The physiohistological effect of pesticide Endosulfan 35% on sexual hormone on female rabbits and its role in Environmental pollution in marsh area south of Iraq

*Khawla B. Al-Jassim  **Ala –Aldeen H. Jawad  **Salih K.Majeed

* Veterinary hospital in Basra / E-mails: dr_vet20076@yahoo.com  ** Basra University-Veterinary Medicine collage.

Abstract

Endosulfan is an insecticide which used in illegal & misused application by fisherman as a mean of fish hunting purpose in marsh area / south of Iraq. This study was undertaken to investigate the effect of oral administration of three different doses of Endosulfan 35% on the sexual hormone level (LH, FSH & Estradiol) in addition to histological study of uterus, ovaries on female rabbits and study their effect on environment pollution. 48 mature female rabbits were randomly divided into 4 groups of equal number (12) on each as follow: first group which administered 0.9 ml NACL which act as control, the second, third, fourth groups which administered Endosulfan 35% as concentration (0.5 ml, 1 ml, 1.5 ml/ kg .B.W ) respectively. After 8 weeks, the one half of animals of each groups (6 rabbits) were sacrificed, the blood sample was taken for hormonal assay (LH, FSH & Estradiol) and histological examination of uterus, ovaries were done. After recovery period (5 weeks) the same parameters mention above was measured. The results showed that Endosulfan 35% regarded as persistent organic pollutant caused endocrine disruptor and causing lowering of LH, FSH & Estradiol level and did not return to normal value after recovery period. Histological changes were noticed in uterus including fibrotic polyp (tumor like) in fourth group (1.5 ml), as well as reduce endometerium in all treated groups as compared with control group. Whereas the ovaries showed primary and secondary follicles in third and fourth groups (1ml, 1.5ml) and did not return to normal structure after recovery period.

Keyword: organic pollutant, Endocrine disrupter, Endosulfan 35%, Histological change.
INTRODUCTION

Endosulfan is a broad spectrum insecticide which belongs to organochlorine group of pesticides, under the cyclodiene sub-group (IPCS, 1975). It was introduced in the 1950s and emerged as a leading chemical used against a broad spectrum of insects and mites in agriculture and allied sector (Nayer, et al., 2002). It is used as a pesticide for vegetables, fruits, paddy, cotton, cashew, tea, coffee, tobacco & timber crop (Herman, 2003). In Iraq, Endosulfan is used as a pesticide in cotton crop (Al-dil, 2006). Al-Helfi, (2005) reported that Endosulfan used in illegal and misuse in fish hunting in marsh area, south of Iraq. Dalsenter, et al. (1999); Sinha, et al. (2001) concluded that Endosulfan has caused a number of adverse effects on male reproductive parameters in rat including fertility: degeneration of seminiferous tubules epithelium, reduce sperm count, altered spermatogenesis, increase abnormal sperm, testicular necrosis & spermatogenesis. Hiremath, and Kaliwal (2002) demonstrated that Endosulfan caused reduced implantation in female mice and increased estrus cycle. Many researchers in different countries found that Endosulfan and its metabolites can be detected in human umbilical cord blood, placental tissue, breast milk, fat, blood, and urine (Saleh et al. (1996); Burke, et al. (2003); Sanghi, et al. (2003); Cerrillo, et al. (2004); Botella, et al., (2004); Fukata, et al. (2005), and Damgarrd, et al. (2006).

Materials & Methods

I- preparation of Endosulfan concentration

Endosulfan 35% was purchased from the local market which manufacture from Khazal chemical company, Iran.

II- Experimental Design

Forty –eight mature female rabbits (lupus cuniculus) were divided into four groups (12 rabbits for each group). These rabbits were given daily oral single dose of Endosulfan 35% for 8 weeks except control group (group I):

- Group I administered 0.9% NACL which act as a control,
- Group II, III, IV which administered 0.5mg, 1mg, & 1.5mg/Kg B.W of Endosulfan respectively.

After 8 weeks of administration, the blood sample (4ml) was collected directly from the heart by using disposable syringe from the half number of animal of each group (6 rabbit) which has chosen randomly and put in screw tube without anticoagulant and then centrifuged at 4000rpm for 10 minutes to get the serum for hormonal assay (LH, FSH, & Estradiol) by using Elisa technique by protocol Luteinizing Hormone Enzyme Immunoassay kit was used (Monobind Inc. lake forest CA 92630, USA).

