Neurochemical And Oxidative Stress Of Rat Offspring After Maternal Exposure To Chlorpyrifos During Gestation

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Abstract: The present work aimed at evaluating the oxidative damage and histopathological alteration in postnatal rats whose mothers were exposed to chlorpyrifos (CPF) during gestation (Ges) and up to delivery. Sprague-Dawely rats used for this experiment were divided into two sets, one was not exposed to CPF while the other exposed to CPF until deliver. The offspring from (set I) was divided into: one served as control and group 2 was exposed to CPF postnatal (PN) for 30 days. The offspring from (set II) were divided into: one allow to grow up to 60 days without any more treatment (Ges) (group 3), and group 4 was re-exposed to CPF for 30 days, (Ges+PN). All animals were sacrificed, the brain was dissected into cortex, midbrain, cerebellum and brainstem. Chlorpyrifos treatment resulted in a significant inhibition (P<0.05) of AchE levels in cortex of Ges+PN group, midbrain of PN & Ges+PN groups and in cerebellum all treated groups when compared to control. NO levels showed a significant increase (P<0.05) in PN group of all different brain regions, Ges group in cortex and brainstem regions, Ges+PN group in the brainstem, when compared to the control. GSH reduced showed a significant decrease (P<0.05) in all brain regions when compared with control. LP levels showed a significant increase (P<0.05) in midbrain groups, cerebellum PN group and brainstem, Ges+PN group, when compared to the control. SOD showed significant decrease (P<0.05) when compared to control. GSHPx showed significant increase (P<0.05) in cortex (Ges+PN) group when compared with control.

On conclusion, chlorpyrifos resulted in oxidative stress and histopathological alterations in pups exposed to it during and after gestation. This data of the study may be consider as a contribution to the problem of pregnant mothers and newly born exposed to CPF.

INTRODUCTION

Organophosphorous (OP) pesticides are regularly used in the control of insects around the home and in agricultural practice. The widespread and the extensive use of pesticides in developmental countries affect the general population specially pregnant women and children and lead to a variety of neurotoxic damage. Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridinyl phosphoro-thioate) is a broad spectrum, chlorinated OP insecticide, which is one of the largest selling in the world and has both agricultural and urban uses (Mansour & Mossa, 2010). CPF exposure can occur via inhalation or through dermal or oral routes from residues on floors and carpets, children toys, food and dust (Fenske et al., 2002). It is known as a developmental neurotoxicant. During gestational or neonatal times, exposure to CPF is associated with neurobehavioral changes (Ricceri et al., 2006) and deficit in locomotor activity, cognitive performance and coordination skills (Levin et al., 2001). In additional, CPF alters synaptic neurotransmission, inhibits neural cell replication and neurite outgrowth, evokes oxidative stress, interfere with signaling cascades and transcriptional events involved in neural cell differentiation (Qiao et al., 2001). Chronic CPF exposure associated with decreased birth weight and birth length and alteration in developmental and psychomotor indices in Mexican-American children (Rauh et al., 2006 and Eskenazi et al., 2007).

CPF is metabolized by P-450 enzymes to either CPF-oxon (which inhibits cholinesterase, ChE) or to 3,5,6-trichloro-2-pyridinol (TCP). Its active metabolite CPF-oxon caused cholinergic hyperstimulation. In contrast, CPF itself damages the developing brain through additional, noncholinergic mechanisms, including interference with neural cell replication and differentiation, and perturbations of the patterns of axonogenesis and synaptogenesis, ultimately producing deficits in synaptic function (Rice & Barone 2000). It was found that, many pesticides including CPF can be accumulated and excreted in human milk, raising the risks to breastfeeding infants toxicity (Marty et al., 2007). Also, it has been reported that milk CPF concentrations were up to 200 times those in blood of pregnant rats dosed with CPF (Mattsson et al., 2000).

