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ASSESSMENT OF CONTRACEPTIVES AS A POTENTIAL CHEMOSTERILANT FOR CONTROL OF RAT POPULATIONS

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SUMMARY

Women contraceptive progestogen (Levonorgestrel) was evaluated as chemosterliant for laboratory & domestic collected female rats in 4 ascending doses as 5.40 µg/rat/day (10 times of rat contraceptive therapeutic doses), 10.8, 16.2 & 21.6 µg/rat/day (as, 20, 30 and 40 time of the rat dose) respectively. These doses were administered for female rats via paraffin bait at a concentration of 154µg, 308µg, 462µg & 616µg Levonorgestrel /kg paraffin block during one month. A direct relationship was recorded between the increase in the administered dose and the increase in mean body weight at the end of exposure period. No adverse effect could be recorded in the total or differential leucocytic count of treated groups in comparison with the control rats.

The used progestogen causes irreversible changes leading to sterility of the exposed female, which is increased with increasing the dose. Atrophy of the ovaries and fibrosis of the endometrium was observed in histological sections after administration to the doses of 16.2 & 21.6 µg/rat/day. While three offsprings were produced after mating of two females treated with16.2 µg/rat/day, no pregnancy could be recorded in domestic and laboratory females treated with 21.6 µg/rat/day. This clarified the efficacy of progestogen derivative as an efficient chemosterilant inducing irreversible sterility in the exposed rats. Levonorgestrel is palatable to rats, also considered as safe for human being and effective approach to regulation of rodent populations as well as to curb rodent numbers.

INTRODUCTION

Rodents are of special interest to public health workers owing to their long association with man.

They have followed man to almost all inhabited parts of the world carrying with them serious zoonotic agents.

In the last few years, rodent populations have markedly increased in some Egyptian governorates to the extent of being a terrible pest to man and his domestic animals. They annually cause considerable losses in quality of almost all kinds of field crops and stored products. Moreover, spectacular outbreaks were occurring from time to time, thereby focusing attention on the ever-present rodent problems in Egypt, (FAO, 1989).

Rodents have become major parasites of all human civilizations. Not only are they vectors of many human diseases, such as plague, leptospirosis, typhus and salmonellosis, but also they destroy an estimated 20% of the harvested crops of the world during storage, and can do major damage to buildings and equipment (Meehan, 1984).

The conventional way of controlling populations of rats or mice is the use of one of a number of commercially available poisons (Meehan, 1984). Under optimal conditions, such poisoning campaigns can be extremely effective. However, there are usually few individuals that survive the poisoning. These survivors can reproduce rapidly to produce a new plague, and there will be a strong selection pressure to favor the spread of genetic resistance to the poison. This has certainly occurred in the case of resistance to warfarin (one of the most widely used anticoagulant poisons), and difenacoum, (one of the second generation anticoagulants), (Greaves, 1985).

An alternative to poisoning would be the use of a chemo-sterilant that prevented the target species from reproducing. Knipling and McGuire (1972) have shown by computer modeling that administration of a poison with a 70% kill rate in both sexes to rats over three successive generations would produce only a transitory decline in the population, whereas administration of an irreversible chemosterilant that was 70% effective in both sexes over three successive generations would result in extinction of the species within 12 generations. In spite of this theoretical advantage of chemosterilants over conventional poisons, little research has been done on chemosterilants in the past two decades (Bomford, 1990). Also, Ma Lin (2004), mentioned that only one gram of contraceptive can sterilize 100 rats.

The optimal strategy for using a chemosterilant for rodent control would be its application in combination with a conventional poisoning campaign (Howard, 1976).The target population would first be poisoned to reduce the numbers as rapidly and effectively as possible. The survivors could then be chronically exposed to the chemosterilant in different bait; thereby not only preventing a population rebound, but also inhibiting the development of strains resistant to the initial poisons Marsh and Howard (1973).

Another way of using a chemosterilant would be to apply it when the population was at a nadir, for example, at the end of winter, thereby preventing numbers from increasing in the spring.

