Hepatotoxicity Induced by Chlorpyrifos in 'Wistar' Rats

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ABSTRACT

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Chlorpyrifos-ethyl is one of the most widely used organophosphorus insecticides for industrial, agricultural and public health purposes. The present work aimed to evaluate the chlorpyrifos-ethyl induced hepatic toxicity on some parameters of oxidative stress in 'Wistar' rats. After daily gavage for 60 days, this insecticide induced cellular damage in exposed rats through the increase in alanine aminotransferease (ALT) and aspartate aminotransferase (AST) activities. In addition, liver parameters showed an increase in hepatic lipid peroxidation as indicated by the malondialdehyde (MDA) contents, as well as a decrease in the catalase (CAT) activity. A reduction in glutathione (GSH) and glutathione-S-transferase (GST) and an increase in glutathione peroxidase (GPx) activities were also noted. These alterations indicated that chlorpyrifos affected the antioxidant system in hepatic tissue, induced hepatic damage and consequently triggered the oxidative stress.

Keywords: Antioxidant system, chlorpyrifos-ethyl, hepatic toxicity, lipid peroxidation, oxidative stress

The widespread use of pesticides in public health and agricultural programs has caused severe environmental pollution and potential health hazards, including acute and chronic cases of human poisoning. Organophosphate insecticides (OPI) constitute one of the most widely used classes of pesticides being employed for both agricultural and landscape pest control (El-Bini Dhouib et al. 2015; Kamath and Rajini 2007). Moreover, residual amounts of pesticides have been detected in the soil, water bodies, vegetables, grains, and other food products (Poet et al. 2004).

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Chlorpyrifos (CPF) is one of the most widely used for domestic, agricultural, and industrial purposes and for public health applications OPI (Akande 2016; Richardson and Chambers 2005). It is able to cause oxidative stress and histopathologic changes in humans and animals, and it can cause embryotoxicity, teratogenicity, immune-logical abnormalities, neurobehavioral changes, developmental and reproductive toxicity, neurotoxicity (Breslin et al. 1996; Ki et al. 2013), hematologic changes (Uzun and Kalender 2013), and testicular toxicity (Joshi et al. 2007). These findings on the various toxicities caused by CPF are of great concern to the general public.

The principle mode of action of CPF is the phosphorylation and subsequent irreversible inhibition of

acetylcholinesterase (Eaton 2008; Wang et al. 2013). This enzyme hydrolyses acetylcholine at cholinergic synapses and neuro-muscular junctions and the persistent inhibition of its activity causes neurotoxic effects (Luis et al. 2015) disrupting cholinergic function in the nervous system (Lassiter and Brimijoin 2008). CPF has also been shown to elicit oxidative stress in biological systems (Ahmed et al. 2010; Alvarez et al. 2008; Vermaet al. 2007).

Oxidative stress caused by reactive oxygen species (ROS) has been reported in membrane lipid peroxidation, DNA damage, mutagenesis and has been associated with the various stages of tumor formation process (Sidhuet al. 2014). Oxidative stress can occur when there is an imbalance between ROS production and antioxidant defenses (Preedy 2013). Acute and chronic exposure to CPF has resulted in considerable liver damage, as evidenced by changes in several liver enzymes (Goel et al. 2000). Liver is the first organ exposed to ingested toxins including pesticides, metals, etc., for biotransformation. Therefore, toxic responses have been reported to occur more in the liver as compared to other organs (Raina et al. 2015) and it is the organ where activation and detoxification of CPF take place. It is demonstrated that OP pesticides like CPF causes hepatotoxicity by changing the profile of liver marker enzymes such as alanine aminotransferase (ALP), aspartate aminotransferase (AST), lactate déshydrogénase (LDH) and histopathological changes (Mansour and Mossa 2009). Therefore, in toxicological studies histopathological lesions have been widely used as biomarkers (Ncibi et al. 2008). For this reason, the aim of this work was to study the alterations in hepatic functions and oxidative damage in hepatocytes following repeated oral exposure to CPF during 60 days in 'Wistar' rats.

MATERIALS AND METHODS

Pesticide.

DURSBAN 4® (Dow agrosciences) containing 480 g chlorpyrifos per liter was used in the present study.

Animals.

