THE EFFECTS OF 4 ,4¯ -(4,5,6,7-TETRAHYDRO-[1,2,3-] SELENADIAZOLO [4,5E] PYRIDINE-4,6-DIYL) BIS(BENZENE-1,3- DIOL) ON FERTILITY, REPRODUCTIVE HORMONES AND OVARIAN HISTOLOGICAL C…

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THE EFFECTS OF 4',4''-(4,5,6,7-TETRAHYDRO- [1,2,3-] SELENADIAZOLO [4,5E] PYRIDINE-4,6-DIYL) BIS(BENZENE-1,3-DIOL) ON FERTILITY, REPRODUCTIVE HORMONES AND OVARIAN HISTOLOGICAL CHANGES IN FEMALE RATS TREATED WITH DIPYRONE.

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Key wards: Selenadiazole, fertility, estrogen.

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ABSTRACT

The current study aimed to evaluate the effects of [4',4'' -(4,5,6,7-tetrahydro-[1,2,3-] selenadiazolo[4,5e] pyridine-4,6-diyl) bis(benzene-1,3-diol)] (T) on female fertility and reproductive hormones, in addition histopathological examination of ovaries in comparison with the effects of Metamizole or Dipyrone (Di). Four groups of mature female rats each group consist of ten healthy female rats. Three groups received T and /or Di dissolved in drinking water 2mL (DW), the forth group received 2 mL of (DW) for 20 days before mating and ten days after mating. The results indicated that there was a significant increase in the number of births of (T) group (13.70±3.34) compared to DW group (9.7±1.06). Also, significant decline in T&Di group to (6.91±0.32), with no pregnancies reported in Di treated group. The pregnancy percentage was in T&Di group basically decline to 40% compare with T and DW groups 100%. Follicle stimulating Hormone (FSH) level showed a significant elevation ($p<0.05$) in T group (5.19mIU/mL

±0.72), and T&Di (5.12 mIU/mL ±0.78) compared with DW (1.61mIU/mL±0.52), and Di (1.46 mIU/mL ±0.60) groups. LH concentration of T (1.88 mIU/mL ±0.48), and T&Di (1.67 mIU/mL ±0.41) groups LH values than in DW (1.75 mIU/mL ±0.67). Only in Di group (0.88 mIU/mL ±0.48) LH value reduced significantly ($p<0.05$) than in other test groups. T group (17.22 mIU/mL ±4.50) progesterone level; also T&Di group progesterone level (10.11 mIU/mL ±2.05) statistically important increase, while Di group there is essentially decreased ($p<0.05$) to (2.69 mIU/mL±0.89) compare to DW (6.31 mIU/mL ±1.41). Histopathological results showed that ovarian section of Di group disclosed large cystic corpus luteum CC.L, absence of Graafian Follicles and follicles at different stages of development. T group section showed normal Graafian follicles and different follicles in developmental stages, as well as there were several persistence corpus luteum. Ovary of T&Di female rats relieves improvement of some Graafian follicles, Cystic corpus luteum (CC.L). Furthermore, there was some of clear C.L. It can be concluded that T compound had a Good effect on the level of reproductive hormones and increase fertility in female rats. While Di long term treatment had bad effects on female fertility, by affecting reproductive hormones levels and pathological change of ovaries.

INTRODUCTION

Selenium was first discovered in 1817 by the Swedish chemist Jons Jacob Berzelius, during sulfuric acid production[1].Vital physiological role of selenium (Se) is due to the potent antioxidant activity selenoproteins. Some of selenoproteins are well known functions such as thioredoxin reductase (TrxR), iodothyronine deiodinases, and glutathione peroxidase (GPx) , the functions of other proteins are not known up till now include selenoproteins T ,X ,Y,[2]. In spite of the rare knowledge of the particular biochemical functions, several attempts have been made to show that inadequate Se, principally Se-proteins, are related with frequent human diseases including malignancy, diabetes mellitus, cardio-vascular, and immune system conditions[3]. However, much uncertainty still exists about the relation between serum selenium levels and reproductive effects.

