Letter to the Editor

Influence of Soy Isoflavone on Lindane Cumulant in Sprague-Dawley Rats*

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Female Sprague-Dawley rats weighing 60-80 g were given different dosages of soy isoflavones and/or lindane for four weeks. Soy isoflavones was added in feed and lindane was given by oral gavage. We found that soy isoflavones could reduce the level of lindane in rat’s serum and brain, but might cause the uterus hyperplasia. Lindane could inhibit the effect of soy isoflavones on uterus and significantly decrease the level of estradiol and testosterone in serum. This study indicated that soy isoflavones could reduce the level of lindane in rat’s body. Lindane could reduce the level of hormones and decreased the effect of soy isoflavones on rat’s uterus.

Lindane (γ-hexachlorocyclohexane) is a broad spectrum environmentally persistent organochlorine pesticide. The organochlorine pesticides in the body could disturb endocrine function, cause reproductive and developmental disorders. As reported, lindane could inhibit uterine contractions by inhibiting myometrial gap junction permeability. Soy isoflavones (SIF) is a kind of phytoestrogen which mainly exists in soybean. Some reports have shown that SIF could alleviate the climacteric symptom and prevent breast cancer. SIF was considered to be a selective estrogen receptor modulators, which may bind to estrogen receptors and selectively stimulate or inhibit estrogen-like function in various tissues.

The interaction analysis of the phytoestrogens and synthetic chemicals has caused scholars attention. You et al. discovered that genistein may change the toxicological characteristics of methoxychlor. Through investigating the relationship between the level of organochlorine pesticides in the serum and diet on community population, Li JY et al. found that the level of organochlorine pesticides was negatively associated with the intake of soy products. So far, there was no report about the interaction of SIF and lindane and the influence of SIF on the accumulation of lindane in body. The aim of the present study was to discover the interaction of SIF and lindane, which confirmed that SIF could protect body from the harmful effects of lindane by reducing the level of lindane in organism.

Female Sprague-Dawley (SD) rats weighing 60-80 g were randomly divided into nine groups (n=9/group) and given different dosages of SIF (900, 600, or 0 mg/kg diet) and/or lindane (8, 4, or 0 mg/kg BW). SIF was added in feed. Lindane was solved in corn oil and the rats were given corn oil containing lindane at dosage of 1 mL/kg BW daily by oral gavage.

The rats were fed for four weeks. After recording the body weight, the tissues (brain, liver, spleen, kidney, uterus, ovary, pancreas, and fat) of the killed rats were removed and weighed. Serum was separated by centrifugation at 3,000 rpm for 15 min and stored at -20 °C until analysis. Other tissues were frozen in liquid nitrogen and then stored at -80 °C freezer. The level of estradiol (E₂) and testosterone in the rat’s serum were analyzed by chemiluminescent immunoassay in Beijing Institute of Biotechnology.

Lindane was determined by gas chromatography (Agilent 7890A; Agilent, USA) equipped with OV-1701 (30 m×0.25 mm×0.25 μm) capillary column and electron capture detection. Pure nitrogen gas (99.999%) was used as a carrier gas at a constant flow rate of ±1 mL/min. The oven temperature was programmed from 160 °C to 250 °C at 30 °C/min, held isothermal for 2 min and finally raised to 280 °C at 10 °C/min, then held for 2 min. 1 μL sample was injected and the split ratio was 20:1.

The serum (0.5 mL) were put into centrifuge

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tubes and mixed with 2 mL hexane. All the samples were given vortex shake for 2 min and ultrasonic treatment for 5 min. After centrifugation at 3 000 rpm for 10 min, the supernatants were transferred into another tube. The residues were repeated with above methods for two times. Combined the extract and concentrated with nitrogen blowing, the extracts were metered volume to 1 mL with hexane.