Adult female rats of 'Wistar' strain, weighing 190-210 g were purchased from the Pasteur Institute of Algiers, Algeria. They were kept in metal cages at 23±2ºC, 40-60% relative humidity, and light/dark cycle of 12 h. These spleens received water and standard diet and were subjected to the force-feeding every day, during a period of 60 days.

Experimental protocol.

The body weight of all the animals was checked every day. The rats were randomly divided into two groups composed each of five rats. The protocol of rat treatment is given below:

- Group I (control): Animals were administered corn oil only.
- Group II (CPF-treated): Animals were administered CPF dissolved in corn oil (1/25 LD_{50}) (Tanvir et al. 2015).

After 60 days of treatment, the blood and liver tissue were collected for biochemical examinations as indicated below.

Biochemical marker enzymes in plasma.

After 60 days, rats were fasted overnight. Then, they were weighed and sacrificed. The blood samples were collected from each rat and a 2 ml-sample was injected in a glass tube without EDTA and left for 20 min to coagulate at

room temperature, and then centrifuged at 3000 rpm for 10 min to obtain serum samples used for biochemical assays.

Measurement of malondialdehyde level.

MDA can be detected by a colorimetric reaction with thiobarbituric acid (TBA). It was detected after degradation of polyunsaturated fatty acids 3 or 4 Double peroxidized bonds. This is a highly sensitive method for determining lipid peroxidation in vitro. The assay of MDA was carried out according to the method of Esterbauer et al. (1992). Optical density has been measured at λ = 532 nm.

Antioxidant enzymes assay.

Glutathione (GSH) level was determined according to the method of Ellman et al. (1959). This assay is based on the measurement of 2-nitro-5-mercapturic absorbance at λ= 532 nm. The latter resulted from the reduction of the acid 5,5'-dithiobis-2-nitrobenzoic acid (reagent Elleman) by groups (-SH) of glutathione. Once prepared, it must undergo homogenate deproteinization (by 0.25% sulfosalicylic acid) to protect the SH-groups of glutathione.

The enzymatic activity of GPx was determined according to Flohe and Gunzler (1984) method using H_2O_2 as substrate. The spectrophotometric assay of catalase (CAT) activity was performed according to Aebi (1984). The decrease of absorbance was noted for 3 min by a spectrophotometer at a wavelength of 240 nm and an extinction coefficient $\varepsilon = 0.036$ $mM^{-1}cm^{-1}$.

The activity of glutathione S-transferase (GST) was determined according to the method of Habig et al. (1974). It is based on the conjugation reaction between GST and a substrate, the 1-Chloro2,4-dinitrobenzene (CDNB) as a cofactor of GST, the conjugation results in the formation of a new molecule: 1-S-glutathionyl 2-4-Di nitrobenzene to measure the activity of GST.

Statistical analysis.

Data were expressed as mean ± SD of five rats in the group. Significant differences between the control and the treated groups were determined by the Student's *t*-test. Statistical calculations were carried out using Minitab 17.1 statistical package and the Excel 16.0 (Microsoft, Inc.). The level of statistical significance was set at $P < 0.05$.

RESULTS

Effect on liver function biomarkers.

There was a significant increase ($P \leq 0.001$) in ALT activity levels in chlorpyrifos (CPF treated rats (58.66 ± 2.08 IU/L) compared to control animals (38.00 ± 4.00 IU/L). Similarly, the value of AST was significantly ($P \leq 0.001$) increased in CPF group (62.00 ± 2.64 IU/L) compared to control (32.33 ± 2.51 IU/L) (Table 1).

Effects of CPF on lipid peroxidation.

Treatment with CPF induced significant ($P \leq 0.01$) increases in MDA levels in the treated group (0.632 ± 0.140 nmol/mg prot) as compared to control group (0.557 ± 0.071 nmol/mg prot) (Table 2).

Effects of CPF intoxication on antioxidant defense system in the liver tissue.