Chemosterilant must be applied via highly palatable bait es-pecially when there may be an abundance of alternative foods. It is also essential for the chemosterilant to achieve a high degree of species specificity. One way of targeting rats and mice very effectively is to incorporate the chemosterilant into paraffin wax blocks which also contain some cereal grains (Bajomi et al, 1976).

Therefore, this study aimed to evaluate the efficacy of irreversible human contraceptives as chemosterilant that could be successfully administered in paraffin blocks chronically to rats in a free choice feeding situation to inhibit their reproduction as well as curb rodent numbers.

MATERIALS AND METHODS

Rats:-

Sexually mature 5 male and 25 female outbreed Albino Swiss rats and 6 female and 2 male of domestic rats (Rattus norvegicus) were used; they were kept in a cycle of 12 hours light, 12 hours dark and given water ad libitum and a standard broilers diet as wax blocks as described later.

Rats under experiment were kept in the laboratory for one month before administration of contraceptive to be sure that, they were non pregnant.

Contraceptive:-

A potent new orally active progestogen (Levonorgestrel) used as women contraceptive under a trade name "Levo-Nor" was obtained from Women & Child Care Unit Ministry of Health and Population, Cairo, Egypt. It was supplied as tablet each containing 0.03 mg levonorgestrel, (synthetic progestogen derived from 19-nortestosterone) to be used as continuous daily tablet without cessation periods. This contraceptive was produced by, Family Care Ltd. For/ ACDIMA International Trading.

Dose calculation:

The women dose (0.03mg/day) was modified to use for rat according to Paget and Barnes (1964) dose conversion table, the rat dose was calculated as multiplying the women dose (30µg)X rat factor (18/1000) = 0.54 µg/day/Rat (as corresponding therapeutic dose to rat).

The estimated rat dose was tested as ten times the therapeutic dose (10X) which is equal to 5.40 µg/day, (20 X) corresponding to 10.8 µg/day, (30 X) equal to 16.2 µg/day and (40X) corresponding to 21.6 µg/day.

Baits and diet:-

In order to estimate the daily amount of food intake by each rat, food was administered at a dose of 35 gram paraffin blocks one week before addition of the drug. These blocks were prepared according to Gyo & Short (1993), as paraffin wax (9.0 gram) was first melted in an oven at 65°C. A mixture of blended grains (24.0 gram) were then added to give a ratio of 26% paraffin, 74% mixed improved broiler ration (wheat meals, yellow corn meals, powdered sugar, dried milk, vitamin A-D3-H & minerals).

The paraffin mixture was then poured into plastic disks 7cm square and 1cm deep and allowed to set to give individual paraffin blocks.

These blocks were suspended inside the cage by wire and the sawdust bedding was excluded from the area beneath the block by plastic partition, so that any chewed but non-ingested block could be weighed. Food intake of the laboratory prepared paraffin block was recorded every other day by weighing the food remaining in the hoppers plus the remaining paraffin blocks.

The rate of daily consumption of the diet in this paraffin form was estimated per each rat.

New paraffin blocks were prepared containing double the calculated amount of food and the required dose of Levo-Nor (progestogen). The drug was added to melted paraffin which was stirred on a hot plate for 20 minutes before addition of the calculated amount of food. Untreated control paraffin blocks were prepared in the same way but without addition of contraceptive for feeding of the control groups.

Experimental design:

Two experiments were performed; the first one using outbreed Albino Swiss rats in order to estimate the lowest progestogen dose able to induce irreversible sterility in the treated rats. The second experiment included the application of these doses on domestic collected rats.

Experiment (1): Twenty five female rats were weighed and assigned at random into five groups, five females per cage, moreover five male were also reared separately. The first female group was fed on paraffin blocks containing 5.40 µg/rat /day of Levo-Nor, the 2nd, 3rd, & 4th groups were fed on paraffin blocks containing 10.8, 16.2 & 21.6 µg/rat/day respectively.

