ENDOCRINAL DISRUPTION EFFECTS OF ATRAZINE ON THE PUBERTY OF JUVENILE ALBINO RATS: A SUB-CHRONIC STUDY

By

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ABSTRACT

Background: Atrazine (ATR) is one of the most commonly used triazine herbicides in the world. It was found that ATR can cause adverse effects on reproductive function in both genders of several mammalian and non-mammalian species. The aim of this work was to evaluate the pubertal hormonal disruption effects of atrazine herbicide during and after its sub-chronic administration in juvenile albino rats of both sexes. Material and methods: One hundred twenty six juvenile albino rats of both sexes were used. They were divided into 3 equal groups as follow: Group I (negative control group), group II (positive control group), group III (ATR group). Each group was subdivided equally into two subgroups; male rats (subgroup a) and female rats (subgroup b). Female rats were monitored daily for vaginal opening. At the end of the 3rd and the 6th week of the study, 7 rats of each subgroup were submitted to estimate the serum levels of estradiol and luteinizing (LH) hormones in all rats of both sexes in addition testosterone in male rats. Then the rats were sacrificed. The testis and epididymis in males, uterus and ovary in females were dissected and subjected to histopathological examination. The remaining rats were left without intervention for another 3 weeks served as follow up group. Results: Atrazine was found to delay puberty in male rat presented by significantly decreased levels of testosterone level and increased in the estradiol levels, impaired spermatogenesis and decrease in number and size of Leydig cells with disorganization. It also was found to delay puberty in females denoted by delayed vaginal opening, underdevelopment of the uterus, impaired folliculogenesis and ovulation in the ovaries. Three weeks of follow up resulted in partial improvement. Recommendation: more efforts are needed to limit exposure to atrazine especially in ground and drinking water.

Key words: herbicide, atrazine, puberty, endocrine disruption.

INTRODUCTION

Herbicides are a class of pesticides that are marketed specifically for the purpose of killing or inhibiting the growth of weeds. Because of widespread use and easy accessibility, poisoning with herbicides has become a global environmental and health problem (Bhatti et al., 2011).

Atrazine, 2-chloro-4-(ethyl amino)-6-(isopropyl amino)- s-triazine, is a widely used herbicide for the control of grass and broadleaf weeds in crops such as maize, sugarcane, and pineapple plantations. Although it has been banned in the European Union in 2004 because of its persistent ground water contamination, it is still one of the most widely used herbicides in the world (Ackerman, 2007).

As a result of its indiscriminate use, residues of ATR are found not only in plants, soil, water, and cultivated ground but also in agricultural products such as fruits, milk, butter, and sugar beet (Purcell et al., 2001).

Human exposure can occur through ingestion of these products, drinking contaminated water, consumption of contaminated fish, also through both inhalation and dermal absorption during its manufacture, its formulation and its application by spraying (Singh et al., 2011).

Atrazine was considered as an endocrin disruptor as it was found that ATR can cause adverse effects on reproductive function in both genders of several mammalian and non-mammalian species. Moreover, it has been reported to inhibit androgen receptor function in vitro. On the other hand, it can affect thyroid gland function in mammalian animals and lead to delayed sexual maturation (Hayes et al., 2002; Betancourt et al., 2006; Oka et al., 2008 and Rey et al., 2009).

The aim of this work was to evaluate the pubertal hormonal disruption effects of ATR herbicide during and after its sub-chronic oral administration in juvenile albino rats of both sexes through daily examination for vaginal opening, assay of testosterone, estradiol and lutetinizing (LH) hormones. Histopathological examination of testis, epididymis, uterus and ovary were also evaluated.

MATERIAL AND METHODS

(I)Material:

a) Chemical:

Atrazine in the commercial product gesaprim 80WP (an 80% wettible powder) was obtained from Syngenta Crop Protection / Basel, Switzerland, import and refilling by El-Nasr company for intermediate chemicals, in the form of white powder. Corn oil was obtained in the...
form of oily solution as solvent agent from commercial sources.

b) Animals:

Juvenile albino rats of both sexes with average age of 3-4 weeks (Stoker et al., 2000b), each weighed about 50-75 gm were obtained from animal’s house, Faculty of Medicine, Zagazig University.  

**Group I** (negative control group): These rats were separated from each other for another 3 weeks for follow up. At the end of the 3rd and the 6th week of the study, blood samples were obtained from the retro-orbital plexus as described by Joslin (2009) from each subgroup to estimate the serum levels of estradiol and luteinizing hormone (LH) and significant increases in the mean value of estradiol compared to those of positive control male group (Table 3).

