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EFFECT OF IVERMECTIN ON FEMALE FERTILITY AND TERATOGENICITY IN RATS

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ABSTRACT

Ivermectin has gained a good reputation as the drug of choice having a broad spectrum nematocidal, insecticidal and acaricidal activities. Nevertheless, the seemingly promising future use of Ivermectin, could be highly precluded by its side effects. The study pointed out that, ivermectin prolonged the oestrus phase in 40% of the examined females in the first group and 60% in the second group the observed morphological deformities in response to ivermectin injection were mostly the presence of red patches in different parts of the body, swelling above the eyes and protrusion of the intestine from the abdomen. The most frequently encountered desirable internal malformations in response to ivermectin injection at a dose of 0.2 and 0.4 mg/kg b.wt. were; cleft palate, thickening of the ventricular wall of the heart, haemorrhage around brain and dilatation of renal pelvis. Skeletal malformations in response to both doses of ivermectin were; incomplete ossification of the cranium, absence of the last coccygeal vertebrae, rudimentary or missing sternebrae and tiphisternums and absence of phalanges of both the fore and hind limbs.

INTRODUCTION

Ivermectin, a potent broad spectrum antiparasitic drug, is a mixture of two natural fermentation products of *Streptomyces avermititis*. The avermectins are a group of chemically related anthelmintics produced by fermentation of an actinomycete, newly named *Streptomyces avermititis*, was originally isolated from a soil sample in Japan.

The drug mobilizes internal nematodes by blocking the excitatory motor neurons in parasites which contain gamma amino butyric acid (GABA) in their nervous system[1].

Ivermectin was an antiparasitic agent that paralyzed nematodes without causing hypercontraction or flaccid paralysis using selective stimulation techniques. It has been shown that ivermectin blocked transmission between interneuron and excitatory motorneurons in the ventral nerve cord of Ascaris. The drug also inhibited transmission between inhibitory motorneuron and muscle [2].

Moreover, the drug has an ovicidal as well as larvicidal effects. Nevertheless, the extremely potent antiparasitic activity of ivermectin is greatly averted by its frequently encountered adverse effects in horses, goats and rabbits [3].

Given this concept, it is noteworthy to conjecture that the female reproductive system could be highly prone to the unwholesome effects of the drug. The previous forethought was attested by studying the possible effects of ivermectin on the rat foeti, and neonates.

MATERIAL AND METHODS

Animals :

A total number of fifteen female albino rats, displaying regular oestrus cycles, were selected for investigating the effect of ivermectin on the oestrus cycle. The animals were grouped into 3 equal groups each of five. The first group was injected S.C. with therapeutic dose of ivermectin; 0.2 mg/kg b.wt. , the second group was injected S.C. with double therapeutic dose; 0.4 mg/kg b.wt. The last group received no treatment and considered as a control group.

The effects of ivermectin on foetal development were investigated on fifty five mature female albino rats, using thirty mature male albino rats for mating purpose. Five female rats recieved no treatment and considered as a control group. The other fifty were divided into five main equal groups each of ten. The main groups were further divided into two equal subgroup each of five. The first subgroups were S.C. injected with the therapeutic dose of ivermectin; 0.2 mg/kg b.wt. The second subgroups were S.C. injected with double therapeutic dose; 0.4 mg/kg b.wt. The first main group was injected on the 1st day, the second on 6th day, the third on 10th and the fourth on the 15th day of gestation. The last main group was injected on both the 1st and the 15th day of gestation. Daily vaginal smears were examined and the female proved to be in oestrus was paired with a fertile male rat in a separate cage.

The method used for morphological examination of foeti is previously described [4]. The number of alive and dead foeti, foetal body weight and foetal crown -rump length were recorded. Visceral

examination of foeti preserved in Bouin's fixative and rinsed with cold water were examined grossly. Skeletal examination of eviscerated foeti were conducted [5].

Culturing of the bone marrow was carried out according to previous rport [6].

RESULTS AND DISCUSSION

The number of implantation sites, number of resorbed, dead and viable foeti were recorded in Table (1) and shown in Figs. (1 through A). The obtained data clearly demonstrated that ivermectin evoked a significant increase in foetal resorbtion rate in dams injected ivermectin, 0.2 mg/kg b.wt. on the 1st, 10th and both the 1st and 15th day of gestation and in dams injected with 0 4 mg/kg b.wt. on the 1st, 6th, 10th and both the 1st and 15th day of gestation. Ivermectin provoked a marked increase (0.2±0.02 and 0.4±0.04) of the mean values of the dead foeti from dams injected with 0.2-0.4 mg/kg b.wt. respectively on the 15th day of gestation and 1.2±0.7 in foeti from dams injected with 0.4 mg/kg b.wt. on both the 1st and the 15th day of gestation compared with the control. Ivermectin elicited a significant increase in both the pre-implantation deaths in dams injected with 0.4 mg/kg b.wt. on the 1st and on both the 1st and 15th day of gestation and post-implantation death in dams injected with 0.2 and 0.4 mg/kg b.wt. on the 1st and on both the 1st and 15th day of gestation.

The possible teratogenic effects of ivermectin evaluated by morphological, visceral and skeletal examination of the foeti. Ivermectin, 0.2 and 0.4 mg/kg b.wt. provoked a significant decrease in the average foetal body weight and foetal crown rump length of the obtained foeti compared with the untreated control group (Fig.5).

Red patches with varying sizes was seen externally on different parts of the foetal body especially in the head, limbs and back (Fig. 6). Ivermectin induced swelling above the eyes, in 15% of the examined foeti obtained from dams subcutoneously injected with 0.4 mg/kg b.wt. on both 1st and 15th day of gestation, (Fig.7). Ivermectin elicited protrusion of the intestine from the abdomen, in 10%, of examined foeti obtained from dams subcutaneously injected with, 0.4 mg/kg b.wt. on both 1st and 15th day of gestation (Fig.8).

