Fenthion, an organophosphorus pesticide, induces alterations in oxidant/antioxidant status and histopathological disorders in cerebrum and cerebellum of suckling rats

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Fenthion (FEN) is an organophosphorus pesticide known for its wide toxic manifestations. In this study, the effects of FEN were evaluated on the cerebrum and cerebellum oxidant/antioxidant status and histopathological disorders in the suckling rats. Pregnant rats were divided into two groups: control group received pure water, while FEN group received daily by their drinking water 551 ppm of FEN from the 14th day of pregnancy until day 14 after delivery. Acetylcholine esterase (AChE) activity was inhibited in both the cerebrum and cerebellum of suckling rats whose mothers were treated with FEN. The cerebrum and cerebellum oxidative damage was demonstrated by a significant increase of malondialdehyde (MDA), advanced oxidation protein product and glutathione (GSH) levels and disturbance in the antioxidant enzyme activities. A significant decline of non-protein thiol and vitamin C levels was also observed. These changes were confirmed by histopathological observations which were marked by pyknotic neurons in the cerebrum and apoptotic cells in the cerebellum of FEN-treated rats. In the cerebellum of FEN-treated rats, the most conspicuous damage was the absence of external granular layer, indicating growth retardation. These data suggested that exposure of pregnant and lactating rats to FEN induced oxidative stress and histopathological disorders in the cerebrum and cerebellum of their pups. Thus, the use of FEN must be under strict control, especially for pregnant and lactating mothers.

Keywords: Fenthion, Neurotoxicity, Suckling rats, Cerebrum, Cerebellum, Pycknotic neurons, Apoptotic cells, Oxidative stress.

Fenthion (FEN) [O,O-dimethylO-[3-methyl-4-(methyl-thio)phenyl]phosphorothioate] is a systemic organophosphorus (OP) pesticide, which is widely used in agriculture against a broad range of insects and mites as well as for indoor control of houseflies1. Due to its extensive application to crops and cattle, presence of FEN residue in food and the environment has been widely reported2. It has been classified by the U.S. Environmental Protection Agency as a restricted use pesticide because of its toxic effects in birds, reptiles and fish. Toxic effects of FEN includes structural changes in the testes of gobiid fish, reduction in larval production in sand fiddler crabs, chronic and acute toxicity in hens, birds, reptiles and fish3-6. In addition, FEN exerts toxic effects on many tissues and organs of experimental animals, including pancreas7,8, liver9-11, kidney10,11 and brain12-14.

Previous studies have demonstrated that FEN can induce toxicity via the generation of reactive oxygen species (ROS) and hence results an increased expression of oxygen free radical scavenging enzymes in the liver of rats15. In in vitro and in vivo studies, FEN is also reported to provoke induction of hepatic and brain lipid peroxidation (LPO), production of chemiluminescence, increase of DNA single-strand breaks and lactate dehydrogenase (LDH) leakage, suggesting that ROS and/or free radicals may be involved in the toxic manifestations of this insecticide16. This study has suggested LPO as the one of the molecular mechanisms involved in FEN-induced oxidative damage. In fact, excessive ROS generation leads to damage of cellular components like lipids, proteins and nucleic acid.
The relationship between the toxicity and metabolism of FEN has been widely examined in human neuroblastoma cell lines, birds and fish\(^{17-19}\). FEN exerts its principal biological effects by phosphorylation of the enzyme acetylcholinesterase (AChE), resulting in subsequent accumulation of acetylcholine and continuous stimulation of the nervous system\(^{20}\). In fact, brain exhibits distinct variations of antioxidant defenses\(^{21}\). Thus, neural cells and/or brain regions respond differentially to changes in metabolic rates associated with the generation of ROS\(^{22}\). Among brain regions, the cerebrum and cerebellum of suckling rats are more susceptible to oxidative damage after xenobiotics treatment\(^{23,24}\).

To our knowledge, only one study has been carried out on the hippocampus of adult rats exposed to FEN\(^{25}\), and reports are lacking on brain of suckling rats. Thus, in this study, we have investigated potential effects of FEN on the cerebrum and cerebellum of suckling rats, whose mothers have been exposed to FEN. Brain oxidative impairment in terms of LPO, protein oxidation, response of antioxidants and their histopathological aspects have been examined.