The toxicity of many xenpiotics is associated with the production of oxygen-free radicals (ROS) which are not only toxic themselves but are also implicated in the pathophysiology of many disease (Abdollahi et al., 2004). The harmful effects of ROS are balanced by the antioxidant action of nonenzymatic antioxidants and...
antioxidant enzymes (Halliwell, 1996). CPF was found to generates reactive oxygen radicals acutely in neural cells in vitro (Garcia et al., 2001) producing lipid peroxidation during specific phases of replication and differentiation (Qiao et al., 2005). Oxidative stress is a major mechanism for cellular damage associated with a wide variety of neurotoxicants (Gitto et al., 2002 and Gupta, 2004). It has been postulated to represent a noncholinergic mechanism by which CPF or other organophosphates can damage the developing brain (Gupta, 2004). Moreover, the oxidative stress from generation of reactive oxygen species (ROS) is a potential mechanism, through which the reactive oxygen interacts with receptors, second messengers and transcription factors lead to alter gene expression and influencing the cell growth and survival (Palmer & Paulson, 1997). ROS may provide a common pathway for different neurotoxicants that elicit apoptosis of brain cells (Sarfian, 1994) an outcome also present with gestational CPF exposure (Roy et al., 1998).

The brain is the most vulnerable organs because of its high oxygen consumption and because its cell membrane lipids are high in oxidizable polyunsaturated fatty acids (Gupta, 2004). The developing brain may be especially sensitive not only because of the increased metabolic demand associated with growth but also because it has lower reserves of protective enzymes and antioxidants such as glutathione (Gupta, 2004 and James et al., 2005) and relative to the adult brain, is deficient in glia which ordinary protect neurons from oxidative molecules (Tanaka et al., 1999). Evakuation of the had evaluate the relative susceptibility of the immature brain as compared to other fetal or neonatal tissues was done by Jett & Navoa (2000) and they found that the oxidative stress in the developing brain has been demonstrated only at doses well above the threshold for systemic toxicity.

The objective of this study was to examine the relative neurochemical changes and the oxidative stress in developing neonate following lower-level repeated exposure of pregnant rat to CPF during gestation (GD 2-20) and post-gestation up to 60 days.

MATERIALS AND METHODS

Materials:
Chlorpyrifos (~ 99%; O.O-diethyl-O-3,5,6-trichloro-2-pyridinyl phosphorothioate) was obtained from Dow Elanco (Indianapolis, IN, USA). Butyrylthiocholine iodide, acetylthiocholine iodide, thiobarbeturic acid (TBA), N-(1-naphthyl) ethylenediamine HCl, 5,5’-Dithiobis (2-nitro-benzoic acid, DTNB), 1,1,3,3 tetra-ethoxypropane standard, glutathione standard, pyrogallol, NADPH, diethylene triamine penta acetic acid (DTPA), glutathion peroxidase, sodium azid, hydrogen peroxide were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other reagents were of highest purity available commercially.

Animals:
Healthy male and female of Sprague-Dawely rats (200-250 g) were obtained from the Animal Breeding House of National Organization for Drug Control and Research (NODCAR), Egypt. Rats were allowed to acclimate to laboratory conditions for 1 week before breeding. Twenty virgin female were distributed into 10 cages One male was placed in each cage for overnight and the presence of spermatozoa was checked in the vaginal smear the following morning. This day was considered as gestation day 0 (GD 0). The pregnant females were individually housed in clean cages in the laboratory animal room (23°C±2°C) with free accesses to food and tap water ad-libitum. and a 12-h dark/light cycle. The experimental work was done in the Department of Zoology, faculty of Science, Cairo University. All treatment and procedures were approved of the Animal Care and Experimental Committee, Faculty of Science, Cairo University and according to the guidance for care and use of Laboratory animals (NRC, 1996).

Experimental Design:
Control Treatments: Set 1, each pregnant rat was received a daily dermal 0.1 ml vehicle allowed to deliver. Ten of the offspring (control animals) (group 1), and the other ten offspring were treated with CPF (group 2).
CPF Treatment: Set 2, each pregnant rat was received a daily dermal 10mg/kg in 70% ethanol up to day of delivery. Ten of the Offspring (group 3) allow to grow up to 60th day without any more treatment, while other ten Offspring (group 4) exposed to CPF from day 30th up to day 60th .

Group 1:
Offspring from the 1st group received only saline.

Group 2:
Offspring from the 1st group and receive repetitive doses (30 doses) of CPF dermal (10 mg/kg/day) started after 30 days from gestation.

