Reproductive toxicity of quinalphos on female albino rats: Effects on ovary and uterus

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Received: 12-08-2015 Accepted: 20-05-2016 DOI:10.18805/ijar.10771

ABSTRACT
Quinalphos is a toxic organophosphate pesticide having a wide applicability to control pests in a number of vegetable crops like ladyfinger, brinjal, cauliflower etc. due to its good penetrative properties and long residual action in animals. The present investigation was carried out to determine its toxic effects on reproduction in the female albino rats. Technical quinalphos (2 mg/kg body weight) was administered orally to female albino rats for 15 and 30 days respectively. The weight of ovary decreased significantly in 30 day treated group. In the same group the corpus luteum also decreased significantly. The histologic observations of the ovary revealed the presence of less number of healthy follicles and more number of atretic follicles. There was a non significant decrease in the surface follicles and corpus luteum of treated rats. Gross morphology of uterus had shown significant increase in epithelial cell height and changes in lumen length even. There was a decline in the number of uterine glands with decreased thickness of myometrium. Hence quinalphos at dose concentration of 2mg/kg body weight is toxic for female reproduction.

Key words: Female albino rats, Organophosphate, Ovary, Pesticide, Quinalphos, Uterus.

Abbreviations: FSH = follicle stimulating hormone; LH = leutinizing hormone; QP = quinalphos

INTRODUCTION
Agriculture in developing countries has become strongly dependent on the use of chemical substances (Ejaz et al 2004). They may affect human health in general and reproductive outcome in particular by occupational exposure, food residues, and contamination of air and water (Krieger 2001). Women who worked with pesticides suspected of being hormonally active had a 60-100% increased odds of experiencing long cycles, missed periods and inter menstrual bleeding compared with women who had never worked with pesticides (Farr et al 2004 and Abell et al 2000). The pollutants cause structural and morphological damage to endocrine glands, impair reproductive function and even disturb endocrine metabolism by disruption of steroidogenesis (Swarup and Balagangatharathilagar 2002). Many organophosphates have been reported as toxic to reproductive system (Akbarsha and Sivasamy 1998; Sarkar et al 2000; Brucker 1998). Among organophosphates quinalphos is a widely used pesticide on crops (Suvardhan et al 2005). There are several studies on the effect of quinalphos on male reproduction but the information regarding the effect of quinalphos on female reproduction is lacking hence a study was designed to investigate the effect of quinalphos on ovary, oviduct and uterus.

MATERIALS AND METHODS
Three months old female albino rats (Rattus norvigicus) weighing 150-200 g were maintained under conditions of controlled temperature (75±2°F) and humidity (40-60%). The animals were housed in groups of three rats per cage. The rats were acclimatized for 15 days before using them for experimentation. They were provided with standard diet containing pelleted food and water ad libitum. The cyclicity of rats was confirmed by examining their vaginal smears daily and the ones showing two or more regular 4-5 days estrous cycle were selected for the experiment. The rats were divided into 2 groups of 8 animals each and treated for 15 and 30 days with their respective control groups. This experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Exposure of quinalphos to rats: The commercial quinalphos having 25% EC, diluted with peanut oil to get the test concentration of 2 mg/kg/day. Rats were tube fed orally with quinalphos for two and four weeks. Simultaneously same amount of vehicle i.e. peanut oil was also administered orally to control group of rats. All the animals (control and quinalphos treated) were observed daily for clinical symptoms like salivation, activity, irritability, faecal pellet conditions, diarrhea, eyeball movement, weakness, coarse tremor, paralysis of limb, wounds and mortality etc.

Necropsy of animals: One day after the last dose i.e. after two and four weeks respectively, the animals were mildly anaesthetized using chloroform and were dissected.

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Weights of reproductive organs viz.; ovaries, oviduct and uteri were taken in treated and control rats after they were cleaned off the adhering tissues. The surface follicles and corpus luteum were also counted in the ovary.

**Histomorphological studies:** Ovaries and uterus of dissected animals were cleared and fixed in Bouin’s fixative for 24 hours. After processing, paraffin sections were cut at 6µm thickness and stained with haematoxylin and eosin for microscopic examination. Slides were studied under an optical light microscope and morphological characteristics of normal follicle, atretic follicle and corpora lutea were examined in ovary. Atretic follicles were distinguished from normal follicles either by the presence of pycnotic bodies or by the disruption or disintegration of granulosa cell layers, uneven thickness of granulosa cells and invasion of oocyte by granulosa cells. Various parameters like length and width of uterine cavity, height and type of uterine epithelium, vacuolization in uterine epithelium, abundance of endometrial glands and thickness of myometrium were observed in the slides of uterus of control as well as quinalphos treated rats.

