Histopathologic Studies on the Effect of Repeated Doses of Dectomax on Some Genital Organs of Female Guinea Pigs

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Abstract:

Histopathologic studies were carried out in order to detect the adverse effect and tissue alterations in the genital organs of healthy female guinea pigs after weekly repeated injections of variable dose levels of Dectomax (Doramectin). A total of 72 adult female guinea pigs were used for this purpose. They were divided into four groups; non-treated control and other three groups treated with therapeutic dose (0.2 mg/kg. b.wt), double therapeutic dose (0.4 mg/kg. b.wt) and triple therapeutic doses (0.6 mg/kg. b.wt). Microscopic examination revealed minimal or no changes in the genital organs of the female guinea pigs which received a total of 6 weekly repeated therapeutic doses. On the other hand, the repeated injections of double or triple therapeutic doses resulted in remarkable histopathologic alterations dominated by follicular degeneration and necrosis in the ovaries. In addition, there was interstitial edema, congestion and degenerations of the endometrial glands, especially after the 5^{th} and 6^{th} repeated triple doses. It was concluded that the repeated as well as duplication for the therapeutic dose levels lead to some adverse effects on the female genital organs.

Introduction :

Doramectin (Dectomax) is a member of the Avermectin class of compounds which includes Abamectin and Ivermectin. It is a semi-synthetic product that has a structural similarity to Abamectin and Ivermectin. The drug is used for treatment of ectoparasites as well as endoparasites in various animals such as cattle (*Float, et al., 2001, Taylor, et al., 2001 and Sallovitz, et al., 2005*), canines (*Lavy, et al., 2003*) and rabbits (*Voyvoda, et al., 2005*). Previous studies have shown a long-term persistence of unwanted residues of Ivermectin and Doramectin in treated animal tissues and fluids (*El-Sadek, et al., 1985 and Crook, et al., 2000*). Studies on maternal / embryotoxic and teratogenic effects of Doramectin were performed in many animals including rats, mice and rabbits. The detection of Doramectin in rat and mouse fetuses as well as the embryo mortality in rabbits indicated that Doramectin passes the placental barrier in these species (*The European Agency for the Evaluation for Medicine Products, 1997*). Some studies on Avermectins were also reported on female cattle (*Rehbein, et al., 2002*), goats (*Dupuy, et al. 2001 and Imperiale, et al. 2003*), and rats (*Souza, et al. 2000*) but no reports on their effects on the female genital organs or

reproductive performance in either rabbits or guinea pigs are currently available. The present work was thus aimed to study the possible adverse effects and tissue alterations in the ovaries and uteri of female guinea pigs following repeated administration of variable dose levels of Doramectin.

Material and Methods :

- **Animals:** A total of 72 adult female guinea pigs, weighing 400 - 450 g., were used. The animals were grouped and housed in separate metal cages and fed on standard pelleted rabbit food. Both food and water were supplied *ad libitum*.

- **Drugs:** Dectomax (Doramectin, 10 mg/ml), a sterile injectable drug, colorless to pale yellow solution contained 1 % (W/V) Doramectin (Pfizer). The drug was administered through intramuscular route of injection in the neck region.

- **Experimental design:**

- ***Treatments:*** The animals were divided into 4 groups, each of 18 female guinea pigs. Animals of the first group (GI) were the non-treated control. Animals of the second group (GII) were weekly injected with the therapeutic dose of Dectomax (0.2 mg/kg) for 6 weeks. Animals of the third group (GIII) were weekly injected with double of the therapeutic dose (0.4 mg/kg) for 6 weeks and the fourth group (GIV) was further injected with triple of the therapeutic dose (0.6 mg/kg) also for 6 weeks (Table, 1). The animals of various groups were kept under closed observation. Three animals from each group were sacrificed at weekly intervals and subjected for postmortem examination and specimen collection from the uteri and ovaries for the histopathologic examination.

