Bifenthrin (BIF) is a pyrethroid (PYR) insecticide. The target point for PYR’s toxic action are voltage sensitive sodium channels in the central nervous system (CNS). Intoxication with PYRs results in motor activity impairment and death in insects. Although PYRs are considered to be safe for mammals, there were numerous cases of pyrethroid poisoning in humans, animals and pets described. The general population is chronically exposed to PYRs via grain products, dust and indoor air. Therefore new questions arise: whether PYRs act in a dose-additive fashion in the course of subacute poisoning, are there other target organs (but brain) for BIF and if there is one common mechanism of its’ toxic action in different organs. The objective of this work was to characterize the effect of BIF at the doses of 4 or 8 mg/kg injected intraperitoneally (i.p.) daily for 28 consecutive days on memory and motor activity, hematological, biochemical and histopathological parameters in mice. BIF at the doses of 8 mg/kg or 4 mg/kg of body mass was administered i.p. daily to the mice for 28 consecutive days. Motor function was measured on day 1, 7, 14 and 28 and memory retention was tested in a passive avoidance task on day 2, 7, 14 and 28. BIF significantly impaired memory retention on day 2. BIF decreased locomotor activity at every stage of the experiment in a single dose depending manner. No behavioral cumulative effect was observed. Subacute poisoning with the higher dose of BIF caused anaemia, elevated white blood cell count (WBC), elevated alanine transaminase (ALT), superoxide dismuthase (SOD), and decreased glutathione peroxidase (GPx) activity. Lymphocyte infiltrates were visualized in the livers. In conclusion: subacute poisoning with BIF decreases locomotor activity in a single dose proportionate manner. BIF damages also the liver and alters blood morphology. The possible common mechanism of these effects can be oxidative stress.

Key words: bifenthrin, pyrethroid, locomotor activity, memory, oxidative stress, liver function, blood morphology

INTRODUCTION

Bifenthrin (BIF) is a pyrethroid (PYR) insecticide. BIF is a cyclopropanecarboxylate ester of alcohol. It is characterized with strong insecticidal properties due to its ability to alter functioning of nerves by modifying the kinetics of voltage-sensitive sodium channels in insects (1, 2). BIF and other synthetic PYRs are considered to be safe for mammals because they are readily cleaved at the central ester bond to relatively non-toxic metabolites in the liver, which are passed with urine (3-5). The relative resistance of mammalian to PYRs is attributed to their higher body temperature than of insects, faster metabolism in the liver and lower sensitivity of sodium channels (6). However, there were numerous cases of PYR poisoning in non-target organisms described (7-9). Acute poisoning with BIF alters gait and other motor functions in rodents (1, 10, 11).

For long time it was believed that PYRs acted only via fast disregulation of the nervous system, without any significant cytotoxic effect. However, there is evidence, that exposure to PYRs may also produce neuron death in adult animals (12), inhibition of nervous system development in rodent newborns (13) hepatic toxicity, changes in blood morphology, disrupt the endocrine system (14, 15), produce reproductive toxicity (16), as well as oxidative stress (OS) (17-19).

PYRs are used in medicine and veterinary medicine against vectors (ticks and mosquitoes) and ectoparasites (scabies), as well as in agriculture for crop control, and to fight domestic pests. Due to the global climate change significant changes in global disease patterns are expected (20). The incidence of malaria, Lyme disease and tick-borne encephalitis is to increase. Therefore residents and tourist to the endemic areas are expected to be chronically exposed to PYRs. Farmers and greenhouse workers can be chronically exposed to the insecticides at workplace in their efforts to increase crops (21, 22). The use of PYRs in agriculture increases as they replace organophosphorous insecticides. In Poland current annual use of PYRs is estimated to be over 80,000 kg of active ingredient (23), and one of them is BIF. Members of the public are exposed to traces of PYRs via food of plant origin, water, indoor air and textiles (24, 25). Therefore a new questions arise: whether PYRs act in a dose-additive fashion in the course of subacute poisoning, are there other target organs for BIF apart from brain and if there is one common mechanism of its’ toxic action in different organs.
The objective of this work was to characterize the effect of BIF at the doses of 4 or 8 mg/kg injected intraperitoneally (i.p.) daily for 28 consecutive days on memory and motor activity, hematological, biochemical and histopathological parameters in mice.