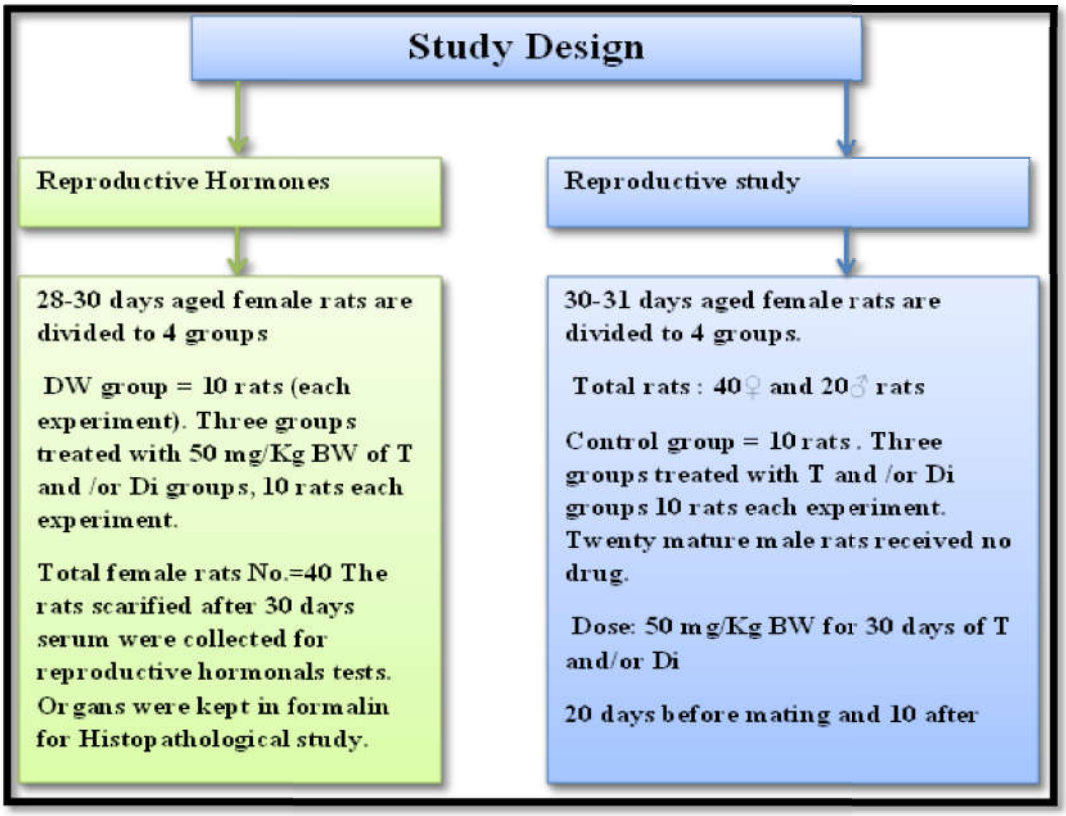
Dipyrone (Metamizole or Novalgin) used in both human as well as veterinary medicine. It is a Pyrazolone derivative, first it produced in 1920 by Hoechst AG, a German company and in 1922 Dipyrone has been global use. In several countries Dipyrone withdraw due to serious complication of agranulocytosis, whereas in others like Germany it obtainable as prescription drug and over the counter in India, Spain, Russia[4]. Dipyrone decreases endogenous glutathione levels and inhibits GPx action in dose dependent manner[5]. Research on the Dipyrone has been mostly restricted to limit comparisons of infertility effects, most research's emphases on the effects of Dipyrone on pregnancy and fetal malformations; to the best of our knowledge quite a few studies on infertility effects of Di were done. Therefore, the present study accentuated mainly on two significant items. A novel selenadiazole derivative (T) was used to detretmine its effects on reproduction, reproductive hormones, and an overy histopathological changes.