**Statistical analysis:** Statistical analysis between experimental and control values were calculated according to Fishers’ student t-test (1950).

**RESULTS AND DISCUSSION**

**Toxicological effects of quinalphos on rats:** In the first and second week of treatment no toxicological effect of pesticide was observed. However for the third and fourth week, in the present study it was observed that the rats were hyperactive and aggressive with loose faecal pallets.

**Effect on organ weight:** After four weeks of treatment, the ovarian weight of treated rats decreased significantly as compared to the treated ovaries of 15 days (Table 1). The oviduct and uterus showed non-significant decrease in weight at both two and four weeks of treatment as compared to their relative weights in control groups (Table 1, Plate 1). The present study revealed that the ovarian weight decreased with decrease in corpus luteum along with increase in atretic follicles. Kaur (2003) also found decrease in ovarian weight when organophosphates like methyl parathion, dimethoate and monocrotophos was given to female albino rats. There was significant decrease in weight of ovary of mice when treated with carbofuran (Baligar and Kaliwal 2002). Decrease in ovary weight was also observed when rats were treated with cypermethrin for 15 and 30 days (Kaur 2008). Similar observations were made in rats treated with monocrotophos and have reported that decrease in weight and size of the ovaries is due to extensive fibrosis and atretic follicles (Adilaxmamma et al 1994, Radhika and Kaliwal 2002).

**Surface follicles and corpus luteum:** No significant difference was observed in the surface follicles and corpus luteum of control and treated rats at two and four week treatment, although there was slight increase in the number of corpus luteum and surface follicles in 30 day control rats. It was also observed that the number of surface follicles decreased in 4 week treatment in comparison to their control group, though the decrease was non significant indicating that perhaps long term exposure will decrease the number of follicles (Table 1).

**Micromorphology of ovary and corpus luteum:** When the follicular kinetics in serial sections of the ovaries of control and quinalphos treated rats were studied under the light microscope, the atretic and normal follicles were counted. It was observed that the numbers of normal follicles were higher in control groups and their number decreased in the ovaries on treatment with quinalphos (Fig 1). It was also observed that the number of atretic follicles were more (p < 0.05) in treated group of rats as compared to control in 30 day treatment thereby indicating increased atresia in treated rats. No information is available on the effect of quinalphos on follicular kinetics of ovary. However it has been reported

![Fig 1: Antral follicles, atretic follicles and corpus luteum in ovary of control rats and treated rats after two and four week treatment with quinalphos.](image)

**Table 1:** Changes in weights (g/100g bw) of reproductive organs in control and quinalphos treated rats after two and four weeks of treatment.

<table>
<thead>
<tr>
<th>Organ (g/100g of bw)</th>
<th>After 2 Weeks</th>
<th>After 4 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.0148±0.02</td>
<td>0.0183±0.05</td>
</tr>
<tr>
<td>Oviduct</td>
<td>6.084×10⁻³±0.45</td>
<td>5.814×10⁻³±0.87</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.21±0.30</td>
<td>0.18±0.19</td>
</tr>
<tr>
<td>Corpus Lutea*</td>
<td>10.75±2.18</td>
<td>11.94±1.44</td>
</tr>
<tr>
<td>S. Follicles</td>
<td>7.75±1.32</td>
<td>12.25±1.77</td>
</tr>
</tbody>
</table>

All the values are Mean ± SE values of 8 animals in each group. *per ovary

b = p<0.05
that rats treated with carbamate fungicide mancozeb, showed a decrease in the number of healthy follicles with increased atretic follicles (Baligar and Kaliwal 2001, Mahadewaswami et al. 2000). It has been reported that chlorinated pesticides induced follicular toxicity by reducing the pool of healthy, large and medium sized follicles with increase in the atretic follicles (Jadaramkunti and Kaliwal 1999).

Organophosphates like methyl parathion, dimethoate and monocrotophos given to female albino rats have also resulted in increased atresia of follicles in treated rats as compared to control rats (Kaur 2003). Quinalphos might have exerted deleterious effects on ovaries in terms of increased atresia in ovarian follicles which may perhaps be because of some hormonal alterations induced by quinalphos treatment.

It was observed that there was no significant difference in the number of normal growing corpora lutea in the quinalphos treated rats as compared to that in control rats.

Plate A: Portion of ovary showing, Graafian Follicle (GF), Corpus Luteum (CL) and Antral Follicle (An F) in control rat (x20X).

