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Assessment of genotoxicity of pyrethrin in cultured human lymphocytes

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Abstract
Pyrethrin is an insecticide that is obtained from the Chrysanthemum flower (Pyrethrum). In this study, we examined the genotoxic effects of pyrethrin on cultured human lymphocytes using sister chromatid exchanges (SCEs) and 8-hydroxy deoxyguanosine (8-OHdG) assays. Cultures were treated with different concentrations of pyrethrin (25, 50, and 100 μg/mL), which was dissolved in dimethyl sulfoxide (DMSO). The results showed that treatment of cultured lymphocytes with pyrethrin at 50 μg/mL and 100 μg/mL induced significant elevation in SCEs (p<0.05). In addition, the 100 μg/mL concentration significantly affected both mitotic and proliferative indices (p<0.05). Finally, pyrethrin induced a significant elevation in the oxidative stress marker 8-OHdG in a dose-dependent manner (p<0.001). In conclusion, the results suggest that pyrethrin is genotoxic as measured by two independent assays on genetic toxicity.

Keywords
Pyrethrin, DNA damage, 8-OHdG, sister chromatid exchange

Introduction
Pyrethrin is a natural insecticide obtained from the flowers of Chrysanthemum. Pyrethrin is a derivative of (+)-trans-chrysanthemic acid (Matsuda, 2012). Pyrethrin is considered among the safest insecticides used in edible plants. The mechanism of action of pyrethrin involves modulation of the flow of sodium ions out of the nerve cells that causes persistence firings of the nerves and the subsequent death of the insects (Costa et al., 2008). Outside body, pyrethrin has been shown to be very sensitive to sunlight and air and thus, it is degraded easily and disappeared from the area being treated (Bradberry et al., 2005). When pyrethrins entered the body via the digestive system, they get hydrolyzed and destroyed in the stomach in most mammals (Bradberry et al., 2005). However, exposure to high doses of pyrethrins causes toxicity to respiratory and nervous system. The symptoms include breathing problems, nasal stuffiness and itching, headache and incoordination, and muscle trimming (Taguchi et al., 2006). In animal studies, exposure to high doses of pyrethrins can lead to liver tumors in rats (Price et al., 2007). This toxicity is mediated by induction of oxidative stress in liver cells and the subsequent DNA damage (Muguruma et al., 2006). Similar toxicity was reported in thyroid gland of pyrethrin-exposed rats (Finch et al., 2006).

In this study, we proposed to examine the effects of pyrethrin on the spontaneous frequency of sister chromatid exchanges (SCEs) and 8-hydroxy deoxyguanosine (8-OHdG) in cultured human blood lymphocytes. SCEs are developed from reciprocal DNA exchange in homologous loci of sister chromatids during DNA replication (Wilson & Thompson, 2007). SCEs occur spontaneously at certain frequencies in all cells, and the frequency may be enhanced by certain treatments or associated with diseases (Karaman & Aliagaoglu, 2006; Norppa et al., 2006; Ray et al., 2001). 8-OHdG on the other hand is a strong predictor of oxidative stress DNA damage. The results enhanced our knowledge about the health effects of pyrethrin to human body.

Material and methods

Subjects
A total of five blood donors were recruited to participate in the study. The donors were healthy nonsmokers and nonalcoholic adult males. Blood samples (10 mL) were obtained in in heparinized tubes and were used within less than 1 h of withdrawing. Prior to start of the study, written informed consents were obtained from all donors according to the institutional review board of Hashemite University (approval number: 16-2013).

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Cell cultures

Blood cultures were set up by inoculating 1 mL of freshly withdrawn blood into 50 mL tissue-culture flask containing 9 mL of ready to use complete karyotyping media (PB-Max media, Gibco-Invitrogen, Paisley, UK) (Khabour et al., 2014). This medium contains RPMI-1640 medium with 15% fetal bovine serum, 1% penicillin-streptomycin, and 3% of phytohemagglutinin. Cultures were incubated in CO₂ incubator at 37°C for 72 h (Alzoubi et al., 2014a). Pyrethrin (molecular formula: C₄₃H₅₆O₈, purity: 99%, CAS No. 8003347) was obtained from Shaanxi Sinuote Biotech Co., Ltd. (Shanghai, China). Cultures were treated with different concentrations of pyrethrin (25, 50, and 100 µg/mL), which was dissolved in dimethyl sulfoxide (DMSO) in the last 24 h of the incubation period. These concentrations were selected based on previous studies (Amrutha et al., 2013; Kakko et al., 2004a). Control cultures were treated with vehicle (Alzoubi et al., 2014b).

