SEXUAL HORMONES AND PATHOLOGICAL CHANGES IN FEMALE ALBINO RATS *RATTUS NORVEGICUS* TREATED WITH ABAMECTIN BIOCIDE

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Abstract

Effect of 1/4 LD_{50} of abamectin biocide was studied on sexual hormones and histopathology of ovary, oviduct and uterus of mature female albino rats, *Rattus norvegicus*. Follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, estradiol (E_2) and progesterone were determined in plasma and brain tissue after three and seven days of treatment. The results revealed that plasma FSH and LH were significantly decreased, while prolactin hormone was significantly increased in treated animals after three and seven days. Estrodiol (E_2) and progesterone hormones were significantly decreased in brain tissue after three and seven days. The decrease and increase in plasma and brain tissue hormones after seven days were more than after three days. Sever congestion was noticed in the medullary blood vessels of the ovary after three days, and also it was proliferation in the interstitial stromal cells at the medullary portion after seven days of treatment. The lining mucosal epithelium showed cellular hypertrophy in oviduct after three days, also hypertrophy was observed in the lining mucosal epithelial cells as well as in the muscularis after seven days. Hypertrophy with vacuolization were detected in the lining mucosal epithelium and associated with congestion in the blood vessels of the muscularis in uterus after three days, the same result, there were focal vacuolization and degeneration as well as focal stratification in the lining mucosal epithelial cells after seven days compared to normal histological structure. In conclusion, abamectin biocide was toxic on sexual hormone and sex organs of mature female rats. This action affected on the fertility of female rats leading to severe reducing for rat population, so abamectin biocide could be use as rodenticide for rodent control program.

Key words: abamectin, female rats, sexual hormone, histopathology.

INTRODUCTION

Rodent pests are controlled by chemical compounds that cause health hazards and environmental pollution in addition to the toxic effect to non-target animals. These problems provided an impetus to get poisonous natural materials for using as rodenticides. Natural product as Vertimic biocide (abamectin) proved promising efficiency for control of rodent species (Gabr *et al.,* 2012). Also, ivermectin revealed significant decreased in FSH hormone in male red sokoto (Onakp *et al.,* 2010). El-Ashmawy *et al.* (2011) found that ivermactin caused significantly decrease in weights and numbers of fetal in albino rats. The European Agency for the Evaluation for Medicine Products (1997) reported that doramectin passed the placental barrier of rat, mouse and rabbit lead to dead of fetuses.

The present work aims to study the effects of the abamectin biocide on sexual hormones in (plasma and brain tissue) and histopathological effect on ovary, uterus and oviduct of mature female albino rats (*Rattus norvegicus*) to affecting the fertility of females leading to reduction of rats population.

MATERIALS AND METHODS

I- Pesticides:

Abamectin (Vertimic 1.8% EC) is a natural product produced by the soil microorganism *Strptomyces avermitillis.* It was obtained from Syngenta Agro. Co. Switzerland.

II- Animal groups:

The present experiment was carried out on 40 mature female rats *Rattus norvegicus,* (180-200gm) obtained from animal farm (Ministry of Health). Rats were kept at constant environmental conditions throughout the period of the experiment; water and food were supplied *ad libitum.* The rats were divided into three groups. The first group acted as control. The second and third groups administrated 1/4 LD_{50} (2.18mg/kg) of abamectin for three and seven day as a single dose.

Blood sampling and analysis: -

Blood samples were collected by heparinized syringes from the orbital vein of rats after three and seven days of abamectin treatments.

Plasma was separated after centrifugation (3000 rpm for 15 min.) and stored at -20 °C until used for hormones determinations.