Materials and Methods

Chemicals

A commercial formulation of fenthion (FEN), named Lebaycid 50 EC was purchased from Crop Science (Ariana-Charguia, 2035, Tunis-Carthage). All the remaining chemicals were of high commercially available grade.

Animals

The experimental procedures were carried out according to the general guidelines on the use of living animals in scientific investigations\(^{25}\) and approved by the Ethical Committee of Sfax Faculty of Sciences.

Male and female rats of Wistar strain, weighing about 180 g were purchased from the Central Pharmacy (SIPHAT, Tunisia). They were kept in polypropylene cages in normal housing conditions at ambient temperature 22 ± 3°C with a 12-h light/dark cycle and a minimum relative humidity of 40%. Food (standard diet, supplied by SICO, Sfax, Tunisia) and water were available \textit{ad libitum}. The amount of diet ingested was calculated as the difference between the weight of feed that remained in the food bin (Da) and the amount placed one day before (Db). These data were then used to calculate a daily average feed intake according to the formula:

\[
\text{Average feed intake} = (Db - Da)
\]

The same procedure was used for the determination of daily drinking water.

After one-week of acclimatization in the laboratory conditions, pairs of male and virgin female rats were kept overnight in each cage. Pregnant female rats were inspected daily by the presence of the vaginal plug, which indicated day zero of pregnancy.

Experimental setup

Twelve pregnant rats were randomly divided into two groups of six each: Group 1 or controls and Group 2 (FEN) which received 551 ppm of FEN (equivalent to 61 mg/kg bw) through drinking water from the 14\textsuperscript{th} day of pregnancy until day 14 after delivery.

In our experiments, different doses of FEN were tested. No toxic effects and no oxidative stress were observed in pups, whose mothers were treated with FEN at doses between 400 and 550 ppm. From 551 ppm, oxidative stress was observed in mothers and their suckling pups without lethal effects. This dose represented \(\frac{1}{4}\) of LD\(_{50}\). A FEN dose over 551 ppm provoked abortion or reduced the number of pups.

The rats were allowed to deliver spontaneously 3 weeks after coitus. At delivery, the number of pups born and their sex were recorded. Each litter was culled to eight pups (four males and four females, if possible) as this procedure is shown to maximize the lactation performance\(^{26}\). At the end of experimental period, 96 pups (controls and treated rats) and 12 dams were anesthetized with chloral hydrate by intra-abdominally. After sacrifice, cerebrum and cerebellum of control and FEN-treated pups were collected, cleaned and weighed. Some samples were rinsed, homogenized (10%, w/v) in phosphate buffer (pH 7.4) in an Ultra Turrax homogeniser in ice-cold, centrifuged at 10,000 \(\times\) \(g\) and the resulting supernatants were used for biochemical assays. Other samples were immediately fixed in 10% formalin solution for histological studies.

Biochemical parameters

Determination of AChE activity

AChE activity was measured immediately in tissue homogenates according to the method of Ellman \textit{et al}\(^{27}\), using acetylthiocholine iodide as a substrate. The reaction mixture contained: phosphate buffer (0.1 M; pH 8) and 0.01 M 5,5-dithiobis-2
nitrobenzoic acid (DTNB). The hydrolysis rate of acetylthiocholine iodide was measured at 412 nm through the release of thiol compound, which when reacted with DTNB produces the colour-forming compound nitrobenzoic acid (TNB). The reaction was initiated by adding 0.075 M acetylthiocholine iodide.

Estimation of LPO levels
LPO was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) in tissues by the method described previously.

Protein estimation
Protein content in cerebral and cerebellar homogenates was measured by the method of Lowry et al. using bovine serum albumin as standard.

Estimation of advanced oxidation protein product (AOPP)
AOPP levels were determined according to the method of Kayali et al. The absorbance was recorded at 340 nm. The concentration of AOPP for each sample was calculated using the extinction coefficient of 261 cm$^{-1}$ mM$^{-1}$.

