EFFECT OF WITHANIA SOMNIFERA ON CHLORPYRIFOS INDUCED NEUROTOXICITY IN RATS

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ABSTRACT

Withania somnifera, a medicinal herb having potential antioxidant activity, has been explored to ameliorate chlorpyrifos induced neurotoxicity in adult Wistar rats. The root extract of the plant was given i/p @ 25 mg/kg and 50 mg/kg daily for 14 days after i/p administration of chlorpyrifos @ 85 mg/kg. This study indicated no beneficial effect of the plant extract at given doses on chlorpyrifos-induced neurobehavioral alterations or on brain and plasma acetylcholinesterase inhibition. It rather aggravated these effects.

Key words: Chlorpyrifos, Withania somnifera, neurotoxicity, acetylcholinesterase, rats

Chlorpyrifos, o-o-diethyl-o-(3, 5, 6-trichloro-2-pyrindinyl) phosphorothionate, is a very widely used organophosphate insecticide. In veterinary medicine it is used as an acaricide and is an active ingredient of sprays, dips, collars, ear tags etc. for the control of various ectoparasites of livestock and poultry. Its continuous use is known to cause various toxic effects in non-target species including man and animals. Like other organophosphates, it also exerts its action by inhibiting acetylcholinesterase enzyme in the central and peripheral nervous system with subsequent accumulation of acetylcholine at synaptic terminals and hyperstimulation of postsynaptic cells, thereby causing various neurotoxicological effects. The toxic manifestation of chlorpyrifos has been associated with intracellular oxidative stress (Bebe and Panemangalore, 2003). A few studies using exogeneously administered antioxidant have been reported to ameliorate chlorpyrifos-induced oxidative stress (Gultekin et al., 2001). Cithiolone, an antioxidant, has been reported to possess prophylactic potential against some organophosphate-induced neurotoxic effects (Naqvi and Hasan 1992). But no attempt has yet been made to explore the medicinal plants possessing high antioxidant activity in ameliorating the neurotoxic effects of chlorpyrifos. The present study describes the effects of Withania somnifera possessing high antioxidant potential (Bhattacharya et al., 2001) on chlorpyrifos-induced neurotoxicity in adult Wistar Rats.

MATERIALS AND METHODS

Withania somnifera roots were procured from the Medicinal, Aromatic and Underutilized Section of CCS Haryana Agricultural University, Hisar in the month of March and dried in shade. The aqueous extract was prepared as per the method of Bhattacharya et al. (1997) and administered i/p @ 25 mg/kg and 50 mg/kg body weight (0.5 ml) in each rat. Chlorpyrifos formulation (Dhanvan-20) obtained from Northern Minerals Ltd. Gurgaon was used to produce toxicity in rats.

Seventy eight adult male Wistar rats weighing 150-200g obtained from Disease Free Small Animal House of the University, were randomly divided into three groups each containing 24 rats and six rats were kept as control. The rats of groups 1, 2 and 3 were given chlorpyrifos i/p @ 85 mg/kg (80% of MTD as determined earlier). Simultaneously from the same day, the rats of groups 2 and 3 were given Withania somnifera root extract in normal saline i/p @ 25 mg/kg and

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50 mg/kg, respectively daily for 14 days.

After chlorpyrifos exposure, all the rats were tested following the Functional Observational Battery (FOB) of Nostrandt et al. (1997) and Moser and Padilla (1998) which consisted home cage, handling, open field and manipulative neurobehavioral evaluation. These observations were recorded at frequent intervals of 5 min in the beginning followed by 10-15 min up to 12 h then at 24 h, 36 h, 48 h, 72 h, 5 d and 14 d. The peak time of all the neurobehavioral observations was noted separately in each case and the scores of different neurobehavioral parameters were measured as per the scale mentioned against each. The values for a normal rat are given in parenthesis. [For smacking, tremors, gait, ataxia 0 to 4 scale (0), arousal, tail pinch response 0 to 4 scale (4), handling reactivity, click response 0 to 8 scale (4), all other parameters subjective and noted for presence (+) or absence (-)].

The spontaneous locomotor activity (SLA) in animals of all groups was recorded immediately after FOB testing at peak time, 1h, 6h, 12 h, 18 h, 1 d, 2 d, 3 d, 5 d, 7 d and 14 d post chlorpyrifos administration using photocell activity cage (Actophotometer) by keeping each rat for a period of 5 min.

The acetylcholinesterase (ACHE) activity in brain and plasma of each rat was determined at peak time and on day 3, 7 and 14 after chlorpyrifos exposure in different treatment groups and 0 h in control group following microassay procedure of Correll and Ehrich (1991) with ELISA plates. The activity of enzyme was calculated as difference between initial absorbance and the absorbance after 15 min and was expressed as enzyme activity/min/ml plasma or g tissue. The comparison of activities was made as per cent of control activity at 0 h.