Each organ of ovary, oviduct and uterus were homogenized under cooling in homogenizer instrument for 3 min. with 10 ml of 0.0006M phosphate buffer, pH= 7, at 1-4°C and centrifuged (3500 r.p.m.) for 10 min. and supernatant was taken from samples and stored at -20°C until used (Bermeyer, 1963)

FSH, LH, prolactin, estradiol (E_2) and progesterone were measured by Radioimmunoassay kit from Monobind Inc. USA according to **Pierce and Parsons (1981), Passing and Bablok (1983) and Quinn (2005).**

V- Histopathological analysis:

Autopsy samples were taken from the ovaries, uterus and oviducts of rats in different groups and fixed in 10% formol saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for examination through the light electric microscope (**Banchroft *et al.*, 1996**).

IV- Statistical analysis:

The obtained results were statistically analyzed by one way ANOVA and Least Significant Difference (LSD) at ($p< 0.05$) using Costat program **(Cohort, 2005)**.

RESULTS

-Effect of pesticide on sexual hormones:

Plasma FSH and LH were significantly decreased ($p< 0.05$) in animal group treated with abamectin after three and seven days compared to control (Table, 1). There was more significantly decreased in treated animals with abamectin after seven days than treated animals after three days on FSH and LH hormones.

Plasma prolactin hormone was significantly ($p< 0.05$) increased in animal groups treated with abamectin after three and seven days compared to control group. There

was more significantly increased in treated animals with abamectin after seven days than treated animals after three days on prolactin hormone (Table, 1).

Data in Table (2) E_2 and progesterone were significantly decreased after treatment compared to control in brain tissue. There were higher significantly decreased in treated animals with abamectin after seven days than treated animals after three days on E_2 and progesterone hormones.

As shown in Figs. (1&2) the ovary was sever congestion was noticed in the medullary blood vessels after three days, but it was proliferation was noticed in the interstitial stromal cells at the medullary portion after seven days Figs. (3&4) compared to the normal histological structure Figs. (5&6).

The lining mucosal epithelium showed cellular hypertrophy after three days in oviduct Fig. (7), while Hypertrophy was detected in the lining mucosal epithelial cells as well as in the muscularis in oviduct after seven days Fig. (8) compared to normal histological structure Figs. (9&10).

Hypertrophy with vacuolization was detected in the lining mucosal epithelium and associated with congestion in the blood vessels of the muscularis in uterus after three days Figs. (11,12&13), while the focal vacuolization and degeneration as well as focal stratification in the lining mucosal epithelial cells in uterus after seven days Figs. (14) compared to normal histological structure Fig. (15).

DISCUSSION

Obtained results revealed that FSH was significantly decreased in the treated rats may be due to dose of abamectin. Similar results were reported that decrease in the level of FSH with abamectin in male red sokoto (Onakpa *et al.,* 2010). El- Shafey *et al* (2011) found that abamectin effect on sex hormone, sperm count and motility of sperm of male albino rats due to effect of pesticide on sex gland. El-Betieha and Isa (2003) reported that the rat treated with abamectin which could be explained by the fact that the pesticide acted directly on the testis and effected the androgen biosynthesis pathway.

LH was significantly decreased and prolactin was significantly increased may be due to attributed in pituitary gland. On the other hand Celike *et al.* (2012) recorded non significantly decreased in serum LH and FSH in men's exposure to abamectin. El-Ashmawy *et al.* (2011) found that albino rats due to increase different types of

chromosomal aberrations in embryos cells. Molinari *et al.* (2009) reported that ivermectin caused damaged in ovary cells of hamster due to accumulation of pesticide.

E_2 and progesterone were significantly decreased after treatment may be due to effect of biocide on pituitary gland and damage of uterus cells.

In the present study abamectin treatment revealed sever congestion was noticed in the medullary blood vessels after three days, but it was Proliferation was noticed in the interstitial stromal cells at the medullary portion in ovary after seven days. The lining mucosal epithelium showed cellular hypertrophy after three, while Hypertrophy was detected in the lining mucosal epithelial cells as well as in the muscularis in oviduct after seven days. Hypertrophy with vacuolization was detected in the lining mucosal epithelium and associated with congestion in the blood vessels of the muscularis in uterus after three days, while the focal vacuolization and degeneration as well as focal stratification in the lining mucosal epithelial cells in uterus after seven days. These changes in sex organs of female rats may be due to dose of pesticide. Similar results were reported by Al-Hizab *et al.* (2010) who mentioned that significant pathologic alterations that mainly affect the genital organs of female guinea pigs. The European Agency for the Evaluation for Medicine Products (1997) recorded dead of fetus of rats due to passes of dormactin of placental barrier.

