Effect of Ginger Extract on Deltamethrin Induced Histomorphological and Immunohistochemical Changes in Testes of Albino Rats

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Abstract: The current study investigated the effect of ginger (Zingiber officinale Roscoe) extract on deltamethrin induced testicular damage in albino rats. Treating animals with deltamethrin at a dose level of 1/10 LD50, 3 days weekly for 6 weeks caused a decrease in body and testes weights. Remarkable decreases were also noted in sperm cell concentrations and sperm motility. In addition histopathological results revealed degeneration of spermatogenic cells, congestion of blood vessels and destruction of Leydige cells. The diameters of the seminiferous tubules and heights of their germinal epithelium were significantly reduced. Immunohistochemical results showed that Bax expression was increased in Leydige cells and p53 expression increased in spermatocytes of testes of deltamethrin treated rats. According to these results, deltamethrin induced oxidative stress and caused apoptosis in testes of albino rats. Treating animals with deltamethrin and ginger revealed an improvement in the histological changes observed in animals treated with deltamethrin and increased sperm concentration and motility. Moreover, ginger treatment leads to a decrease in the expression of p53 and bax. This effect of ginger extract may be attributed to its antioxidant activity.

Keywords: Deltamethrin, testes, ginger, apoptosis, p53, Bax.

1. Introduction

Insecticides are frequently used in agriculture for the eradication of insects and the heavy use of chemical insecticides resulted in lethal effects on non-target organisms inagroecosystem, and has direct toxicity to users (Ansari and Kumar 1988, Kalavathy et al., 2001). Synthetic Pyrethroids are manufactured analogues of naturally occurring pyrethrins found in the flowers of Chrysanthemum cinerariaefolium (Luty et al., 2000). These insecticides are commonly divided into two types: Type I compounds or (T-syndrome pyrethroids), which lack an alphacyano substituent, and Type II compounds or (CS-syndrome pyrethroids), which contain an alphacyanophenoxbenzyl substituent (Naumann, 1990). Moreover, the use of pyrethroid insecticides has been documented since 1970s, preliminary evidence suggested that its usage has been increasing and the pyrethroid insecticides are replacing the organophosphorus insecticides for residential control (Sudakine, 2006). So, human exposure to the pyrethroid insecticides was increased (Khan et al., 2008). Deltamethrin is a synthetic pyrethroid with potent insecticidal property. The technical grade deltamethrin comprises of eight stereomeric esters (four cis and four trans isomers) of the dibromo analogue of chrysanthemic acid, 2,2-dimethyl-3-cyclopropanecarboxylic acids. Deltamethrin is extensively used as an ectoparasiticide in animals and as insecticide in crop production and public health programme (Tuet al., 2007). Deltamethrin was found to cause various adverse effects in experimental animals. Treating pregnant rats from day 6 to day 15 of pregnancy with deltamethrin caused retardation of growth, hypoplasia of the lungs, dilation of the renal pelvis and increase in placental weight (Abdel-Khalik et al., 1993). Lukowicz-Ratajczak and Krechniak (1992) reported that deltamethrin suppress immune system in Balb/c mice. It inhibited the mitotic index and increased the frequency of chromosomal aberrations in the bone marrow of rats (Agarwalet al., 1994). Reproductive toxicity and endocrine disruption, effects related to deltamethrin exposure have been reported in numerous studies (Abdallah et al., 2010).

Herbal and natural products represent one of the most common forms of complementary and alternative medicines. Many natural product extracts have been found to have a variety of pharmacological effects. Ginger (Zingiber officinale Roscoe) is an example of plants which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally and culinary useful part (Mascolo et al., 1989). Ginger was used in popular in relief the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Gilani and Rahman, 2005). Many
pharmacological effects were reported on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects demonstrated are anti-platelet, antioxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxicity and anti-arthritic effect (Fisher-Rasmussen et al., 1991, Sharma et al., 1991, Kamtchovinget al., 2002). Khaki et al., (2009) reported that ginger extract possesses a protective effect against DNA damage induced by H₂O₂ and enhanced sperm healthy parameters in rats. The effect of ginger on male reproduction was studied by some investigators (Hafez 2010, Zahediet al., 2010, Sakr and Badawy, 2011). The current study was designed to investigate the effects of ginger extract on histomorphological and immunohistochemical changes induced in testes of albino rats by deltamethrin.

