Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society of Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

The Journal publishes original research papers and reviews from any zoological discipline or from directly allied fields in ecology, behavioral biology, physiology & biochemistry.

www.eajbs.eg.net

Effects of Acute Exposure of Diazinon on the Ovarian Steroidogenesis in Chicken, *Gallus gallus*

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**ARTICLE INFO**

**ABSTRACT**

Diazinon (DZN) is an organophosphate insecticide has been used widely in agriculture to control pests. DZN has been found to induce reproductive dysfunctions in animals, particularly, avian species. This current study evaluated the lethal effects of acutely ingested DZN on the ovaries of laying hens. Twenty white leghorn laying hens were divided equally into two groups. Group one was kept as a control, and the second group was administrated orally with a single dose of DZN (6 mg/kg B.W) for twenty one days. At end of the experiment, serum and tissue samples (ovaries) were collected for hormonal and histopathological analysis. The results revealed that body weight was not affected significantly by DZN treatment. However, DZN treated group exhibited destructive changes in folliculogenesis, degeneration of ovarian follicles, and stromal hemorrhage in comparison with the control group. Additionally, significant changes of steroidal hormonal levels were observed with a significant increase in serum progesterone and decrease in estrogen in the treated group as compared to the control group. Therefore, the current reduction of serum estrogen in our study could be due to the destructive effects of DZN on ovarian tissues and/or its ability to alter the expression of P450 aromatase, the enzyme which is involved in estrogen production. In the contrary, serum progesterone levels increased significantly. This increase might be due to the activation of some steroidogenic enzymes such as 3β-hydroxy-steroid dehydrogenase (3β-HSD) by ovarian secondary follicles.

In conclusion, this current study showed that acute oral administration of DZN induces destructive structural changes in ovary and changes in female sex steroid hormone levels in blood. This lethal effect of DZN may lead to the infertility in laying hens, thus economic lose and health impact.

**INTRODUCTION**

Pesticides are employed extensively as an essential technological tool all over the world in agriculture and horticulture not only to control pests, but also to increase crop yield and to improve human nutrition and economy. However, a host of unfavorable environmental adverse effects of life forms and human health have been reported (Johnson, 1968; Goodman *et al.*, 1992). Pesticides adverse effects might cause direct or indirect effects on life forms depending on toxicant grade, toxicant concentration, species sensitivity, age, sex, size, duration of exposure, temperature,
and various other environmental parameters (Johnson, 1968; Kingsbury and Kreutzweiser, 1987; Goodman et al., 1992).

Several studies have shown that organophosphate pesticides (POs) may induce various toxic effects on reproduction in many different animal species (Hanna and Kerr, 1981). Administration of methyl parathion in rat resulted in decreased in ovarian weight, number of healthy follicles, and the number of estrous cycle (Asmathbanu and Kaliwal, 1997). Similarly, methyl parathion causes deterioration in structural changes in reproductive organs and in the biochemical parameters in the epididymis male rats (Prashanthi et al., 2006). Other study showed that exposure to dimethoate decreased serum testosterone levels, testicular weight, sperm mortality, and increased the percentage of dead and abnormal sperms in rats and rabbits (Salem et al., 1988; Afifi et al., 1991). Exposure to quinalphos resulted in increased serum LH, FSH, prolactin, and testosterone levels, and severe disruption in spermatogenesis in male rats (Sarkar et al., 2000). On the other hand, some studies showed that exposure to DZN induced lipid peroxidation, altered antioxidant enzymes, damaged DNA (Altuntas et al., 2004), and increased the risk of cancers (Gandhi and Snedeker, 1999). In avian species, exposure of parathion to female bobwhite quail decreased egg production, plasma progesterone, cortisone, LH levels, and inhibited follicular development (Rattner et al., 1982a; Rattner et al., 1986b).

The organophosphate diazinon (0,0-diethyl 0-[[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate], like many other organophosphate insecticides, has been used widely to control insects in crops, ornamentals, lawns, fruits, and vegetables, as well as many other pests through its ability to inhibit the enzyme acetylcholinesterase activity, thus, impair the reproductive systems of animals (VDH, 2001; Nabi and Tabrizi, 2015; Hanna and Kerr, 1981; Akbarsha et al., 2000) and inflicting harm to animals before death (Neishabouri et al., 2004; VDH, 2001). In fish, DAZ exposure was found to alter reproductive behavior, delayed sexual maturity (Moore and Waring, 1996), reduced growth, disrupted ovarian structure (Dutta and Meijer, 2003), and reduced egg production (Burkepil et al., 2000). Fattahi et al., (2009) showed that administration of DZN to experimental mice caused degenerative changes in testes, including reduction in testicular weight, seminiferous tubules diameter, sperm counts spermatogenesis, leydig and sertoli cells, and decreased in serum testosterone. Dikshith et al., (1975) observed that administration of DAZ caused structural and functional changes in liver and testes in male rats.