The fat and brain samples (0.15-0.35 g) were placed in glass tubes and added 1 g anhydrous sodium sulfate. After adding 2 mL hexane and being homogenate, the samples were given vortex shake for 2 min and ultrasonic treatment for 5 min. After centrifugation at 3 000 rpm for 10 min, the supernatants were transferred into another tube. The residues were treated with the same method for two times. The fat extracts were concentrated with nitrogen blowing to 1 mL and diluted (1:20) before analysis. The brain extracts were metered volume to 6 mL with hexane and filtrated by organic membrane (0.22 μm) and prepared for gas chromatography analysis.

The uterus samples were embedded in paraffin. 3 μm thick sections were cut, mounted, and stained with hematoxylin and eosin (H&E) for microscopic analysis. For morphologic analyses, we recorded known estrogens induced features including shape, determination of hypertrophy and hyperplasia of glands and endometrial epithelium.

All the data were expressed as arithmetic means±SD. The data was analyzed by Two-way ANOVA with lindane and SIF. The accepted level of significance was P≤0.05.

There was no significant difference in body weight among the rats in nine groups. We calculated the daily SIF intake was about 75 mg/kg BW or 50 mg/kg BW according to the intake of feed (data not shown). Two-way ANOVA analysis showed that different dosage of SIF and lindane could influence the weights of spleen (SIF, P=0.015; lindane, P=0.012). Huang J et al.[5] found that the weight of spleen significantly increased at the dosage of SIF >50 mg/kg BW, which is consistent with our study. Compared with control group, SIF (900 mg/kg feed) increased the weight of spleen while lindane (8 mg/kg BW) decreased the weight of spleen. Zhou TL et al.[6] found that DDT could induce hepatomegaly in rats. As shown in Table 1, lindane could cause hepatomegaly in rats (P=0.051). The interaction of SIF and lindane had significant influence on the weight of liver (P=0.033). SIF (900 mg/kg feed) increased the influence of lindane on the weight of liver, while at the dosage of 600 mg/kg feed inhibited the influence of lindane on the weight of liver.

SIF had no significant effects on the level of estradiol and testosterone in serum (estradiol, P=0.159; testosterone, P=0.74), but Zhou ZJ et al.[7] found that SIF at the dosage of 100 mg/kg BW could increase the level of E2 in aged rats. The discrepancy of the two studies results might be due to (1) different age of the rats-weanling rats were used instead of aged rats in this study, so their response to SIF. might be different. (2) different concentration of SIF-93.3% SIF was used instead of 40% SIF in this study, so the estrogen effect of SIF might be influenced. Recent researches suggested that lindane was considered to be estrogen-like action as a kind of organochlorine pesticide. Changes in concentration of sex hormone and activity of estrogen receptor partly reflected exogenous compounds to (anti) estrogen effect. Liu GH et al.[8] found that the level of E2 in the group in which lindane was detected was lower than control group. As showed in Table 1, lindane could significantly decrease the level of estradiol and testosterone in serum (estradiol, P=0.003; testosterone, P=0.003). This might explain why organochlorine pesticide cause sterilitas virilis and female infertility.

Researches have proved that lindane had some negative effects on nervous system development. Two-way ANOVA analysis showed that giving lindane could significantly influence the level of lindane in brain, serum and fat tissue (brain, P=0.000; serum, P=0.000; fat tissue, P=0.000). High dosage of lindane

<table>
<thead>
<tr>
<th>Lindane (mg/kg BW)</th>
<th>Spleen (g)</th>
<th>Liver (g)</th>
<th>Estradiol (pg/mL)</th>
<th>Testosterone (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.457±0.021</td>
<td>7.382±0.132</td>
<td>33.64±2.39</td>
<td>1.45±0.22</td>
</tr>
<tr>
<td>4</td>
<td>0.493±0.022</td>
<td>7.060±0.135</td>
<td>36.45±2.39</td>
<td>1.66±0.22</td>
</tr>
<tr>
<td>0</td>
<td>0.548±0.021</td>
<td>6.927±0.132</td>
<td>45.37±2.51</td>
<td>2.30±0.22</td>
</tr>
</tbody>
</table>