These effects were more prominent after 6 weeks of ATR administration. In the follow up group, although the mean values of testosterone and LH showed significant increases in comparison with those of positive control male group, these mean values showed significant increases when compared to those after 6 weeks of ATR administration. Also, the mean value of estradiol showed a significant increase after the follow up in comparison to that of positive control male group, but this mean value showed a significant decrease when compared to that after 6 weeks of ATR administration (Tables 3, 4).

Regarding female rats, ATR female group showed a significant increase in the mean value of LH in comparison with that of positive control female group. This effect was more prominent after 6 weeks of ATR administration. In the follow up group, although the mean value of LH showed a significant decrease compared to that of positive control female group, this mean value showed a significant increase when compared to that after 6 weeks of ATR administration (Tables 5, 6).

Moreover, ATR female group showed non-significant changes in the mean values of estradiol compared to that of positive control female group after 3, 6 weeks of ATR administration and after the follow up period (Tables 5, 6).

**Daily examination for complete vaginal opening:**

In this study ATR female group showed a highly significant delay in the mean value of the age at complete vaginal opening when compared to the mean value of the age at complete vaginal opening of positive control female group (Table 7).

**Histopathological results:**

Microscopic examination of the testicular specimens of the male rats revealed reduction in layering of the germinal epithelial lining, maturation arrest, hypospermia, dissociation and

Endocrinal Disruption Effects of Atrazine on........
separation of germ cells, intraluminal sloughing of germ cells and apoptosis in germ cells. The interstitial tissue showed edema with few disorganized small vacuolated Leydig cells with strip like configuration.

The appearance of these histopathological changes was time dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery noticed in follow up group (Table 8) & (Plate I, Figs B,C,D,E).

Microscopic examination of the epididymal specimens of the male rats showed no sperms and hypospermia. The appearance of these histopathological changes was time dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery had occurred after the follow up (Table 9) & (Plate II, Figs B,C,D).

Microscopic examination of the uterus showed underdeveloped structure in the form of absence of apparent endometrial glands which was regressive in the follow up group (Table 11) & (Plate III, Figs B,C, D).

By the end of the 3rd week of ATR administration, microscopic examination of the ovarian sections showed numerous primary follicles separated by cellular stroma with no mature graafian follicles and no histopathological changes (Table 11) & (Plate IV, Fig B).

By the end of the 6th week of ATR administration, the same histo-pathological changes as those described by the end of the 3rd week of ATR administration were noticed. In addition, appearance of secondary follicle, atretic follicles were observed (Table 11) & (Plate IV, Fig C).

After the follow up, the ovarian sections showed mature graafian follicles and corpus luteum were formed with decrease in numbers of atretic follicles (Table 11) & (Plate IV, fig D).

### Table (1): Student (t) test for comparison of the mean values of serum testosterone (ng/ml), estradiol (pg/ml) and LH (mIU/ml) between –ve control male group and +ve control male group (daily gavaged with 0.5ml of corn oil) after 3, 6 and 9 weeks of the study in male albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>-ve control male “Ia” Mean ± SD</th>
<th>+ve control male “IIa” Mean ± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>3 weeks</td>
<td>0.96±0.04</td>
<td>0.94±0.2</td>
<td>0.13</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>2.1±0.2</td>
<td>2.03±0.3</td>
<td>1.109</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>9 weeks</td>
<td>2.77±0.23</td>
<td>2.67±0.9</td>
<td>0.37</td>
<td>0.71</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>3 weeks</td>
<td>9.14±0.7</td>
<td>9.35±1.0</td>
<td>0.45</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>9.20±0.63</td>
<td>9.3±1.97</td>
<td>0.09</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>9 weeks</td>
<td>10.7±0.76</td>
<td>10.6±1.3</td>
<td>0.25</td>
<td>0.8</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>3 weeks</td>
<td>0.21±0.04</td>
<td>0.24±0.03</td>
<td>1.58</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>0.32±0.11</td>
<td>0.33±0.06</td>
<td>0.11</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>9 weeks</td>
<td>0.77±0.1</td>
<td>0.73±0.17</td>
<td>0.8</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Number of sacrificed rats for each group was 7 rats.
SD : Standard Deviation. p>0.05 : non-significant