Finally, abamectin biocide was toxic on sexual hormone and sex organs of mature female rats. It reduced the fertility of female rats, so abamectin biocide is very important to use for rodent control.

Table 1. Effect of oral single sublethal dose (1/4 LD50) of abamectin biocide (2.18 mg/kg) on plasma FSH, LH and prolactin hormones of female albino rats.

Parameters / Days after treatment	FSH (mIU/ml)	LH (mIU/ml)	Prolactin (mIU/ml)
Control (0)	$0.05^{a} \pm 0.01$	$0.51^{a} \pm 0.01$	$0.68^{c} \pm 0.02$
3days	$0.03^{b} \pm 0.00$	$0.21^{a} \pm 0.03$	$1.61^{b} \pm 0.11$
7 days	$0.02^{b} \pm 0.00$	$0.02^{b} \pm 0.00$	$1.85^{a} \pm 0.08$
LSD	0.01	0.01	0.19

Values are expressed as means (10 rats) ± standard errors ^{abc} values in column with different letters are significantly different at ($P \leq 0.05$).

Table 2. Effect of oral single sublethal dose (1/4 LD_{50}) of abamectin biocide (2.18 mg/kg) on brain tissue E_2 and progesterone hormones of female albino rats.

Parameters / Days after treatment	E_2 (pg/ml)	Progesterone (ng/ml)
Control (0)	$80.61^{a} \pm 0.27$	$0.84^{a} \pm 0.02$
3days	$48.05^{b} \pm 0.02$	$0.52^{b} \pm 0.002$
7 days	$36.61^{c} \pm 0.04$	$0.31^{c} \pm 0.009$
LSD	1.16	0.05

Values are expressed as means (10 rats) ± standard errors ^{abc} values in column with different letters are significantly different at ($P \leq 0.05$).

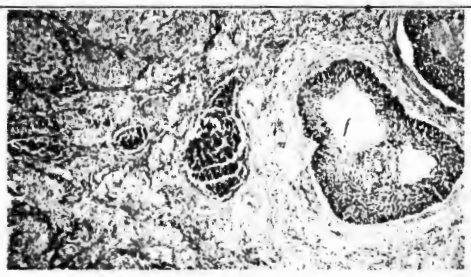


Fig (1): Ovary of rat in treated groups with 1\4 LD_{50} of abamectin after 3 days showing sever congestion was noticed in the medullary blood vessels(v). (H&E* 16).

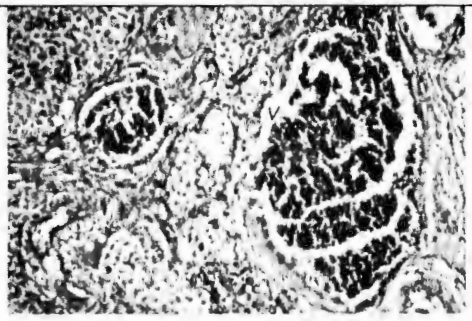


Fig (2): Ovary of rat in treated groups with 1\4 LD_{50} of abamectin after 3 days showing the magnification. (H&E*40).

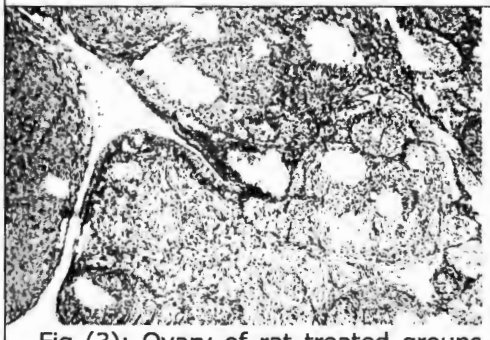


Fig (3): Ovary of rat treated groups with 1\4 LD_{50} of abamectin after 7 days showing proliferation was noticed in the interstitial stromal cells (t) at the medullary portion. (H&E* 16).