2. Materials and Methods
Preparation of ginger aqueous extract
Ginger (Z. officinale R.) rhizome was purchased from the local market. One kilogram fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried and powdered. 125 g of this powder were macerated in 1000 ml of distilled water for 12 h at room temperature and were then filtered. The concentration of the extract is 24 mg/ml. Each experimental animal in the present study was orally given 1 ml of the final aqueous extract (Kamtchovinget al., 2002).

Animals and treatments
Adult (150±10 g body weight) male rats of Wistar strain were housed in groups of two per cage, maintained under controlled conditions of temperature (22 ± 2°C) and light (12 :12L : D) and maintained under controlled conditions of temperature (22 ± 2°C) and light (12 :12L : D) and provided with rodent food and water ad libitum. Animals were divided into 4 groups:

Group 1: Animals of this group (10 rats) were considered as control and were given 0.5 ml corn oil.

Group 2: Each animal of this group (20 rats) was orally given 0.5 ml of final aqueous extract of ginger (24 mg/ml) 3 days weekly for 6 weeks.

Group 3: Animal of this group (20 rats) were orally given deltamethrin at a dose level of 1/10 LD₅₀ (0.6 mg/kg body weight) (Odaet al., 2011) in corn oil, 3 days weekly for 6 weeks.

Group 4: Animals in this group (20 rats) were given the same dose of deltamethrin given to animals of group 2 followed by 0.5 ml of final aqueous extract of ginger (24 mg/ml) 3 days weekly for 6 weeks.

Epididymal sperm concentration and motility

The left epididymis of each rat was used for the determination of epididymal sperm concentration using the Neubauer haemocytometer, while % sperm motility was determined as described by Sönmez et al., (2005). Fluid was obtained from the epididymis with a pipette and diluted to 2 ml with tris-buffer solution. The percentage of motility was evaluated at ×1000 magnification.

Histological Study
Immediately after decapitation animals were dissected after 3 and 6 weeks, testis were removed from treated and control groups and fixed in Bouin’s solution. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. For histological examination, sections were stained with Ehrlich’s hematoxylin and counterstained with eosin. Seminiferous tubules diameter and germinal epithelial height were measured from the spermatogenic cells on the inner surface of the basement membrane through the most advanced cell types lining the lumen of the tubules.

Immunohistochemical Study
From each testis block, 4 microns thick sections were cut on Neoprene-coated slides. The immunostaining was performed using the avidin-biotin complex (ABC) method and an automatic autostainer (CODE-ON Immuno/DNA slide stainer: Biotek solution, Santa Barbara, CA). Slides were deparaffinized and blocked for endogenous peroxidase with 1.75% hydrogen peroxide in methanol for 20 mm, antigen retrieval for 15 mm using Biogenex Antigen Retrieval Citra solution in 90°C water bath for 30 mm. The slides were allowed to cool for 20 min before continuing. Slides were then blocked by normal horse serum for 5 mm at 37°C. The monoclonal antibody was applied overnight in humid medium at room temperature followed by the biotinylated secondary antibody for 15 min at 37°C and the ABC complex for 15 min at 37°C (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA). Diaminobenzidine (DAB) was applied for 20 min at room temperature as chromogenic slides were counterstained with hematoxylin, dehydrated, and covered by coverslips. In negative control slides, the same system was applied with replacement of the monoclonal antibody by diluted normal bovine serum. Baximmunostaining was performed using polyclonal rabbit-anti-human (A3533 Ig fraction; DAKO, Glostrup, Denmark) at a dilution of 1:50. Monoclonal antibody which recognizes both wild type and mutant p53 was used.

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Statistical Analysis

Data were expressed as mean values ± SD and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at P < 0.05. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® 4 Inc., USA).