The administration of CPF caused significant ($P \leq 0.05$) decreases in CAT (0.099 ± 0.015 µmol/min/mg prot), GSH (1.158×10^5 ± 1.093×10^7 µmol/min/mg prot) and GST (0.003 ± 0.048×10^2 µmol/min/mg prot) activities in liver compared to the control group (0.190 ± 0.005 µmol/min/mg prot; 2.469×10^5

±2.376×10^6 µmol/min/mg prot; 0.006 ± 0.001 µmol/min/mg prot, respectively). A significant ($P \leq 0.05$) increase in GPx activity was also noted following oral administration of CPF to rats (0.574 ± 0.056 µmol/min/mg prot) relative to the control ones (0.422 ± 0.076 µmol/min/mg prot) (Table 2).

Table 1. Effect of treatment with chlorpyrifos (CPF) on hepatic injury markers in the plasma of 'Wistar' rats

Treatment	Liver function biomarker	
	ALT (IU/L)	AST (IU/L)
Control	38.00 ± 4.00	32.33 ± 2.51
Chlorpyrifos (CPF)	58.66 ± 2.08***	62.00 ± 2.64***

Values given are means ± SD of the results obtained from 5 rats. Means with at least one common superscript do not differ significantly at $P \leq 0.05$. ALT: Alanine aminotransferease, AST: Aspartate aminotransferase
*** Very highly significant at $P \leq 0.001$

Table 2. Effect of chlorpyrifos (CPF) on lipidperoxidation, reduced glutathione and activities of enzymatic antioxidants in the liver of experimental animals.

Treatment	Control	Chlorpyrifos (CPF)
MDA	0.557 ± 0.071	0.632 ± 0.140**
GSGST	0.006 ± 0.001	0.003 ± 0.048×10^{2*}
GSH	2.469×10^5 ±2.376×10^6	1.158×10^5 ± 1.093×10^{7*}
CAT	0.190 ± 0.005	0.099 ± 0.015***
GPx	0.422 ± 0.076	0.574 ± 0.056

MDA (nmol/mg prot), GST (µmol/min/mg prot), GSH (µmol/min/mg prot), CAT (µmol/min/mg prot), GPx (µmol/min/mg prot). Values given are mean ± SD of the results obtained from 5 rats. Means with at least one common superscript do not differ significantly at $P \leq 0.05$.
* Significant at $P \leq 0.05$; ** Highly significant at $P \leq 0.01$; *** Very highly significant at $P \leq 0.001$.

DISCUSSION

In the present study, we demonstrated that CPF administrated to rats provoked a marked elevation in serum AST and ALT activities. These findings are in accordance with various previous studies (Ambaliet al. 2011; Geolet al. 2005; Heikal 2013; Mansour and Mossa 2011; Ncibi et al. 2008). These increases could potentially be attributed to the release of these enzymes from the cytoplasm into the blood

circulation indicating a necrosis and inflammatory reactions (Acker et al. 2012; Tanvir et al. 2015), and reflect the alteration of the membrane permeability of the hepatocytes (Raina et al. 2015).

Various studies indicate the production of reactive oxygen species (ROS) as secondary means of toxicity (Sidhu et al. 2014). We showed that rats exposure for 60 days to CPF caused an increase in malondialdehyde (MDA) level, which is the major end products of lipid peroxidation, in liver of rats. This effect may be ascribed to an excessive production of ROS by a pesticide-induced oxidative cell (Akande et al. 2014). This result is in agreement with those of other studies showing an increase in lipid peroxidation after treatment with CPF (Gultekin et al. 2000; Kalender et al. 2012; Ojha et al. 2011; Saulsbury et al. 2009; Uzun and Kalender 2013). We have demonstrated that CPF-induced injury is caused by the induction of oxidative stress which was suggested to be an additional factor inducing apoptosis. Tuzmen et al. (2008) demonstrated previously that rats developed lipid peroxidation and liver damage subsequent to CPF exposure.

In the present study, the liver rats treated with CPF showed significant decreases in GSH and GST levels and in CAT activity but there was an increase in the GPx activity. The perturbation of these enzymes may be due to oxidative stress of pesticide intoxication. This is in accordance with previous works (Aggarwal et al. 2014; Ajay et al. 2005; Mansour and Mossa 2011; Raina et al. 2015). Therefore, Adedara et al. (2015) revealed a decrease of catalase and GST activities in treated *Drosophila melanogaster* following CPF treatments. Other researchers also recorded an elevation in CAT activity (Ozkan et al. 2012; Tuzmen et al. 2008; Uzun and Kalender 2013) and in GST level (Cacciatore et al. 2015). However, Aly et al. (2010) recorded an increase in GSH level and GPX activity was unchanged. Thus, CPF increased rate of hepatic lipid peroxidation (MDA), with the depletion in the defense system, suggesting that this alteration induced by CPF involved an oxidative stress.

