	Urea (mg/dl)		Creatinine (mg/dl)	
	3 weeks	6 weeks	3 weeks	6 weeks
Control	30.5+3	31.5+3	2.1+0.6	2.4+0.3
Curcumin	31.6+4	32.5+3	2.2+0.4	2.3+0.4
CPF	33.2+5	58.3+4*	2.8+0.4	4.2+0.9*
CPF+Curcumin	32.5+6	42.6+6	2.4+0.5	2.9+0.7

(*) Significant at p< 0.05

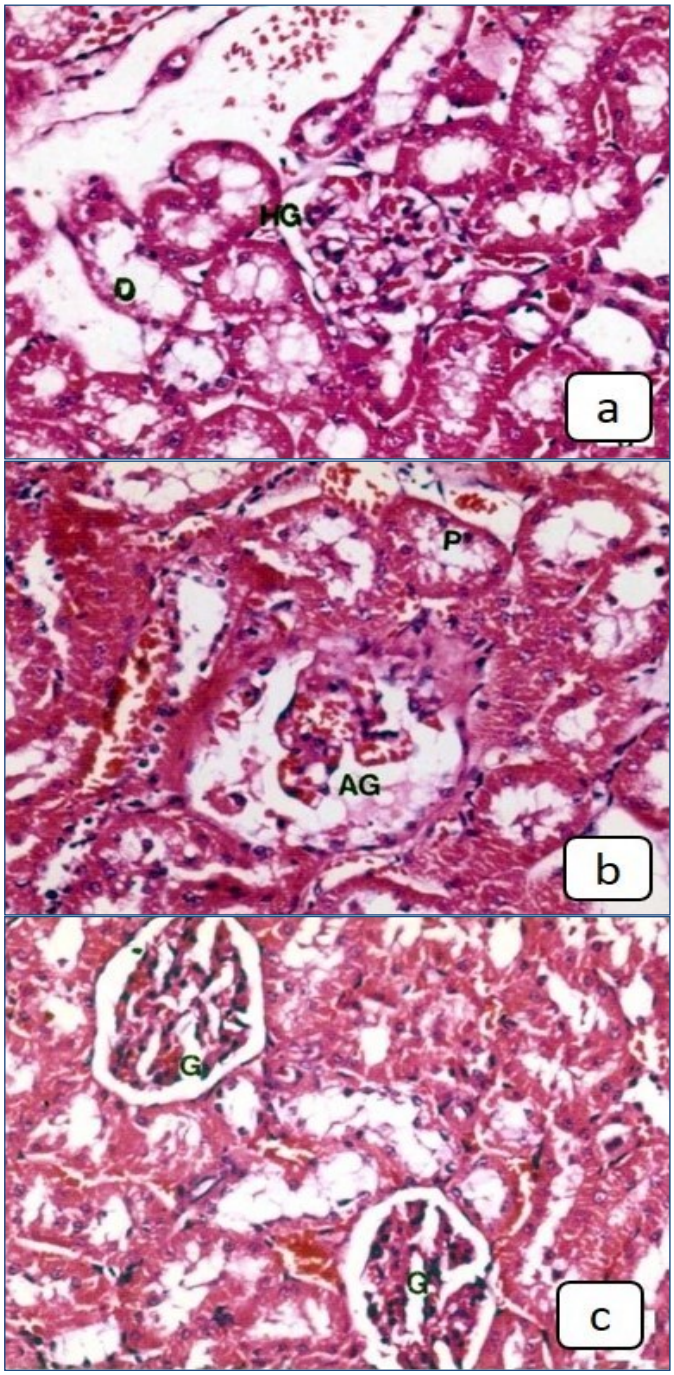

Fig. 2- Sections of rat kidney show **(2a):** Degenerated tubules in CPF treated kidney (D) and hypertrophied glomerulus (HG). **(2b):** Atrophied glomerulus (AG) and degenerated renal tubules, (P) pyknotic nucleus, **(2c):** Improvement in tubules and glomeruli (G) structure in kidney treated with CPF and Curcumin, (X 300).