3. Results

Morphmetrical results

Results in table 1 revealed that rats intoxicated with deltamethrin showed significant decrease in the body and testes weights after 6 weeks of treatment. Treatment with ginger caused apparent increase in body and testes weights (table1). Epididymal sperm concentration of 4.50±0.5 million/ml obtained in the control rats was significantly higher (p<0.05) than those of rats treated with deltamethrin (2.3±0.4) after 6 weeks. Sperm motility in the control group (60.2±3.5%) was significantly decreased in deltamethrin group. On the other hand, sperm concentration and motility increased in rats given deltamethrin and ginger extract (table2). Data in table 3 showed that treatment with deltamethrin caused atrophy of the seminiferous tubules. The diameter of seminiferous tubules was significantly decreased in deltamethrin treated rats. A decrease in epithelial height of seminiferous tubules is also recorded in compare with control ones. However, treatment with ginger extract caused an increase in diameters and epithelial heights of the seminiferous tubules.

Histological observations

Histological examination of testis of control rat showed normal appearance of seminiferous tubules and interstitial tissue. Sertoli cells and spermatogenic cells (Spermatogonia, 1ry and 2ry spermatocytes and sperm) appeared normal. Interstitial tissue and Leydige cells can be recognized (Fig.1A).Animals intoxicated with deltamethrin showing many histomorphological changes. After 3 weeks, the seminiferous tubules lost its shape and appeared with irregular outline and widely separated from each other (Fig.1B). The germ cells were degenerated and exfoliated in the lumen center (Fig.1C). Intertubular blood hemorrhage was observed with abnormal appearance of germ cells which showed pyknotic nuclei (Fig.2A). These alterations became severe after 6 weeks. In these specimens, the intertubular tissue was degenerated and showed many vacuoles with blood hemorrhage and most of the seminiferous tubules were devoid of germ cells (Fig.2B). Examination of testes of animals treated with deltamethrin and ginger revealed less prominent histopathological changes when compared with deltamethrin group. Most of the seminiferous tubules appeared with increase of spermatogenic cells and an increase in the number of sperm bundles was seen (Fig.2C).

Immunohistochemical results

Immunohistochemical examination of testes of control rats revealed that p53 was expressed in germ cells (spermatogonia, primary and secondary spermatocytes) (Fig.3A). Animals treated with deltamethrin showed an increase of p53 expression in these cells (Fig.3B). Treating animals with deltamethrin and ginger showed a decrease of p53 expression. The percentage of p53 expression was 14% in control rats compared with 46% and 22% in rats intoxicated with deltamethrin and deltamethrin plus ginger, respectively (Fig.4). Figure 5 (A & B) showed the expression of Bax in Leydige cells. The number of the Bax positive staining cells increased in Leydige cells of rats treated with deltamethrin compared with control and decreased after treatment with deltamethrin and ginger (Fig.6).

Table 1.Change in mean value of the body and testes weights in rats of different groups after 6 weeks.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Body weight(g)</th>
<th>Testes weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>167 ±3.8</td>
<td>2.52 ± 0.5</td>
</tr>
<tr>
<td>Ginger extract</td>
<td>171 ± 4.5</td>
<td>2.80± 0.3</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>101 ± 2.4*</td>
<td>1.67± 0.2*</td>
</tr>
<tr>
<td>Deltamethrin+ ginger</td>
<td>152± 1.2</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

(*). Significant at P < 0.05

Table 2. Mean sperm concentration and % sperm motility in rats of different groups.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Sperm concentration (×10^9)</th>
<th>Sperm motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.5 ±0.5</td>
<td>60.2 ± 3.5</td>
</tr>
<tr>
<td>Ginger extract</td>
<td>4.1 ± 0.2</td>
<td>58.5± 2.2</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>2.3 ±0.4*</td>
<td>22.6± 3.7*</td>
</tr>
<tr>
<td>Deltamethrin+ ginger</td>
<td>3.5 ±0.2</td>
<td>41.5± 2.3</td>
</tr>
</tbody>
</table>

(*). Significant at P < 0.05

Table 3. Mean value of the diameter and epithelial height of seminiferous tubules in rats of different groups.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Diameter of tubules</th>
<th>Germinal epithelial height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>223 ±5.6</td>
<td>99.5 ± 6.2</td>
</tr>
<tr>
<td>Ginger extract</td>
<td>211 ± 3.7</td>
<td>86± 3.2</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>144 ± 3.4*</td>
<td>56± 4.2</td>
</tr>
<tr>
<td>Deltamethrin+ ginger</td>
<td>180±6± 2.8</td>
<td>72±4.2</td>
</tr>
</tbody>
</table>

(*). Significant at P < 0.05
Fig. 1. (A): Section in testis of a control rat showing seminiferous tubules with active spermatogenesis, S: sperm, IT: interstitial tissue, (B): After 3 weeks of treatment with deltamethrin showing irregular seminiferous tubules with reduced spermatogenic cells and damaged interstitial tissue (IT), (C): exfoliated degenerated spermatogenic cells in the lumen (arrow), P: pyknotic nuclei, X 300.