Foeti obtained from the treated and control groups and latex kept in Bonin's solution were macroscopically examined by the aid of magnifying hand lens. The obtained results are recorded in Table (2) and revealed in Figs. (9-13). Subcutaneous injection of ivermectin; 0.2 mg/kg b.wt. to pregnant rats on the 1st day of gestation period led to mainfold visceral abnormalities in the foeti; palate in 21.4 % . Under the influence of the drug injection on the 6th day of gestation, palate in 55.6%, and kidney in 16.7%. Ivermectin administration in 10th day, palate in 33.3%, heart in 26.7% on response to the drug administration on both 1st and 15th day of gestation, visceral abnormalities comprised, palate in 71.4%, brain in 43%, kidney in 29% and heart in 27.1%.

Subcutaneous injection of ivermectin; 0.4 mg/kg b.wt. evoked also visceral abnormalities.

Detailed examination of the visceral abnormalities revealed cleft palate (Fig. 9 and 10), echonosis of blood around brain and suborbital area (Fig. 10) thicking of the ventricular wall of heart (Figs. 11 and 12), and slight dilation of renal pelvis (Fig. 13).

Mutagenic effects:

The mutagenic activity of ivermectin in female rats clearly depicted in Table (3). In the present work, it has been obvious that in pregnant female rats the subcutaneous administration of both therapeutic dose of ivermectin and its two-fold did evoked a significant increase in foetal resorption rodes which correlated with that results previously reported [7]. The foetal body weight and the crown-rump length faithfully echo, the foetal development and neonatal mortality coupled with the concept that chromosomal abnormalities induced by many chemicals affect cellular DNA and consequently the foetal growth [8].

Given this background, it is tempting to suggest that the decreased foetal body weight and length is a forthright outcome of the chromosomal abnormalities. It is intriguing to suggest that ivermectin induced teratogenesis in multifactorial in origin. This concept is best explained by the fact that ivermectin is directly toxic to foetus, exerts indirect toxicity to the foetus probably by alteration of its endocrine balance, affects the endocrine balance, affects the endocrine status of the mother and brings about chromosome damage.

Given this forethought, one is enticed to propose that the neonatal toxicity of ivermectin in rats is probably the result of a combination of excessive exposure through maternal milk and the increased permeability of the blood brain barrier during the early postnatal period in these species. This conclusion is highly substituted by the notion that high drug levels are found in the plasma and brain of offsprings relative to adult rats. In particular, there is a greater concentration of the drug in the brain of neonatal rats during the early neonatal period of enhanced sensitivity and this may explain the increased toxicity. This early neonatal period of increased sensitivity to ivermectin toxicity in rats correlates well with the postnatal completion of the blood brain barrier in these species[9].

Further support for the importance of postnatal exposure in the toxicits observed in neonatal rats is

Table (1): Morphological changes and mortality rate in foeti from dam rats subcutaneously injected during gestation, with the therapeutic dose of ivermectin (a); 0.2 mg/kg B.wt. and its twofold (b).

Treatment	Injection day	Number/mother								Foetal B.wt. (gm)	Foetal length (cm)	Deaths	
		Number of corpus luteum	Number of implantation sites	Viable foeti		Dead foeti		Resorbed foeti				Pre-implantation	Post-implantation
				Mean ± S.E.	%	Mean ± S.E.	%	Mean ± S.E.	%				
0	--	10.2±0.3	10±0.3	9.4 ±0.3*	94	0	0	0.6±0.2	6	4.2±0.08	3.8±0.04	1.02±0.02	0.15±0.04
a	1st	7.4±0.5*	6±0.4**	3.2±0.6*	54	0	0	2.8±0.5*	46	3.8±0.1*	3.4±0.1*	1.3±0.11	0.54±0.1*
b		8±0.4**	7±0.3**	3.6±0.8*	52	0	0	3.4±0.6**	48	2.8±0.1**	2.8±0.1**	1.44±0.1*	0.7±0.1**
a	6th	11±0.1**	10.2±0.06	8.2±0.2*	80	0	0	2±0.7	20	3.9±0.07*	3.5±0.04**	1.08±0.02	0.18±0.06
b		8.4±0.4**	7.6±0.46**	5.6±0.8*	73	0	0	2±0.5*	27	3.8±0.08*	3.4±0.05**	1.02±0.03	0.3±0.04
a	10th	9.6±0.2**	9.0±0.2	6.6±0.4*	75	0	0	2.2±0.2*	25	3.6±0.05**	3.4±0.03**	1.08±0.02	0.14±0.04
b		9.2±0.21**	9.2±0.2*	5.2±0.6*	57	0	0	3.8±0.5**	41	3.5±0.06**	3.3±0.06**	1.02±0.02	0.56±0.18
a	15th	6.8±0.3**	6.6±0.3**	5.6±0.2*	86	0	0	0.8±0.3	12	3.6±0.1**	3.3±0.06**	1.02±0.02	0.09±0.04
b		7.2±0.5**	7±0.5**	5.2±0.6*	75	0.4±0.04	6	1.2±0.2	17	3.2±0.1**	3.2±0.03**	1.02±0.2	0.17±0.03
a	both 1st	8.2±0.4**	7±0.6**	2.8±0.5*	40	0.2±0.02	3	4±0.3**	57	3±0.1**	2.7±0.1*	1.28±0.09	0.58±0.12*
b	and 15th	7.6±0.3**	8.2±0.3**	2.4±0.5*	30	1.2±0.7	15	4.2±0.3**	52	2.5±0.1**	2.5±0.1**	1.3±0.08*	0.6±0.12*

* Significant at P < 0.05 ** Significant at P < 0.01

Table (2): Visceral malformations in foeti from dam rats subcutaneously injected during gestation, with the therapeutic dose of ivermectin (a); 0.2 mg/kg B.wt. and its twofold (b).