### Table (2): Student (t) test for comparison of the mean values of serum estradiol (pg/ml) and LH (mIU/ml) between -ve control female group and +ve control female group (daily gavaged with 0.5ml of corn oil) after 3, 6 and 9 weeks of the study in female albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>-ve control female “Ib” Mean ± SD</th>
<th>+ve control female “IIb” Mean ± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/ml)</td>
<td>3 weeks</td>
<td>12.29±1.5</td>
<td>12.8±2.4</td>
<td>0.47</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>14.6±2.4</td>
<td>14.3±2.7</td>
<td>0.1</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>9 weeks</td>
<td>19.16±3</td>
<td>19.8±3.7</td>
<td>0.23</td>
<td>0.81</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>3 weeks</td>
<td>0.21±0.02</td>
<td>0.24±0.04</td>
<td>1.77</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>0.32±0.04</td>
<td>0.3±0.07</td>
<td>0.64</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>9 weeks</td>
<td>0.6±0.11</td>
<td>0.55±0.13</td>
<td>0.78</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Number of sacrificed rats for each group was 7 rats.
SD : Standard Deviation. p>0.05 : non significant.
Table (3): Student (t) test for comparison of the mean values of serum testosterone (ng/mL), estradiol (pg/mL) and LH (mIU/mL) between +ve control male group “IIa” and ATR male group “IIIa” after 3, 6 weeks of ATR administration and after the follow up in male albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>+ve control male group “IIa” Mean ± SD</th>
<th>ATR male group “IIIa” Mean ± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/mL)</td>
<td>After 3 weeks of ATR administration</td>
<td>0.94 ±0.2</td>
<td>0.24 ±0.1</td>
<td>7.35</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>After 6 weeks of ATR administration</td>
<td>2.03±0.3</td>
<td>0.15±0.02</td>
<td>16.54</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>After the follow up</td>
<td>2.67±0.9</td>
<td>1.06±0.5</td>
<td>4.07</td>
<td>0.0015*</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>After 3 weeks of ATR administration</td>
<td>9.35 ±1.0</td>
<td>12.6 ±1.4</td>
<td>4.57</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>After 6 weeks of ATR administration</td>
<td>9.3±1.97</td>
<td>19.4±3.3</td>
<td>6.99</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>After the follow up</td>
<td>10.6±1.3</td>
<td>13.4±3.1</td>
<td>2.21</td>
<td>0.047*</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>After 3 weeks of ATR administration</td>
<td>0.24±0.03</td>
<td>0.18±0.06</td>
<td>2.36</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td>After 6 weeks of ATR administration</td>
<td>0.33±0.06</td>
<td>0.12±0.04</td>
<td>7.7</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>After the follow up</td>
<td>0.73±0.17</td>
<td>0.28±0.04</td>
<td>6.82</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Number of sacrificed rats for each group was 7 rats.
ATR: Atrazine
*: significant (p<0.05)
**: highly significant (p<0.001).

Table (4): A statistical comparison between the mean values of serum testosterone (ng/mL), estradiol (pg/mL) and LH (mIU/ml) in ATR male albino rats “IIIa” after 3 and 6 weeks of oral ATR administration (300 mg/kg) and after the follow up by ANOVA and LSD tests:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>After 3 weeks of ATR administration Mean ± SD</th>
<th>After 6 weeks of ATR administration Mean ± SD</th>
<th>After the follow up Mean± SD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone(ng/ml)</td>
<td></td>
<td>0.242±0.1</td>
<td>0.148±0.02</td>
<td>1.065±0.54</td>
<td>17.4</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Estradiol(pg/ml)</td>
<td></td>
<td>12.6±1.4</td>
<td>19.4±3.3</td>
<td>13.4±3.1</td>
<td>12.93</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>LH(mIU/ml)</td>
<td></td>
<td>0.18±0.06</td>
<td>0.12±0.04</td>
<td>0.28±0.04</td>
<td>20.1</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Number of sacrificed rats for each group was 7 rats.
ATR: Atrazine
*: significant (p<0.05)
**: highly significant (p<0.001).
a: significance between 3 & 6 weeks of ATR administration (P< 0.05)
b: significance between 3 weeks of ATR administration & follow up group (P< 0.05)
c: significance between 6 weeks of ATR administration & follow up group (P< 0.05)
Table (5): Student (t) test for comparison of the mean values of serum estradiol (pg/mL) and LH (mIU/mL) between +ve control female group “IIb” and ATR female group “IIIb” after 3, 6 weeks of ATR administration and after the follow up in female albino rats.