Discussion

CPF is widely used in agriculture and domestic life against harmful insects and has been shown to cause a number of toxic effects on many organs and animal tissues. The present results revealed that CPF caused nephrotoxicity in kidney of albino rats. This effects have led to disorder in kidney structure and function including tubular cell necrosis, intertubular hemorrhage, leucocytic infiltrations and degeneration of glomeruli. It also shown that the toxic effect of chlorpyrifos caused up normality in serum level of creatinine and urea in treated rats used in this study. This effect led to reduction in glomerular filtration rate and consequently retention of urea in the blood serum.

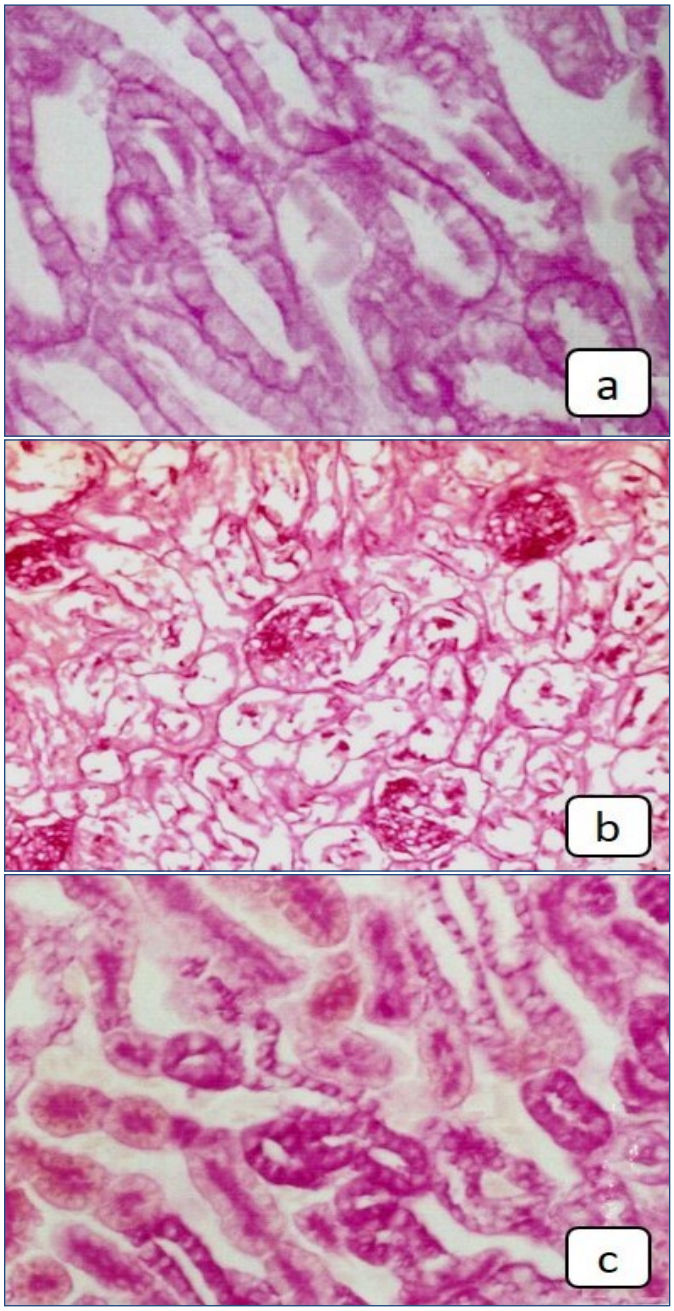

Fig. 3- Sections of rat kidney show **(3a):** Control section shows glycogen in the renal tubules. **(3b):** Section treated with CPF shows decrease in glycogen content in the renal tubules. **(3c):** Section treated with CPF and Curcumin shows increase in glycogen content again in the renal tubules, (X 300).