MATERIALS AND METHODS

Experimental animals

Non-gravid female albino Swiss mice weighing 18–24 g approximately 6 weeks of age purchased from a licensed breeder (T. Gorzkowski, Warsaw, Poland) were used in the study. All animals were given a 7-day acclimation period and maintained on a 12 h light/dark cycle. Food and tap water were provided ad libitum. Temperature was maintained at 21 ± 2°C. The mice were randomly divided into groups of 8 animals each. A total of 48 animals were used in the experiment.

All the experimental procedures were conducted with respect for the law regulations of the European Community and the law regulations of the Republic of Poland. Medical University of Lublin, Lublin. The Local Ethics Committee for Animal Experiments in Lublin had approved the experiment (Opinion No. 4/2009, dated: Jan. 9th 2009). The experiments were performed between 8:00 a.m. and 6:00 p.m.

Chemicals

BIF [2-methylbiphenyl-3-ylmethyl (Z)-(1RS)-cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropanecarboxylate] 99% purity was purchased from the manufacturer (Institute of Industrial Organic Chemistry, Annopol, Warsaw, Poland). Tween 60 (poloxylethylene sorbitan monostearate) purchased from the manufacturer (Institute of Industrial Organic Chemistry, Annopol, Warsaw, Poland) was used to prepare solutions in bidistilled water.

Experimental procedures

Dosing

On the first day of the experiment 16 mice injected i.p. with BIF at the dose of 8 mg/kg bw. BIF at the dose of 8 mg was dissolved in 9.9 ml of saline with 0.1 ml of Tween as pure PYRs poorly dissolve in water. The volume of 10 ml of solution was prepared per 1,000 g of mice body mass. A mouse of body mass 20 g received 0.2 ml solution i.p. in one injection. The injections were repeated once daily for 28 subsequent days. Body mass (b.w.) of each animal was recorded daily before BIF administration. The same number of animals was given BIF at the dose of 4 mg/kg bw. for 28 consecutive days. Controls were 16 animals injected with respective volume of saline i.p. for 28 days.

Locomotor activity

To evaluate the influence of BIF on locomotor activity, 8 animals from each group (control, BIF 8 mg, BIF 4 mg) were tested in Opto-Varimex 4 Activity Meter (Columbus, OH, USA). Each monitoring instrument (Opto-Varimex) consisted of a Plexiglass cover connected to the Auto-Track interface and intercrossed by 16 pairs of horizontal infrared beams equally spaced at 1 cm above the floor. Interruption of a beam generated an electrical impulse, which was subsequently processed and sent to a computer linked to the Auto-Track interface. The Auto-Track system detected behavioral parameters including locomotor activity. Interrupting five or more beams was recorded as locomotor movement activity. Testing was conducted at the time of peak effects of BIF. Locomotor activity monitoring started 30 min after the injection and was continued for 60 min.

Passive avoidance task

The remaining animals (8 from each group: control, BIF 8 mg, BIF 4 mg) were tested in a step-through passive avoidance task (PA). PA is regarded as a good measure of long-term memory retention (26). The task relies on the natural preference of rodents for dark, enclosed spaces. PA is regarded as a good measure of long-term memory retention. Thirty minutes after BIF injection each animal was placed in a well lit box (15 × 12 × 15 cm) adjacent to a darkened one. The dark box had an electric grid floor. Thirty seconds after placing an animal in the centre of the illuminated box, a passage joining the two boxes was opened. After entering the dark box, the mouse was affected with an electric foot shock (2 mA for 2 s). Twenty four hours after the training, memory retention test was conducted. The latency to enter the darkened box was recorded. The test ended when the mouse entered the darkened box or when 180 s elapsed.

Training was repeated on day 6, 13, 27. PA test was done on day 2, 7, 14 and 28.

Laboratory tests

On the 29 day all the animals were decapitated and their blood samples were collected to clot in order to measure alanine transaminase (ALT) activity in the blood sera. Blood samples with EDTA were also collected in order to measure blood morphology. The livers of 50% animals from each group were used for measurement of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity. The livers of the remaining animals were used for histopathological examination.

Blood samples were centrifugated for 4 min at the speed of 3,400 rpm. Cobas Integra 800 apparatus manufactured by Roche, France, was used to measure ALT activity. Sysmex XT 2000i automatic hematologic analyser was used to measure blood morphology.