Treatment	Number of examined foeti	Injection day	Malformations							
			Palate		Brain		Kidney		Heart	
			No	%	No	%	No	%	No	%
0	16	--	--	00.0	--	00.0	--	00.0	--	00.0
a	14	1st	3	21.4	--	00.0	--	00.0	--	00.0
b	20		5	25.0	1	10.0	3	15.0	--	00.0
a	18	6th	10	55.6	--	00.0	3	16.7	--	00.0
b	10		6	60.0	4	40.0	3	30.0	--	00.0
a	11	10th	5	45.5	--	0.00	--	00.0	--	00.0
b	10		6	60.0	4	40.0	3	30.0	2	20.0
a	6	15th	2	33.3	--	00.0	--	00.0	1	26.7
b	9		3	33.3	1	11.1	--	00.0	4	44.4
a	7	both 1st and	5	71.4	3	43.0	2	29.0	4	27.1
b	8	15th	6	75.0	4	50.0	3	37.5	5	62.5

Table (3): Skeletal malformations in foeti from dam rats subcutaneously injected during gestation, with the therapeutic dose of Ivermectin (a), 0.2 mg/kg B.wt. and its twofold (b).

Treatment	Number of examined foeti	Injection day	Malformations									
			Incomplete fusion of the cranium		Absent							
					Coccygeal vertebrae		Sternebrae		Phalanges of fore limbs		Phalanges of hind limbs	
			No	%	No	%	No	%	No	%	No	%
0	16	--	--	00.0	--	00.0	--	00.0	--	00.0	--	00.0
a	7	1st	--	00.0	2	28.6	1	14.3	3	42.8	4	57.1
b	9		2	22.3	4	44.5	5	55.6	4	44.5	5	55.6
a	14	6th	9	64.3	7	50.0	10	71.5	8	57.1	9	64.2
b	18		13	72.3	11	61.0	13	72.3	11	61.0	12	66.7
a	18	10th	10	55.6	6	33.3	11	61.0	10	55.6	11	61.0
b	9		6	66.7	5	55.6	6	66.7	5	55.6	6	66.7
a	10	15th	2	20.0	3	30.0	6	60.0	4	40.0	3	30.0
b	13		6	46.2	5	38.5	9	69.3	6	46.2	5	38.5
a	8	both 1st and	6	75.0	5	62.5	6	75.0	5	62.5	6	65.0
b	7	15th	6	85.7	6	85.7	6	85.7	5	71.4	6	85.7

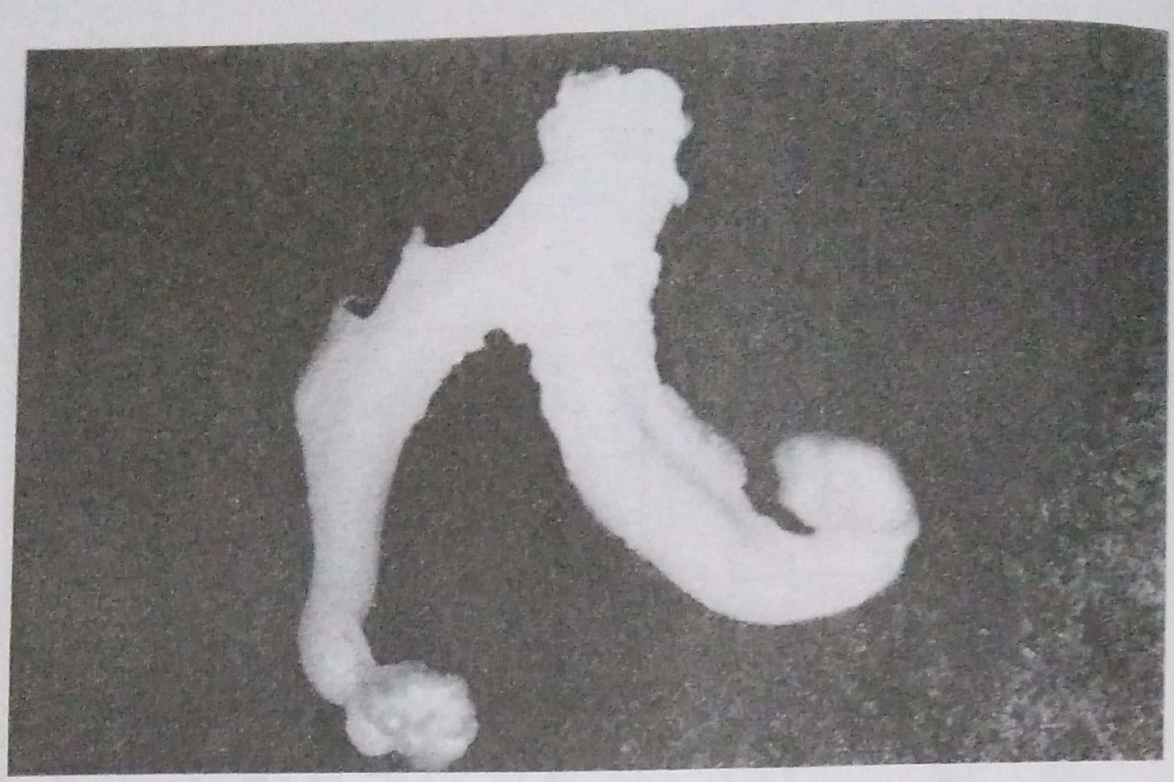

Fig. (1): Uterus of a pregnant female rat subcutaneously injected with ivermectin;0.4 mg/kg B.wt. on the 1st day of gestation, displaying oedema and presence of corpora lutea.

Fig. (2): Uterus of a pregnant female rat subcutaneously injected with ivermectin;0.4 mg/kg B.wt. on the 6th day of gestation,demonstrating oedema and early foetal resorption.