Note. Values are shown as means±SD. * is significantly different from 0 mg/kg BW group, P≤0.05.
accompanied with high level of lindane in brain, serum and fat tissue (Table 2). The founding of lindane in brain indicated that lindane could pass through blood brain barrier and accumulated in brain tissue, and this might be one of the important evidence why lindane influenced the development of nervous system. As showed in Table 3, SIF at the dosage of 900 mg/kg feed or 600 mg/kg feed could significantly reduce the level of lindane in brain and serum (brain, P=0.022; serum, P=0.018), which indicated that SIF might have some benefit effects on lessening the damage of lindane. As a storage site for lipophilic persistent organic pollutant, adipose tissue plays an important role in the toxicokinetics of a variety of drugs and pollutants[9]. In this study, we found that SIF slightly decreased the level of lindane in fat tissue (P=0.363). The level of lindane in fat tissue was about 200 times higher than that in brain and serum. So the statistic results showed that SIF had the significant effects on decreasing the level of lindane in lower concentration such as brain and serum, but had no significant effects in fat. The mechanism of SIF decreasing the level of lindane in brain and serum needs further study. Lindane can be detected in control group, which indicated that the feed material or the milk through breast-feeding also contained some lindane.

SIF exposure could cause uterine hypertrophy, and the mechanism might be: (1) that SIF binded to ER-α and stimulated the uterus hyperplasia. (2) and SIF, through the influence on E2 levels in the body and uterine ER expression, indirectly had a proliferative effect on uterus[10]. In Control group, low columnar of endometrial epithelial cells and closer cytoplasms were found. In SIF (900 mg/kg feed) group, the whole uterine structures were hypertrophic and hyperplastic such as hypertrophic and hyperplastic glands, tall columnar of endometrial epithelial cells, large cytoplasms in endometrial cells and vacuolar degeneration. In lindane (8 mg/kg BW) group, we found hypertrophic and hyperplastic glands, lower columnar endometrial epithelial cells and closer cytoplasms. This might be due to the reduction of lindane on the level of E2. In SIF (900 mg/kg feed)+lindane (8 mg/kg BW) group, hypertrophic and hyperplastic uterine structures were also found, but its intensity was weaker than SIF (900 mg/kg feed) group.

This study demonstrated that SIF could decrease the accumulation of lindane in brain and serum. Furthermore, we found that lindane (8 mg/kg BW or 4 mg/kg BW) could reduce the level of sex hormones. Further research on the mechanism of SIF on the reduction of lindane in organism is needed.

Note. Values are shown as means±SD, * is significantly different from 0 mg/kg BW, # is significantly different from 8 mg/kg BW, P≤0.05.

<table>
<thead>
<tr>
<th>The dose of Lindane (mg/kg BW)</th>
<th>Brain (μg/g)</th>
<th>Serum (μg/mL)</th>
<th>Fat Tissue (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2.66±0.167*</td>
<td>0.23±0.009*</td>
<td>388.63±15.58</td>
</tr>
<tr>
<td>4</td>
<td>1.64±0.162*</td>
<td>0.15±0.009*</td>
<td>307.50±14.90</td>
</tr>
<tr>
<td>0</td>
<td>0.01±0.174*</td>
<td>0.00±0.009*</td>
<td>8.45±21.44</td>
</tr>
</tbody>
</table>

Note. Values are shown as means±SD, * is significantly different from 0 mg/kg diet, P≤0.05.

<table>
<thead>
<tr>
<th>The dose of SIF (mg/kg feed)</th>
<th>Brain (μg/g)</th>
<th>Serum (μg/mL)</th>
<th>Fat Tissue (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>900</td>
<td>1.20±0.167*</td>
<td>0.11±0.009*</td>
<td>224.85±17.14</td>
</tr>
<tr>
<td>600</td>
<td>1.27±0.162*</td>
<td>0.12±0.009*</td>
<td>223.97±17.32</td>
</tr>
<tr>
<td>0</td>
<td>1.84±0.174</td>
<td>0.15±0.009*</td>
<td>255.77±18.14</td>
</tr>
</tbody>
</table>

ND: vacuolar degeneration. In group, the whole uterine structures were also found, but its intensity might be different from 8 mg/kg BW, P≤0.05.
REFERENCES