Group 3:
Offspring from the 2nd group and receive no treatment up to 60 days.
**Group 4:**
Offspring from the 2nd group and re-treated with repetitive doses (30 doses) of CPF dermal (10 mg/kg/day) after 30 days from gestation.

At day 61th, the animals were sacrificed, brain was removed, washed thoroughly with ice-cold saline (0.9% sodium chloride). Brains were dissected into cortex, midbrain, cerebellum and brainstem on ice and rapidly stored at -80°C for AChE, ROS and antioxidant detection. Piece of the cortex of each animal was kept in formalin 10% for histopathological studies. Thus, n=5 for biochemical and pathological evaluations each, represents offspring from five different mothers.

**Biochemical Assays:**
1. Determination of AChE: was determined according to the method of Ellman et al. (1961). The absorbance was recorded at 412 nm using Microplate Reader. Protein concentration was determined according to Smith et al. (1985) method. The enzyme activities are expressed as µmoles/min/mg protein.
2. Nitric Oxide (NO): was determined according to the photometric method of Green et al. (1982) as described by Canals et al. (2001) by measuring nitrite. The absorbance recorded at 562 nm. The concentration are expressed as µM/mg protein, (UV-Visible Spectrophotometer, Shimadzu, UV-1601)
3. Total Glutathione (GSH): was determined according to the modified method of Beutler et al. (1963). The absorbance was recorded at 412 nm. The data was expressed as µM/g wet tissue/mg protein.
4. Malondialdehyde (MDA) (Lipid Peroxidation): was carried out according to the modified method of Draper & Hadley (1990). The absorbance of the samples and standards were determined at 535 nm against blank. The MDA value was expressed as µM/g wet tissue/mg protein.
5. Superoxide dismutase (SOD): was determined according to the pyrogallol method of Woolliams et al. (1983). The absorbance was recorded at 420 nm and the change in optical density was recorded for 3 mins.
6. Glutathione peroxidase (GSHPx): was performed according to the method of Woolliams et al. (1983). The absorbance at 340 nm was recorded for 5 min.
7. Histopathological analysis: The offspring brain tissues fixed in formalin 10% for 18-24 h, were then blocked and embedded in paraffin according to the standard histological techniques. Six micrometer-thick coronal sections were cut through the cortex, midbrain, cerebellum and brainstem (n=5). Sections were stained with hematoxylin and Eosine for light microscopic examination (Banchroft et al., 1996).
8. Statistical analysis: The results were expressed as mean ± SEM. Data were statistically analyzed using Neman-Kelus test to evaluate the comparisons between means at P<0.05( Steel and Torrie,1980)

**RESULTS AND DISCUSSION**

**Effect Of CPF On Biochemical Parameters:**

**Table(I) and Fig. (1)** showing a significant inhibition (P<0.05) of AChE levels in the cortex region Ges+PN group only by 27%; P<0.05, midbrain region in the groups PN and Ges+PN by 21% and 14%, respectively, (P<0.05), while, in cerebellum region there is a significant inhibition in all treated groups (PN, Ges & Ges+PN) by 20%, 14% & 19%, respectively. However, in brainstem region there was no significant change at least more or less the control. As a result of CPF administration, the nitric oxide (NO) levels showing a significant increase (P<0.05) in the different brain regions (cortex, midbrain, cerebellum and brainstem) of PN group by (47%, 33%, 50% and 49%) respectively, while the Ges group showing significant increase (P<0.05) of NO level in the cortex and brainstem regions by 39% and 29%, respectively, on the other hand, the group of Ges+PN showing significant increase (P<0.05) of NO level in the cerebellum and brainstem by 33% and 30%, respectively, (P<0.05) when compared to the control group (Fig. 2). The GSH content showing a significant decrease (P<0.05) in all brain regions in all the groups of treated animals PN, Ges & Ges+PN when compared with control by (cortex, 22%, 15% & 21%, midbrain, 37%, 41% & 45%, cerebellum, 27%, 29% & 28%, and brainstem, 30%, 26% & 24%, respectively), (Fig. 3). The LP as a result of CPF showing significant increase (P<0.05) in midbrain region in all groups (PN, Ges. & Ges+PN) by 25.5%, 26% and 29%, respectively, while in cerebelum only the PN group showing a significant increase by 42%, (P<0.05) while in brainstem, the group of Ges+PN showing a significant increase (P<0.05) by 27%, (P<0.05) when compared to the control group (Table 1 and Fig. 4). Table (2) and Fig. (5) showed that, administration of CPF significantly decrease (P<0.05) the level of SOD in cortex region in PN, Ges and Ges+PN groups by 24%, 21% and 27% respectively, when compared to the control group. While the GSHPx was increased by 33%, 22% and 78% respectively in cortex region when compared with control group (Table, 2 and Fig. 5).
Fig. 1: Effect of dermal administration of chlorpyrifos (10 mg/kg/day) on the level of acetylcholine esterase (AChE) in different brain region of offspring rats.