Zagazig J. Pharm. Sci. December 1996
Vol. 5, No. 2, pp.113-124

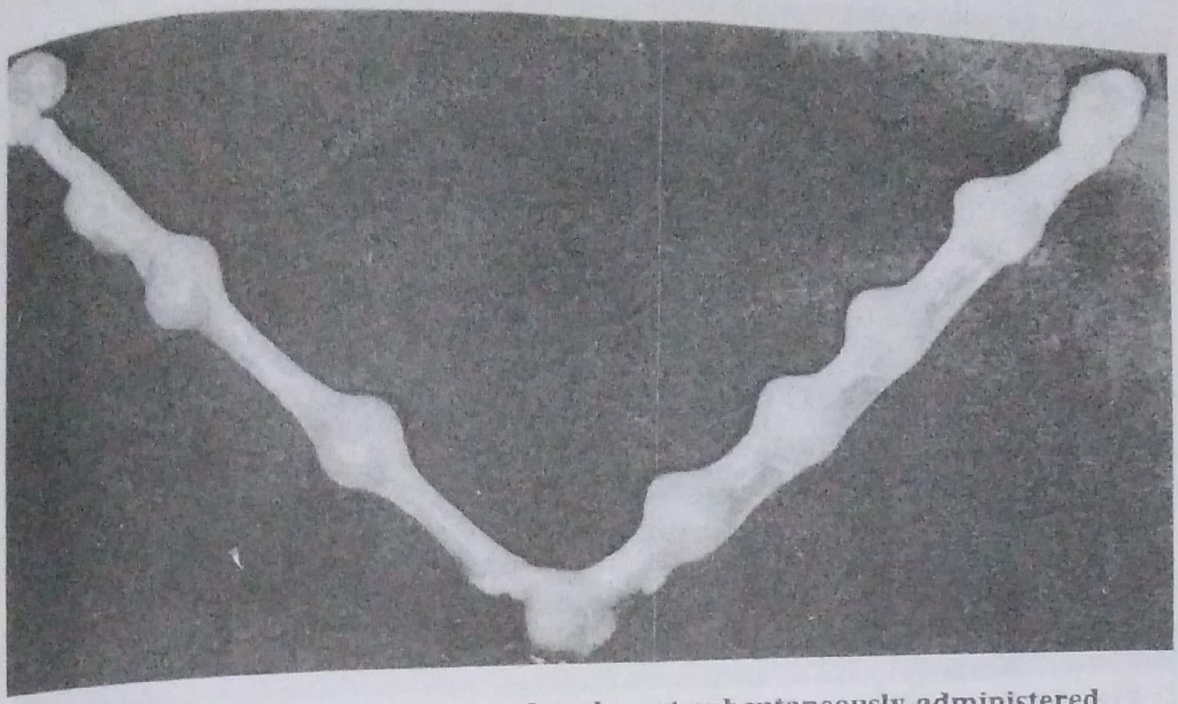

Fig. (3): Uterus of a pregnant female rat subcutaneously administered ivermectin; 0.4 mg/kg B.wt. on the 10th day of gestation, revealing early foetal deaths.

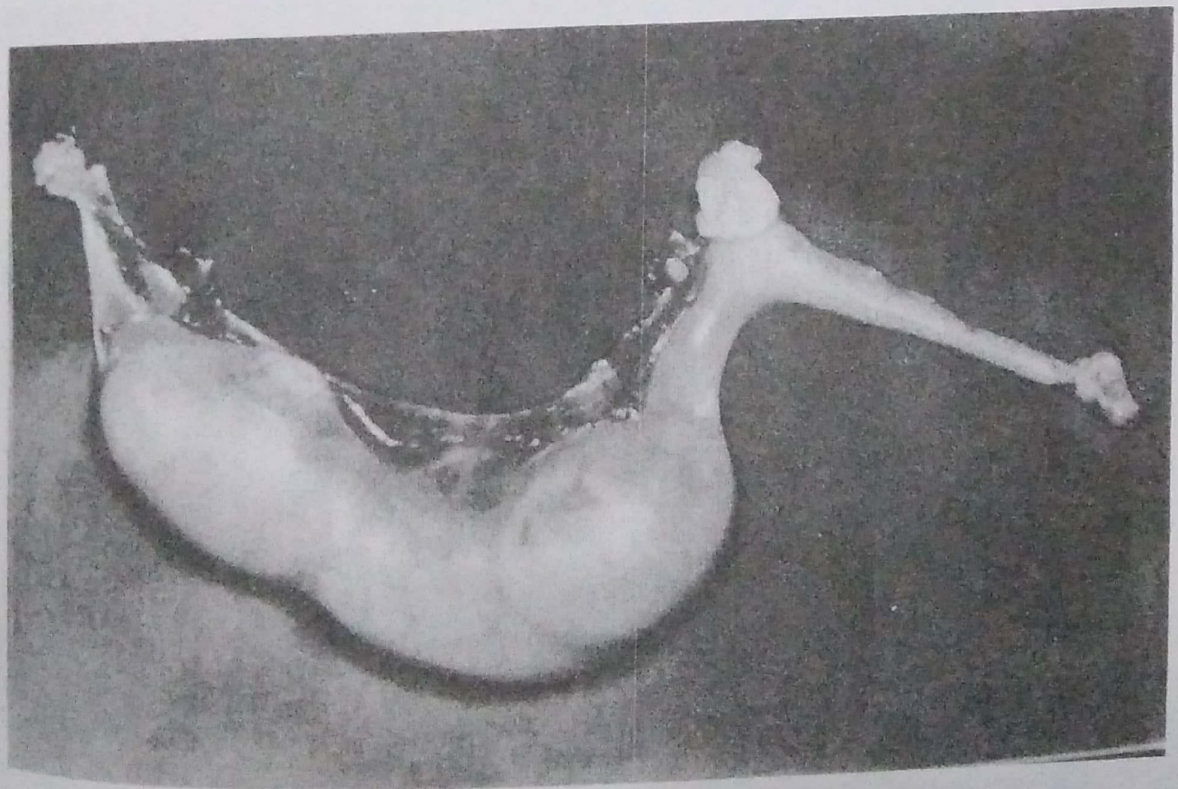

Fig. (4): Uterus of a pregnant female rat subcutaneously administered ivermectin, 0.4 mg/kg B.wt. on both the 1st and 15th day of gestation, exhibiting foetal resorption (a) and a dead foetus (b).