Plate B: Section of ovary of treated albino rats for 15 days, showing the ruptured Ovarian Wall (OW) (x20X).

Plate C: Section of ovary of albino rats treated for 30 days, showing the damaged Follicular Epithelium and the Germinal Epithelium contracting toward the antrum (x20X).

Plate D: Section of ovary of albino rats treated for 30 days, showing the Eccentric Oocyte (EO) with Granulosa Cells contracting towards the antrum (x20X).
rats at 15 day treatment, but the corpora lutea decreased significantly (P ≤ 0.05) at 30 day treatment in treated rats (Fig 1). Organophosphate dimethoate given to female albino rats had also resulted in significant increase in number of regressing corpora lutea (Kaur 2003). The treatment of rats with carbofuran resulted in less number of corpus lutea (Baligar and Kaliwal 2002). The effects on follicles and corpora lutea in ovaries may be due to reduced synthesis of steroids caused by treatment with pesticide. In the present study there is a possibility that decreased healthy follicles with concomitant increase in atretic follicles in rat may also be due to disrupted gonadotropin secretion via central nervous system mechanism as it was observed in rat following administration of dithiocarbamates (Goldman et al 1997).

Plate A: Portion of Uterine Wall of two weeks control albino rats, showing Uterine Epithelial Cell height (UE) (x40X).

Plate B: Uterine Wall of control albino rats showing Uterine Lumen (UL), Endometrium (ED) and Myometrium (MY) (x20X). Note the presence of abundant Uterine Glands (UG) in the Endometrium (Fig B) (x10X).

Plate C: Section of four week control albino rats, showing Uterine Lumen Width (UL W) and Uterine Epithelium (UE) (x20X).

Plate D: Portion of Uterine Wall of two weeks control albino rats, showing thickness of Myometrium (MY) (x40X).
Abnormal pattern of Uterine Lumen (UL) with absence of Uterine Glands (UG) and increased Uterine Epithelial Cell height (UE) (x20X).

Effect of quinalphos on uterus: It was observed that in uteruses of almost all the rats, luminal epithelium was of columnar type with nuclei located at the base. Height of luminal epithelium increased (p<0.01) at two weeks, which further increased (p< 0.05) in quinalphos treated rats at four weeks as compared to their values in respective control rats. The lumen length even increased (p< 0.05) in treated rats at two weeks and then decreased in four week treatment. There was no effect on the lumen width of control and treated rats at both two and four week period. In quinalphos treated rats there was less number of endometrial glands as compared to their abundance in control groups. Myometrium was normal
Table 2: Histological changes in the uterus of treated and control rats during two and four week treatment with quinalphos.

<table>
<thead>
<tr>
<th>Uterine components</th>
<th>After 2 Weeks</th>
<th></th>
<th>After 4 Weeks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>10.83±1.09</td>
<td>13.97±1.32</td>
<td>23.75±3.16</td>
<td>21.41±2.69</td>
</tr>
<tr>
<td>Lumen length</td>
<td>593.25±11.04</td>
<td>925.24±14.86</td>
<td>1798.12±25.97</td>
<td>882.28±25.21</td>
</tr>
<tr>
<td>Lumen Width</td>
<td>75.25±6.21</td>
<td>158.62±10.86</td>
<td>256.12±13.46</td>
<td>190.27±13.26</td>
</tr>
<tr>
<td>Type of Luminal epithelium</td>
<td>CL</td>
<td>CLCLCL</td>
<td>CLCLCL</td>
<td>CLCLCL</td>
</tr>
<tr>
<td>Endometrial Glands</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Myometrium Thickness</td>
<td>115.42±4.96</td>
<td>128.41±4.90</td>
<td>121.87±3.95</td>
<td>113.07±5.01</td>
</tr>
</tbody>
</table>

All the values are Mean ± SE values of 8 animals in each group. + = present, ++ = less, +++ = numerous, CL= columnar epithelium. a= p<0.01, b=p< 0.05 intraperitoneal treatment of insecticide orthopedic DDT (o-p'-DDT), an organochlorine compound. After injection of o-p'-DDT for 10 days it was observed that endometrial epithelium was of tall columnar type with numerous endometrial glands (Raifa and Garieb 2001). Kang et al (2004) observed no change in uterine epithelial height of rats after treatment with chlorpyrifos-methyl. As we had obtained no effect of quinalphos on uterine weight but increase in epithelial cell height, decrease in uterine gland and decrease in myometrium thickness with vacuolization shows that given substance can be selective in its toxicity. It may not have any detrimental effect on size or weight but it may induce gross morphological defects.

REFERENCES