Fig. 2. (A): Section in testis of a rat after 3 weeks of treatment with deltamethrin showing degenerated germ cells and intertubular hemorrhage (H), (B): Section in testis of a rat after 6 weeks of treatment with deltamethrin showing highly degenerated interstitial tissue with vacuoles (V) and blood hemorrhage. The seminiferous tubules appeared with complete absence of germ cells, (C): Section in testis of a rat after treatment with deltamethrin and ginger showing increase of germ cells and sperm bundles (S), X 300.
Fig. 3. Seminiferous tubules of (A): a control rat, (B): deltamethrin-treated rat showing p53 expression in germ cells (arrows), X 300

Fig. 4. Percentage of p53 positive staining germ cells in different animal groups, (**) significant at P<0.05

Fig. 5. Seminiferous tubules of (A): a control rat, (B): deltamethrin-treated rat showing Bax-positive staining Leydig cells (arrows), X 300

Fig. 6. Percentage of Bax positive staining cells in different animal groups, (*) significant at P<0.05
4. Discussion

Administration of deltamethrin caused a decrease in body as well as testes weights of rats. The sperm concentration and sperm motility was also decreased. Similarly, Abdel-Khalik et al., (1993) found that maternal weight gain was reduced in Sprague-Dawley rats intoxicated with deltamethrin. Abd el-Aziz et al., (1994) reported that deltamethrin significantly decreased the weight of testes, seminal vesicle, and prostate glands. Significant decreases were also noted in sperm cell concentrations, percentage of live cells and sperm motility. Male rats administered with deltamethrin for 65 days at doses of 1 or 2 mg/kg showed significantly lower testicular, prostate gland, and seminal vesicle weight. The mating success of treated rats was reduced by 50% during the study and for two months afterwards at both doses (Bradberry et al., 2005). Takahashi and Oishi, (2001) mentioned that the weight of the testis is basically dependent on the mass of the differentiated spermatogenic cells; the reduction in the weight of the testis may be due to decreased number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity. The obtained results support this speculation; deltamethrin induced histopathological alterations in the testes of treated rats and lead to inhibition of spermatogenesis. The effect of deltamethrin on male reprodution was studied by (Abdallah et al., 2010; Oda and El-Maddawy, 2011).

Immunohistochemical results revealed that deltamethrin increased expression of p53 and Bax in testes of rats. Several investigators reported that p53 expression is confined to the primary spermatocytes within the testicular seminiferous tubules (Almonet et al., 1993; Sjöblom and Lähdetie, 1996; Stephan et al., 1996). These studies support the present results in which P53 was detected within the seminiferous epithelium and not in the Leydigee cells or other interstitial cells. However, Bax protein was detected in interstitial cells suggesting that Bax gene expression is not under the exclusive control of p53 (Taylor et al., 1998). There are two main pathways known to cause apoptosis (Fadeel and Orrenius, 2005). One is intrinsic (mitochondrial) and the other is extrinsic pathway. Intrinsic pathway triggers by stress-caused reasons in the cell as irradiation, toxins and oxidative stress. These stress sources can affect the members of Bcl-2 family, which stabilizes or destabilizes the mitochondrial membrane by proapoptotic or antiapoptotic (Bax and Bcl-XL) factors. Extrinsic pathway triggers by extracellular ligands (Fas-ligand [FasL]). Apoptosis of germ cells may be induced with both extrinsic and intrinsic pathways.