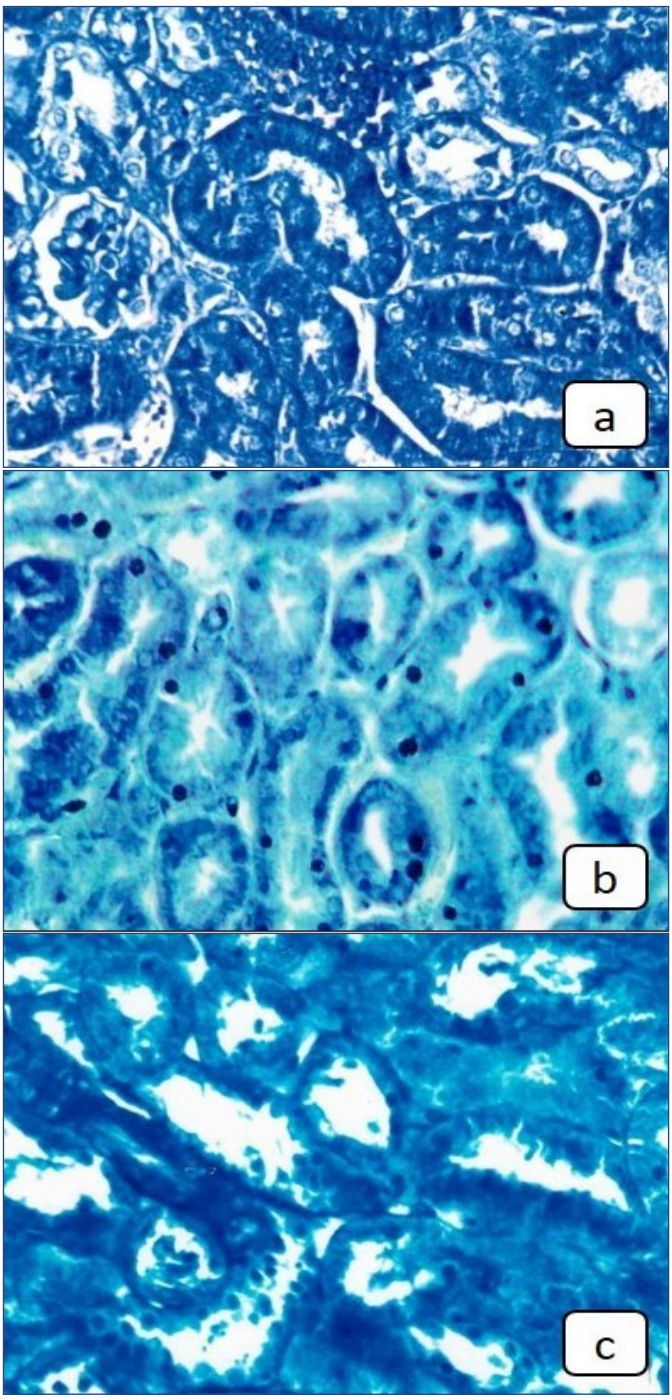

Fig. 4- Sections of rat kidney show **(4a):** Control section shows total proteins in the renal tubules. **(4b):** Section treated with CPF shows decrease in total proteins content. In the renal tubules. **(4c):** Section treated with CPF and Curcumin shows increase in proteins content again in the renal tubules, (X 300).

These findings are closely similar to those observations obtained by Johnson [22], in which using chlorpyrifos caused a significant increase in liver weight, blood creatinine and urea levels. In addition a remarkable decrease were noticed in body weights, kidney and testis weights of those animal under investigation. It has been found by Oncu, et al [23] that exposing rats to CPF caused noticeable histopathological alterations in kidney including infiltration in mononuclear cells at perivascular and peritubular areas, hydropic degenerations in tubule epithelium and glomerular sclerosis. Oral admin-

istration of chlorpyrifos caused remarkable degeneration of renal corpuscles, proximal distal and convoluted tubules, leading to decrease in size of glomeruli and marked increase in size of Bowmans spaces together with elevation of creatinine and urea [24]. Also, Heikal, et al [5] have found that the main characteristic findings in kidney of CPF-treated rats were the appearance of vacuolization and swelling in the endothelium of glomerular tuft**,** swelling in the lining epithelium of tubules and inflammatory cells infiltration. In addition of degeneration of tubules with fibrosis and hyalinosis between the tubules in focal manner and caused elevation in creatinine and blood urea nitrogen.