FSH enzyme immunoassay test kit was used (Gesellechalf für Biochemical and diagnostic mbH ,Germany ). Estrogen enzyme
Immunoassay Kit was used (DRG instrument GmbH, Germany).

After sacrificed animal (6 rabbit), the uterus and ovary were taken out and preserved in formalin 10% to study the histopathological changes, the organ samples were taken (ovary and uterus). The preserved organs were taken and the dehydration was done by passing specimens in ascending concentration of Ethanol, infiltrated with xylene and then was embedded in paraffin. Five-micron thick sections of paraffin-embedded tissue were cut by using microtome and mounted on glass slides, then affixed of ribbon by Mayer’s albumin on glass slide, then dehydrated at hot plate overnight and later stained with hemotoxlyin-eosin stain. The sections were examined by using light microscope.

The reminder of animal (6 rabbits/group) were left without administration for 5 weeks to study the effect of Endosulfan after recovery period by estimating the same parameter mention above.

**Statistical analysis**

The results were analyzed by one-way ANOVA test in whole study. All statistical calculations were carried out by the aid of the statistical SPSS V. 16 (SPSS Inc). The data were expressed as means ± standard error (X ± SE). Least significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) SPSS (1998).

**Results**

I- Effect of Endosulfan 35% on female rabbit hormone (LH, FSH and Estradiol) after 8 weeks of treatment.

The results of oral administration of Endosulfan 35% (0.5mg, 1mg and 1.5mg/kg B.W) on female rabbit's hormones (LH, FSH, and Estradiol) after 8 weeks is displayed in Table (1).

The LH level in the group II and III which administered 0.5mg & 1mg/kg B.W Endosulfan showed no significant differences present as compared with control group, while the group which treated with 1.5mg / K g B. W. Endosulfan showed significant decrease (P<0.05) in serum LH hormones as compared with control group.

The FSH level in the 2nd and 4th groups revealed no significant differences present as compared with control group while 3rd group showed significant decrease (P<0.05) in serum FSH as compared with control groups. All treated groups showed depression in Estradiol level but didn’t reach the significant level.
Table (1): The effect of Endosulfan 35% on LH, FSH, and Estradiol after 8 weeks of treatment on female rabbits (*Lepus cuniculus*).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LH ng/ ml</th>
<th>FSH ng/ml</th>
<th>Estradiol ng/ ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Nacl (First group)</td>
<td>0.565 ± 0.044 a</td>
<td>0.884 ± 0.267 a</td>
<td>24.093 ± 1.98</td>
</tr>
<tr>
<td>0.5mg/kg Endosulfan (Second group)</td>
<td>0.401 ± 0.057 a</td>
<td>0.431 ± 0.070 ab</td>
<td>20.45 ± 3.53</td>
</tr>
<tr>
<td>1mg/kg Endosulfan (Third group)</td>
<td>0.379 ± 0.100 a</td>
<td>0.384 ± 0.122 b</td>
<td>19.93 ± 0.53</td>
</tr>
<tr>
<td>1.5mg/kg Endosulfan (Fourth Group)</td>
<td>0.316 ± 0.036 b</td>
<td>0.324 ± 0.069 b</td>
<td>17.67 ± 1.91</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. n=6/ group.
The different letters denote differences between groups, P<0.05.

II-Effect of Endosulfan 35% on female rabbit hormone (LH, FSH and Estradiol) after recovery periods (5 weeks).

Table (2) showed the effect of Endosulfan (0.5mg, 1mg and 1.5mg/ Kg B.W) on female rabbit's hormone (LH, FSH, and Estradiol) after recovery period (5 weeks). There was no significant difference in luteinizing hormone (LH) level among all treated groups. Follicle stimulating hormone (FSH) level revealed significant increase (P<0.05) in all treated groups as compared with control group.