Estimation of glutathione (GSH)
GSH in the cerebrum and cerebellum was determined by the method of Ellman modified by Jollow et al. The method is based on the development of a yellow colour when DTNB is added to compounds containing sulphydryl groups. The absorbance was measured at 412 nm.

Estimation of non-protein thiols (NPSH)
Cerebral and cerebellar NPSH levels were determined by the method of Ellman. Absorbance of colorimetric reaction was measured at 412 nm.

Estimation of ascorbic acid (Vitamin C)
Ascorbic acid determination was performed as described previously. The reaction product was determined using colour reagent containing 4.5 mg/ml dinitrophenylhydrazine and CuSO$_4$ (0.075 mg/ml).

Antioxidant enzyme activities
Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich. The developed blue colour in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%.

Glutathione peroxidase (GPx) activity was measured by the procedure of Flohe and Gunzler. The supernatant was assayed for GSH content using DTNB reagent (10 mM).

Catalase (CAT) activity was assayed by the method of Aebi. Changes in absorbance were recorded at 240 nm.

Histological studies
Some cerebra and cerebella of 14-day-old rats were used for histological studies. They were fixed in 10% buffered formalin solution and embedded in paraffin. Blocks were sliced at 5 µm, stained with hematoxylin-eosin or toluidine blue and examined under light microscopy. Six slides were prepared from six cerebra or cerebella for each group. Each slide was examined and assigned for severity of changes using scores on a scale of none (−), mild (+), moderate (++), and severe (+++) damage.

Statistical analysis
The data were analyzed using the statistical package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Statistical analysis was performed using one-way ANOVA test. Differences were considered significant at p value less than 0.05.

Results
Clinical signs of toxicity
There were no signs of mortality or abortion during the experimental period (21 days). The animals were observed for behavioral performance and symptoms of possible intoxication and were also placed in an open field for observation of tremors and gait abnormalities. We evaluated the presence of weakness, agitation, as well as drowsiness and lethargy in suckling rats treated with FEN.

Evaluation of body, cerebrum and cerebellum weights
Body weight (22.76 ± 2.14 g), cerebrum (768.80 ± 59.08 mg) and cerebellum (111.59 ± 13.88 mg) absolute weights of 14-day-old rats were decreased by 32, 10 and 14% (p ≤ 0.001), respectively, while their relative weights (34.00 ± 3.54 mg/g of bw), (4.93 ± 0.70 mg/g of bw) were increased, respectively by 32 and 27% (p ≤ 0.001). Furthermore, the reduced absolute weights of these organs were associated with a decrease in protein content (15% and 37%, respectively, p ≤ 0.001), when compared to controls (Fig. 1).

Estimation of food intake and water consumption
Daily food intake by FEN-treated rats was (17.41 ± 1.09 g/day/dam), similar to that of controls rats (18.82 ± 1.58 g/day/dam), while a marked
increase (34%) in the daily water intake was observed (p ≤ 0.001), when compared to controls (24.10 ± 0.46 ml/day/dam). The quantity of FEN ingested daily by dam was 18.21 ± 1.63 mg.

**Effect of FEN on AChE activity**

FEN treatment of pregnant and lactating rats caused a significant inhibition of AChE activity in cerebrum and cerebellum of pups (61 and 83%, respectively, p ≤ 0.001) (Fig. 2).

**Oxidative stress parameters**

Our results showed that malondialdehyde (MDA), a LPO marker increased by 58% in the cerebrum (p ≤ 0.001) and by 97% in the cerebellum (p ≤ 0.001) in FEN-treated rats, compared to those of controls. In addition, AOPP, an index of protein oxidative damage, increased by 74% in the cerebrum (p ≤ 0.001) and by 127% in the cerebellum (p ≤ 0.001), when compared to controls (Table 1).