Fig. 2: Effect of dermal administration of chlorpyrifos (10 mg/kg/day) on the level of Nitric oxide (NO) in different brain region of offspring rats.

Histopathological Effect of CPF:

The brain tissue showed no histopathological alteration observed and the normal histological structure of the meninges, cerebrum, hippocampus and cerebellum were recorded in (Fig 6 a,b,c). Administration of CPF for 30 days (group 2) showed focal encephalomalacia in the midbrain, associated with neuronal degeneration and gliosis in the cerebrum. The hippocampus showed congestion in the blood capillaries with perivascular oedema (Fig. 7.a,b,c). Group 3 showed congestion in the blood vessels and oedema in association with focal encephalomalacia in the underlying cerebral cortex. Congestion in the blood capillaries with diffuse as well as focal gliosis were detected in the cerebrum (Fig.8 a,b,c,d). There was encephalomalacia in the med brain, while the medulla oblongata showed lose of the Nissels granules with degeneration in the neuronal cells, and congestion in the blood capillaries (Fig. 9.a,b,c,d).
Fig. 3: Effect of dermal administration of chloropyrifos (10 mg/kg/day) on the Glutathione content (GSH) in different brain region of offspring rats.

Table 1: Influence of chlorpyrifos (10 mg/kg/day, dermal) on acetylcholinesterase (ChE) and reactive oxygen species (ROS) in different regions of offspring brain rats.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Groups</th>
<th>AChE</th>
<th>NO</th>
<th>GSH</th>
<th>LP</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>2.63±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.61±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.84±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Cortex</td>
<td>PN</td>
<td>2.22±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.69±0.07&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>6.68±0.21&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>3.33±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Gestation</td>
<td>2.31±0.11&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>1.60±0.06&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>7.35±0.16&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>2.81±0.36&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Gestation+</td>
<td>1.91±0.09&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.19±0.04&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>6.81±0.19&lt;sup&gt;c,b&lt;/sup&gt;</td>
<td>3.36±0.46&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Mid-brain</td>
<td>Control</td>
<td>1.84±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.15±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.78±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>PN</td>
<td>1.46±0.03&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.86±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.71±0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.49±0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Gestation</td>
<td>1.83±0.03&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>1.58±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.13±0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.51±0.48&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Gestation+</td>
<td>1.58±0.09&lt;sup&gt;c,b&lt;/sup&gt;</td>
<td>1.38±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.74±0.52&lt;sup&gt;c,b&lt;/sup&gt;</td>
<td>3.59±0.46&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Cerebelum</td>
<td>Control</td>
<td>0.85±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.32±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.66±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.30±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>PN</td>
<td>0.68±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.60±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.55±0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.38±0.39&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Gestation+</td>
<td>0.69±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.71±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.63±0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.46±0.42&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Brain-Stem</td>
<td>Control</td>
<td>1.83±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.13±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.95±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>PN</td>
<td>1.69±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.58±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.25±0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.49±0.44&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Gestation</td>
<td>1.72±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.37±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.71±0.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.46±0.48&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>Gestation+</td>
<td>1.82±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.41±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.63±0.65&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.75±0.42&lt;sup&gt;d&lt;/sup&gt;</td>
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Means with the same letter at the same column are significantly different at P<0.05