There was a significant increase (P<0.05) in Estradiol level in the 3\textsuperscript{rd} and 4\textsuperscript{th} groups in comparison with the control group, while the 2\textsuperscript{nd} group did not show significant differences.
Table (2): The effect of Endosulfan35% on female rabbit hormone (LH, FSH, and E2) after recovery period (5 weeks).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LH ng/ ml</th>
<th>FSH ng/ml</th>
<th>E2 ng/ ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.356± 0.057</td>
<td>2.391±0.275b</td>
<td>25.063± 1.927b</td>
</tr>
<tr>
<td>0.9 Nacl (1st Group)</td>
<td>1.535 ± 0.141</td>
<td>3.033 ± 0.086a</td>
<td>36.360 ±9.949ab</td>
</tr>
<tr>
<td>0.5 mg/kg Endosulfan (2nd Group)</td>
<td>1.498 ± 0.059</td>
<td>3.135 ± 0.112a</td>
<td>48.806 ± 11.342a</td>
</tr>
<tr>
<td>1 mg/kg Endosulfan (3rd Group)</td>
<td>1.386 ± 0.089</td>
<td>2.970 ± 0.065a</td>
<td>54.428 ± 3.639a</td>
</tr>
<tr>
<td>1.5 mg/kg Endosulfan (4th Group)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Value were express as (mean ± SE), n=6 / group.
The different letters denote difference between groups P< 0.05.

III- Histopathological changes

A- During Treatment

Uterus

The examination of uterus of control rabbits showed well developed endometrium with papillary invagination mucosal epithelial of endometrium Figure (1). The histological examination of uterus in 2nd and 3rd group showed reduced endometrium Figure (2), (3) while the 4th group revealed fibrotic polyp (tumor like) as well as reduced endometrium Figure (4), (5).

Ovaries

The microscopical findings on ovaries section related to control rabbits showed numerous primordial follicles, primary and secondary follicles, graviian follicles and corpus lutum Figure (6). The histological examination of ovaries in the 2nd group showed existence of primary and secondary follicles , graviain follicle and absence of corpus lutum Figure (7),while the 3rd and 4th group revealed only primary and secondary follicles , and no graviain follicle and corpus lutum Figure (8 and 9).
Figure (1): Normal rabbit’s uterus stained with (H&E) X50. The pointer indicates villi(V) and myometrium(M).

Figure (2): Uterus of rabbit treated with 0.5mg/kg Endosulfan stained with (H&E) X50. The pointer indicates reduce endometrium. Smooth muscle(SM), villi (V), lumen (L).

Figure (3): Uterus of rabbit treated with 1mg/kg Endosulfan stained with (H&E) X50. The pointer represent reduce endometrium. Myometrium(M), Endometrium (E).

Figure (4): Uterus of rabbit treated with 1.5 mg/kg Endosulfan stained with (H&E) X 50. The pointer indicates reduce endometrium.
Figure (5): Uterus of rabbit treated with 1.5mg/kg Endosulfan stained with (H&E) X 50. The pointer represent fibrotic polyp (tumor like).

Figure (6): Normal ovary of rabbit stained with (H&E) X50. The pointer indicate primary and secondary follicles A, B, gravian follicles C.

Figure (7): Ovary of rabbit treated with 0.5mg/kg Endosulfan stained with (H&E) X50. The pointer indicate primary and secondary follicles A, B and gravian follicles C.
B-After recovery period

Uterus

Figure (10) showed the uterus of rabbit within normal values. The macroscopic examination of uterus of rabbit after recovery period revealed reduce endometrium in 2\textsuperscript{nd} and 3\textsuperscript{rd} group Figure (11), (12). Moreover, the 4\textsuperscript{th} group showed fibrotic of endometrium as well as reduced endometrium Figure (13).

Ovaries

Figure (14) represent ovary of the control rabbit with 3-5 corpus lutum and gravian follicles. The histological finding of ovary of 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} group after recovery period (5 weeks) showed only primary and secondary follicle and absence of gravian follicles and corpus lutum Figure (15), (16) and (17).
Figure (10): Uterus of rabbit within normal value stained with (H&E) X125.

Figure (11): Uterus of rabbit treated with 0.5mg/kg Endosulfan after recovery period (5 weeks) stained with (H&E) X 125. The pointer indicates reduce endometrium.