**Table 1—Evaluation of oxidative stress biomarkers (MDA, AOPP, GSH, NPSH and vitamin C) in the cerebrum and cerebellum of suckling rat controls and treated with FEN**

<table>
<thead>
<tr>
<th>Parameters &amp; treatment</th>
<th>MDA (nmol/g tissue)</th>
<th>AOPP (nmol/mg protein)</th>
<th>GSH (µg/g tissue)</th>
<th>NPSH (µmol/g tissue)</th>
<th>Vitamin C (µmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cerebrum</strong></td>
<td></td>
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</tr>
<tr>
<td>Controls (n = 10)</td>
<td>96.76 ± 12.05</td>
<td>0.17 ± 0.03</td>
<td>81.05 ± 1.47</td>
<td>1.41 ± 0.07</td>
<td>130.68 ± 8.9</td>
</tr>
<tr>
<td>FEN (n = 10)</td>
<td>153.01 ± 17.83***</td>
<td>0.3 ± 0.03***</td>
<td>89.19 ± 1.48***</td>
<td>0.9 ± 0.1***</td>
<td>93.97 ± 9.96***</td>
</tr>
<tr>
<td><strong>Cerebellum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 10)</td>
<td>86.77 ± 6.21</td>
<td>0.16 ± 0.02</td>
<td>81.08 ± 2.10</td>
<td>1.69 ± 0.07</td>
<td>163.42 ± 5.08</td>
</tr>
<tr>
<td>FEN (n = 10)</td>
<td>171.18 ± 17.56***</td>
<td>0.38 ± 0.04***</td>
<td>87.99 ± 1.16***</td>
<td>1.13 ± 0.04***</td>
<td>114.24 ± 12.06***</td>
</tr>
</tbody>
</table>

The number of determinations is indicated between parentheses.

FEN treated vs controls: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

GSH levels were significantly increased in both the cerebrum (10%) and cerebellum (8%) of FEN-treated rats, as compared to those of controls (Table 1).

After FEN treatment, NPSH and vitamin C, the non-enzymatic antioxidants were significantly decreased in the cerebrum by 35 and 28% (p ≤ 0.001) and the cerebellum by 32 and 30% (p ≤ 0.001), compared to those of controls (Table 1).

In FEN-treated rats, SOD and GPx activities decreased by 12 and 22% (p ≤ 0.01; p ≤ 0.001) in the cerebrum and by 18 and 64% (p ≤ 0.01; p ≤ 0.001) in the cerebellum, respectively, while CAT activity increased by 41% in the cerebrum (p ≤ 0.001) and decreased by 56% in the cerebellum (p ≤ 0.001), when compared to those of controls (Table 2).

**Histopathological studies**

In control rats, the cerebral and cerebellar tissues presented normal histoarchitecture, upon histological examination (Fig. 3A1, A2; Fig. 4A; Fig. 5A and Fig. 6A). Exposure to FEN provoked degenerative changes in these organs. The cerebrum sections in FEN-treated rats, stained with hematoxylin and eosin.
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Table 2—Antioxidant enzyme activities (SOD, CAT and GPx) in cerebrum and cerebellum of suckling rat controls and treated with FEN

<table>
<thead>
<tr>
<th>Parameters &amp; treatment</th>
<th>SOD (U/mg of protein)</th>
<th>CAT (nmol GSH/min/mg protein)</th>
<th>GPx (µmol H₂O₂/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 10)</td>
<td>274.43 ± 22.36</td>
<td>7.18 ± 0.28</td>
<td>5.06 ± 0.29</td>
</tr>
<tr>
<td>FEN (n = 10)</td>
<td>241.32 ± 16.10 ++</td>
<td>12.19 ± 0.56 ***</td>
<td>3.91 ± 0.46 ***</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 10)</td>
<td>276.63 ± 35.94</td>
<td>18.07 ± 0.67</td>
<td>1.69 ± 0.30</td>
</tr>
<tr>
<td>FEN (n = 10)</td>
<td>226.67 ± 15.49 ++</td>
<td>11.53 ± 0.25 ***</td>
<td>0.60 ± 0.07 ***</td>
</tr>
</tbody>
</table>

The number of determinations is indicated between parentheses. FEN treated vs controls: * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