Furthermore, diazinon was blamed to be able of reducing fertility due to its action on pituitary and interfering with the synthesis of natural hormones as an endocrine disrupter, thence leads to a variety of reproduction problems (Nabi and Tabrizi, 2015; NCAP, 2000; McLachlan, 2001). Diazinon was reported (Dutta and Maxwell, 2003; Maxwell and Dutta, 2005) to function as an endocrine disrupter with changes in the ovarian follicles and estradiol levels. Khawaja et al., (2011) reported diazinon to be toxic to ovarian tissues in general and developing oocyte and endocrine para-follicular, which cause a decline in oogenesis ovulation and fertility index in virgin females. Neishabouri et al., (2004) stated that oxidative stress has been indicated to be an essential component to the mechanism of toxicity of organophosphate insecticides leading to generation of free radicals and alteration in antioxidants or reactive oxygen species scavenging enzymes.

The diazinon toxicity like many other organophosphate insecticides might be mediated through stimulation of lipid peroxidation, changes in the antioxidant enzymes, DNA damage, and free radicals, which are involved in cell apoptosis (Ibrahim and El-Gamal, 2003; Izumi et al., 2005). Generally, female reproductive
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The system is under the control of hormones through the hypothalamus, pituitary, and gonad axis that regulate its various functions including folliculogenesis, ovulation, lutenization, luteolysin, pregnancy, and parturition (Bhardwaj and Saraf, 2014). Therefore, organophosphate of which diazinon has been indicated to disrupt the structural and functional aspects of female reproduction altering the molecular, endocrinological, cytological, and biochemical aspects as well, causing ovarian cycle irregularities and fertility. Furthermore, diazinon has been reported to be highly very toxic to avian species than are mammals.

Although, there are many available studies concerning the toxic effects of DZN on the reproduction of many animal species including wild animals, vertebrates, mammals, birds, and humans, only limited information is available about its effects on the structure of ovary and sex steroid hormone in female chicken. Therefore, since scarce studies are available on wild animals, but non on the impacts of DZN to laying chicken, hence the aim of this study is to investigate the toxic impacts of the available commercial formulation of diazinon at the biochemical and histomorphological levels on the ovaries of laying hens.

**MATERIALS AND METHODS**

**Chemicals**

Diazinon (DZN), a commercial formulation (60% active ingredient) was obtained from local market, Saudi Arabia.

**Experimental Animals**

Twenty white leghorn laying hens weighing 1200-1250 g were used in this study. Hens were obtained from Aljazeera poultry farm (ABHA, Saudi Arabia). Hens were housed in separate cages (5 birds per cage), and kept under 14 h:10 h light and dark cycle, at a room temperature of 24±2°C and provided with commercial layer feed and water *ad libitum*. The hens were then divided into two groups, 10 birds each: Group I was used as a control, while group II was administered orally with sublethal doses of DZN (6 mg/kg b.w) daily for 21 days.

**Blood Collection and Histopathological Examination**

At the end of the treatment, blood samples were collected from the wing vein of each bird using vacuum syringes, centrifuged at 3500 rpm for 10 min, and sera were separated and stored at – 80°C until used for determination of blood sex steroid hormones (estradiol and progesterone) by ELISA using commercial kits. Birds were sacrificed by decapitation, and the ovaries were dissected, washed in cold saline solution (NaCl 0.9 % w/v), and the number of preovulatory follicles were recorded. The dissected ovary was immediately fixed in 10% neutral buffered formalin (NBF) overnight at room temperature for general histological examination. The fixed tissues were dehydrated through ascending series of ethanol, cleared in xylene, and embedded in paraffin wax. Paraffin-embedded ovarian blocks were then serially sectioned at 5- 8 μm thick using rotary microtome. Sections were then dried on glass slides, processed for staining with hematoxylin & eosin (Bancroft and Steven, 1996), and examined under light microscope (Olympus) equipped with digital camera for histomorphological changes.

**Statistical Analysis**

The data were statistically analyzed using SPSS software. The values were expressed as mean ± standard deviation. Differences between the control and treated groups were determined by one-way analysis of variance (ANOVA). *P*-value < 0.05 was considered as statistically significant.
RESULTS

The toxic effects of diazinon (DZN) administered orally to laying hens daily for 21 days on body weights are presented in (Table 1). There were no significant differences in weight between the control and DZN-treated groups (Table 1 and Fig. 1).