<table>
<thead>
<tr>
<th>Duration</th>
<th>ATR female group “IIIb” Mean ± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 3 weeks of ATR administration</td>
<td>12.4±2.4</td>
<td>0.3</td>
<td>0.76</td>
</tr>
<tr>
<td>After 6 weeks of ATR administration</td>
<td>15.72±4.2</td>
<td>1.92</td>
<td>0.07</td>
</tr>
<tr>
<td>After the follow up</td>
<td>18.6±4.2</td>
<td>1.11</td>
<td>0.28</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 3 weeks of ATR administration</td>
<td>0.19±0.03</td>
<td>2.64</td>
<td>0.02*</td>
</tr>
<tr>
<td>After 6 weeks of ATR administration</td>
<td>0.12±0.06</td>
<td>5.16</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>After the follow up</td>
<td>0.33±0.07</td>
<td>3.76</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Number of sacrificed rats for each group was 7 rats.
SD : Standard Deviation.                           ATR: Atrazine
*: significant (p<0.05)                               **: highly significant (p<0.001).

Table (6): A statistical comparison between the mean values estradiol (pg/ml) and LH (mIU/ml) in ATR female albino rats “IIIb” after 3 and 6 weeks of oral ATR administration (300 mg/kg) and after the follow up by ANOVA and LSD tests.

<table>
<thead>
<tr>
<th>Duration</th>
<th>After 3 weeks of ATR administration Mean± SD</th>
<th>After 6 weeks of ATR administration Mean± SD</th>
<th>After the follow up Mean± SD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol(pg/ml)</td>
<td>12.4±2.4</td>
<td>15.72±4.2</td>
<td>18.6±4.2 a</td>
<td>4.9</td>
<td>0.02*</td>
</tr>
<tr>
<td>LH(mIU/ml)</td>
<td>0.19±0.03</td>
<td>0.12±0.06 b</td>
<td>0.33±0.07 a c</td>
<td>26.33</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Number of sacrificed rats for each group was 7 rats.
A TR: Atrazine                                        SD : Standard Deviation.
*: significant (p<0.05)                               **: highly significant (p<0.001).
a: significance between 3 weeks of ATR administration and follow up group (P< 0.05)
b: significance between 3 and 6 weeks of ATR administration (P< 0.05)
c: significance between 6 weeks of ATR administration & follow up group (P< 0.05)

Table (7): Student (t) test for comparison of the mean value of the age at complete vaginal opening between +ve control female group “IIb” and ATR female group “IIIb” along duration of the study in peripubertal female albino rats.

<table>
<thead>
<tr>
<th>+ve control female “IIb” Mean ± SD</th>
<th>ATR female group “IIIb” Mean ± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at complete vaginal opening / days</td>
<td>34.7±2.56</td>
<td>45.4±2.7</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Number of rats = 21 rats for each group
SD : Standard Deviation.                           ATR: Atrazine
**: highly significant (P<0.001)
### Table (8): Frequency of histopathological changes in the testis specimens after 3 and 6 weeks of atrazine administration and after the follow up.

<table>
<thead>
<tr>
<th>Histopathological parameter</th>
<th>After 3 weeks of ATR administration</th>
<th>After 6 weeks of ATR administration</th>
<th>After the follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Seminiferous tubules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maturation arrest</td>
<td>6</td>
<td>85.7</td>
<td>1</td>
</tr>
<tr>
<td>Hyposperma</td>
<td>1</td>
<td>14.3</td>
<td>6</td>
</tr>
<tr>
<td>Dissociation and separation of germ cells</td>
<td>3</td>
<td>42.9</td>
<td>4</td>
</tr>
<tr>
<td>Intra luminal sloughing of germ cells</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Apoptosis in germ cells</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Interstitial tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edema &amp; widening</td>
<td>2</td>
<td>28.5</td>
<td>5</td>
</tr>
<tr>
<td>Congested blood vessels</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Leydig cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease in size &amp; number</td>
<td>7</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>Vacuolation</td>
<td>1</td>
<td>14.3</td>
<td>5</td>
</tr>
<tr>
<td>Strip like configuration</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

N= number of rats
% = percentage

ATR: Atrazine

### Table (9): Frequency of histopathological changes in the epididymal specimens after 3 and 6 weeks of atrazine administration and after the follow up.