These were corresponding to 154µg, 308µg, 462µg & 616µg Levo-Nor/kg paraffin block for the above four ascending doses respectively. The fifth female group was fed on paraffin blocks free from drugs. The male group were fed normal ration and kept for induction of natural mating only at the end of medication period. Feeding of fe-

males on medicated paraffin blocks continued for one month (according to Gyo & Short (1993), without periods of cessation as the rate of food consumption by the rats, did not decline with continuous administration of the same baits.

In order to ensure ingestion of the required dose by exposed rats per day, daily drug consumption was estimated by weighing of un-consumed food. At the end of medication period (30 days), change in the body weight was estimated, non-coagulated blood samples were collected, where the changes in total & differential leucocytic counts was taken as a guide for the immune defense mechanisms of the body. Two females from all groups were sacrificed; their ovaries & uteri were accurately separated from the surrounding tissues then weighed and fixed in Bouins solution for histological study.

One untreated male was caged with three females 14 days according to (Gyo & Short (1993). The females were then caged separately and observed daily for signs of pregnancy or birth dates and numbers of offspring were recorded as the index of female fertility. Also rate of mortalities was estimated on the 15th day old, in the treated and control groups.

Experiment (2):

Six field collected domestic female rats (Rattus norvegicus) were weighed and assigned into 2 groups, one was fed on paraffin blocks containing progestogen at dose of 21.6 µg/rat/day (40X) for one month. The other one was fed on un-medicated paraffin blocks. At the end of medication period, blood samples were collected from all rats. Males were caged with females for two weeks and the experiment was continued as before.

Narcosis of rats using ether under glass funnel was adopted during handling of rats.

Estimation of the contraceptive efficacy

According to the producer, progestogen, induces its contraceptive effects via inhibiting the ovarian activities, prevent arrival of spermatozoa to the ovum, moreover it interferes with implantation of the fertile ovum in the endometrium. For these reasons, evaluation of the drug effect depended on the number of the produced litters as well as the changes occurring in the genital system. It neglects the weight of the produced litters and the length of pregnancy period where these parameters may be affected by individual factors especially on the laboratory level.

RESULTS AND DISCUSSION

Using of modern contraceptive for eradication of rodent population is a safe method. The use synthetic chemicals friend to the nature, as these chemicals was mainly patent for prolonged use by women.

Aside from the fact that chemosterilants can be important in regulating disease bearing rodent populations, it is also fortunate that they permit this to be done without destroying great numbers of rodent. This means that, unlike with lethal rodenticides, there will be no sudden appearance in the environment of fleas, mites and ticks that have abandoned poisoned rodents. Such release of ectoparasites increases the risk that the vectors will further spread the rodent borne diseases, Marsh and Howard (1973)

As mentioned by Pajomi et al; (1976), paraffin blocks are ideally suited to chronic administration since the chemosterilant is not leached out by rain, or decomposed by exposure to sunlight, moisture, oxidation or bacterial contamination, and the block is unlikely to be consumed by humans, carni-vores, or birds.

Progestogen (Levonrgestrel) is an effective contraceptive inhibiting successful fertilization by inducing changes in the cervical mucus by which sperm migration and ascent are rendered difficult or blocked. It inhibits nidation via causing changes in the endometrium throughout the cycle. The drug prevents ovulation, because it suppresses secretion of LH hormone, and finally it causes fibrosis of uterus and thin endometrium and so prevents implantation of the fertile ovum.

The present study evaluated the efficacy of administration of ascending doses from this type of progestogen as a method inducing irreversible changes in the genital organs of female rat to achieve permanent sterility of these females as a mean of eradication on the long run.

Their effect on treated rats was estimated from different aspects includes its effect on the mean body weight, cellular immune mechanism, pathological changes in the genital organs and its effect on pregnancy and parturition after mating by normal male.