- ***Histopathologic studies:*** The collected specimens of the ovaries and uteri were fixed in 10 % neutral buffered formalin, washed in water and passed through the routine technique of the paraffin-wax embedding and preparation of paraffin blocks. The blocks were sectioned at 3-5 microns thickness, mounted on microscopic glass slides and subsequently stained with hematoxylin and eosin (H&E) according to the methods of Culling (1983) and then subjected for the light microscopy.

Table (1)
Experimental design

Groups	No.	Doses			Schedule for sacrificed animals/week					
		T	2T	3T	1W	2W	3W	4W	5W	6W
GI	18	-	-	-	3	3	3	3	3	3
GII	18	+	-	-	3	3	3	3	3	3
GIII	18	-	+	-	3	3	3	3	3	3
GIV	18	-	-	+	3	3	3	3	3	3
	72	-	-	-	12	12	12	12	12	12

No. : Number of guinea pigs **T:** Therapeutic dose **W:** Week
2T: Double therapeutic dose **3T:** Triple therapeutic dose

Results :

- ***Histopathologic finding in organs of the control group:***
  Microscopic examination of the ovaries and uteri of GI animals revealed normal histologic structures throughout the experiment period.

- ***The effect of repeated weekly administration of therapeutic dose:***
  No microscopic changes were seen in the examined genital organs of GII animals following the repeated weekly administration of the therapeutic dose of Dectomax throughout the experiment period.

- ***The effect of repeated weekly administration of double therapeutic dose:***
  Microscopic examination of the genital organs of GIII animals revealed the following changes: The uterus showed some degrees of endometrial edema on the 3rd week post inoculation. Following subsequent weeks of administration, the changes of endometrial edema were further accompanied with degenerative changes and atrophy of the endometrial glands (Fig. 1), especially after the administration of the 6th dose (Final week of the experiment). No abnormal changes were seen in the ovarian tissue, except of some vascular changes of capillary congestion that were seen at the last 2 weeks of the experiment.

- ***The effect of repeated weekly administration of triple therapeutic doses:***
  After administration of 3 doses to GIV animals, the uteri showed some degrees of endometrial infiltrations and aggregation of mononuclear cell with congestion of the perimetrial blood vessels (Fig. 2). After the 4th

dose, the ovaries were affected with degeneration of the oocytes of the small growing follicles, while some of the large mature follicles were apparently damaged (Figs. 3 & 4). The endometrial glands in the uterus of those cases appeared degenerated, atrophied and widely separated by the edema and cellular reactions (Fig. 5). After the administration of 5 doses, the ovaries appeared with excess atretic and damaged growing follicles (Fig. 6). In addition the uterus showed some degrees of glandular atrophy and endometrial edema. The administration with the 6^{th} doses was further associated with similar but more progressive changes in the ovarian tissue. The ovaries contained large numbers of the degenerated and atretic growing follicles (Fig. 7). Some of these degenerated follicles contained degenerated, shrunken and wrinkled oocytes (Fig. 8). Also the uterus showed several areas of degenerated and atrophied endometrial glands, edema and cellular reaction.

Discussion

The above mentioned pathologic alterations in the genital organs of female guinea pigs were in line with some previous reports on Avermectin (*Cousens et al., 1997*). However, these findings were not in agreement with the reports of *Lanks, et al., (1989)* that indicated the absence of adverse effect of Ivermectin on the reproductive performance of female rats.

The adverse effect of Doramectin on the genital organs of female guinea pigs observed in the present work was probably due to the cumulative effect of the six weekly repeated triple therapeutic doses of the drug. Such effect is well attributed to the high bioavailability of Doramectin and its known long duration of action (*Toutain, et al., 1997 and Williams, et al., 1997*). On this respect it is worth mentioning that a wide safety margin is well documented for this drug (*The European Agency for the Evaluation for Medicine Products, 1997*). The loss of correlation between these results and other reports about the wide safety margins is probably due to either species difference in metabolism and excretion or to some dose dependent factors.