Regarding the histochemical changes observed in this study under CPF administration, the results clearly indicated reduction in carbohydrates and total proteins in the kidney tissue of treated rats. These findings are mostly in agreement with different previous studies which indicated that the administration of pesticides caused histochemical alterations in experimental animals. Alhady [25] have reported that aldicarb led to a decrease of carbohydrates concentration in renal tubules of rats treated with CPF. Moreover, it has been shown that lannate induced a marked decrease in glycogen content in liver of guinea pig [26]. Rady [27] has recorded significant decrease in the total protein and general carbohydrates in the lung and intestine of guinea pig exposed to diazinon. Some studies have found that Inhalation of other chemical such as tetramethrin led to decrease in glycogen, proteins and RNA of rats' liver [28]. Shady & Noor El-deen [29] have reported that CPF decreased PAS positive material in thyroid gland of rats. In addition, treatment with carbosulfan for 30 days has caused significant decrease in DNA, RNA, protein and glycogen in kidney tissue of mice [30,31]. The reduction in the carbohydrate and protein contents observed in the present study could be due to the release of hydrolytic enzymes from ruptured lysosomes under the toxic effect of CPF. This finding confirmed the increase in lysosomal enzymativ activities in response to organophosphate insecticide [32]. On the other hand Mishra & Devi [7] and Liu, et al [8] have confirmed that Parathion (PS) and chlorpyrifos (CPF) effect neurotransmission and cause neurodegeneration by inhibiting acetylcholinesterase (AChE) Endocannabinoids (eCBs, N-arachidonoylethanolamine, AEA; 2-arachidonoylglycerol, 2AG).

Besides inhibiting cholinesterase, oxidative stress has been recently proposed as a main toxicity mechanism for organophosphates [33]. Oxidative damage is the central mechanism of organophosphates toxicity occurs primarily through production of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide that are generated during the reaction with biological molecules causing damaging in membranes and other tissues [33].

CPF found to increased oxidative stress in the body, as evidenced by enhanced lipid peroxidation accompanied with concomitant decrease in the levels of SOD, CAT and GPx in liver, kidney and spleen [34]. It has been shown by Ahmed & Zaki [35] that CPF may have properties to induce oxidative stress indicated by enhancement of MDA production, decrease in GSH content, GST and CAT activities in rat tissues. However, accumulation of lipid peroxides has been recorded after exposure to chlorpyrifos in rat liver [31], kidney [23] and brain [36]. Thus, CPF may have toxic properties to induce oxidative stress leading to kidney injury as observed clearly in the results of current work.

The present results indicated that treating CPF-intoxicated rats with curcumin attenuated the severe alterations in histological and histochemical observed in the kidney or treated rats. It also helped normalized the serum level of creatinine and urea. These results are in agreement and confirming the findings of some investigators who reported the protective effect of curcumin against nephrotoxicity.

It has been revealed by Venkatesan, et al [11] and Ahmida [37] that the protective effect of curcumin against nephrotexicity of Adriamycin and Vancomycin was accompanied by significant reduction in urinary excretion of glycosaminoglycan and influence the integrity of glomerular basement membrane. Tirkey, et al [38] have illustrated that curcumin improved the renal dysfunction induced by cyclosporine-in rat kidney, as it improved creatinine clearance and decreased the elevated levels of serum creatinine and BUN. In addition, Abdel, et al [39] has found that curcumin prevented histopathological alterations in kidney of rats induced by cyclosporine. Some evidences have been reported by El-Zawahry & Abu El Kheir [40] showing that curcumin suppressed renal toxicity of gentamicin as evident by a remarkable improvement of renal function parameters and blocking oxidative injury in kidney and restore the antioxidant enzymatic profile. Curcumin was found to have nephroprotective effects against cisplatin and improved creatinine and urea clearance [12].