<table>
<thead>
<tr>
<th>Histopathological parameter</th>
<th>After 3 weeks of ATR administration</th>
<th>After 6 weeks of ATR administration</th>
<th>After the follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>No sperm</td>
<td>6</td>
<td>85.7</td>
<td>2</td>
</tr>
<tr>
<td>Hyposperma</td>
<td>1</td>
<td>14.3</td>
<td>5</td>
</tr>
</tbody>
</table>

N= number of rats
% = percentage

ATR: Atrazine

### Table (10): Frequency of histopathological changes in the uterus after 3 and 6 weeks of atrazine administration and after the follow up.

<table>
<thead>
<tr>
<th>Histopathological parameter</th>
<th>After 3 weeks of ATR administration</th>
<th>After 6 weeks of ATR administration</th>
<th>After the follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Immature uterus (absence of endometrial glands)</td>
<td>5</td>
<td>71.5</td>
<td>4</td>
</tr>
<tr>
<td>Mature uterus</td>
<td>2</td>
<td>28.5</td>
<td>3</td>
</tr>
</tbody>
</table>

N= number of rats
% = percentage

ATR: Atrazine

### Table (11): Frequency of histopathological changes in the ovarian specimens after 3 and 6 weeks of atrazine administration and after the follow up.

<table>
<thead>
<tr>
<th>Histopathological parameter</th>
<th>After 3 weeks of ATR administration</th>
<th>After 6 weeks of ATR administration</th>
<th>After the follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>No mature graafian follicle</td>
<td>5</td>
<td>71.5</td>
<td>4</td>
</tr>
<tr>
<td>Mature graafian follicle</td>
<td>2</td>
<td>28.5</td>
<td>3</td>
</tr>
<tr>
<td>Atretic follicle</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

N= number of examined tissues
% = percentage

ATR: Atrazine
Plate (I) : sections in the testes of male albino rats showing:

- **Fig. (A)**: normal mature testis (control group) (H&E x400).
- **Figs. (B,C,D,E)**: testicular tubules with reduction in layering of the germinal epithelial lining
  - **Fig. (B)**: maturation arrest(→), marked dissociation and separation of germ cells(▲), and few and small Leydig cells (L) (atrazine administration for 3 weeks) (H&E x200).
  - **Fig. (C)**: hypospermia(→), edema in the interstitial tissue(▶) and degenerated and vacuolated Leydig cells (L) (atrazine administration for 6 weeks) (H&E x200).
  - **Fig. (D)**: maturation arrest(→), apoptosis(A) widening and edema in the interstitial tissue(▶) and decrease in number of Leydig cells with strip-like configuration(L) (atrazine administration for 6 weeks) (H&E x400).
  - **Fig. (E)**: hypospermia(→), apoptosis(A), wide interstitial space(▶), and vacuolation with degeneration of Leydig cells(L) (follow up group) (H&E x200).
Plate (II): sections in the epididymis of male albino rats showing:

Fig. (A): normal epididymal ducts distended by seminal fluid containing numerous mature sperms (→) (control group) (H&E x400).

Fig. (B): epididymal ducts containing secretion devoid of sperms (atrazine administration for 3 weeks) (H&E x400).

Fig. (C): Some epididymal tubules are distended by seminal fluid containing few sperms (→). Other tubules are rounded and contain no sperms (●) (atrazine administration for 6 weeks) (H&E x200).

Fig. (D): epididymal ducts distended by seminal fluid containing few sperms (follow up group) (H&E x400).
Plate (III) : sections in the uterus of female albino rats showing:

Fig. (A) : normal mature uterus. The endometrium (E) lined by surface columnar epithelium with the underlying endometrial glands (→) and stroma. Outer to the endometrium is the myometrium (M) formed of bundles of smooth muscle fiber and vascular stroma (positive control female group) (H&E x200).

Fig. (B) : the endometrium (E) has no apparent glandular differentiation (atrazine administration for 3 weeks) (H&E x200).

Fig. (C) : the endometrium (E) with the underlying endometrial glands (→) (atrazine administration for 6 weeks) (H&E x200).

Fig. (D) : the endometrium (E) with the underlying endometrial glands (→) (follow up group) (H&E x200).
Plate (IV) : sections in the ovary of female albino rats showing:
Fig. (A) : normal mature ovary (control group) (H&E x100).
Fig. (B) : numerous primary follicles separated by cellular stroma (atrazine administration for 3 weeks) (H &E x 200).
Fig. (C) : secondary follicle (S) and atretic follicles (→), separated by cellular stroma (atrazine administration for 6 weeks) (H&E x200).
Fig. (D): normal corpus luteum (C) and atretic follicles (→), separated by cellular stroma (follow up group) (H&E x 200).