MATERIAL AND METHODS

Healthy female rats, procured from veterinary medicine college/university of Basrah. The female rats kept in polypropylene cages lined with sawdust (3-4 rats/cage). Rats were provided with usual rat pellet diet and tap water. At the beginning the rats were adapted to laboratory circumstance, natural day and light(12 hours day and 12 hours night). Room temperature 21±4°C[6]. Body weight of all rats was measured. Then the cages were labeled and separated as groups. In present study mature female rats are used, divided in to four groups, each group consists of 10 female rats, cohabited in one cage in ratio 2 treated female: 1 untreated male. Free excess drinking water and rat food. The mating recorded after mainly personal observation or presence of vaginal plug[7]. Fertility test was measured through the following equation:

$$\text{Pregnancy percentage} = \frac{\text{No. of Pregnant Female}}{\text{No. of Mated Female}} \times 100$$

Experimental design



Eighty mature female rats and twenty male rats were obtained from Veterinary Medicine College/University of Basrah. The rats kept in polypropylene cages lined with sawdust, and provided usual rat pellet diet and tap water. At the beginning the rats were adapted to laboratory circumstance, natural day and light 12 hours day and 12 hours night. Room temperature 21±4°C [6]. Then the cages were labeled and separated as groups. Forty of female rats aged 30-31 days, used for hormonal estimation and ovarian histopathological study. The rats divided in to four groups, each group consists of 10 female rats' 3-4 rats in one cage.

The rest female rats were used for reproductive study each group consist of 10 rats. The rats in each group were cohabited in one cage treated females with untreated male

in ratio 2:1.Rats. Each of treated group only females received 50mg/kg BW of T compound (synthesized at pharmacy college-pharmaceutical chemistry) and/ or 50mg/kg of Di (Dipyrone powder from Shaanxi pioneer Biotech-China), while control group received equal volume of DW for 20 days before mating. Compounds are administered orally using a mouth gavage, and the administration continues ten days after mating. The animals were observed carefully for 5days. Presence of sperms in vaginal swaps indicates mating.

Reproductive hormones and Histopathological study

After 30 days of receiving treatments, the rats were anesthetized using chloroform, blood sample were collected directly from the heart. Blood sample kept in Serum-separating tubes on room temperature for 20 -30 minutes. Serum was then collected using centrifuges for 10minutes at 2000 rpm. Serum collected and stored in deep freeze till analysis. Serum levels of estrogens, progesterone, follicle stimulating hormone (FSH), and Luteinizing hormone (LH) hormones were measured using special kits (India) in I Chroma II apparatus.

Ovaries were separated from the body and washed with normal saline. The samples were fixed in 10%formalin, and then sent to Pharmacy College Central Lab. The samples prepared in embedded paraffin for histopathological analysis, and stained with hematoxylin eosin

Statistical analysis

Results are documented as mean values and standard deviations (mean±SD). Differences among groups were evaluated using One-way analysis of variance (ANOVA)at ($p<0.05$)[8]

RESULTS

Number of birth

From the data in (table1) is apparent that there were significant differences among studied groups. The results indicate that the Female rats in Di group did not have

pregnancy according to the work plan in the current study (0.0±0.0) compared to the other groups. The results in the same table indicated highly significant increase ($p<0.05$) in the number of births of T group (13.70±3.34) as regards to DW group (9.7±1.06). Compared with was remarkably declining ($p<0.05$) in number of birth of T&Di group (6.915476±0.320961) correlated to all other groups.

Body weight (BW) of new born

The results as illustrated in the table (1) that was no analytical differences in body weight of new born were recorded between all treated groups. Di group as mentioned before, no pregnancy occurred.

Pregnancy percentage

The results presented that the pregnancy percentage of T and DW group were 100% within the limited period. Surprisingly, Di group pregnancy percentage that recorded within 40 days was 0%. Contrary to expectations T&Di group 40% pregnancy percentage was detected and the labors occurred with the limited time of the study.

Table (1): - The effects of synthesized (T) on mean number of new born and body weight of Di induced toxicity of female rats.

Treatment groups	Parameters		
	Number of new born	Mean of body weight of new born	Pregnancy Percentage %
DW	9.7±1.06 A	6.37±1.27 A	100
Di	No new born	No new born	0
T	13.70±3.34 B	5.80±0.71 A	100
T&Di	7.25±2.06 C	6.91±0.320 A	40
LSD	2.45	NS	

Means bearing different capital letters vertically differs significantly at 0.05 level ($p<0.050$)

Reproductive hormones

Gonadotropic hormone

From the data in table 2, it is apparent that the average of FSH value in both T group (5.19 mIU/mL ±0.72and T&Di group (5.12 mIU/mL ±0.78)) were significantly ($p<0.05$) increase, compared with DW group (1.61mIU/mL±0.52), and Di (1.46 mIU/mL ±0.60).