The SOD activity was measured with use of RANSOD kit manufactured by Randox Laboratories Ltd., with a spectrophotometric method (27). The livers were homogenized with a mechanic blender MPW- 120 in a 0.1 M buffer of Tris-HCl, 7.4 pH (0.5 g of tissue per 5 ml buffer). The homogenates were centrifuged for 15 min twice at the speed of 5,000 rpm. The supernatants were collected for SOD activity measurement. The activity of GPx was measured with use of RANSEL kit manufactured by Randox Laboratories Ltd., with the spectrophotometric method (28). The supernatants for GPs determination were prepared in the same way as for SOD activity measurement.

Livers from the remaining animals were used for microscopic examination. The livers were assessed with a light microscope. The livers were buffered in 10% formalin, dehydrated in alcohol-acetone series, subjected to xylene, embedded in paraffin blocks, cut into sections and stained with hematoxillin and eosin (H + E). They were assessed in light microscopy, magnification ×40.

Statistical analysis

The PA results were shown as medians ± 25th and 75th percentile and results obtained in the remaining tests as means ± S.E.M., and evaluated by one-way analysis of variance ANOVA followed by Dunnett’s test. The P value < 0.05 was considered statistically significant.
RESULTS

Locomotor activity was significantly reduced in a single dose proportionate manner in animals exposed to BIF on day 1 (Fig. 1), 7 (Fig. 2), 14 (Fig. 3) and 28 (Fig. 4) in comparison with control group. No cumulative effect of BIF was observed. The results obtained in the step-through passive avoidance task are shown in Fig. 5. BIF significantly impaired memory.

Fig. 1. Effect of exposure to a single dose of BIF on locomotor activity in mice (mean ± S.E.M.). N = 8, *P<0.05 vs. control, Anova, Dunnett’s test.

Fig. 2. Effect of a 7-day exposure to BIF on locomotor activity in mice (mean ± S.E.M.). N = 8, *P<0.05 vs. control, Anova, Dunnett’s test.

Fig. 3. Effect of a 14-day exposure to BIF on locomotor activity in mice (mean ± S.E.M.). N = 8, *P<0.05 vs. control, Anova, Dunnett’s test.
Fig. 4. Effect of a 28-day exposure to BIF on locomotor activity in mice (mean ± S.E.M.). N = 8, *P <0.05 vs. control, Anova, Dunnett’s test.

Fig. 5. The effect of BIF intoxication on memory retention in the step-through passive avoidance task (median ± 25th and 75th percentile). N = 8. *P <0.05 vs. control, Anova, Dunnett’s test.

Fig. 6. Light microscopy examination of the liver of a mouse exposed to BIF at the dose of 8 mg/kg for 28 days. Lymphocyte infiltrates around blood vessels and biliary ducts (arrow). Haematoxillin and eosin staining. Magnification ×40.
The results of blood morphology, ALT activities in the blood sera, SOD and GPx activities in the livers are shown in Table 1. Subacute poisoning with the higher dose of BIF caused anaemia, elevated white blood cell count (WBC), and lymphocyte percentage (LYM), elevated alanine transaminase (ALT), superoxide dismuthase (SOD), and decreased glutathione peroxidase (GPx) activity. In the light microscopy examination in the livers of mice receiving BIF at the dose of 8 mg/kg for 28 days lymphocyte infiltrates around blood vessels and biliary ducts were seen (Fig. 6). Less infiltrates and widened blood vessels were visualized in the livers of mice receiving the lower dose of BIF. Body mass gain was recorded daily (Fig. 7). Animals treated with BIF gained weight at a slower rate than controls.

**DISCUSSION**

Pyrethroids are neurotoxins. The primary target sites underlying PYR’s toxicity are voltage-gated sodium channels. PYRs enhance sodium channel activity by shifting activation to more negative membrane potentials and by inhibiting channel inactivation. The altered sodium channels cause repetitive firing and depolarizing block in neurons (1). Type II PYRs typically produce longer delays in channel inactivation than do type I compounds (29). In target organisms acute poisoning with PYRs results in motor activity impairment. In insects moderate doses of PYRs produce hyperexcitability, an increase in motor activity with altered mode of flying or walking (30). Higher doses cause immobility of walking insects and falling down among flying insects. The highest doses cause immediate hindlimb paralysis, movement incoordination and eventually prostration and death (31).