DISCUSSION
Atrazine is an agricultural pesticide used widely all over the world to control grasses and weeds. Its extensive use and its detection in surface and ground water have made ATR the subject of a number of studies that are designed to determine its possible adverse effects on endocrine parameters and reproductive function in both females and males (Rosenberg et al., 2008).

Puberty is an important milestone in reproductive life as it is a transition between childhood and the adult reproductive stage. Puberty development is a multifaceted process that is under control of different hormonal regulatory mechanisms. It is a vulnerable stage of life and changes in the timing of puberty have been associated with health and psychosocial problems. So, it is considered as an area of great research interest (Maranghi and Mantovani, 2012).

As regard male rats in this study, ATR male rats showed significant progressive and time dependent decreases in the serum levels of testosterone and LH hormones in comparison with those of the positive control male rats. At the same time, these rats showed a significant progressive and time dependent increase in the serum level of estradiol in comparison with that of the positive control male rats. The follow up group showed partial recovery in these hormones.

These biochemical results coincided with those of Stoker et al. (2000a); Victor-Costa et al. (2010) and Jin et al.(2013) who demonstrated that ATR exposure in male rats caused significant decreases in serum testosterone and LH levels and significant increases in serum estradiol levels.

Also, the results of the present study were in a harmony with the studies performed by Feyzi-Dehkhargani et al. (2011)and (2012) on mature male rats received ATR orally for 12, 24 and 48 days. The serum levels of testosterone and LH declined dramatically in time dependent manner.

The partial improvement present in the current study after ATR cessation could be attributed to ATR deposition in adipose tissue as described by Revised et al. (1996).

Stoker et al.(2000a) stated that decreased testosterone synthesis by the Leydig cell after exposure to ATR was secondary to suppression of LH release.
Hayes et al. (2002) hypothesized that decreases in testosterone associated with ATR exposure could be driven by an atrazine-induced increase in aromatase enzyme activity. This enzyme is responsible for catalyzing the conversion of testosterone to estradiol, so leading to depleted testosterone levels via increased estradiol synthesis.

Pogrmic et al. (2009) and Jin et al. (2013) reported that when juvenile male rats and mice respectively exposed to ATR, the transcription of several genes responsible for steroidogenesis such as 17β hydroxysteroid dehydrogenase (17β-HSD) and cytochrome p450 (CYP)17A1 were reduced significantly in the Leydig cells which is accompanied by decreased testosterone concentration.

Normally, testosterone levels rise gradually from postnatal day (PND) 20 to 40, and abruptly double by PND 50 in rats (Monosson et al., 1999). So, the present research suggested that ATR can delay puberty in male rats.

Cooper et al. (2007) demonstrated that the concentration of the GnRH decapetide was increased in the median eminence of the atrazine-received rat. This finding suggested that ATR works at the level of the hypothalamus to inhibit GnRH release decreasing LH hormone level.

Dooley et al. (2013) suggested that ATR metabolites forms adducts on proteins involved in GnRH-induced calcium signaling and subsequent decreased release of LH.

Estrogens appear to play an important role in the regulation of puberty in both girls and boys (Delemarre-van de Waal et al., 2001). Estradiol is the most potent, biologically prevalent and active compound of estrogens (McCarthy, 2008).

Saksena and Lau (1979) showed that, prior to PND 22, serum estradiol and estrone concentrations were high in the male rats. However, there is a dramatic decrease in the serum levels of these two hormones between PND 22 and PND 32. Anderson et al. (1995) and Chapin et al. (1997) found that natural and environmental estrogens typically delay puberty in male rats if administered prior to puberty. Thus, one might expect that a persistent elevation in serum estradiol contributed to the atrazine-induced pubertal delay (Stoker et al., 2000a).

As regard female rats in the present study, ATR produced a significant progressive and time dependent decrease in the mean values of LH hormone in comparison with that of positive control female rats. After the follow up period, there was a partial recovery in the serum level of LH.

These results were in a harmony with that of Gojmerac et al. (2004). They found that administration of ATR slows progression of the estrous cycle and suppresses circulating concentrations of LH in female pigs.

This can be supported by the study of Cooper et al. (2000). They demonstrated that ATR, like aging, caused a dose- and time-dependent decrease in the amplitude of the LH release in female rats.

Foradori et al. (2009a,b and 2011) found that the inhibition of LH pulses in female rats received ATR are likely to be mediated by interference with central mechanisms controlling GnRH release from the hypothalamus.

The results of Foradori et al. (2013) study suggest that ATR acts to inhibit the secretory dynamics of GnRH pulses without interfering with GnRH mRNA and protein synthesis.