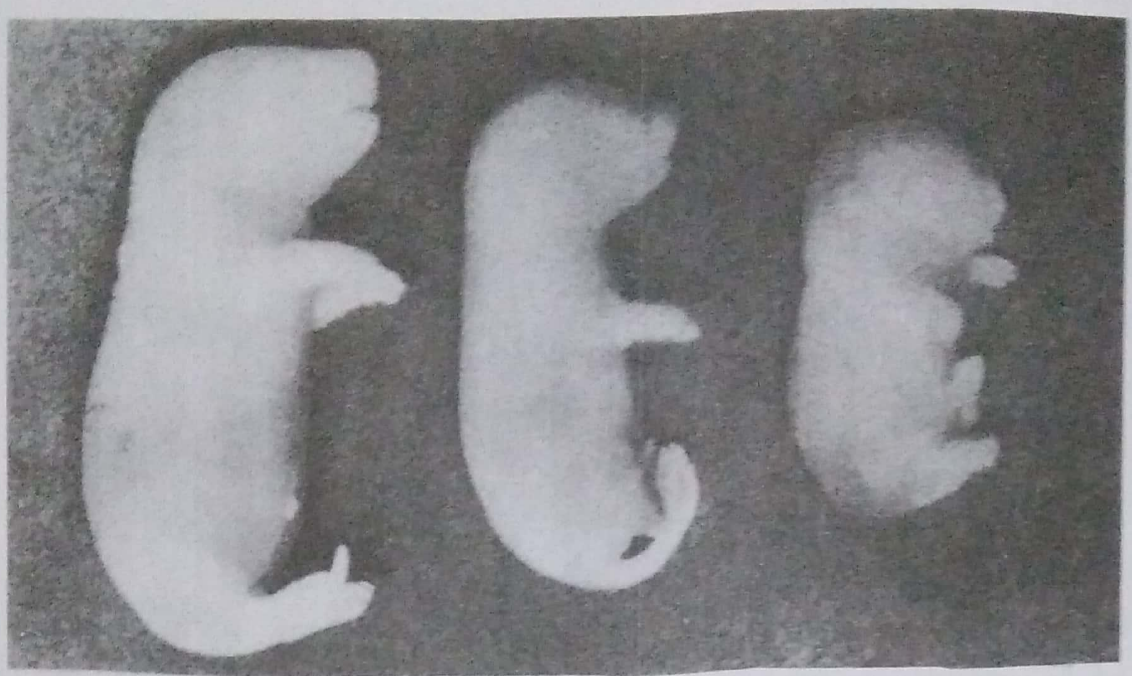

Fig. (5): Rat foeti from dams subcutaneously administered with ivermectin; 0.4 mg/kg B.wt. in a single dose (a) or two doses with 2 weeks interlude, (b) during the gestation period, unveiling a decrease in the foetal body length compared with the control (c)

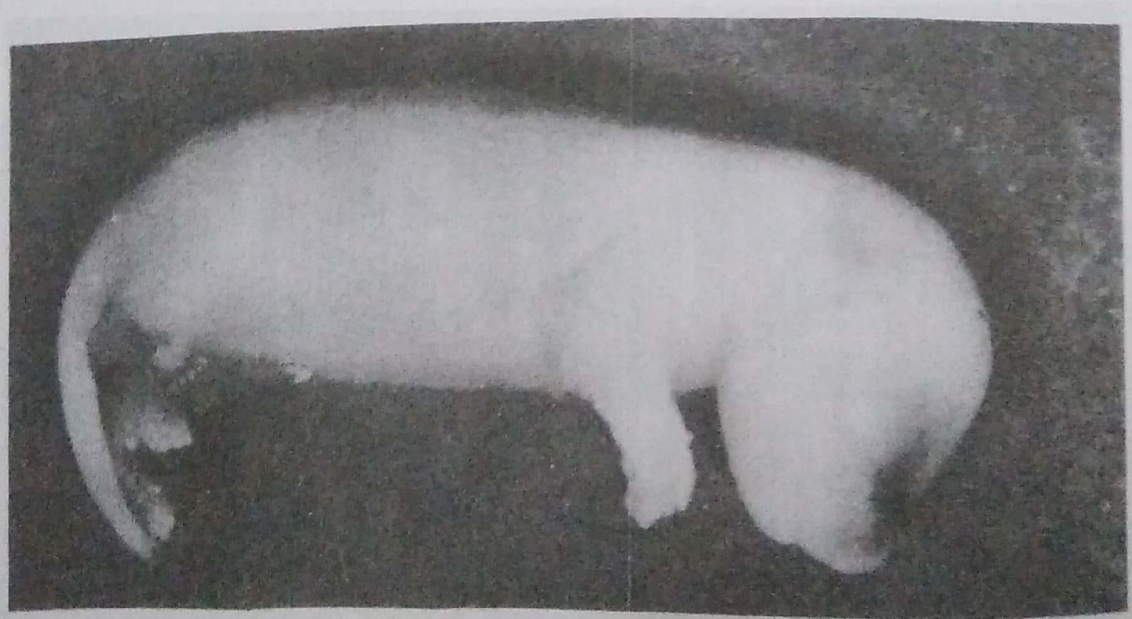

Fig. (6): A rat foetus from a dam subcutaneously injected with ivermectin; 0.4 mg/kg B.wt. on the 6th day of gestation, showing red patches in the head and limbs.