**RESULTS AND DISCUSSION**

The results are presented as mean ± S.E. and statistical analysis was done using Duncan’s Multiple Range Test (Snedecor and Cochran, 1967). The effects on various neurobehavioral changes in all the experimental groups were evaluated according to Functional Observational Battery (FOB) as shown in Table 1.

Smacking, tremors, gait abnormalities and ataxia were the most prominent neurobehavioral changes observed following single dose administration (i/p) of chlorpyrifos @ 85 mg/kg in adult Wistar rats in group 1. The onset of these effects was at 9 h post chlorpyrifos exposure except for smacking which appeared at 3 h. All these effects disappeared at 24 h. The peak time for each effect was 12 h and for smacking it was 9-12 h. The peak score for all these parameters was 2. Slight depression in handling reactivity and arousal, crouched posture and loose defeation in one animal out of six was also observed at peak time. However, no exophthalmia, lacrimation and alteration in corneal and tail pinch response was seen in these animals as also reported by other workers (Nostrandt et al., 1997, Moser and Padilla 1998, Verma et al., 2002). Treatment of *Withania somnifera* root extract following chlorpyrifos exposure in groups 2 and 3, resulted in aggravation of neurobehavioral toxicity as evident by increased severity and prolongation of all these effects (Table 1). Some of the additional end points like loose defeation, exophthalmia, lacrimation, tail pinch response and corneal reflex which were not present in chlorpyrifos control group 1 were also altered in groups 2 and 3. In these the behavioral alterations appeared earlier and persisted for longer duration. The duration of peak time was also increased and was 7-18 h and 12-20 h in groups 2 and 3, respectively in comparison to a peak time of 9-12h in group 1. Handling activity and click response initially showed depression as in chlorpyrifos treated group 1, but was followed by a period of excitement in dose dependent manner in groups 2 and 3.

Chlorpyrifos administration in group 1 rats caused reduction in spontaneous locomotor activity (SLA). The maximum reduction of 79.5% was observed on day 1 (mean counts 97.5 ± 5.65) with complete recovery on day 14 (mean counts 463 ± 3.49). Similar observations have also been reported by Pope *et al.* (1992), Nostrandt *et al.* (1997) and Moser and Padilla (1998). In *Withania somnifera* treated groups 2 and 3, an early and severe reduction of 83.4% and 83.9% (mean count 66 ± 14.05 and 68 ± 13.09, respectively) SLA was recorded which
Table 1
Effect of *Withania somnifera* treatment on chlorpyrifos induced changes in neurobehavioral parameters in rats

<table>
<thead>
<tr>
<th>Neurobehavioral parameters</th>
<th>Group-1 (CPF)</th>
<th>Group-2 (CPF-WSI)</th>
<th>Group-3 (CPF-WSII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smacking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset (h)</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Disappearance (h)</td>
<td>24</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Peak time (h)</td>
<td>9-12</td>
<td>12-18</td>
<td>12-20</td>
</tr>
<tr>
<td>Score</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Tremors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset (h)</td>
<td>9</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Disappearance (h)</td>
<td>24</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Peak time (h)</td>
<td>12</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Score</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gait abnormalities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset (h)</td>
<td>9</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Disappearance (h)</td>
<td>24</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Peak time (h)</td>
<td>12</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Score</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ataxia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset (h)</td>
<td>9</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Disappearance (h)</td>
<td>24</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Peak time (h)</td>
<td>12</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Score</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Handling reactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score (h)</td>
<td>3(12)</td>
<td>3(9-18), 6 (24-36)</td>
<td>2(4-24), 6-8 (36-72)</td>
</tr>
<tr>
<td>Click response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score (h)</td>
<td>4(12)</td>
<td>2-3 (6-24), 5 (36-48)</td>
<td>5 (24-36)</td>
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<tr>
<td>Arousal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score</td>
<td>3</td>
<td>2-3</td>
<td>2-3</td>
</tr>
<tr>
<td>Body posture</td>
<td>Crouched</td>
<td>Crouched</td>
<td>Crouched</td>
</tr>
<tr>
<td>Loose defication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals affected/Total</td>
<td>1/6</td>
<td>4/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Exopthalmia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals affected/Total</td>
<td>0/6</td>
<td>4/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Lacrimation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals affected/Total</td>
<td>0/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>Corneal reflex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals affected/Total</td>
<td>0/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>Tail pinch response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals affected/Total</td>
<td>0/6</td>
<td>5/6</td>
<td>5/6</td>
</tr>
</tbody>
</table>

was in agreement with increased duration of ataxia and gait abnormalities and persistent higher level of brain ACHE inhibition in these groups. Complete recovery from reduced SLA could not be achieved till day 14 in both groups 2 and 3 treated with *Withania somnifera* indicating no beneficial effect of the plant extract on chlorpyrifos-induced alteration in SLA. Reduction in SLA reported in the present study might be due to depression in central nervous system activity and motor incoordination observed in the form of ataxia and altered gait (Sarkar, 1990).