In this study, there were non-significant changes in the mean values of estradiol after 3 and 6 weeks of ATR administration and after the follow up in comparison with that of the positive control female group.

Wilhelms et al. (2006) did not support the hypothesis that ATR possesses overt estrogenic activity or can enhance endogenous estrogen production in sexually immature quail.

Tennant et al. (1994) reported that administration of very high doses of ATR expressed anti estrogenic activity in uteri of female rats without expressing intrinsic estrogenic activity.

Moreover, Zorrilla et al. (2010) studied the effects of a 21- and 41-day exposure to simazine, an example of chlorotriazine herbicides, on pubertal development in juvenile/peripubertal female rat. In both the 21-day and 41-day exposure, delayed vaginal opening and non significant changes in serum estradiol levels were reported.

The onset of puberty in the female rats is a transitional process and encompasses the period of vaginal opening (VO) and first ovulation (Rivest, 1991). Vaginal opening is an external marker of puberty onset in the female rats (Mayer et al., 2010).

In the present study, female rats gavaged with ATR 300 mg/kg/day showed a highly significant delay in the age of complete VO by 9.2 days when compared to the positive control female group. This delay in the age at complete VO indicates that ATR delays puberty in female rats.

This result agreed with the results of Laws et al. (2000) and Ashby et al. (2002). The authors found that atrazine significantly delay VO in juvenile female rats.
Laws et al. (2000) demonstrated that the cause of delay in female rat sexual maturation induced by ATR, was due to inhibition of hypothalamic GnRH release and subsequently decreased LH release. 

Testicular specimens of the male rats in this study revealed reduction in layering of the germinal epithelial lining, maturation arrest, hypospermia, dissociation and separation of germ cells, intraluminal sloughing of germ cells and apoptosis in germ cells. The interstitial tissue showed edema with few disorganized small vacuolated Leydig cells with strip like configuration.

The appearance of these histo-pathological changes was duration dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery had occurred after the follow up.

These histopathological lesions were described by Luangpirom and Junsanjuk (2008); Feyzi-Dehkhargani et al. (2011); Milena et al. (2012) and Severi-Aguir and Silva-Zacarin (2012) who support our findings.

Atrazine altered sex organs affecting spermatogenesis process. This is attributed to its oxidative stress and increased reactive oxygen species (Abarikwu et al., 2010; Adesiyan et al., 2011; Feyzi-Dehkhargani et al., 2012 and Abarikwu et al., 2013).

In the present study, epididymal specimens of the male rats showed no sperms and hypospermia. The appearance of these histopathological changes was time dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery had occurred after the follow up.

These results coincided with the results of Stoker et al. (2000a) and (2002) who reported hypospermia in the histopathological evaluation of peripubertal male rats gavaged with ATR and ATR metabolites respectively. This observation was probably a result of the delay in puberty in ATR group.

Uterine tissues of the female rats in the present study revealed underdeveloped structure in the form of absence of apparent endometrial glands which was regressive after in the follow up group.

This result was consistent with the findings of Laws et al. (2000). The authors found underdevelopment of the uterine structure in the form of decreased myometrial development, absent endometrial glands, and immature endometrial stroma.

McMullin et al. (2004) reported that ATR does have anti-estrogenic properties in vitro and in vivo and inhibited binding of estrogen to its receptors. So, the presence of underdeveloped uterus in spite of non significant changes in estradiol levels in the present research, may be attributable to ATR’s inhibition binding of estrogen to its receptors.

By the end of the 3rd week of ATR administration, Ovarian sections in this study showed numerous primary follicles separated by cellular stroma with no mature graafian follicles and no histopathological changes.

By the end of the 6th week of ATR administration, the same histo-pathological changes as those described by the end of the 3rd week of ATR administration were noticed. In addition, appearance of secondary follicle, atretic follicles were observed.

In the follow up group, the ovarian sections showed mature graafian follicles and corpus luteum were formed with a decrease in the number of atretic follicles. This means that there was an improvement in the ovary and ovulation had occurred.

These results were consistent with the results of Laws et al. (2000) and (2003). They observed that juvenile female rats exposed to ATR had absence or decrease in corpus luteum development.

The histopathological results of ovaries of the present study coincide with those of Shibayama et al. (2009). They suggesting that ATR had an anovulatory effect through suppression of the luteinizing hormone.