The study evidenced that, there is a direct relation between the increase in the administered dose and the increase in mean body weight of laboratory rats as in (table 1). This may be attributed to the observation that contraceptive drugs may lead to water retention in the body. Moreover, fat deposit was recorded around ovaries of sacrificed females at the end of the exposure period. On the contrary, there was a decrease in mean body weight of domestic collected rat. This decrease may be related to the nature of these rats as they did not accommodate on captivity.

The obtained results in table (2) demonstrated that the different doses of Levonorgestrel didn't induce adverse effect on the cells of the body defense mechanism. Total white blood corpuscles and lymphocytes, neutrophils, monocytes, eosinophiles & basophiles were still in the normal range

as described by (Rastogi, 1982) for rats. This may be related to the high potency of rat immune mechanisms and in the same time these types of products (contraceptives) were designed for prolonged use by women, so it must be safe toward the immune mechanism of the body and have a broad safety margin with no cumulative effect as mentioned by the producer company.

In order to estimate the direct effect of progestogen on the function of female genital organs, the results in table (3) and the histological sections cleared that the dose of (5.4 µg/rat/day, 10X) did not cause marked changes in ovaries and structure of the uterus. Decrease in the weight of the ovaries have started with increasing the dose to (10.8 µg/rat/day, 20X). Atrophy of the ovaries and fibrosis of the endometrium became clear at the dose of (16.2 µg/rat/day, 30X), while the target objective of irreversible changes was achieved using the dose of (21.6 µg/rat/day, 40X) as demonstrated in histological sections.

The mean weight of the ovaries have decreased about half of the control one using 30X & 40X dose. In the same time the control ovary section as shown in (figure 1) has normal health active follicles with normal corpora lutea. On the other hand, changes gradually appeared in treated uterus with increasing of the administered contraceptive doses. The adverse changes have started at dose of (10.8 µg/rat/day, 20 X), increased with the increase of the dose to (16.2 µg/rat/day, 30X). The maximum atrophy was recorded after administration to the dose of (21.6 µg/rat/day, 40X). The histological section of the ovaries showed that follicles persisted suffered from degeneration with few number of corpora lutea (figure (2).

The obtained results as cleared in table (3) did not evidence marked decrease in the mean weight of the uterus at the end of exposure period. This may be due to replacement of fibrous tissue in endometrium by active epithelial lining tissue, a matter which reflects changes in the function of uterus rather than in its weight.

Histological sections of uterus using the highest dose of contraceptive, evidenced presence of thin endometrium, associated with fibrosed stroma accompanied with atrophied uterine glands, (figure, 4), in comparison with the non treated uterus which appeared has normal in the uterine stroma with active dark stained normal uterine glands (figure 3).This came in agreement with Gyo & Short (1993). It is worthy to mention that, presence of fibrosis in the endometrium ascribed the irreversible nature of these changes in this vital pregnancy organ.

These changes gave accurate explanation for the rate of decrease in number of litters per female as recorded in table (4). The obtained results cleared that there is an indirect relation between increase

of the dose and decrease in the number of the produced offsprings per female. High decrease was started at dose of 16.2 µg/rat/day and reached its maximum with increasing the dose to 21.6 µg/rat/day where complete sterility of the treated female was achieved associated by the previous described adverse effects in histological structures of ovaries and uteri. This came in agreement with (Knipling and McGuire, 1972) and Sorenson (1997).

From present observations of rodent behaviors, no aggression was observed in the control female rats when males were introduced to them. In contrast, the treated females with the highest dose showed aggression to the introduced males, these behaviors were previously described in similar work done by Gyo & Short (1993).

It may be attributed the concept that this compound should be thought to inhibit the production of sexual phermones. For an animal lacking this basic communication it might not elicit typical sexual responses from the opposite sex (Whitten, 1965).

It is important to mention that, in this study 3-5 days delay which was recorded in time of parturition between different females, also the recorded rate of mortalities in the neonates during the first two weeks of life were not considered as parameters in the efficacy of the drug where this may be affected by general health condition of each female. Moreover and according to mode of action of this contraceptive, it must be accepted that it prevents occurrence of successful ova fertilization and inhibit successful implantation to the juvenile zygote. So formation of offspring meaning fewer efficacies for the tested dose.