BIF is classified as producing T syndrome (aggressive sparring, sensitivity to external stimuli, tremor progressing to prostration) or type I pyrethroid (32, 33). BIF is considered to be of moderate toxicity in vivo (10) and low efficacy on the sodium channels in vitro (34).

In the present study we have recorded reduced locomotor activity in mice exposed to BIF for 28 subsequent days. These results come in line with results published by other authors, however, majority of articles show description of acute BIF neurotoxicity. In rats acute oral exposure to BIF at the dose of 75 mg/kg causes whole-body tremors, twitching, staggered gait, uncoordinated movement/ataxia, splayed hindlimbs, abnormal posture, clonic convulsions, and abdominogenital staining (1). Wolansky (10) wrote that the threshold dose of BIF of 1.28 mg/kg was enough to reduce motor activity in rats. Wolansky reported that BIF’s LD50 was 55 mg/kg. In his experiments the PYR was administered to rats in corn oil by gavage. The author indicated the following potencies of PYRs in vivo: esfenvalerate > lambda-cyhalothrin > beta-cyfluthrin > tefluthrin > deltamethrin > BIF > fenpropothrin > cypermethrin > permethrin > s-bioallethrin > resmethrin. In our experiment BIF was administered i.p. to mice at the dose of 4 or 8 mg/kg. The i.p. LD50 for BIF calculated at our department (the Hygiene Department of the Medical University in Lublin) was 16.1 mg/kg (35). In our former study BIF at the dose of 1.61 mg/kg was administered to mice in a single injection and the dose was high enough to impair memory tested in a Y-maze and impair

<table>
<thead>
<tr>
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<th>Control</th>
<th>BIF 4 mg/kg</th>
<th>BIF 8 mg/kg</th>
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<tbody>
<tr>
<td>RBC [million/mm³]</td>
<td>9.49 ± 0.1 (N = 16)</td>
<td>8.4 ± 0.1 (N = 16)</td>
<td>8.1 ± 0.4* (N = 16)</td>
</tr>
<tr>
<td>WBC [mm³]</td>
<td>8,071 ± 1,108 (N = 16)</td>
<td>12,103 ± 2,226* (N = 16)</td>
<td>12,805 ± 2,675* (N = 16)</td>
</tr>
<tr>
<td>LYM [%]</td>
<td>60 ± 4 (N = 16)</td>
<td>68 ± 11 (N = 16)</td>
<td>75 ± 8* (N = 16)</td>
</tr>
<tr>
<td>PLT [thousand/mm³]</td>
<td>640 ± 149 (N = 16)</td>
<td>789 ± 134 (N = 16)</td>
<td>613 ± 210 (N = 16)</td>
</tr>
<tr>
<td>HGB [g/dl]</td>
<td>14.08 ± 0.2 (N = 16)</td>
<td>13.9 ± 0.4 (N = 16)</td>
<td>11.1 ± 0.8* (N = 16)</td>
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<tr>
<td>ALT [U/l]</td>
<td>50 ± 11 (N = 16)</td>
<td>78.4 ± 10.3 (N = 16)</td>
<td>133.4 ± 38.2* (N = 16)</td>
</tr>
<tr>
<td>SOD-liver [U/g of tissue]</td>
<td>1,420 ± 85 (N = 8)</td>
<td>1,312 ± 180 (N = 8)</td>
<td>1,648 ± 307 (N = 8)</td>
</tr>
<tr>
<td>GPx-liver [U/g of tissue]</td>
<td>63.6 ± 11.6 (N = 8)</td>
<td>49.9 ± 13.6 (N = 8)</td>
<td>28.1 ± 15* (N = 8)</td>
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**Fig. 7.** Effect of BIF on body mass gain. N = 16,*P <0.05 vs. control, Anova, Dunnett’s test.
movement co-ordination but there was no significant effect on locomotor activity (35). Therefore this time we’ve made the decision to use a higher dose of the PYR. Scollon et al. (36) demonstrated that BIF reduced motor activity in rats. The doses used in their experiment were even higher than used in our present study. In Scollon’s experiment motor activity was measured 4 and 7 hours after exposure to BIF at the dose of 0–16 mg/kg or 0–9 mg/kg. And they have found out that the relationship between motor activity and brain concentration of BIF was not significantly different between the two time points. To sum up majority of studies confirm that PYRs affect motor activity in rodents. Another question remains open: if this is due to sodium channel activation.