Zagazig J. Pharm. Sci. December. 1996
Vol. 5, No. 2, pp.113-124

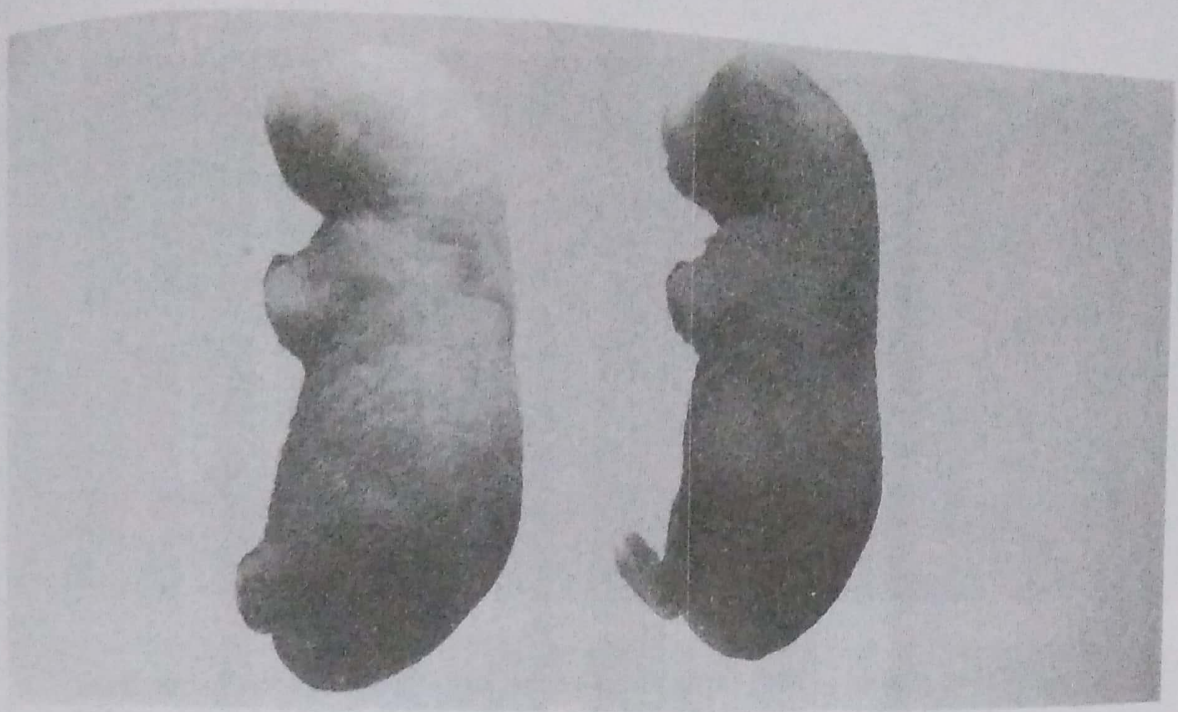

Fig. (7): A rat foetus (a) from a dam subcutaneously administered with ivermectin; 0.4 mg/kg B.wt. on both the 1st and 15th day of gestation. The animal demonstrates a swelling in the head compared with the control (c).

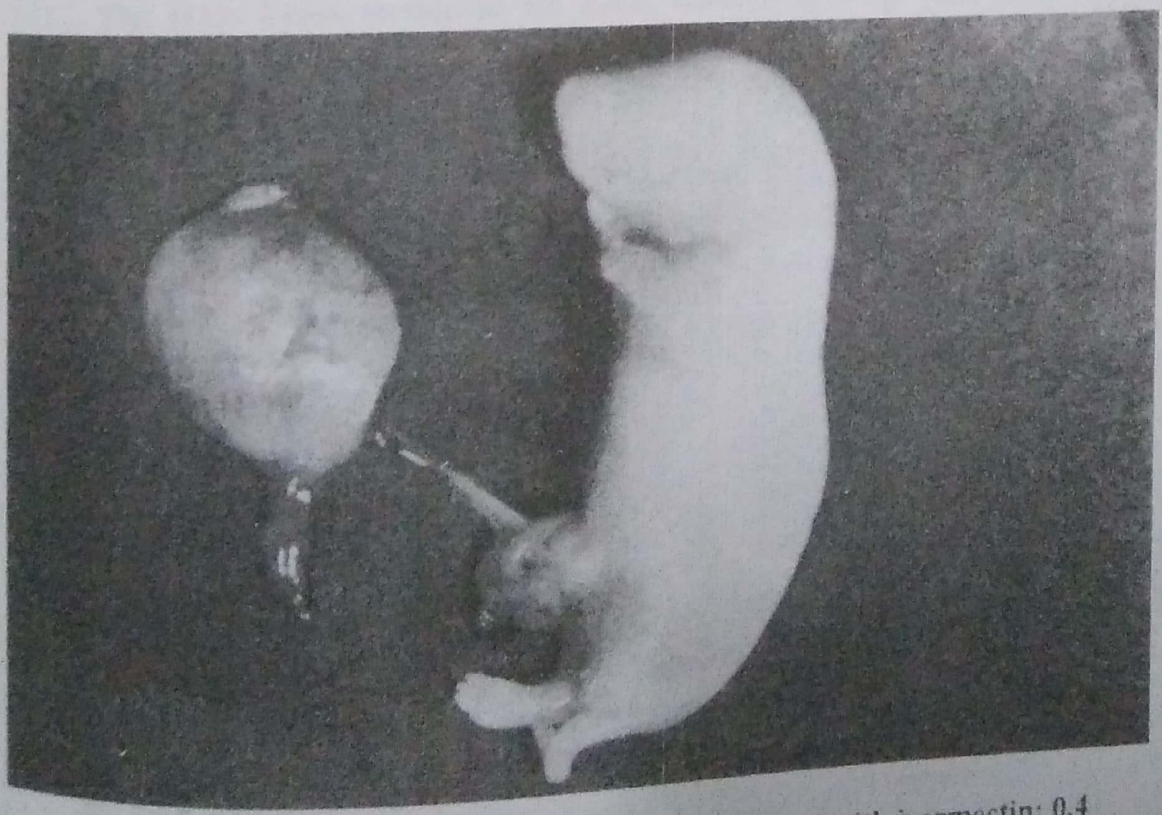

Fig. (8): A rat foetus from a dam subcutaneously injected with ivermectin; 0.4 mg/kg B.wt. on both the 1st and 15th day of gestation. Notice the protrusion of the intestine from the abdomen.