The dose of (21.6 µg/rat/day, 40X) was further evaluated as a mean of control on domestic rats. The results in table (4) cleared that this dose induces sterility in the exposed females as no offspring was obtained after mating. Death of the produced offspring in the control rat of this group may be due to a trial from their dams to swallow them as they did not accommodate to this type of housing.

The present study cleared that progestogen (Levo-Nor) appears as an effective female rat chemosterilant able to induce irreversible changes in the uteri of the exposed rats at a low doses of 16.2 and 21.6 µg/day. These estimated doses were appearing lower than those mentioned by Gyo & Short (1993) for Ethinyl oestradiol, methyl testesterone or Org 5933, a synthetic gestagen. They cleared that Methyl testosterone (5000 mg/kg paraffin block), was effective in inducing almost immediate infertility in female rats and mice at an ingested dose of about 35 µg/kg body weight/day. This infertility persisted for several weeks after the cessation of treatment. Org 5933 (4 mg /

kg/ paraffin block) at dose of about 420 µg/kg body weight/ day was effective in inhibiting ovulation in rats within 3 to 4 days after the start of treatment. They concluded that these synthetic gestagen in paraffin blocks could be used as a chemosterilant for the control of rat and mouse populations.

In conclusion, two progestogen (Levo-Nor) women tablets per day are able to induce irreversible sterility in three rats if they still feed on them which continues for one month during their life. So, it will be most useful in controlling rodent populations in crop-producing and wild land area. They will also be valuable for rodent control along waterfronts, on the banks of canals and rivers, in sewer systems and other situations where few rats can be tolerated or where total elimination of the population is economically unattainable by other means.

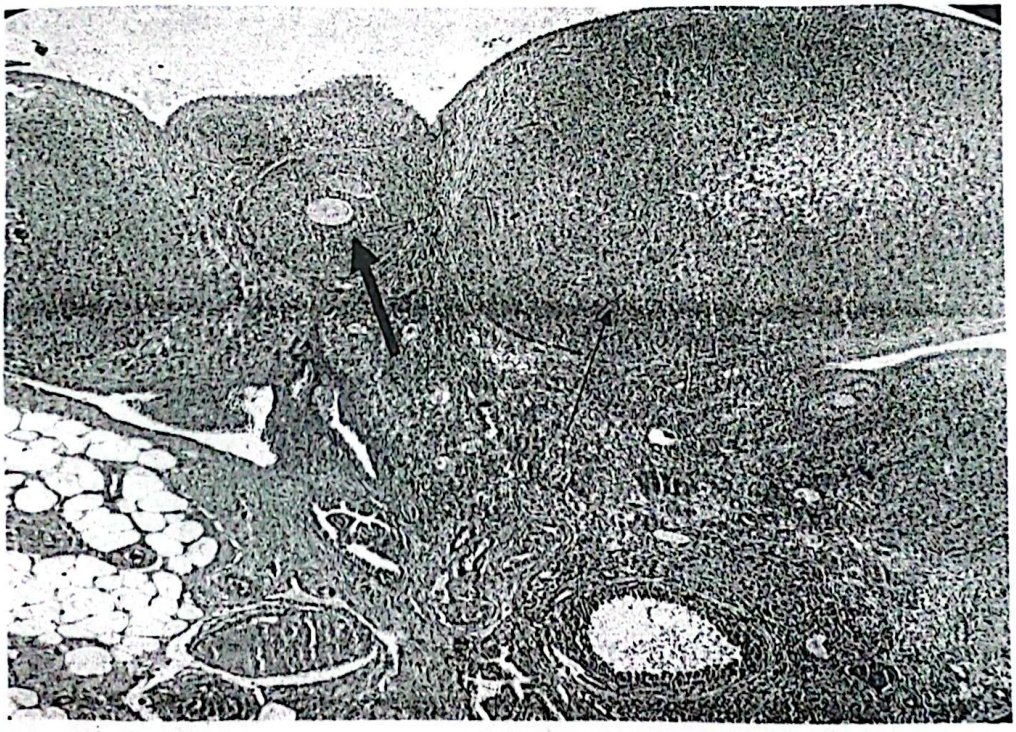

Figure (1): Control ovary,, normal functional ovary,, notice normal foollicles (thick arrows) with normal corpora lutea (thin arrows). H & E X 100.

