Chronic Exposure to Chlorpyrifos Alters Spontaneous Motor Activity and Skeletal Muscles Contractility in Young Rats

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***Abstract*—Chlorpyrifos (CPF) is one of the most common Organophosphorus Pesticides (OP) used worldwide. The toxicity of CPF results from the phosphorylation of the acetylcholinesterase enzyme at nerve endings leading to a cholinergic poisoning in both central and peripheral nervous system. Neurobehavioral alterations, such as cognitive deficits and locomotor impairment, have been extensively reported in studies following an acute exposure to CPF, but to less extent following a chronic exposure. The aim of this study is to examine the effects of chronic dietary exposure to CPF for 6 weeks on the spontaneous motor activity and the physiological parameters of two skeletal muscles, soleus and *extensor digitorum longus (edl)*, involved in motor activities in young rats. Young rats were fed diets containing 1 mg/kg/day CPF (CPF1) or 5 mg/kg/day CPF (CPF5) for six weeks. Behavioral testing to assess the locomotor activity has been performed on weekly basis. The fibers from soleus and *edl* muscles were dissected and used to study contractile properties. Our results showed that animals treated with CPF suffered from hypolocomotion dose independent, which have been clearly manifested as an increase in latency time as well as the number of errors assessed by the beam walking test and a significant decrease in the beam balance time. CPF5 exposure showed an increase in twitch tension comparing to control groups for both soleus and edl. However, CPF1 exposure induces a decrease in twitch contraction in edl muscle. Repeated low level exposure to CPF1 and CPF5 impaired the fatigability index of soleus but not *edl* muscle. Those dose independent decrements reported in the behavioral tests came along a significant effect on the muscle's contractile parameters. Thus, the greater level of contraction in both soleus and edl muscles studied in vitro might be linked to the alterations shown in the locomotor activity of rats.**

Keywords-pesticides chlorpyrifos; spontaneous motor activity; muscle contractility.

I. INTRODUCTION

Even though lots of governmental regulations are being engaged to limit the market growth of chlorpyrifos (CPF) over the next seven years (up to 2022), it remains one of the most common Organophosphorus Pesticides (OP) used worldwide [1]. Its toxicity results primarily from the inhibition of the AcetylCholinesterase Enzyme (AChE) at nerve endings that will lead to the accumulation of the acetylcholine (Ach) in the synaptic cleft. This will overstimulate the effector organ, such as neurons in the Central Nervous System (CNS) and the skeletal muscle cells and will lead to cholinergic poisoning [2]. Neurobehavioral alterations, such as cognitive deficits and locomotor impairment, have been extensively reported in previous studies following an acute exposure to CPF via two main routes subcutaneous [3] [4] or intragastric administration [5], but to less extent following a chronic exposure via one of the main route "the ingestion of contaminated food". The aim of this study is to investigate whether the repeated exposure of young rats for six weeks to low doses CPF will affect 1) the spontaneous motor activity assessed by the beam walking test and prehensile traction test and 2) the physiological parameters of two skeletal muscles involved in the motor activities: the soleus, a slow-oxidative muscle and the *edl* (*extensor digitorum longus*), a fast-glycolytic muscle. The paper proceeds as follows: Section II describes the experimental design, data are analyzed in Section III, and, finally, Section IV presents the discussion and draws the conclusions.

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II. MATERIALS AND METHODS

A. Experimental protocol

30 adults male Sprague-Dawley rats (6 weeks old, average body weight 200 g) were housed in a plastic cage and maintained on a 12L:12D cycle and temperature maintained at 23°C. They were randomly divided into three groups of 10 each: control, CPF1 and CPF5. Rats in CPF1 and CPF5 treated groups were fed a diet containing the CPF at 1 mg/kg/day and 5mg/kg/day respectively for 6 weeks. The control group was given only the standard pellet diet with the vehicle (corn oil).

B. Behavioral experiments

The locomotor activity was assessed on a weekly basis during the treatment period. In the beam walking test, the rat had to cross the beam readily in order to reach a box placed at the other end. Traversing latency and number of hind-limb slips were measured. In the prehensile traction test, rats were assessed for their ability to grasp with their forepaws a horizontal rod, for a one minute. Latency from when the animal mounted the rod to when it fell from it was measured.

C. Measurement of muscle contractility parameters

After 6 weeks of exposure to CPF, the rats were sacrificed. Soleus and *edl* were removed and the preparation was mounted as described by Joumaa and Leoty [6]. The preparation was stimulated by square electrical pulses at 0.1 Hz. After equilibration, single twitches were elicited. For each twitch the peak twitch tension was determined. The fatigue resistance was evaluated using a protocol consisting of a 2 Hz train of supramaximal stimuli for a 5 min period. The fatigue index was calculated as the percentage of initial force remaining after 5 min of muscle stimulation.