Figure (12): Uterus of rabbit treated with 1mg/kg Endosulfan after recovery periods (5 weeks) stained with (H&E) X125. The pointer indicates reduce endometrium

Figure (13): Uterus of rabbit treated with 1.5mg/kg Endosulfan after recovery periods (5 weeks) stained with (H&E) X 125. The pointer indicates fibrotic of endometrium.

Figure (14): Normal ovary of rabbit after recovery period stained with (H&E) X50. The pointer indicates (A) corpus lutum (B) gravian follicle.

Figure (15): Ovary of rabbit treated with 0.5mg/kg Endosulfan after recovery period (5 weeks) stained with (H&E) X 50. The pointer indicates primary follicles (A) and secondary follicles(B) .
Discussion

I- The Effect of oral administration of Endosulfan 35\% on hormone level (LH, FSH, and E2) in female rabbits.

The results of the present study revealed significant decrease in luteinizing hormone (LH) in the 4\textsuperscript{th} group, and FSH level showed significant decrease in the 3\textsuperscript{rd} group, while Estradiol decrease but didn’t reach the significant level. This finding is in agreement with Anderson, et al. (2002); Hodges, et al. (2000) and Soto, et al (1995). These authors suggested that endosulfan acts as endocrine disrupter and have been reported to possess estrogenicity in vivo. When Endosulfan or its metabolites bind and activate the estrogen receptor, it imitates the hormone 17-\beta-estradiol and acts as an agonist. This result leads to decrease the production of GnRH by the hypothalamus (negative feedback mechanism) and of LH, FSH by the pituitary gland, as a result, the level of LH, FSH will drop and finally lead to a lack of Estradiol. Under normal circumstances the hypothalamus will then be triggered to produce more GnRH, but this will be prevented by the endocrine disruptor. As a result the hormone cycle may be disrupted. Wilson, et al (1990) postulated that any environmental compound mimicking or antagonizing steroid hormone action could presumably alter the glycosylation of LH & FSH thereby reducing their biological activity. On the other hand, Chitra, et al (1990) claimed that endosulfan has been shown to reduce plasma LH, FSH and testosterone in rats.

II-Effect of Endosulfan on hormone level (LH, FSH, and Estradiol) of female rabbits after recovery period (5 weeks).

The results of the present study indicated the level of LH remain in normal and an elevation of FSH level in all treated groups while Estradiol showed significant
increase in the 3rd and 4th group. No other studies suggest the effect of endosulfan on hormone level (LH, FSH, and Estradiol) on recovery periods. This result may be due to disruption of endocrine system which elongated to farther extent which was explained previously and may be because endosulfan caused damage to liver cell which lead to increase in the activity of estrogen. The liver convert the potent estrogen (Estradiol & Estrone) into almost totally impotent estrogen Estriol, therefore diminished liver function actually increases the activity of estrogen in the body and causes hyperestrinism (Guyton and Hall, 2006). The amount of estrogen which secreted in the recovery period may be inactive and may be different in normal secretion, as a result the amount of LH, FSH increased (negative feedback mechanism). The hyperestrinism may effect on ovarian function and cause damage to cell and tissue.

Guillette, et al. (1994) Concluded that female alligators from Lake Apopka polluted with Dicofol & DDT exhibit abnormal ovarian morphology with large numbers of polyvular follicles and polynuclear oocytes, also their Estradiol level were almost twice as high as in female alligators from a control lake.

Changes III-Histopathological

The histological changes in uterus after endosulfan treatment showed reduce endometrium in all treated groups. This result may be due to hormonal disturbances especially estrogens. Estrogen caused marked proliferation of the endometrial stroma and greatly increased development of the endometrial gland (Guyton and Hall, 2006). During treatment because endosulfan possesses estrogenicity which caused decrease in estrogen level this may lead to reduce endometrium. The results of the present study in high dose demonstrated fibrotic polyp (tumor like) as well as reduced endometrium. This result is in agreement with Guillette, et al. (1994) and Lemaire, et al. (2006) which emphasized that Endosulfan ability to act as a xeno-estrogen may also cause to contribute to cervical cancer & endometriosis. As well as, it causes activation of the estrogen receptor alpha but weakly antagonizes estrogen receptor beta, this will increase its harmful action because estrogen receptor beta can oppose the cell proliferation effects of estrogen receptor alpha, antagonizing estrogen receptor beta increases the risk of cell proliferation. On the other hand, Foster and Agrawal, (2002) reported that endosulfan is estrogenic interfering with the steady state levels of estrogen receptor and causing proliferation of MCF-7 human estrogen sensitive breast cancer cell.