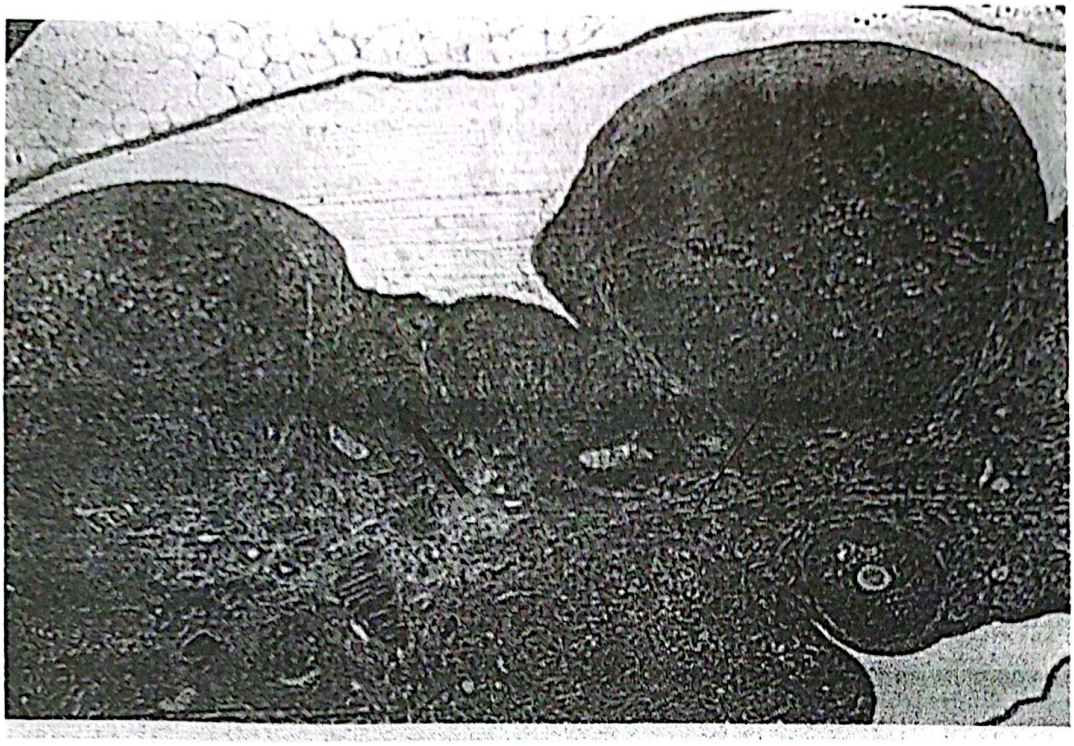

Figure (2): Treated ovary (21.6ug/rat/day), notice persisted follicle suffering from degeneration (thick arrow) and few number of corpora lutea (thin arrows). H & E X 100.