Table 1: Effect of ingested diazinon (6 mg/kg b.w) administered orally daily over 21 days on body weight of laying hens.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight(g)</th>
<th>Body Weight Difference (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1231 ± 14.31</td>
<td>1314.2 ± 28.41</td>
<td>82</td>
</tr>
<tr>
<td>Diazinon</td>
<td>1223.4 ± 6.28</td>
<td>1296.2 ± 14.08</td>
<td>72</td>
</tr>
</tbody>
</table>

Values were expressed as means ± SD of six birds in each group. *P* < 0.05 was considered as significant difference in body weight between diazinon and control group.

The results of the biochemical analysis of the blood samples of laying hens exposed to 6 mg/Kg b.w. DZN over 21 days demonstrated significant decrease in serum estradiol levels (*P* < 0.05) in the treated group (212 ± 5.9 pg/ml) as compared to the control group (232 ± 12.2 pg/ml), (Fig. 2A). Similarly, the levels of serum progesterone (Fig. 2B) increased significantly (*P* < 0.001) in DZN-treated group (4.8 ± 0.69 ng/ml) as compared to the control group (0.73 ± 0.17 ng/ml).

The histological examination of the ovarian tissues of the control group showed normal ovarian structure with normal folliculogenesis and many distinct stages of normal follicles in the ovarian cortex (Figs. 3A & B). However, ovaries of DZN-treated group showed various degrees of histopathological alterations including disturbances in follicular development, degeneration in stromal connective tissues, hemorrhage, increased atretic follicles, and the absence of developed stages of follicles in the ovarian cortex (Figs. 4A & B).
Fig. 2: Effects of oral ingestion of diazinon (DZN) on: (A) Estradiol (E2) and (B) progesterone levels in laying hens.

**DISCUSSION**

In this current study, the body weight of leghorn chicken given 6 mg DZN kg\(^{-1}\) has not been affected (Table 1 and Figure 1). This is in consistent with some other studies in baboon (Ibrahim and El-Gamal, 2003) and in rats (Habiboallah et al., 2010).

Ovary which plays an important role in reproductive functions by synthesizing steroid hormones and producing oocyte can be affected by pesticides inducing ovarian toxicity by either direct action through binding to ovarian tissue macromolecules or indirect action disturbing the HPG axis. In this current study, when leghorn chicken were given DZN (6 mg/kg) orally for twenty one days, the ovary exhibited many histopathological changes including disturbances in follicular development, degeneration in stromal connective tissues, hemorrhage, and an increased atretic follicles (Figs. 4A & B) as compared to the control group (Figs. 3A & B). Several other studies have shown that DZN like many other organophosphates can induce structural changes in gonads in various experimental animals. Bluegill ovary exposed to DZN resulted in adhesions of primary follicles, cytoplasmic retraction in oocyte II, cytoplasmic degeneration, increased atretic oocytes, damage to the oocyte IV, partial destruction of the ovigerous lamellae, vitelligenic membrane destruction of follicles, increased intrafollicular spaces, and necrosis in cytoplasm (Dutta and Maxwell, 2003). The testis of male rats exhibited decreased reproductive tissue weight, increased dead
abnormal sperms, and decreased plasma testosterone levels when DZN was orally administered (Abd El-Aziz et al., 1994). Similar results were found by Sanjoy Deka and Rita Mahanta (2012) in freshwater catfish ovarian tissues after exposure to the organophosphate Malathion.

Diazinon like many other pesticides can cause a significant decrease in oocytes survival, which indicated the toxicity of OPs on mammalian oocytes during the early oogenesis (Bonilla et al., 2008). DZN might induce its effects on ovarian tissue structures by different mechanisms including apoptosis (Habibollah et al., 2010). One study showed that DZN and other organophosphates can induce ca++ release from intracellular structures and promote caspase 12 activation, which could be the mechanism for apoptosis in ovarian tissues (Nakagawa and Yuan, 2000). DZN has been stated to accelerate the synthesis of interleukin-II, which in turn can elevate prostaglandins leading to corpus luteum degeneration (Aluigi et al., 2005). Additionally, oxidative stress has been shown to be an essential component to the mechanism of toxicity of OPs leading to generation of free radicals and alteration in antioxidants enzymes Neishabouri et al., (2004). Zinat Sargazi et al., (2015) showed that DZN induced lipid peroxidation, increased oxygen free radicals in gonads of male and female rats and caused infertility. Furthermore, pesticides have the potential to act as endocrine disrupters exerting their effects by mimicking estrogens, androgens, and progestins (Newbold et al., 2007). The antagonadal effects of OPs might be due to their ability to inhibit steroids synthesis. The current study showed that oral ingestion of sublethal dose (6 mg/kg B.W) of diazinon caused a significant reduction in estradiol levels and increased serum progesterone significantly (Figure 2A & B). Various other studies showed in consistent results with our studies that DZN altered ovarian steroidogenesis (Ohi et al., 2004; Oduma et al., 1995). Therefore, the current reduction of serum estrogen in our study could be due to the destructive effects of DZN on ovarian tissues and/or its ability to alter the expression of P450 aromatase, the enzyme which is involved in estrogen production. In the contrary, serum progesterone levels increased significantly (Figure 2B) in our study due to DZN effects. This increase might be due to the activation of some steroidogenic enzymes involved in the production of progesterone such as 3β-hydroxy-steroid dehydrogenase (3β HSD) by ovarian secondary follicles. According to several other studies, OPs can significantly reduce plasma level of progesterone (Habibollah et al., 2010; Parkash et al., 1992), interfere with STAR protein gene expression, and reduce its production level (Walsh et al., 2000). Organophosphate such as dichlorvos also can stimulate dopamine level, which leads to decreased prolactin production. Hence, the decreased prolactin production can reduce the quantity of LH receptors which are involved in progesterone synthesis (Goodman, 1994). Thus, affecting ovarian structure and functions.