III. RESULTS

A. Behavior measurements

Our results show that at weeks 5 and 6, there was a significant increase in time to cross the beam in both CPF1 and CPF5 compared to control group (Fig. 1).

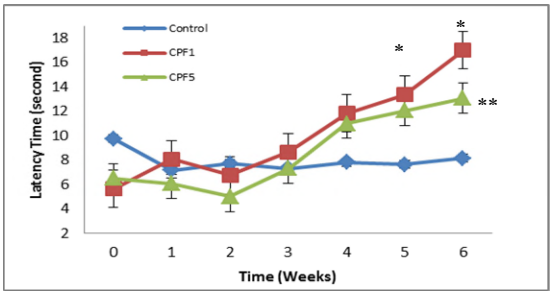

Figure 1. Beam walking test/ latency time. The data are depicted as mean ± SEM. *: $p < 0.05$ for CPF1 vs control at week 05 and 06 and CPF5 vs control at week 05. **: $p < 0.01$ for CPF5 vs control at week 06.

Also, in the hind limb slips test, there was a significant increase in the mean number of errors in both CPF1 and CPF5 groups compared to control group. Regarding the prehensile traction test, there was a significant decrease in latency time from when the animal grasped the horizontal rod with its forepaws to when it fell from it between treated groups (CPF1, CPF5) and control group at weeks 5 and 6 (Fig. 2). No significant difference was observed between CPF1 and CPF5 groups at weeks 5 and 6.

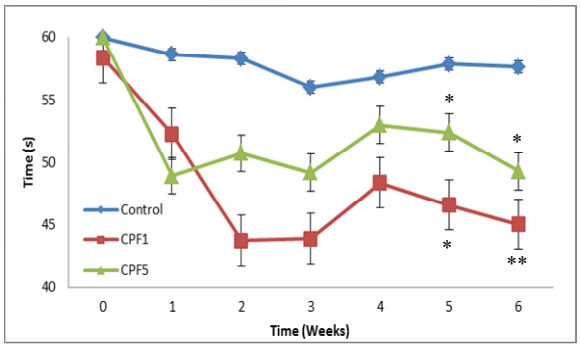

Figure 2. Beam balance test. The data are depicted as mean ± SEM. *: $p < 0.05$ for CPF1 vs control at week 05 and CPF5 vs control at week 05 and 06. **: $p < 0.01$ for CPF1 vs control at week 06

B. Contractility parameters

For the *edl* muscle, our data showed a significant decrease in twitch of contraction (Pt) in CPF1 rats compared to control rats. However, a significant increase in twitch of contraction was observed in rats exposed to CPF5 (Table I). In Soleus muscle, twitch of contraction significantly increase in both CPF1 and CPF5 rats compared to control rats (Table I). Repeated low level exposure to both CPF1 and CPF5 significantly increases the fatigability index of soleus but not *edl* muscle.

TABLE I. EFFECTS OF CPF1 AND CPF5 EXPOSURE ON CONTRACTILE CHARACTERISTICS OF EDL AND SOLEUS MUSCLES. PT PEAK TWITCH TENSION. *: P < 0.05. THE DATA ARE DEPICTED AS MEAN ± SEM.

	Pt (g/cm2)	
	EDL	Soleus
Control (n=10)	150.38 ± 7.96	106.09 ± 4.72
CPF1 (n=10)	96.55 ± 7.32*	133.64 ± 5.08*
CPF5 (n=10)	185.56 ± 9.19*	173.44 ± 6.86*

IV. DISCUSSION AND CONCLUSION

The effect of CPF on locomotor activity has been described previously but mainly during acute exposure and to less extent during chronic repeated low level of exposure. The present study revealed that animals treated with CPF showed deficits during locomotion dose independent

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following beam walking test, which have been clearly manifested as an increase in latency time, as well as the number of errors through walking the horizontal rod. Impairment in the motor function was also recorded following the prehensile test showing a significant decrease in the time spent and the ability for hanging on the rope compared to the control group. This alteration in locomotors activity can be referred either to an effect on CNS or peripheral skeletal muscles. In our study, the dose independent decrements reported in the behavioral tests of treated rats came along a significant effect on the soleus and edl muscle's amplitude of contraction and fatigability. The greater level of contraction and the decrease in fatigue resistance in vitro for soleus muscle at the two different doses might be linked to the alterations shown in the locomotors activity of rats. Our present findings are consistent with the documented effects of repeated low level exposure to CPF on another slow oxidative skeletal muscle which is the diaphragm [7]. The increase in twitch tension is explained by the decrease of AchE activity in the studied muscle [7]. In conclusion, the significant variations in motor activity and contractile parameters suggest a possible effect of CPF on skeletal muscle function by affecting the expression of some of the main actors of contraction, such as Ryanodine receptor and SERCA pump that are responsible for calcium homeostasis. This study demonstrates that exposure to CPF has an impact on the vital physiological functions of vulnerable populations. These deleterious effects could have long-term consequences in maintaining body homeostasis and human health.

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