The result of histological examination of ovaries revealed absence of corpus lutum and gravian follicles in intermediate and high dose (1mg & 1.5mg /Kg B.W) of Endosulfan study both after treatment and recovery period (5weeks). This result is in disagreement with Grunfeld and Bonefeld, (2004) which
reported that endosulfan and Malathion cause damage in ovarian tissue by affecting a number of primordial primary & prenatral follicle, but it increases atretic follicle and corpus lutum. The ovaries were considerably smaller and the color was light. Koc, et al. (2009); Alvarez, et al. (2000); Baligar and Kaliwal, (2001); Chapin, et al. (1997); Gray, et al. (1989); Ishmael and Lithfield, (1988) and Kaur and Dhanju, (2005). Those authors suggest the same pesticide when administered to females caused degeneration in ovaries related to dose. On the other hand, Plowchalk, et al. (1993) have concluded that quantitative assessment of follicle number is an indicator of the normal function as well as the most important controllers of follicular development are follicle stimulating hormone (FSH) & luteinizing hormone (LH) produce from the pituitary and the ovarian steroid Estradiol produced by granulose cells. The chlorinated pesticides induces follicular toxicity in reducing the pool of healthy, large sized follicles with increase in the atretic follicles Jadaramkuntic and Kaliwal, (1999); Martinez and Swartz (1991) and Swartz and Mall, (1989).

REFERENCES


9- Chapin, R.E.; Harris, M.W.; Davis, B.J.; Ward, S.M. and Wilson, A.C. (1997). The


22- IPCS, (1975). Data sheets on pesticides No. 15. WHO.


التأثّرات الفسيولوجية النسيجية لمبيد الاندوسفان 35% على الهرمونات الجنسية للإثاث
الأرانب ودوره في تلوث البيئة في أهوار جنوب العراق

خوله بن الجاسم ** علاء الدين حسن جواد *** صالح كاظم مجيد
المستشفى البيطرى في البصرة*
جامعة البصرة دكتور الطيب البيطرى**

الخلاصة
الاندوسفان مبيد حشري يستخدم في عملية الصيد الجائر للأسماك من قبل صيادي الأسماك في منطقة الاهوار جنوب العراق والذي يؤثر على صحة الحيوان والإنسان. أجريت هذه الدراسة لمعرفة تأثير إعطاء ثلاث جرعات مختلفة من الاندوسفان 35% عن طريق الفم على معدل الهرمونات الجنسية (الهرمون اللوتيني، الهرمون المحفز لنمو الحويضات ، والاسترادابيل) والتأثّرات النسيجية على المبيض والرحم لأثاث الأرانب بالإضافة إلى معرفة درجة تأثيره في تلوث البيئة. 48 اثاث أرنب ناضجه اختيرت وقسمت بالتساوي إلى أربع مجموعات كل مجموعة تجري على انزلاق الهرمونات الجنسية (LH,FSH & Estradiol) بالإضافة إلى الدراسات النسيجية للمبيض والرحم. بعد فترة التقييم (5 أسابيع) تم قياس نفس الاختبارات اللفة الذكر.

اظهرت النتائج ان الاندوسفان 35% ملغ يبين دايم سبب تعطيل في وظائف الغدد صماء وادي إلى تقليل من معدل الهرمون اللوتيني، الهرمون المحفز للجريبات والاسترادابيل ولم ترجع إلى معدلاتها الطبيعي بعد فترة التفحة. التغييرات النسيجية لوحظ في الرحم ووضعت تحت نمو سرطاني حيوي في المجموعة الرابعة بالإضافة إلى حدوث انكماش في بطانة الرحم لكل المجموعات المعاملة مقارنة بمجموعة السيطرة. بينما المبيض لوحظ فيها الحويضات الأولى والثانية بالمجموعة الستين ثلاثة والرابعة (0.5 ملغ. 1ملغم، 1.5 ملغ.) ولم ترجع إلى تركيبها الطبيعي بعد فترة التفحة.