Cao et al. (34) described his experiments carried on primary cultures of cerebrocortical neurons. He showed that BIF produced just a modest increase in sodium ions influx. The relative potencies of different PYRs in his in vitro study were not identical to those reported by Wolansky et al. (10). According to Cao et al. (34) the rank order of in vitro potency was deltamethrin > s-bioallethrin > beta-cyfluthrin > lambda-cyhalothrin > esfenvalerate > tefluthrin > fenpropatrin > cypermethrin > bifenthrin while permethrin and resmethrin were inactive. However the in vitro system lacks significant metabolic capability and consists of a single tissue, so the results obtained this way not always are able to explain the results obtained in vivo. In vivo the xenobiotic can interfere with multiple neuronal pathways, act of wider variety of ion channels and can affect neurotransmitter release.

It is known that PYRs also decrease chloride currents through voltage-dependent chloride channels (1). At high concentrations PYRs can also act on GABA-gated chloride channels inhibiting chloride influx (37-39), which is responsible for neurological effects; the seizures seen in severe acute poisoning and the altered motor activity. Exposure to PYRs can affect cholinergic/dopaminergic neurochemistry (40). However, the early studies on PYRs’ structure-toxicity relationships, that gave basis for the current classification of these pesticides into type I and II, were carried after an acute mice poisoning with high doses of intracerebrally injected PYRs (33). Such dosing had little relevance to the possible environmental exposure of humans and other non-target organisms to PYRs.

A newly studied target points for PYRs are voltage-gated calcium channels. Cao et al. studied the activation of voltage-gated calcium channels by PYRs (41). They have demonstrated that PYRs stimulated Ca$^{2+}$ entry into neurons subsequent to their action on the voltage-gated sodium channels. However BIF (at concentrations 10 μM, 30 μM, 50 μM and 100 μM) produced very little Ca$^{2+}$ influx response in neocortical neurons. In that study of Cao et al. the rank order of PYRs’ efficacy was: tefluthrin = deltamethrin > lambda-cyhalothrin > beta-cyfluthrin > esfenvalerate > fenpropatrin > cypermethrin > bifenthrin > s-bioallethrin. In a recent study of Cao et al. (42) the authors investigated whether nanomolar BIF (EC$_{50}$ = 58 nM) altered synchronous Ca$^{2+}$ oscillations necessary for activity-dependent dendritic development. Acute exposure to BIF increased the frequency of synchronous Ca$^{2+}$ oscillations and decreased their amplitude. These changes were independent on voltage-gated sodium channels since 100 nM BIF had no effect on the sodium current and did not influence neuronal resting membrane potential. The metabotropic glutamate receptor antagonist was demonstrated to normalize the BIF-triggered increase in synchronous Ca$^{2+}$ oscillations frequency without changing the baseline synchronous Ca$^{2+}$ oscillations. The authors concluded that BIF amplified metabotropic glutamate receptor signaling independent of sodium channel modification. This is the possible mechanism of reducing motor activity in mammals after exposure to pyrethroids. However, further studies are needed to check if the effects demonstrated in vitro can be confirmed in vivo.

As the bifenthrin’s action on the neuronal firing is irreversible the mechanism of amplifying the metabotropic glutamate receptor signaling may explain why BIF demonstrates long term toxicity although it is quickly metabolized and cleaned.

The data about the influence of BIF on memory processes in mammals are scarce. Our former experiment demonstrated, that a single administration of BIF at the dose of 1.6 mg/kg administered i.p. caused movement incoordination and impaired memory retention in PA in mice after brain oligemia (43). In the present study only during the first test in PA memory retention was impaired. It is possible, that in the course of subacute poisoning the brain structures responsible for memory retention ‘acclimatize’ to BIF. Hornyuchowa et al. also detected memory impairment in rodents after subacute poisoning with PYRs (44). Similar memory deficits were detected in experiments with deltamethrin (45). Such behavioral changes confirm that the central nervous system is the main neurotoxicity target for BIF and other PYRs. Subacute poisoning with PYR causes neurodegeneration and neuroinflammation (46). PYRs applied for 4 weeks to rats cause nerve cell loss in layer III of frontal cortex, dentate gyrus, and hippocampus, cause dopamine and dopamine transporter decreased in hippocampus and striatum (40). The neurodegenerative effect of PYRs in the nigrostriatal pathway resembles the changes occurring in human brains in the course of Parkinson’s disease (PD). Another similarity between toxic effects of PYRS and PD is that deltamethrin reduces the level of dopamine (DA) in rat brain (47).