It has been suggested by Cohly, et al [41] that curcumin inhibits hydrogen peroxide induced oxidative injury in a renal cell line. It found to suppress renal toxicity of adriamycin by blocking oxidative injury in the kidney and prevented rise in lipid peroxide. Also, it increased the glutathione content and glutathione peroxidase activity of kidney tissues [11]. Rukkumani, et al [42] reported the protective effect of curcumin on circulating lipids in plasma and lipid peroxidation products in alcohol and polyunsaturated fatty acid-induced toxicity. Administration of curcumin to rat attenuated puromycin induced renal oxidative damage and improved the levels of the antioxidant enzymes SOD and catalase [43].

It has been documented by Kumar & Singh [44] that curcumin is a powerful scavenger of the superoxide anion, hydroxylradicals and nitrogen dioxide and protects DNA against singlet oxygen-induced strand breaks. It has significantly decreased the elevated tissue malondialdehyde levels, superoxide dismutase, and glutathione peroxidase enzyme activities in acute lung injury induced by intestinal ischaemia/ reperfusion [45]. Pre-treatment of rat with curcumin significantly replenished the renal mitochondrial lipid peroxidation levels and protein carbonyl content induced by cisplatin [46]. Also, it has been reported by García-Niño, et al [14] that curcumin effects liver oxidative damage induced by K2Cr2O7 were associated with prevention of mitochondrial dysfunction. Finally, we suggest that the effects of curcumin on nephrotoxicity, induced by CPF, is possibly due to its antioxidant properties.

Conflict of Interest: None declared.

References

[1] Jamal J.A. (1997) *Adverse Drug React. Toxicol. Rev.,* 16(3), 133-170.

[2] Chen C.C., Sureshbabul M., Chen H.W., Lin Y.S., Lee J.Y., Hong Q.S., Yang Y.C., Yu S.L. (2013) *Evid Based Complement Alternat Med*., 541695.

[3] Wang H.P., Liang Y.J., Long D.X., Chen J.X., Hou W.Y., Wu Y.J. (2009) *Chem. Res. Toxicol.,* 22(6), 1026-1033.

[4] Tripathi S., Srivastav A.K. (2010) *Pesticide Biochemistry and Physiology*, 97(1), 60-65.

[5] Heikal T.M., Mossa A.T.H., Marei G.I.K., Abdel Rasoul M.A. (2012) *J. Environ. Anal. Toxicol*., 2(5), 1-7.

[6] Mansour S.A., Mossa T.H. (2009) *Pesticide Biochemistry and Physiology*, 93, 34-39.

[7] Mishra A., Devi Y. (2013) *Acta Histochem*., 13, 00140-00142.

[8] Liu J., Parsons L., Pope C. (2013) *Toxicol Appl Pharmacol.*, 13, 00334-00337.

[9] Pari L., Tewas D., Eckel J. (2008) *Arch. Physiol. Biochem.*, 114, 127-149.

[10] Aggarwal B.B., Kumar A., Bharti A.C. (2003) *Anticancer Res*., 23(1A), 363-398.

[11] Venkatesan N., Punithavathi D., Arumugam V. (2000) *Br. J. Pharmacol.*, 129(2), 231-234.

[12] Kuhad A., Pilkhwal S., Sharma S., Tirkey N., Chopra K. (2007) *J. Agric. Food Chem*., 55(25), 10150-10155.

[13] Abd El-Ghany M.A., Ramadan A.M., Ghozy S.F. (2012) *World Applied Sciences Journal*, 16(5), 646-655.

[14] García-Niño W.R., Tapia E., Zazueta C., Zatarain-Barrón Z.L., Hernández-Pando R., Vega-García C.C., Pedraza-Chaverrí J. (2013) *Evid Based Complement Alternat Med*., 424692.

[15] Cho Y.J., Yi C.O., Jeon B.T., Jeong Y.Y., Kang G.M., Lee J.E., Roh G.S., Lee J.D. (2013) *Korean J. Physiol. Pharmacol*., 17 (4), 267-274.

[16] Sakr S.A., Mahran H.A., El-Deeb M.M. (2013) *Oxidants and Antioxidants in Medical Science*, 2(1), 29-35.

[17] Henry R.J. (1974) *Clin. Chem. Principles and Techniques* 2nd, Happer and Ow Publishers, 525.