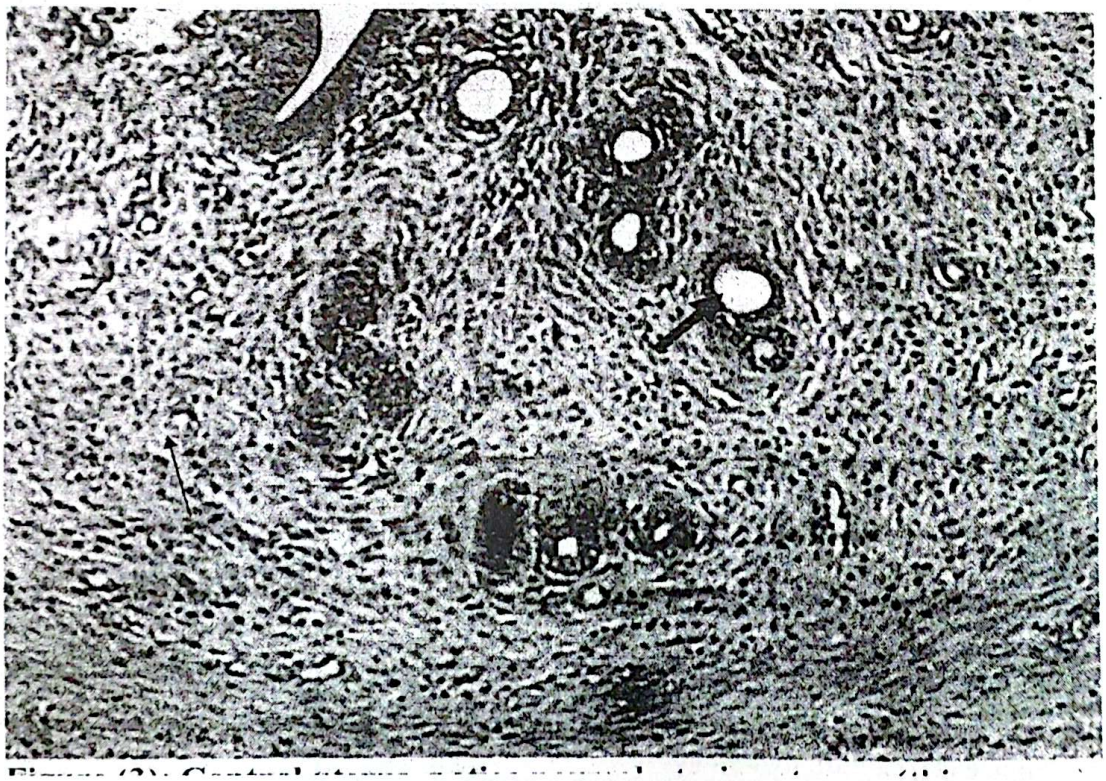

Figure (3): Control uterus, notice normal uterine stroma (thin arrow) with normal uterine glands (thick arrow) . H & E X 200.