Table 2: Influence of chlorpyrifos (10 mg/kg/day, dermal) on superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) in cortex brain region of offspring rats.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Groups</th>
<th>SOD</th>
<th>GSHPx</th>
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</thead>
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<td></td>
<td>Control</td>
<td>10.07±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18±0.019&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Cortex</td>
<td>PN</td>
<td>7.62±0.25&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.24±0.02&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<td></td>
<td>Gestation</td>
<td>7.92±0.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.22±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Gestation+</td>
<td>7.32±0.15&lt;sup&gt;c,b&lt;/sup&gt;</td>
<td>0.32±0.03&lt;sup&gt;c,b&lt;/sup&gt;</td>
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</table>

Means with the same letter at the same column are significantly different at P<0.05
Fig. 4: Effect of dermal administration of chlorpyrifos (10 mg/kg/day) on the lipid peroxidase (LP) in different brain region of offspring rats.

Fig. 5: Effect of dermal administration of Chlorpyrifos (10 mg/kg/day) on the superoxide dismutase (SOD) and glutathione peroxidase (GSHPX)

Fig. 6: Control group showing normal histological structure of the menings (m), cerebral cortex (c), hippocampus (hp) and cerebellum (CT), H&E stain.

Discussion:
The organophosphorus chlorpyrifos is a non-persistent insecticide widely employed in domestic, agricultural and non-agricultural (i.e. schools, golf courses, parks) settings. The exposure of the pregnant woman to pesticides is of major concern since a majority of the pesticides are neurotoxicants and the fetus is at greater risk, compared to the adult, to the toxic effects of these chemicals due to the rapid state of growth of its brain at this stage of development (Barone et al., 2000). Most of the maternal exposures to environmental pesticides are
Fig. 7: Brain of rat group 2 showing focal encephalomalacia of the med brain (arrow), neuronal degeneration (arrow) with gliosis (g), congestion with perivascular oedema (arrow) in the hippocampus, H&E stain.

Fig. 8: Brain of rat group 3 showing congestion in blood vessels of menings (m) accompanied with odema and encephalomalacia in the cerebral cortex, congestion in blood capillaries (V) with diffuse and focal gliosis (arrow) in cerebrum, H&E stain.

Fig. 9: Brain of group 4 showing encephalomalacia in the med brain (arrow), congestion in blood capillaries, neuronal degeneration with lose of Nissel’s granules in neuronal cells cytoplasm (arrow) of medulla oblongata (V), H&E stain.

probably subtle and result in little or no recognizable effects in the pregnant woman. Yet, serious concerns have been raised about their adverse effects on the fetus and of their potential role in subsequent developmental, learning and behavioral difficulties in children (Grandjean et al., 2006).

Dermal exposure to organophosphate pesticide is important because of its popular use. Mitra et al. (2008) observed inhibition of serum AChE by 97% on dermal application of ½ LD50 of CPF for 3 weeks in adult mice. Latuszyńska et al. (2003) found similar (79%) suppression of serum AChE levels in 3-month-old rats following dermal application of CPF. These findings agree with those obtained in this study in which neurotoxic, hepatotoxic and oxidative stress were detected in mice off springs exposed perinatally to dermal application of CPF.