Sister chromatid exchange assay

SCE assay on white blood cells was done as described previously (Alsatari et al., 2012; Khabour et al., 2011). Bromodeoxyuridine (BrdU) (Sigma-Aldrich, St. Louis, MO) was dissolved in distilled water and was added to cultures to a final concentration of 20 µg/mL (Khabour et al., 2013). Cultures were then centrifuged at 300 × g for 5 min, decanted, and the cellular pellets were gently re-suspended in 10 mL hypotonic solution (0.075 M KCl) at 37°C for 20 min. The cellular suspensions were then centrifuged at 300 × g for 5 min, the supernatants were removed and the pellets were fixed with three changes of ice-cold methanol:acetic acid (3:1). The cellular suspension was then dropped on pre-chilled microscope slides to obtain metaphase spreads (Al-Sweeden et al., 2012). The slides were allowed to air dry and were subsequently stained with the fluorescent-plus-Giemska technique as described previously (Azab et al., 2009). The slides were analyzed using light microscopy at 1000× magnification. The person who scored SCEs was blind to the treatment. For SCE analysis, 50 M2 metaphase spreads were scored for per donor per treatment (Al-Sweeden et al., 2012).

Determination of mitotic index and proliferative index

The mitotic index (MI) and proliferative index (PI) were determined as shown previously (Sadiq et al., 2000). For MI, at least 1000 cells from each donor per each concentration were analyzed and the percentage of cells that were in metaphase was determined. The PI was determined by scoring 100 metaphase cells from each donor per each concentration using the following equation: $PI = \frac{(1 \times M1 + 2 \times M2 + 3 \times M3)}{100}$, where M1, M2, and M3 are the number of cells at the first, second, and third metaphase, respectively.

8-OHdG assay

To assay the oxidative stress biomarker 8-OHdG, human blood lymphocytes were cultured as described earlier. After 68 h of incubation, cultures were centrifuged and cells were washed 5 times with serum-free media as previously described (Alzoubi et al., 2012). Cells were then treated with pyrethrin for 4 h. After that, cultures were centrifuged and 8-OHdG was measured in the supernatant using commercially available kit obtained from Abcam Inc., UK according to the protocol provided by the manufacturer. Plates were read at 405 nm using an automated reader (ELx 800/Universal Microplate Reader, Bio-Tek, Winooski, VT). Levels of 8-OHdG were estimated from the standard provided by the kit (Azab et al., 2015).

Statistical analysis

The collected data were analyzed using GraphPad Prism software (version 5) (GraphPad Software Inc., La Jolla, CA). Statistical testing includes ANOVA followed by Tukey’s post-hoc test. The level of significance of hypothesis testing was $p < 0.05$.

Results

To assess the genotoxicity of pyrethrin to cultured human lymphocytes, SCE assay was used. In this assay, pyrethrin stock solution was prepared in RPMI-1640 medium and three different concentrations of pyrethrin (25, 50, and 100 µg/mL) were examined on the spontaneous frequency of exchanges. The results showed significant elevation in the frequency of exchanges induced by treatment with 50 µg/mL (4.82 ± 0.16 in the control versus 5.58 ± 0.20 in the 50 µg/mL group, $p < 0.05$, Figure 1) and 100 µg/mL (4.82 ± 0.16 in the control versus 6.03 ± 0.19 in the 100 µg/mL group, $p < 0.01$, Figure 1) pyrethrin. However, treatment with 25 µg/mL did not affect spontaneous frequency of exchanges ($p > 0.05$).

We also examined cytotoxicity of pyrethrin on cultured human lymphocytes. The results showed significant decrease in MI (Figure 2, $p < 0.05$) and PI (Figure 3, $p < 0.05$) induced by treatment of cultures with 100 µg/mL pyrethrin. However, no effect on such indices was observed after treatment of cultures with 25 or 50 µg/mL pyrethrin ($p > 0.05$, Figures 2 and 3).