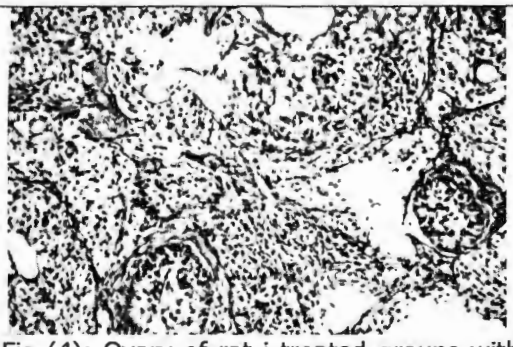


Fig (4): Ovary of rat i treated groups with 1\4 LD_{50} of abamectin after 7 days showing the magnification. (H&E*40)

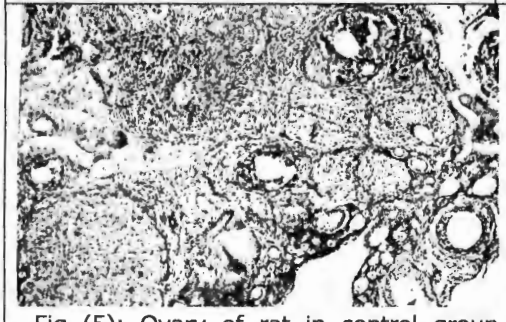


Fig (5): Ovary of rat in control group showing different stages of ovarian follicles (f), corpus luteum (c) and interstitial stromal cells(t). (H&E* 16)

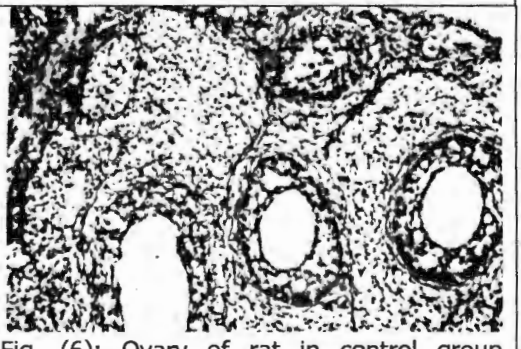


Fig. (6): Ovary of rat in control group showing the magnification. (H&E* 4o)

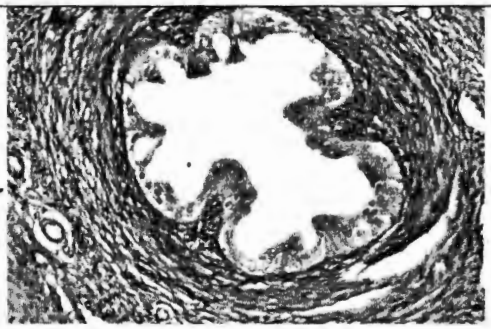


Fig (7): Oviduct of rat treated group with 1\4 LD_{50} of abamectin after 3 days showing the lining mucosal epithelium showed cellular hypertrophy (mu) (H&E*40)

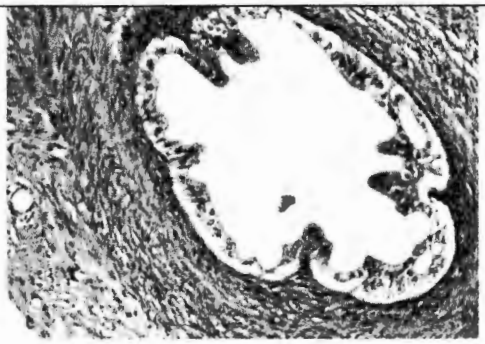


Fig (8): Oviduct of rat treated group with 1\4 LD_{50} of abamectin after 7 days showing the lining mucosal epithelial cells(mu) as well as in the muscularis(ml) (H&E*40)