Finally, it was concluded that the repeated administration of therapeutic doses of Doramectin has a wide margin of safety and leads to minimal or absence of adverse effects on the genital organs of female guinea pigs; while the repeated administration of higher doses would eventually cause significant pathologic alterations that mainly affect the genital organs of female guinea pigs and so may affect their reproductive performance.

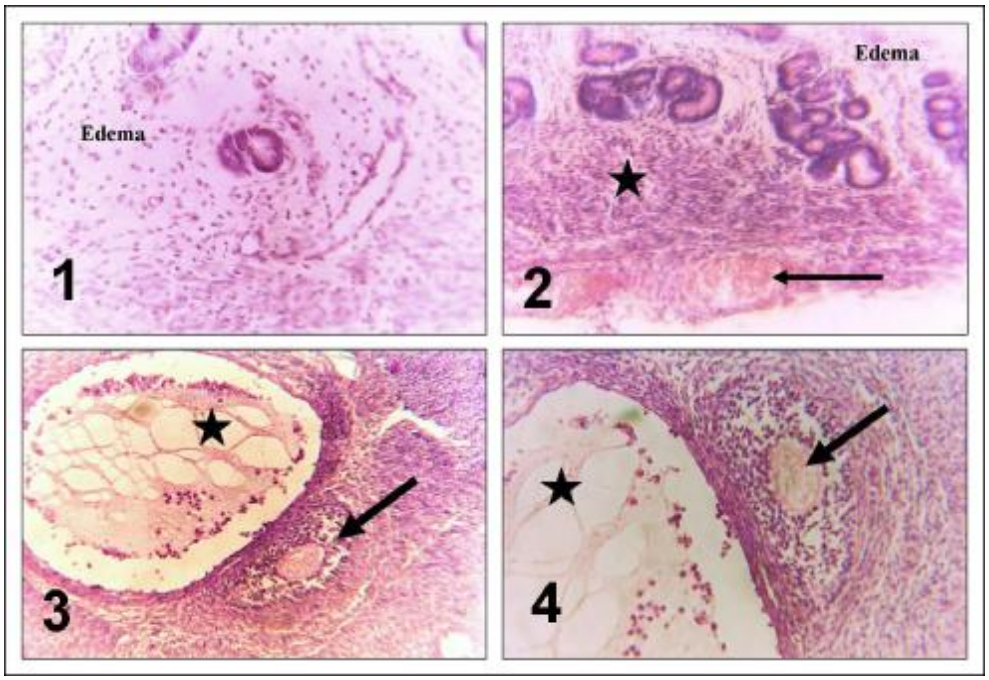

Fig. 1: Uterus of female guinea pig received six therapeutic doses, showing small atrophied endometrial glands widely separated by edema. H and E. X 250.

Fig. 2: Uterus of female guinea pig received three triple therapeutic doses, showing endometrial edema and mononuclear cell reaction (asterisk) with congestion of the perimetrium blood vessels (arrow). H and E. X 160.

Fig. 3: Ovary of female guinea pig received four triple therapeutic doses, showing one small follicle with degenerated oocyte (arrow) and other large damaged mature follicle (asterisk). H and E. X 250

Fig. 4: Higher magnification of fig. 3 to show the degenerated and necrotic oocyte (arrow) in addition to the luminal contents of necrotic debris inside the large damaged mature follicle (asterisk). H and E. X 400