It is known that Bax, Bad, Bcl-xl, and Bcl-2 are expressed in rodent testes (Krajewskiet al., 1996). The tumor suppressor p53 is a potent inducer of apoptosis (Symonds et al., 1994) and is found in unusually high concentration in germ cells (Almonet et al., 1993). During spermatogenesis, apoptosis in testicular germ cells is recognized as an important physiologic mechanism to limit the germ cell population to numbers that the Sertoli cells can support (Billiget al., 1995). Regulation of germ cell apoptosis in the normal testis is controlled by the Bcl-2 family, p53 and Fas-signaling pathway (Woolveridge and Morris, 2000). Many toxicants were reported to induce germ cells apoptosis such as MEHP, 2,5-hexandione, nitrobenzene, deltamethrin, and hydroxyurea (Shinoda et al., 1998; El-Gohary et al., 1999; Shin et al., 1999). Increased apoptosis of testicular cells coincided with increased expression of the apoptosis-promoting proteins Bax and p53 was recorded in rats following combined exposure to Pyridostigmine Bromide, N,N-diethyl m-toluamide and permethrin (Abou-Donia et al., 2003). Excessive or inadequate apoptosis of testicular cells result in abnormal spermatogenesis, azoosperma and severe oligoozoospermia (Lin et al., 1997).

The production of ROS is a normal physiological event in various organs including the testis. On the other hand, overproduction of ROS can be harmful to sperm and subsequently to male fertility (Akiyama, 1999). Pyrethroids are known to generate reactive oxygen species (ROS) and result in oxidative stress in intoxicated animals (Kale et al., 1999). Oda and El-Maddawy (2011) has reported that deltamethrin induced lipid peroxidation in testes of rats. Lipid peroxidation is a marker of oxidative damage, which plays an important role in the toxicity of many pesticides. El-Gohary et al., (1999) reported the deltamethrin induced lipid peroxidation and nitric oxide production in plasma of rats. Aitken et al., (1989) has shown that increase of lipid peroxidation can lead to oxidative damage to sperms DNA, alter membrane functions, impair motility and possibly have a significant effect on the development of spermatoptea. The link between oxidative stress and apoptosis was recorded (Buttke and Sandstorm, 1994). Thus, deltamethrin may induce oxidative stress and resulted in the recorded histomorphological alterations and apoptosis in testes of albino rats which involved expression of p53 and Bax.

The current results showed that ginger extract ameliorates the histopathological alterations caused by deltamethrin in testes of albino rats. Moreover, it caused decrease of apoptosis as indicated by decrease of expression of p53 and bax. Similarly, Sakr and Badawy (2011) reported that ginger improve the
histological alterations and reduce apoptosis in testis of mice treated with metiram fungicide. Amin and Hamza (2006) demonstrated that Z. officinal extract reduced the extent of cisplatin-induce sperm abnormality, enhanced sperm motility and testicular damage by increase the activities of testicular antioxidants. Ginger rhizome was found to overcome reproductive toxicity of gentamicin and induced spermatogenesis through the elevation of testosterone levels (Zahediet al., 2010). Hafez (2010) reported that intake of ginger roots as a drink may be beneficial for diabetic patients who suffer from sexual impotency as their extracts induce antidiabetic activity and enhance male fertility in diabetic rats. Amin et al., (2008) reported that ginger attenuated the testicular damage and decreased apoptotic damage both in testes and sperms. It also retained the control value of p53 protein expression in the testicular tissue. Morakinyo et al., (2010) reported that co-administration of aqueous ginger extract with arsenite was found to protect against adverse change in the reproductive organ weight, attenuate the decrease in sperm functions, enhance plasma reproductive hormones level along with increased antioxidants activities and reduced peroxidation Qureshiet al., (1989) reported that ginger extract significantly increased the sperm mortality and sperm contents in the epididymis and vas deference without producing any spermatoxic effect. Aqueous extract of Z. officinal was found to increase weight of testes, the serum testosterone level and epididymal α-glucosidase activity. in male rats (.Kamotchivinget al., 2002). Khaki et al., (2009) reported that administration of ginger significantly increased sperm percentage, viability, motility and serum total testosterone in rats.

The effect of ginger and its extracts were attributed to antioxidant activity of its major ingredients namely Zingerone, gingerdiol, Zingiberene, gingerols and shogoals( Zancanet al., 2002). It is concluded from the obtained results that one or more constituents of the used ginger extract may ameliorate the testicular abnormalities induced by deltamethrin in rats.

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References