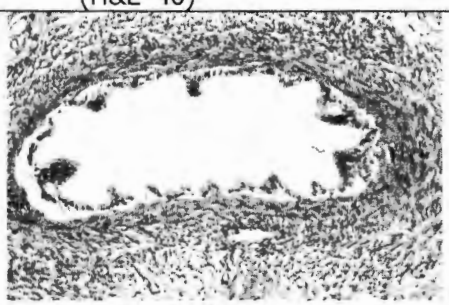


Fig (9): Oviduct of rat in control group showing the normal histological structure of the mucosa (mu) ,submucosa (sm) and muscularis (ml) (H&E*16).

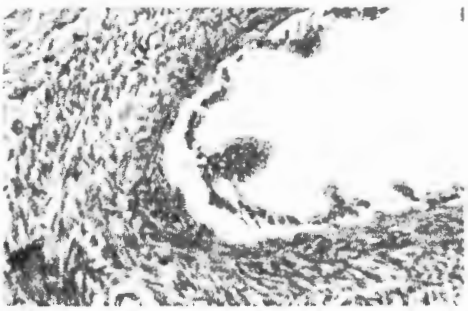


Fig (10): Oviduct of rat in control group showing the magnification to identify the mucosa (mu) and submucosa (sm) (H&E *40).

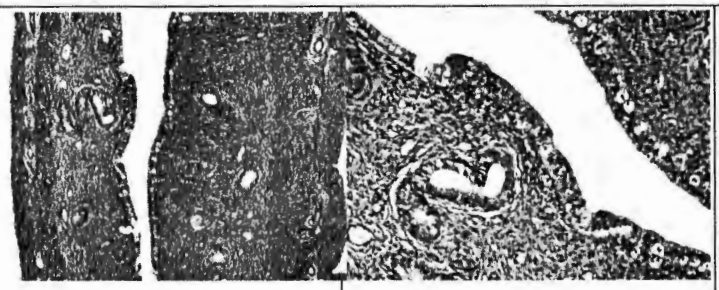


Fig (11&12): Uterus of rat treated group with 1\4 LD_{50} of abamectin after 3 days showing the Hypertrophy with vacuolization were detected in the lining mucosal epithelium (mu).

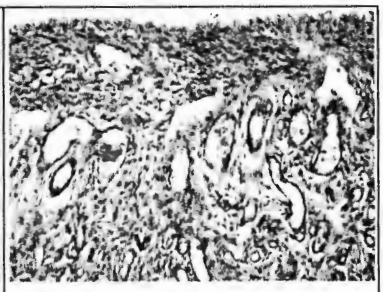
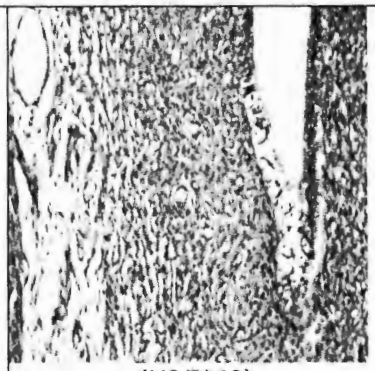
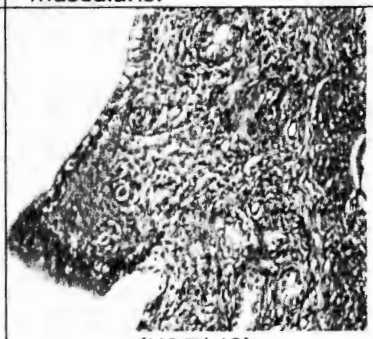


Fig (13): Uterus of rat treated group with 1\4 LD_{50} of abamectin after 3 days showing the associated with congestion in the blood vessels (v) of the muscularis.