Our results show not only that subacute poisoning with BIF reduces locomotor activity at every stage of testing in a dose-proportionate manner, but also that there is no dose-additivity. The lack of cumulative effect is probably due to fast pyrethroid metabolism in the liver. Pyrethroids are metabolized mainly by the liver esterases and cytochrome P450 (3).

The OS is an imbalance between reactive oxygen species (ROS) and antioxidants. The ROS can damage DNA, proteins and lipids, change cell’s metabolism, affect gene expression and posttranslational modifications of proteins, which accelerates ageing, neurodegeneration and development of atherosclerosis, hypertension, type II diabetes as well as cancer (48). There is also data that ROS can cause cytoplasmic calcium influx by phospholipase C activation and phosphorylation of 1,4,5-inositol triphosphate-sensitive calcium channels, potentially affecting neurotransmitter release, memory processes and motor activity (49). Some antidepressant drugs together with chromium trichloride at the dose of 32 mg/kg were shown to increase motor activity in rodents via interaction with monoaminergic (noradrenergic, dopaminergic and serotonin) systems in rodents (50). Moreover, ROS induce activity of cyclooxygenases leading to inflammation and vasculitis (49). Vascular pathology often leads to significant impairment of learning and memory (51).

The SOD and GPx are antioxidant enzymes ubiquitous in living organisms acting as an endogenous defense against ROS (52, 53). Dar et al. showed in their study that repeated oral administration of BIF at the dose of 5.8 mg/kg daily for 20 or 30 days led to an increase in lipid peroxidation, and a decrease in SOD activity (17). Dubey et al. reported significant hepatic OS and hepatic damage in rats exposed to deltamethrin, which is also a pyrethroid, administered with fluoride (18).

Krzepilko (54) studied the effect of exposure of yeast cell extracts to BIF on their oxidative-antioxidative system. They demonstrated that the investigated PYR insecticide reduced concentration of thiol groups in the yeast cell extracts. It confirms that PYRs cause OS. The results obtained in our experiment show that repeated exposure to BIF may cause hepatic OS and affect liver structure. In the present study significant changes in animals’
blood morphology were recorded and these results come in line with data reported by other authors (19, 46, 55-57). The OS stress is also very likely to be responsible for bone marrow damage and changes in blood cells of mammals in the course of intoxication with (19, 56). Elevation of neutrophil and lymphocyte counts after 15-day exposure of mice to deltamethrin and nevirapine in long duration (30-day) study were recorded by Tewari and Gill (57). The leucocytes play an essential role in ROS mediated injury through extracellular release of superoxide free radical, which is cytotoxic. The lymphocytes infiltrates were found in liver samples of mice examined in our study, and the lymphocytes visualized may be responsible for release of free radicals. It indicates that the liver is another target point for BIF’s toxicity, possibly via metabolites as its’ metabolism occurs mainly in the liver. The elevated ALT activities in the blood sera of experimental animals exposed to the highest dose of BIF in our study are in agreement with results obtained by other authors (57) and seem to confirm the notion that BIF shows significant liver-toxicity.

The brain functioning, haematopoesis and BIF’s metabolism in the liver require energy. The oxidative stress triggered by pesticides seems to be one common mechanism of damaging all theses organs, as pesticides cause depletion of mitochondrial energy. They inhibit Na, K-ATPases and causes induction of proteolytic enzymes. This leads to DNA fragmentation and apoptosis (58).

Many authors provided evidence that vitamin C and E, selenium as well as SOD supplementation or nimesulide have the ability to ameliorate the overwhelming OS (8, 52, 56, 58-61).

In our experiment animals exposed to BIF gained weight at significantly slower rate than controls. This is in agreement with results of other experiments (62). The xenobiotic apparently altered metabolism in the liver require energy. The oxidative stress induced by pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens. Environ Health Perspect 2011; 119: 794-800.


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