The inhibition of ACHE activity is generally accepted as an indicator of organophosphate insecticides exposure. Although plasma ACHE plays no important physiological role, it may reflect status of brain ACHE. In the present study, significant inhibition of brain and plasma ACHE activity was observed following chlorpyrifos treatment in group 1 at peak time, and maximum inhibition was noted on day 3 (Table 2). Complete recovery of ACHE activity was not achieved even on day 14, however it was faster in plasma than in brain. Chlorpyrifos induced inhibition of cholinesterase activity and delayed recovery even after four weeks has also been reported by other workers (Pope *et al.*, 1992, Liu *et al.*, 1999, Moser and Padilla 1998, Verma *et al.*, 2002).
### Table 2

Effect of *Withania somnifera* treatment on chlorpyrifos induced changes in acetylcholinesterase activity in brain and plasma of rats

<table>
<thead>
<tr>
<th>Groups (Treatment)</th>
<th>Samples</th>
<th>Acetylcholinesterase activity</th>
<th>Time (post CPF exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peak time</td>
<td>3 d</td>
</tr>
<tr>
<td>Group 1 (CPF)</td>
<td>Brain</td>
<td>1.126 ± 0.082 (↓32.45)</td>
<td>0.840 ± 0.098 (↓49.61)</td>
</tr>
<tr>
<td></td>
<td>Blood plasma</td>
<td>1.319 ± 0.124 (↓34.11)</td>
<td>1.047 ± 0.245 (↓47.70)</td>
</tr>
<tr>
<td>Group 2 (CPF-WSI)</td>
<td>Brain</td>
<td>1.085 ± 0.048 (↓36.91)</td>
<td>0.783 ± 0.049 (↓53.02)</td>
</tr>
<tr>
<td></td>
<td>Blood plasma</td>
<td>1.138 ± 0.102 (↓43.15)</td>
<td>1.210 ± 0.130 (↓39.56)</td>
</tr>
<tr>
<td>Group 3 (CPF-WSII)</td>
<td>Brain</td>
<td>1.043 ± 0.037 (↓37.43)</td>
<td>0.651 ± 0.045 (↓60.94)</td>
</tr>
<tr>
<td></td>
<td>Blood plasma</td>
<td>1.063 ± 0.074 (↓46.90)</td>
<td>1.230 ± 0.129 (↓38.56)</td>
</tr>
</tbody>
</table>

Values are mean ± SE of optical density of enzyme activity (n=6). Values bearing different superscripts within row (capital) or within column (small) differ significantly (p<0.05). Values of brain and plasma acetylcholinesterase activity at 0h: 1.667 ± 0.019 and 2.002 ± 0.029 respectively in Group 1, Group 2 and Group 3. Values in parentheses are per cent inhibition from 0h values. Peak time: Group 1, 12 h; Group 2 and Group 3, 18 h.
Withania somnifera treatment @ 25mg/kg and 50 mg/kg for 14 days after chlorpyrifos treatment in Groups 2 and 3 caused increased inhibition of brain and plasma ACHE activity at peak time, which was maximum on day 3 in brain and at peak time in plasma (Table 2). The recovery of enzyme activity was faster in brain than in plasma but was not complete both in brain and plasma even on day 14. In plasma, the enzyme activity remained inhibited more than in chlorpyrifos treated group 1 while in brain it was less.

The present investigation indicated that treatment of Withania somnifera root extract enhanced chlorpyrifos induced neurobehavioral effects and inhibition of brain and plasma ACHE. The enhanced neurotoxicity in Withania somnifera treated groups is in agreement with the observations of Schliebs et al. (1997) who reported that i/p administration of equimolar mixture of sitokinoside VII-X and withaferin-A obtained from Withania somnifera roots @ 400 mg/kg for 7 days in male Wistar rats caused enhancement of M1 and M2 receptor binding sites in different cortical and subcortical regions of brain and reduced ACHE activity in vertical diagonal band of basal nuclei. However, they also reported slight increase in ACHE activity in lateral septum and globus pallidus. Thus Withania somnifera caused aggravation of neurotoxicity indicating that either the antioxidant potential of the plant was not sufficient to overcome the neurotoxic effects of chlorpyrifos or both the effects have their independent mechanisms.

REFERENCES