Fig. 3—Cerebrum histological sections of 14-day-old rats, controls (A1, A2) and whose mothers were treated by FEN (B1, B2, B3) from the 14th day of pregnancy until the 14th day after delivery [BV: Blood vessel; Optic microscopy: HE (×200); (A1) and (×400): (A2, B1, B2, B3). Arrows indicate: ••••• A network of finely branching small blood vessels (+++); —— Cerebrocortical necrosis (encephalomalacia) (+++) Σ Vacuolated neurons (++++)]

(H&E) showed severe distortions in cellular architecture (Fig. 3B1, B2, B3; Fig. 5B). A network of finely branching small blood vessels and more vacuolated neurons than those of controls (Fig. 3B1, B2) were consistent with oligodendroglioma. In addition, there was a cerebro-cortical necrosis illustrated by encephalomalacia, characterized with indefinite outlines filled by cellular cytoplasmic debris (Fig. 3 B2). In the cerebellum of FEN-treated rats, the most conspicuous damage was the absence of external granular layer (Fig. 4B1, B2, B3). In addition,
FEN treatment led to a reduction of Purkinje cells number with more apoptotic cells than controls. There was also edema, accounting for a widening of the Purkinje cell layer (Fig. 4B3). Under light microscopic examination, brain histological sections of FEN-treated pups, stained with blue toluidine showed dark pyknotic neurons in the cerebrum (Fig. 5B) and pyknotic Purkinje cells in the cerebellum (Fig. 6B).

**Discussion**

In the current study, the exposure of adult rats to FEN during late pregnancy and early post-natal periods provoked a reduction of body and absolute cerebrum and cerebellum weights of their pups. Besides, no appreciable changes in the diet intake by dams following FEN treatment were observed during the experimental period. Thus, these reductions could be explained by a decrease of the cerebrum and cerebellum protein contents. This was in agreement with previous studies, which indicate that organophosphorus compounds like chlorpyrifos interfere with DNA and protein synthesis in different regions of suckling rat brain, leading to neurological disorders. On the other hand, inhibition of AChE activity after OPs exposure is an indicator of poisoning. OPs generally elicit their effects by inhibition of AChE, leading to accumulation of acetylcholine at neuron/neuron and neuron/muscle (neuromuscular) junctions or synapses. Our results demonstrated that the effects of FEN were more accentuated on the cerebellum than on the cerebrum. This could be due to the immaturity of the cerebellum during the early post-natal period, a critical period of life.

Neurological disorders observed in our findings could be attributed to oxidative damage, probably caused by FEN. Although the mechanism of action of FEN seemed to be very complex, previous study has hypothesized that FEN metabolites are involved in the hepatic and brain LPO and DNA-single strand breaks in rats. Due to its lipophilicity, FEN can be absorbed through the gastrointestinal and respiratory tracts and also confers preferential distribution into lipid-rich internal tissues, including body fat, skin, lungs, the central and peripheral nervous systems, causing oxidative damage. The brain is extremely vulnerable to oxidative stress, due to non-heme iron involved in the production of oxygen free radicals. In the present study, exposure rats to FEN resulted in a significant increase in LPO and protein oxidation, as indicated by the significant increase in MDA content.
and AOPP levels, suggesting that FEN activated the formation of free radicals in the cerebrum and cerebellum tissues. Oxidative damage was more accentuated in the cerebrum than cerebellum. In fact, neural cells and/or brain regions respond differentially to oxidative stress.\textsuperscript{22} Our results corroborated with previous findings\textsuperscript{45} showing that FEN stimulates the generation of ROS in brain of adult rats. The current findings established for the first time that FEN caused oxidative stress in the brain of suckling rats.

The cells have various mechanisms to combat oxidative stress and repair damaged macromolecules. The defence is offered by the enzymatic (SOD, CAT, GPx) and non-enzymatic antioxidants (GSH, NPSH, vitamin C) which have been shown to scavenge ROS. To substantiate the mechanisms underlying FEN neurotoxicity, the antioxidant status in the cerebrum and cerebellum was evaluated. SOD is considered as the first line of defense against the deleterious effects of oxy-radicals in the cell by catalyzing the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. Generally, SOD protects CAT against inhibition by superoxide anion. Thus, the enzymatic antioxidant system may be essential to get rid of ROS generated in the brain.