The oxidative DNA damage induced by pyrethrin was examined using 8-OHdG biomarker. This marker is considered the golden marker to assess this type of DNA damage.
The results are shown in Figure 4. Pyrethrin induced significant elevation in the 8-OHdG biomarker in all examined concentrations (25, 50, and 100 μg/mL). The effect of pyrethrin is dose-dependent \((p < 0.01, \text{Figure 4})\). Thus, in addition to SCEs, pyrethrin induces oxidative DNA damage to cultured human lymphocytes. Table 1 summarizes results of the current study.

Discussion

The results of this investigation showed that pyrethrin is genotoxic to human cultured lymphocytes as indicated using SCE assay and 8-OHdG biomarker.

Pyrethrins are natural insecticides derived from chrysanthemum flowers. Pyrethrins are approved pesticides by the US Environmental Protection Agency for use in and around homes, businesses, and on some agricultural crops and is considered minimally toxic to humans, with known respiratory, neurologic, and gastrointestinal effects (Hudson et al., 2014). In addition, pyrethrin is widely used in veterinary medicine for agricultural and domestic purposes (Anadon et al., 2009).

The toxic effects of pyrethrin to humans have been shown by several studies. For example, prolonged exposure of a 66-year woman to pyrethroid has been reported to induce toxic acute tubular necrosis (Bashir et al., 2013). Ingestion of pyrethroids by a 19-month female caused recurrent tonic–clonic seizures and coma (Giampretti et al., 2013). Anosmia after exposure to a pyrethrin-based insecticide has been also reported (Gobba & Abbacchini, 2012). Between 2001 and 2005, 407 cases of illnesses due to exposure to pyrethrin or pyrethroid insecticides exposure were reported in Oregon and Washington states. Among these cases, the most common category of clinical signs and symptoms of illness was respiratory (52% of cases), followed by neurological (40% of cases) (Walters et al., 2009).

The results of the present investigation showed that pyrethrin is genotoxic to human lymphocytes. Pyrethrin induced significant elevation in SCEs and the increase is dose-dependent. Thus, in addition to respiratory and neurological injury caused by exposure of humans to pyrethrin, the drug might be associated with DNA damage. This damage might be due to oxidative stress induced by pyrethrin, which is evident the strong elevation in the oxidative stress biomarker 8-OHdG. These results are supported by the findings that in rats cypermethrin, the synthetic derivative of pyrethrin induced astrocyte injury via modulation in Ca(++) and induction of reactive oxygen species and the subsequent activation of JNK and P38 matrix metalloproteinase expression (Maurya et al., 2014). In addition, lambda cyhalothrin, a synthetic pyrethroid insecticide, has been shown to induce chromosomal damage and to increase the frequency of micronucleated erythrocytes in bone marrow cells in a dose-dependent manner (Celik et al., 2003; Fahmy & Abdalla, 2001). Moreover, in rats, high doses of pyrethrins have been shown to induce liver tumors (Price et al., 2007). This induction of liver tumors might be due to induction of oxidative stress in liver cells and the subsequent DNA damage (Muguruma et al., 2006). Similar effects of pyrethrin has been reported in thyroid gland of exposed rats (Finch et al., 2006). Since in humans, expected half-life of pyrethrin is 50 h (Wester et al., 1994) and the present study showed
genotoxicity of pyrethrin, more precautions should be taken into consideration when using pyrethrin at homes.

The results presented in this study showed cytotoxicity of pyrethrin to cultured human lymphocytes at high doses as measured by the decrease in MI and PI. This result is supported by previous literature that showed cytotoxic effect of pyrethrin to the cultured MCF7 human breast carcinoma cell line (Kakko et al., 2004a) and to SH-SY5Y neuroblastoma cells (Kakko et al., 2004b). The synthetic pyrethroid insecticide lambda cyhalothrin has been shown to cause cytotoxicity to polychromatic erythrocytes in vivo using rat model (Celik et al., 2003). More studies are required to identify the mechanism of this cytotoxicity which may be related to interference with mitochondrial function via inhibition ATPases in the membranes (Kakko et al., 2000).

In conclusion, pyrethrin is genotoxic and cytotoxic to cultured human lymphocytes. This toxicity might be related to induction of oxidative stress inside the cells.

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Declaration of interest

The authors declare that there are no conflicts of interest.

References