Atrazine is known to suppress the secretion of LH from the pituitary gland (McMullin et al.,2004). Luteinizing hormone is known to stimulate the development of ovarian follicles in cooperation with follicle- stimulating hormone from the pituitary gland (Shibayama et al., 2009). So, immature ovaries and increased numbers of atretic follicles observed in this study may have been induced by lower LH secretion caused by ATR.

CONCLUSION

From the above mentioned results, it can be concluded that:

Atrazine administration at a dose of 300 mg/kg/day (1/10 LD50) for 6 weeks had resulted in a delayed puberty in the juvenile male albino rats that represented by: significantly decreased levels of testosterone and increased in the estradiol, impaired spermatogenesis and decrease in number and size of Leydig cells with disorganization. These previous effects may be due to central and peripheral effects of atrazine.
• Atrazine administration by the same dose for 6 weeks had resulted in a delayed puberty in the juvenile female albino rats that represented by delayed vaginal opening, underdevelopment of the uterus, impaired folliculogenesis and ovulation in the ovaries. This endocrine disruption effect of atrazine may be due to central effect as LH hormone was decreased.

• Toxic effects induced by atrazine were time dependent.

• Follow up periods for 3 weeks resulted in partial improvement of the reproductive effects of atrazine in both sexes.

RECOMMENDATIONS

More attention should be given to sources, environmental impact of atrazine and its metabolites in ground and drinking water together with more efforts to limit exposure to atrazine which may be a significant contributory factor to the delay puberty leading to health and psychosocial troubles.

REFERENCES


تأثيرات الاضطرابات الهرمونية للأثرازيه على البلوغ في الجرذان البيضاء الغير بالغت: دراسة مسمى قصيرة المدى

المؤثرات في البحث

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مقدمة البحث: يعتبر الأثرازيه واحدا من مبيدات الأعشاب ترتبط الأثرازيه الأكثر استعدادا في العالم. وقد بدت أن الأثرازيه يمكن أن يسبب آثارا ضارة على الوظيفة التناسلية في كلا الجنسين في العديد من أنواع الثدييات وغير الثدييات. الهدف من البحث: كان الهدف من هذا البحث تقييم آثار الاضطرابات الهرموني لسن البلوغ أثناء وبعد تناول الأثرازيه لمدة قصيرة المدى في الجرذان البيضاء الصغيرة من كلا الجنسين.

طريقة البحث: تم استخدام مانة ستة عشرون من سلوك الجرذان البيضاء من كلا الجنسين وقد تم تقسيمهم إلى 3 مجموعات متساوية على النحو التالي: المجموعة الأولى (المجموعة الضارة السائدة) المجموعة الثانية (المجموعة الضارة الضارة المماثلة) المجموعة الثالثة (مجموعة الأثرازيه). تم تقسيم كل مجموعة بمساوي إلى مجموعات فرعية من الذكور (مجموعة أ) والاناث (مجموعة ب). تم فحص فتحة المهبل لإناث الجرذان يوميا وتسجيل عمرها عند اكتئاب فتحة المهبل. في نهاية الأسبوع الثالث والسادس تم أخذ عينات من 7 جرذان من كل مجموعة وقياس مستوى هرمونات الاستروالدو وليتريكين في كل جرذان من كلا الجنسين بالإضافة إلى التنستروين في ذكور الجرذان بعد ذبح الجرذان.

و بعد ذبح الجرذان تم استئصال الخصية، البربخ، الرحم، المبيض وفحصها بالميكروسكوب الضوئي لتحديد التغيرات البوليمورفيك التي حدثت بها. الجرذان المتبقي في كل مجموعة تم متابعتها دون علاج لمدة ثلاثة أسابيع. النتائج: من تناول البحث وجد أن الأثرازيه قد أدى إلى تأخر سن البلوغ في ذكور الجرذان ممثلاً في اختفاء شكل محفز في مستويات مستوى هرمون التستروين وزيادة في مستويات استروالدو. ضعف في تكون الحيوانات المنوية مع وجود عدد غير منظم وقليل من خلايا ايدنت. كما وجد أيضا أن الأثرازيه قد أدى إلى تأخر البلوغ في إناث الجرذان ممثلاً في تأخر فتحة المهبل ولم تلاحظ أشياء مجزأة للرحم، مع ضعف الإباحة في المبيض. المتباعدة لعدة أسابيع بعد توقف إعطاء الأثرازيه أظهرت نقص جزئي. التوصيات: تنص هذه الدراسة ببذل المزيد من الجهود للحد من تعرض الأثرازيه خصوصا في الأرض، ومياه الشرب.