Chlorpyrifos (CPF) toxicity, related to inhibition of brain and systemic acetylcholinesterase (AChE), is well documented after acute poisoning of adults. Most of available animal studies indicates that CPF exposure below the threshold for systemic toxicity exerts disruptive effects on CNS development and behavior (Aldridge et al., 2004). Besides, many studies dealt with dermal absorption of chlorpyrifos CPF (Mage, 2006) and the consequent effects of its prenatal exposure (Venerosi et al., 2009). According to Mitra et al. (2010) perinatal dermal application of chlorpyrifos has resulted in reduction in serum AChE, hippocampus and iso-cortex. Our study showed significant decrease of neonatal brain AChE in all regions of the brain of those off springs whose mothers being exposed to CPF dermal toxicity during gestation period. Qiao et al. (2004) concluded that the developing brain, and especially the hippocampus, is adversely affected by CPF regardless of whether exposure occurs early or late in brain development. Developmental neurotoxicity of chlorpyrifos (CPF) involves multiple mechanisms, thus rendering the immature brain susceptible to adverse effects over a wide window of
Chlorpyrifos exhibits greater toxicity during development than in adulthood. Meyer et al. (2004) noticed that developmental exposure to CPF elicits long-lasting alterations in cell-signaling cascades that are shared by multiple neurotransmitter and hormonal inputs. Chlorpyrifos-induced developmental neurotoxicity elicits alterations in adenylyl cyclase signaling in rat brain regions after gestational or neonatal exposure. Our results demonstrate a significantly low level of neonatal brain AChE in all regions of the brain of those offspring that got dermal repeated gestational CPF exposure in maternal and developing rats. Campbell et al. (1997) supported these findings, they administered chlorpyrifos to neonatal rats in doses spanning the threshold for systemic toxicity and examined developing brain regions (brainstem, forebrain, cerebellum) for signs of interference with cell development. Although regions rich in cholinergic projections, such as brainstem and forebrain, may be more affected than noncholinergic regions (cerebellum), the maturational timetable of each region (brainstem earliest, forebrain intermediate, cerebellum last) appears to be more important in setting the window of vulnerability. Chlorpyrifos produces cellular deficits in the developing brain that could contribute to behavioral abnormalities. In addition, adverse effects of CPF were reported on cerebral cortex, iso-cortex (Veronesi et al., 1990), hippocampus (Mitra et al., 2009), hypothalamus (Tait et al., 2009) and ponsmedulla (Morris et al., 1983). The reduction in serum and brain AChE was explained by Slotkin et al. (2004) who illustrated that the main metabolite, CPF oxon, prevents acetylcholine degradation, thus acting as cholinesterase inhibitor. According to Slotkin and Seidler (2005), these alterations in CNS serotonergic mechanisms caused by neonatal chlorpyrifos exposure are permanent.

Chanda & Pope (1996) believe that repeated gestational exposure to chlorpyrifos in maternal and developing rats affects neurochemical and neurobehavioral parameters. On the other hand, Venerosi et al. (2009) mentioned that it is possible that only when CPF exposure covers a large portion of gestation it induces statistically detectable neurodevelopmental delays. The present study showed that repeated exposure to CPF during gestation and postnatally for sixty days has caused statistically significant neurochemical and neurodevelopmental delays.

Increasing evidence from animal and human studies indicates that chlorpyrifos (CPF), similar to other organophosphorus insecticides still widely used, is a developmental toxicant (Braquenier et al., 2010). CPF has been shown to elicit alterations in neurotrophic signaling governing cell differentiation and apoptosis, and to evoke oxidative stress. Differentiation enhanced the ability of CPF to elicit the formation of reactive oxygen species (Qiao et al., 2001). Slotkin et al. (2005) examined the ability of CPF to evoke lipid peroxidation in the developing brain of fetal and neonatal rats. CPF elicited oxidative damage in the developing brain with greater sensitivity in the second postnatal week. Lipid peroxidation was significantly increased, while glutathione s-transferase (GST) and superoxide dismutase (SOD) were significantly decreased compared to control (Mansour and Mossa, 2010). The data of our study agreed with these findings, we detected a significant increase of nitric oxide (NO) and lipid peroxidase (LP) associated with significant decrease of glutathione hydrogenase (GSH) and superoxide dismutase (SOD) in the different brain regions of CPF exposed animal groups. These adverse effects were explained by Slotkin et al. (2005) who mentioned that these results indicate that diverse compounds can exert convergent effects on brain development through their shared potential to elicit oxidative stress. CPF stimulates neuronal cell metabolism through direct actions on beta-adrenoreceptors.

These observed histopathological changes agree with Qiao et al. (2003) who demonstrated that fetal chlorpyrifos exposure led to adverse effects on brain cells. Roy et al. (1998) showed that CPF inhibit replication of brain cells.

Our results confirm that CPF exposure during the perinatal period can induce long-term alterations in rat neonates and suggest that the dermal administration and the duration of CPF exposure during brain development may be factors to consider when studying the development of different brain regions and antioxidants in the brain.

REFERENCES