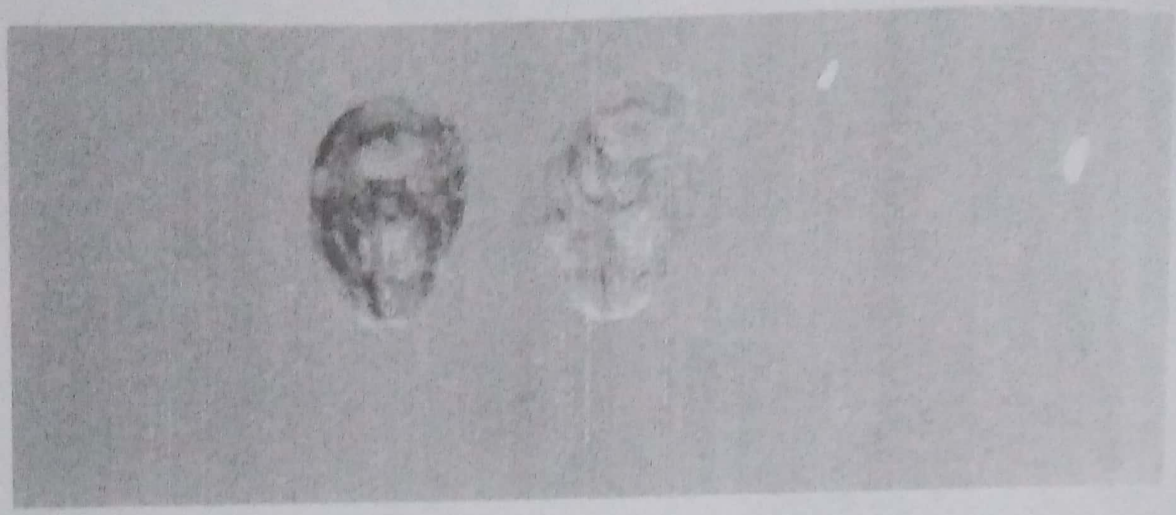

Fig. (9): Cross section (a) at the level of the palate, in the head of a rat foetus from a dam subcutaneously injected with ivermectin;0.2 mg/kg B.wt. on the 6th day of gestation, demonstrating cleft palate compared with the control (c).

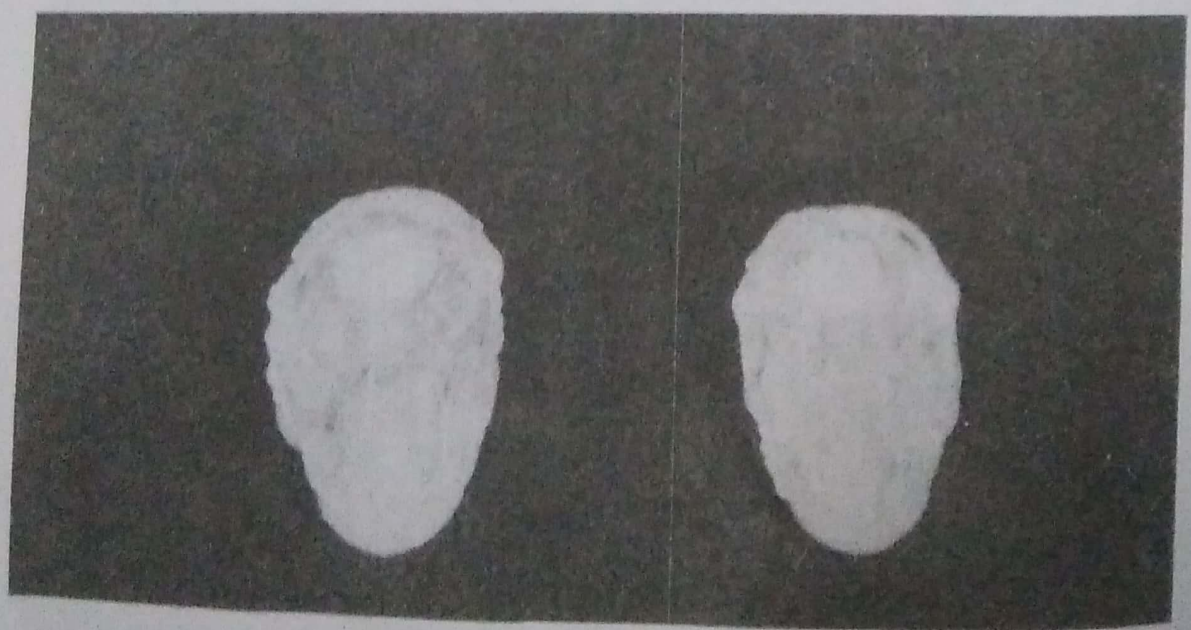

Fig. (10): Cross section (a) in the head of a rat foetus from a dam subcutaneously injected with ivermectin;0.4 mg/kg B.wt. on the 15th day of gestation, Notice diffuse haemorrhages and cleft palate compared with the control (c).

Zagazig J. Pharm. Sci. December. 1996
Vol. 5, No. 2, pp.113-124

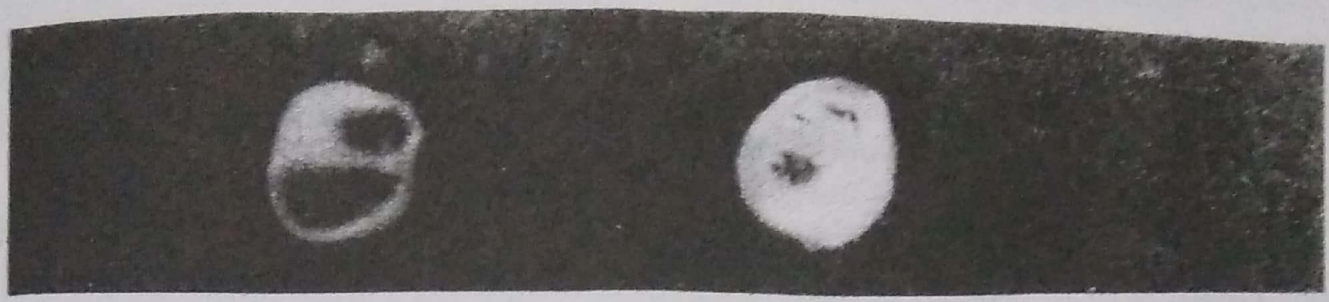

Fig. (11): Cross section (a) in the heart ventricle of a rat foetus from a dam subcutaneously injected with ivermectin; 0.2 mg/kg B.wt. on both the 1st and 15th day of gestation, revealing thickening in the ventricular wall compared with the control (c).