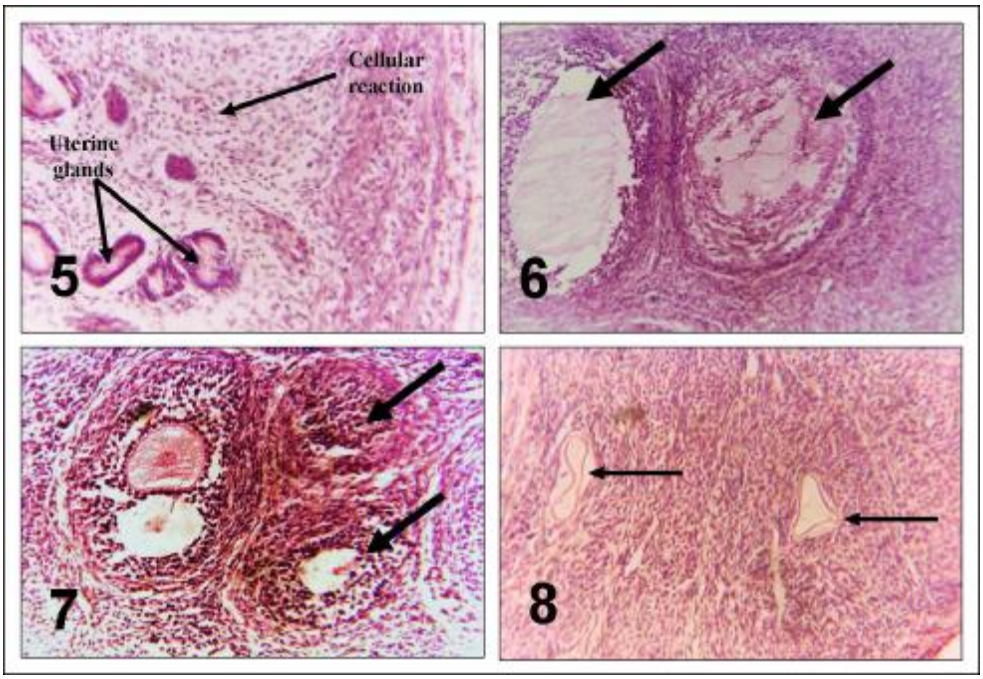

Fig. 5: Uterus of female guinea pig received four triple therapeutic doses, showing degenerated and atrophied endometrial glands widely separated by edema and excess of cellular infiltrations. H and E. X 250.

Fig. 6: Ovary of female guinea pig received five triple therapeutic doses, showing damaged and atretic growing follicles (arrows). H and E. X 160.

Fig. 7: Ovary of female guinea pig received six triple therapeutic doses, showing excess of the degenerated and atretic growing follicles (arrows). H and E. X 400.

Fig. 8: Ovary of female guinea pig received six triple therapeutic doses, showing atretic follicles with degenerated and wrinkled oocytes (arrows). H and E. X 250.

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دراسات نسيجية مرضية على تأثير الجرعات المتكررة للدكتوماكس على بعض الأعضاء التناسلية لإناث خنازير غينيا

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الملخص :

أجريت دراسات نسيجية مرضية بهدف الاستدلال على التأثيرات الجانبية والتغيرات النسيجية المرضية بالرحم والمبيض لإناث خنازير غينيا والممكن حدوثها أثناء العلاج المتكرر لجرعات مختلفة من مركب الكتوماكس (الدورامكتين)، والمستخدم كعلاج مضاد للإصابات الطفيلية.

ولقد دل الفحص المجهري للعينات النسيجية على عدم وجود تغيرات مرضية أو آثار جانبية واضحة للعلاج المتكرر بالجرعات العلاجية. أما في حالة العلاج المتكرر مع مضاعفة الجرعة العلاجية فقد أدى إلي حدوث آثار جانبية وتغيرات نسيجية مرضية متعددة بتلك الأعضاء التي تم فحصها. وقد كان من أهم تلك التغيرات ضمور في غدد بطانة الرحم مع حدوث إرتشاحات للخلايا أحادية النواة وإحتقان الأوعية والشعيرات الدموية وذمات رحمية. كما شملت التغيرات المبيض حيث اتضح حدوث هدم وتنكس في الخلايا والأكياس المبيضية.

ولقد خلصت تلك الدراسة إلى أن تكرار العلاج بالجرعة العلاجية للدكتوماكس لا يؤدي إلى آثار جانبية واضحة في حين أن تكرار العلاج بمضاعفات الجرعة العلاجية يؤدى إلى حدوث آثار جانبية عكسية بالغة بالأعضاء التناسلية وبالتالي فهي يمكن أن تؤثر على الكفاءة التناسلية لإناث خنازير غينيا.