[18] Patton C.J., Crouch S.R. (1977) *Anal. Chem*., 49(3), 464-469.

[19] Bancroft G.D., Stevens A., Turner D.R. (1996) *Theory and Practice of Histopathological Technique*, 4th ed., Churchill, Livingston, New York.

[20] Kiernan J.A. (1981) *Histological and Histochemical Methods: Theory and Practice*, New York, Pergamon Press.

[21] Pearse A.G.E. (1972) *Histochemistry: Theoretical and Applied*, 3rd ed., Churchill-Livingstone, London, 2, 761-1518.

[22] Johnson K.A. (2005) *Toxicology & Environmental Research and Consulting*, Laboratory Project Study 1-35, Midland, MI.

[23] Oncu M., Gultekin F., Karaöz E., Altuntas I., Delibas N. (2002) *Hum. Exp. Toxicol*., 21(4), 223-230.

[24] El-Deeb A.E.A., Abd El-Aleem I.M., Ghaleb S.S. (2007) *J. Egypt. Soc. Toxicol*., 36, 53-60.

[25] Alhady M. (1994) *J. Egypt. Germ. Soc. Zool.*, 15, 177-199.

[26] El-Beih Z.M., Amer M.A., Gamil R.H. (1991) *J. Egypt. Ger. Soc. Zool*., 6c, 87-99.

[27] Rady M.I. (2009) *Brazilian Archives of Biology and Technology*, 52(2), 317-326.

[28] Sakr S.A., El-Mesady F.A., El-Desouki N.I. (2002) *Journal of Medical Sciences*, 2, 24-28.

[29] Shady A.M., Noor El-deen F.I. (2010) *Egypt. J. Histol.*, 33(3), 441-450.

[30] Ksheerasagar R.L., Hiremath M.B., Kaliwal B.B. (2011) *World Journal of Science and Technology*, 1(5), 43-55.

[31] Bagchi D., Bagchi M., Hassoun E.A., Stohs S.J. (1995) *Toxicology*, 104(1-3), 129-140.

[32] Awasthi M., Shah P., Dubale M.S., Gadhia P. (1984) *Environ. Res*., 35(1), 320-325.

[33] Halliwell B., Gutteridge J.M.C. (2012) *Free Radicals in Biology and Medicine*, 3rd ed. Oxford University Press Inc., 105-245.

[34] Bebe F.N., Panemangalore M. (2003) *J. Environ. Sci. Health B*, 38(3), 349-363.

[35] Ahmed M.M., Zaki N.I. (2009) *Nature and Science of Sleep*, 7 (10), 49-61.

[36] Gultekin F., Ozturk M., Akdogan M. (2000) *Arch. Toxicol*., 74(9), 533-538.

[37] Ahmida M.H.S. (2012) *Exp. Toxicol. Pathol*., 64(3), 149-153.

[38] Tirkey N., Kaur G., Vij G., Chopra K. (2005) *BMC Pharmacology*, 5(1), 15.

[39] Abdel F.E., Hashem H.E., Ahmed F.A., Ghallab M.A., Varga I., Polak S. (2010) *Gen. Physiol. Biophys*., 29(1), 85-94.

[40] El-Zawahry B.H., Abu El Kheir E.M. (2007) *The Egypt. J. Hospital Medicine*, 29, 546-556.

[41] Cohly H.H., Taylor A., Angel M.F., Salahudeen A.K. (1998) *Free Radic. Biol. Med*., 24(1), 49-54.

[42] Rukkumani R., Sri Balasubashini M., Menon V.P. (2003) *Phytother Res*., 17(8), 925-929.

[43] Farombi E., Ekor M. (2006) *Food Chem. Toxicol*., 44(9), 1443-1448.

[44] Kumar A., Singh A. (2008) *Phytomedicine*, 15(8), 577-586.

[45] Guzel A., Kanter M., Guzel A., Yucel A.F., Erboga M. (2013) *Toxicol. Ind. Health*., 29(7), 633-642.

[46] Waseem M., Kaushik P., Parvez S. (2013) *Cell Biochem Funct*., 10.1002/cbf.2955.