In the present study, an increase in CAT activity in the cerebrum and a decrease in the cerebellum of suckling rats was observed. The increase of CAT activity in the cerebrum might be linked to the high levels of H$_2$O$_2$ produced by FEN. The induction of CAT activity could be due to the adaptive responses following oxidative stress. Furthermore, FEN exposure decreased SOD and GPx activities in both the cerebrum and cerebellum, suggesting that the production of free radicals exceeded the capacity of detoxification mechanisms.

GSH, a non-enzymatic antioxidant plays an important role in the brain by removing oxidants formed during metabolic processes, like oxygen utilization by the mitochondria. In the present study, the exposure of rats to FEN from the 14$^{th}$ day of pregnancy until day 14 after delivery resulted in a significant increase of GSH level both in the cerebrum and cerebellum of suckling rats. This could be due to the enhancement of its synthesis after induction of γ-glutamylcysteine synthetase and this mechanism has been considered to be an adaptative response.\textsuperscript{46} In most cases, the brain GSH levels have been shown to decrease after treatment with various pesticides, including dimethoate and rotenone.\textsuperscript{24,46}

Other non-enzymatic antioxidants, such as vitamin C and non-protein thiols (NPSH) could also act to overcome oxidative stress, being a part of the total antioxidant system. Our results showed a significant decrease in vitamin C and NPSH levels in the cerebrum and cerebellum of suckling rats after FEN treatment. The reduction of vitamin C levels in the cerebrum and cerebellum was found to be of major importance, due to the crucial role of this antioxidant in protecting the central nervous system under chronic exposure to FEN. Our results were in agreement with a recent study\textsuperscript{24} which reported a decrease of these parameters in the cerebral cortex tissue of rats treated with dimethoate.

Histopathological studies also provided an important evidence for biochemical analysis. FEN treatment for 3 weeks provoked neuronal degeneration and encephalomalacia of the cerebrum. In particular, the presence of dark pyknotic neurons in the cerebrum of FEN-treated rats indicated a chronic neurodegenerative process. In the cerebellum, the most evident damage in suckling rats whose mothers were treated with FEN, was the absence of external granular layer. In addition, FEN treatment led to the apoptotic Purkinje cells, which were rounded and poorly differentiated. We also observed edema in the Purkinje cell layer. This could be due to the increased amount of ROS products which might attack membranes and enhance their permeability, leading to vacuolation, the primary response to cell injury.\textsuperscript{47} Disturbances in lipid inclusions and the accumulation of intracellular water produce cytoplasmic vacuolation.\textsuperscript{48}

Furthermore, in the present study, destruction by FEN of external granular layer could be explained by the fact that neurons of external granular layer were migrated towards the molecular and internal granular layers and the mitosis of these cells was hindered by FEN treatment. Similar perturbations have been reported in the cerebellum of mice treated with fluoride\textsuperscript{49} or methyl azoxymethanol (MAM), an antimitotic drug which affects the cerebellum of mice and rats.\textsuperscript{49,50} In addition, previous studies have shown\textsuperscript{51-53} that granule cells migration depends on the recognition of extracellular neuronal guidance molecule(s) like laminin, a key guidance molecule in the developing brain. In the present study, cerebellar cell formation was depressed during the few days which followed the beginning of FEN administration. The increase in cell death in FEN-treated rats
indicated that some granule cells were degenerated a certain time after lying down because Purkinje cells were unable to establish contact with granule cells at the proper time. Cell damage and cell death were induced by the generation of ROS through the administration of FEN which contributed to the neurotoxicity. These data suggested that ingested FEN was retained by the cerebellum, interfering with its physiology and inducing neurotoxicity, cell damage and even cell death.

In conclusion, the present data showed that exposure of pregnant and lactating rats to FEN caused neurotoxicity in their suckling pups, as evidenced by a decrease in the cerebrum and cerebellum AChE activities, an increase of MDA, AOPP and GSH levels and a disturbance of antioxidant enzymes (SOD, GPx and CAT) activities. Thus, the present study suggests that FEN affects structure and maturation of brain in suckling rats.

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References


Jones M, Yang M & Mickelson O (1972) Fed Proc 31, 1508–1511