GSH plays a key role in the detoxification of free radicals. It directly interacts with high affinity with thiol groups (-SH) of GSH. The GPx is a key antioxidant enzyme, which regulates the level of ROS (GPx is capable not only to reduce the hydrogen peroxide in water, but also the resulting hydroperoxides of oxidation of unsaturated fatty acids). Concerning the glutathione S-transferase (GST), this enzyme plays an important role in detoxification of xenobiotics. Catalase (CAT) is the second step in the enzymatic defense system (Preedy 2013).

To conclude, the results from the current study indicate that oral exposure of CPF produces hepatic damage. Furthermore, the oxidative stress and the altered antioxidant system in liver are due to imbalance between oxidant and antioxidant levels in hepatic tissues.

RESUME

Chenikhar H., Djabri B., Salmi A., Taib C. et Rouabhi R. 2018. Hépatotoxicité induite par le chlorpyrifos chez les rats 'Wistar'. Tunisian Journal of Plant Protection 13 (si): 23-30.

Le chlorpyriphos-éthyl est l'un des insecticides organophosphorés les plus largement utilisés à des fins industrielles, agricoles et des applications de la santé publique. Le présent travail consiste à évaluer la toxicité hépatique induite par le chlorpyriphos-éthyl sur quelques paramètres du stress oxydatif. Après

un gavage quotidien pendant 60 jours, cet insecticide a provoqué des dommages cellulaires chez les rats exposés par l'élévation des activités de l'alanine aminotransférase (ALT) et l'aspartate aminotransferase (AST). En outre, les paramètres hépatiques ont montré une augmentation de la peroxydation lipidique hépatique qui a été évaluée par les teneurs en malondialdéhyde (MDA), ainsi qu'une diminution de l'activité de la catalase (CAT). Une réduction de la glutathion (GSH) et de la glutathion-S-transférase (GST) et une augmentation des activités de glutathion peroxydase (GPx) ont également été notées. Ces altérations ont indiqué que le chlorpyrifos a affecté le système antioxydant dans le tissu hépatique, a induit des lésions hépatiques et a déclenché, par conséquent, le stress oxydatif.

Mots clés: Chlorpyriphos-éthyl, peroxidation lipidique, stress oxydatif, système antioxydant, toxicité hépatique

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ملخص

شنيخر،هاجر وجابري بلقاسم وسالمي آية وتايب شهيناز وروابحي رشيد. التسمم الكبدي الناجم عن كلوربيريفوس (chlorpyrifos) عند الفئران 'ويستار'. Tunisian Journal of Plant Protection 13 (si): 23-30.

كلوربيريفوس-إثيل (Chlorpyriphos-éthyl) هو أكثر المبيدات الفوسفورية استعمالا للأغراض الصناعية والزراعية والصحة العامة. يهدف هذا العمل إلى تقييم التسمم الكبدي الناجم عن استعمال كلوربيريفوس-إثيل على بعض معاملات الإجهاد التأكسدي. بعد تزقيم الفئران يوميا لمدة 60 يوم أدى هذا المبيد الحشري إلى تلف الخلايا بارتفاع أنزيمات Aminotransferase (ALT) و Aspartate aminotransferase (AST). كذلك المعاملات الكبدية بينت زيادة مستوى الاكسدة الدهنة الكبدية وذلك من خلال تقييم تركيز Malondialdehyde (MDA)، إضافة إلى انخفاض في نشاط أنزيم كاتالاز(CAT). سجل أيضا انخفاض في Glutathion (GSH) و Glutathion-S-transferase (GST)، مع ارتفاع في مستوى Glutathion peroxydase (GPx). أكدت هذه الاضطرابات أن كلوربيريفوس يؤثر على النظام المضاد للأكسدة في الكبد مؤديا الى جروح في الكبد وبالتالي يسبب الإجهاد التأكسدي.

كلمات مفتاحية: إجهاد تأكسدي، أكسدة دهنية، تسمم كبدي، كلوربيريفوس-إثيل، مضاد للأكسدة

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