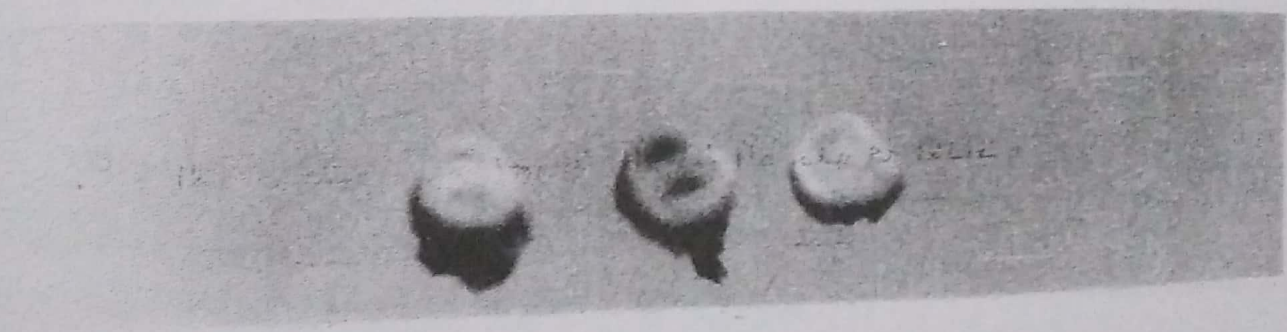

Fig. (12): Cross section in the heart ventricle of a rat foeti from dams subcutaneously injected with ivermectin; 0.4 mg/kg B.wt. on the 15th (a) and on both the 1st and 15th day of gestation (b) disclosing marked thickening of the ventricular wall of heart compared with the control (c).

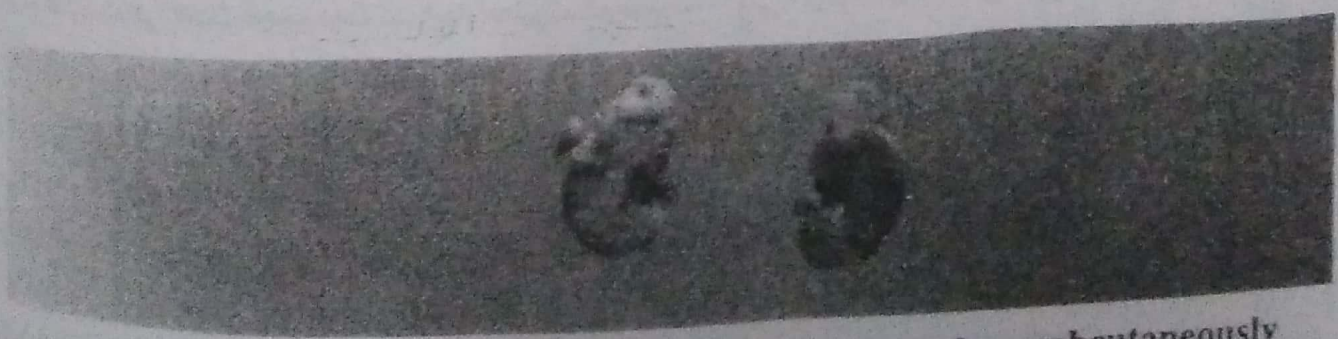

Fig. (13): Cross section of kidney of rat foetus from dam subcutaneously given ivermectin; 0.4 mg/kg B.wt. on both the 1st and 15th day of gestation, revealing slight dilation of the renal pelvis compared with the control (c).

derived from a cross fostering study in which control pups cross fostered to ivermectin treated dams exhibited the same degree of neonatal mortality and toxicity as rats exposed *in vitro* and fostered to drug tested dams[10].

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تأثير الايفرمكتين على الخصوبة والتطور الجنينى فى الاناث

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أجريت هذه الدراسة لمعرفة مدى تأثير عقار الايفرمكتين على الخصوبة والتطور الجنينى لإناث الفئران البيضاء كذلك تأثير العقار على نمو الرضع فى فترة الرضاعة وعلى التأثير التب.... للكروموسات. ولدراسة تأثير العقار على الخصوبة استعمل لهذا الغرض عدد ١٥ من إناث الفئران البالغة، قسمت الى ثلاث مجموعات متساوية أعطيت المجموعة الأولى الايفرمكتين بتركيز الجرعة الداوئية المعالجة ٠,٢ مجم/كجم من وزن الحيوان أما المجموعة الثانية فأعطيت الدواء بتركيز مزدوج للجرعة الدوائية المعالجة ٠,٤ مجم/كجم وذلك عن طريق الحقن تحت الجلد فى فترة الاست.... وجد أن عقار الايفرمكتين أطال فترة الشياع فى ٤٠٪ من الاناث المختبرة فى المجموعة و٦٠٪ فى الاناث المختبرة فى المجموعة الثانية ولدراسة تأثير الايفرمكتين على التطور الجنينى والتشوهات الجنينية استعمل لهذا الغرض عدد ٥٥ من اناث الفئران الحوامل قسمت الى خمس مجموعات أساسية متساوية وكل مجموعة أساسية قسمت الى مجموعتين فرعيتين. المجموعة الفرعية الأولى أخذت الدواء بتركيز الجرعة الدوائية المعالجة ٠,٢ مجم/كجم من وزن الحيوان أما المجموعة الفرعية الثانية أخذت الدواء بتركيز مزدوج للجرعة الدوائية المعالجة ٠,٤ مجم/كجم من وزن الحيوان.

وأثبتت الدراسة أن عقار الايفرمكتين يسبب زيادة ملحوظة فى عدد الأجنة الممتصة كما لوحظ بقع حمراء مختلفة الحجم موزعة على أجزاء الجسم المختلفة فى عدد كبير من الأجنة. وبفحص الأعضاء الداخلية للأجنة شوهد شق سقف الحلق وزيادة فى سمك جدار البطن فى القلب واتساع تجويف حوض الكلى.

وبفحص النخاع من عظمة الفخذ لإناث الفئران العشار المحقونة بالايفرمكتين أوضحت النتائج زيادة معنوية فى نسبة الخلايا متناسبه مع زيادة جرعة الايفرمكتين . ومن تلك الدراسة يتضح مدى تأثير استخدام الايفرمكتين على الخصوبه والتطور الجنينى .