In conclusion, based on the results of this current study, DZN like many other organophosphate pesticides can affect the fertility of laying hens by disrupting ovarian steroidogenesis and destruction of ovarian tissues. Thus, for a conclusive evidence of the effects of DZN on the fertility of laying hens, we recommend executing further experiments on hens and other life forms, which are of economic and health values.

**Acknowledgement**

The authors wish to thank the deanship of research of King Khalid University for its financial support.
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Fig. 3(A & B): Photomicrographs of control ovary showing a normal morphological feature and different stages of follicles development. Cortex (C), ovary follicles (F), arrows (cortical follicle development). (H&E) X 10.

Fig. 4(A & B): Photomicrographs of DZN-treated ovary showing histopathological changes indicated by the absence of folliculogensis, degenerative stromal connective tissue, and atretic follicles. Asterisk (atretic follicle). (H&E) X 10.
التأثير السمي الحاد للديازينون على المبيض في الدجاج البياض
خالد بن عبدالعزيز الزيلعى – إبراهيم بن عبدالرحمن مسعد
قسم الاحياء – كلية العلوم – جامعة الملك خالد – أبها - المملكة العربية السعودية

الديازينون كمبيد حشرى عضوي فوسفاتي يستخدم على نطاق واسع في المجال الزراعي للقضاء على الآفات. وقد أصبح من المعروف أن التعرض للديازينون يؤدي إلى خلل في الوظائف التناسلية في العديد من الأجناس الحيوانية ويشكل خاص في أجناس الطيور. ولذا تستهدف هذه الدراسة تقييم التأثير السمي للديازينون على المبيض في الدجاج البياض. عُثر على قطعة بياضة تم تسميةها إلى مجموعتين: المجموعة الأولى تم استخدامها كمجموعة ضابطة، والمجموعة الأخرى تم تجريعها يومياً عن طريق الفم بجرعة واحدة من الديازينون بتركيز 3 ملجرام / كيلو جرام من وزن الجسم لمدة واحدة وعشرون يوماً. بعد انتهاء فترة التجربة، تم جمع عينات السيروم والأنسجة (المبيض) من كلا المجموعتين لإجراء التحليل الهرموني والتحليل النسيجي. أظهرت نتائج الدراسة عدم تأثير الديازينون على وزن الجسم للمجموعة المعالجة، بينما أظهر الفحص النسيجي للمبيض وجود تغيرات نسيجية في المبيض تشمل توقف النمو التطوري للحويصلات في منطقة القشرة، تحلل بعض الحويصلات غير المتميزة، بالإضافة إلى وجود نزيف دموي في منطقة القشرة لمبايض الدجاج المجرع بالديازينون. أظهرت هذه الدراسة أيضاً انخفاض مستوى هرمون الاستروجين وارتفاع مستوى هرمون البروجسترون في الدم عن طريق المجموعة المعالجة بالديازينون مقارنة بالمجموعة الضابطة، وقد نزح انخفاض تركيز هرمون الاستروجين في الدم أما إلى التأثير التخريبي للديازينون على أنسجة المبيض أو إلى تغير النشاط الإلزامي للأورامات المسؤول عن زيادة انتاج هرمون الاستروجين. في المقابل، ارتفاع تركيز هرمون البروجسترون قد يوجد للتغير في نشاط الإنزيمات المسؤول عن إفراز البروجسترون في الحويصلات الثانوية.
وخلاصة هذه الدراسة الحالية تفيد بأن التجربة الحاد للديازينون أحدث تغيرات تركيبية في أنسجة المبيض وكذلك في مستويات الهرمونات الستيرويدية الجنسية في الدم. وهذا قد يؤدي إلى انخفاض الخصوبة في الدجاج البياض وبالتالي تأثيره على المستويين الاقتصادي والصحي.