(H&E*16)

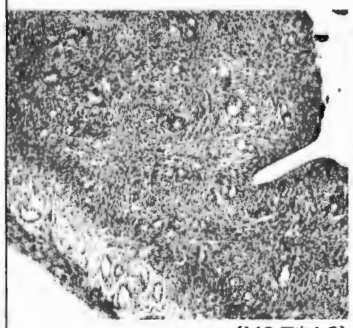


(H&E*40)

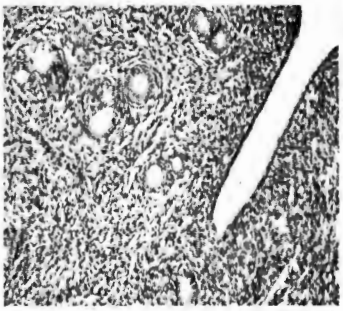


(H&E*40)

Fig (14): Uterus of rat treated group with 1\4 LD_{50} of abamectin after 7 days showing the focal vacuolization and degeneration as well as focal stratification in the lining mucosal epithelial cells



(H&E*16)



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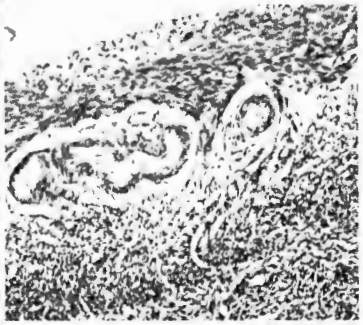

(H&E*40)

Fig (15): Uterus of rat in control group showing the normal histological structure of the mucosa(mu) , glandular structure(g), submucosa (smu), muscularis and serosa

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الهرمونات الجنسية والتغيرات المرضية فى اناث الجرذان البيضاء المعاملة بالمبيد الحيوى الابامكتين

راندا عبد السميع قنديل

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تم دراسة تأثير ٤١١ قيمة الجرعة المميتة للنصف للمبيد الحيوى الابامكتين على الهرمونات الجنسية للمبيض وقناة المبيض و الرحم لاناث الجرذان البيضاء البالغة حيث تم تقدير هرمون المنبة للجريب و الهرمون اللوتينى و البرولاكتين والاستروجين و البروجسترون فى البلازما و نسيج المخ بعد ٣ و ٧ ايام من المعاملة. اشارت النتائج الى حدوث نقص معنوى لهرمون للجريب و الهرمون اللوتينى بعد ٣ و٧ ايام مقارنة بالكنترول بينما حدثت زيادة معنوية لهرمون البرولاكتين فى الحيوانات االمعاملة بعد ٣ و٧ ايام فى البلازما . و فى نسيج المخ لوحظ زيادة معنوية فى كلا من هرمون الاستروجين و البروجسترون بعد ٣ و ٧ ايام.

لوحظ ايضا احتقان شديد فى الاوعية الدموية لنخاع المبيض فى اليوم الثالث مع تزايد ملحوظ فى خلايا التسيج الضام فى اليوم السابع بعد المعاملة . وقد حدث تضخم فى الخلايا المبطنة لانابيب فالوب بعد ٣ ايام و ايضا ظهر هذا التضخم فى الطبقة العضلية لجدار الانابيب بعد ٧ ايام.

ظهور تضخم و فجوات فى الخلايا المبطنة مع احتقان فى الاوعية الدموية فى الطبقة العضلية لجدار الرحم بعد ٣ ايام ، وظهر ايضا تنخر فى الخلايا المبطنة لجدار الرحمع بؤر تعدد الطبقات البطنة للطبقة المخاطية بعد اليوم السابع مقارنة بالنسيج الطبيعى.

الاستنتاج : ان المبيد الحيوى الابامكتين سام جدا على الهرمونات الجنسية و الاعضاء التناسلية لاناث الجرذان البالغة . هذا التأثير الفعال على خصوبة اناث الجرذان ادى الى نقص فى تعداد الجرذان لذلك نستطيع ان نستخدم الابامكتين كمبيد قوارض فى برنامج مكافحة الفئران.