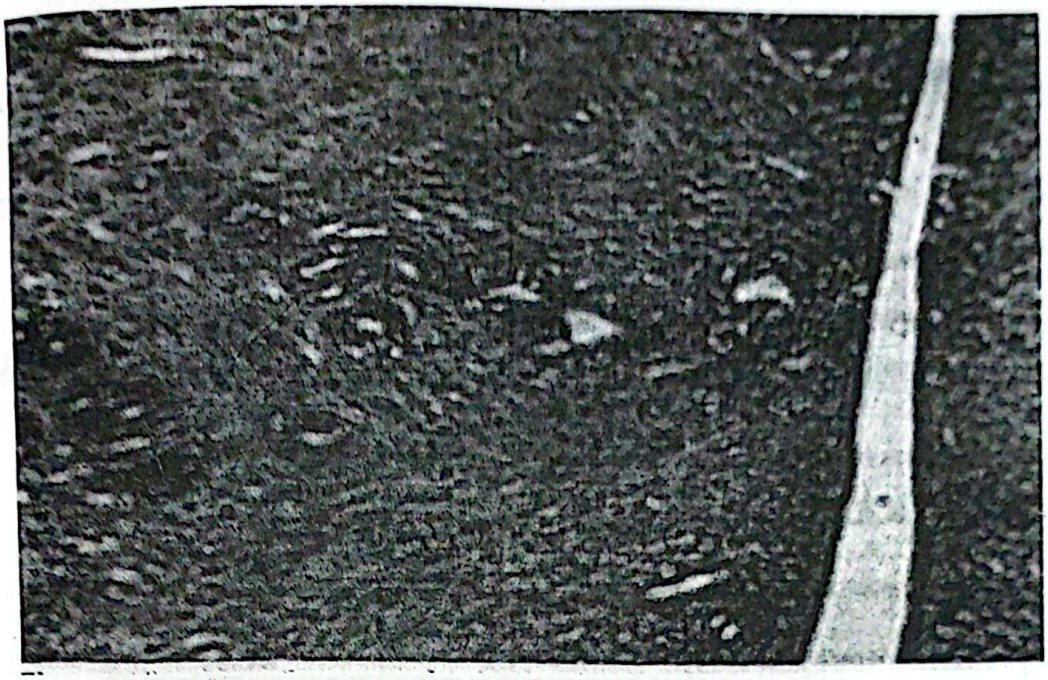

Figure (4): Treated uterus (21.6ug/rat/day), notice thin endometrium, fibrosed stroma with atrophied uterine glands (arrows). H & E X 200.

Table (1): Effect of *progestogen* treatment on mean body weight at the end of exposure period

Rat origin	Tested dose	Mean body weight (gram)		
		At zero day	At end of exposure period	Mean change
Laboratory breed	5.40 µg/day (10X)	140.2	153.6	+ 13.4 (I)*
	10.8 µg/day (20X)	144.4	160.4	+ 16.0 (I)
	16.2 µg/day (30X)	142.2	167.0	+ 24.8 (I)
	21.6 µg/day (40X)	141.6	166.6	+ 25.0 (I)
	Control	141.2	153.4	+ 12.2 (I)
Domestic Rat	21.6 µg/day (40X)	161.4	153.6	- 7.8 (D)**
	Control	165.2	152.2	- 13.0 (D)

** (D) = decrease *(I) = increase

Table (2): Effect of *progestogen* treatment on white blood corpuscles at the end of exposure period

Rat origin	Tested dose	Total leucocytic count	Differential leucocytic count (%)				
			Neutrophils	*Lymphocytes*	*Monocytes*	*Eosinophils*	*Basophils*
Laboratory breed	5.40 µg/day	12200	27.0	66.5	0.5	2.0	0.0
	10.8 µg/day	12000	23.0	72.5	1.0	3.0	0.5
	16.2 µg/day	12400	25.0	75.5	0.5	2.0	0.0
	21.6 µg/day	12200	26.5	74.5	0.2	1.5	00
	Control	12400	28.0	69.0	1.5	1.5	0.0
Domestic Rat	21.6 µg/day	14400	29.0	65.5	1.0	4.5	0.0
	Control	14600	25.0	70.0	0.5	4.5	0.0

Table (3): Effect of *progestogen* treatment on mean weight of ovaries & uteri of treated laboratory rats

Rat origin	Tested dose	Mean weight at end of medication (mg)	
		Each ovary	uterus
Treated rats	5.40 µg/day (10X)	41.5	675.0
	10.8 µg/day (20X)	33.5	686.5
	16.2 µg/day (30X)	25.0	653.0
	21.6 µg/day (40X)	23.5	644.5
Control		43.25	756.5

Table (4): Effect of *progestogen* treatment on fertility rate of treated female rats.

Rat origin	Tested doses	Treated female	No. of letters/female	Mean No./ group	No. still survive after 2 weeks	Mean No.
Laboratory breed	5.40 µg/day (10X)	1	7	6.33	5	5.0
		2	6		5	
		3	6		5	
	10.8 µg/day (20X)	1	6	3.6	5	3.0
		2	5		4	
		3	-		-	
	16.2 µg/day (30X)	1	2	1	1	0.33
		2	1		-	
		3	-		-	
	21.6 µg/day (40X)	1	-	-	-	
		2	-		-	
		3	-		-	
	Control	1	7	7.33	7	6.66
		2	8		7	
		3	7		6	
Domestic Rat	21.6 µg/day (40X)	1	-	-		-
		2	-			
		3	-			
	Control	1	2	2.33	0	0.0
		2	4		0	
		3	1		0	

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