The result of LH value showed that there was a slight numerical change ($P>0.05$) in T group LH value (1.88 mIU/mL ±0.48) and T&Di (1.67 mIU/mL ±0.41) than in DW (1.75 mIU/mL ±0.67). The results of the present study also revealed there was a marked decreased in LH of Di (0.88 mIU/mL ±0.48)($p<0.05$) than in other test groups.

Steroid hormone

Progesterone value of in Di group there was a clear trend of decreasing (2.69 mIU/mL±0.89) at ($p<0.05$) compare to DW (6.31 mIU/mL ±1.41). While a highly significant elevation of T group (17.22 mIU/mL ±4.50) progesterone level; in addition T&Di group (10.11 mIU/mL ±2.05) showed statistically important increase in progesterone level. Table 2 illustrates the reproductive hormones

The results of the present study indicated that estrogen level there was no significant increase in estrogen levels in all treated groups; Di group (54.79±14.86),(T) (57.36±21.32), and T&Di(58.19±16.78) compare with DW group (45.67±9.37).

Table (2):- Illustrates the level of pituitary and gonadal reproductive hormone after administration of Di, and/or T

Treatment groups	Parameters (mIU/mL)			
	FSH	LH	Progesteron	Estrogen
DW	1.61±0.52 A	1.75±0.67 A	6.31±1.41 A	45.67±9.37 A
Di	1.4571±0.72 A	0.86±0.48 B	2.69±0.89 B	54.79±14.86 A
T	5.214±0.60 B	1.67±0.411 A	17.22±4.50 C	57.36±21.32 A
T&Di	5.12±0.78 B	1.88±0.48 A	10.11±2.05 D	58.19±16.78 A
LSD	3.51	0.81	3.61	NS

Ovarian Histopathological Examination

Ovary section in figure (1) of DW treated female rats group showing mature Graafian Follicles (MG) and follicles of different developmental stages. In comparison with DW, ovarian section of Di group rats showed large cystic corpus luteum CC.L, and clear follicular cyst and absence of Graafian Follicles and follicles at different stages of development figure (2). While, section of ovary in (T) group rats showing several structures include normal Graafian follicles and different follicles in developmental stages, as well as there are several persistence corpus luteum figure (3). Ovary of T&Di female rats revealed improvement of some Graafian follicles (figure 4), cystic corpus luteum (CC.L.) was observed, furthermore some of clear C.L. was also seen

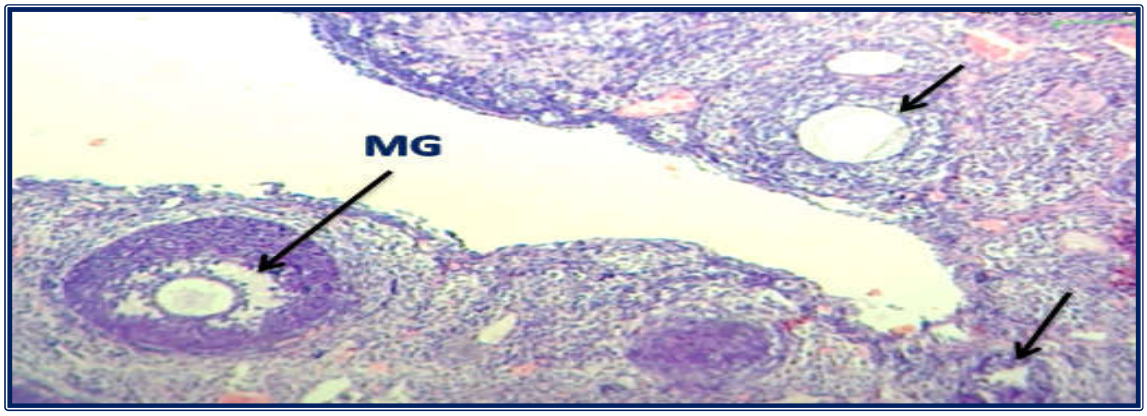


Figure (1):-Cross section in the ovary of DW treated female rats group showing mature Graafian Follicles (MG) and follicles of different developmental stages (arrows). (H and E) stain. 400X.

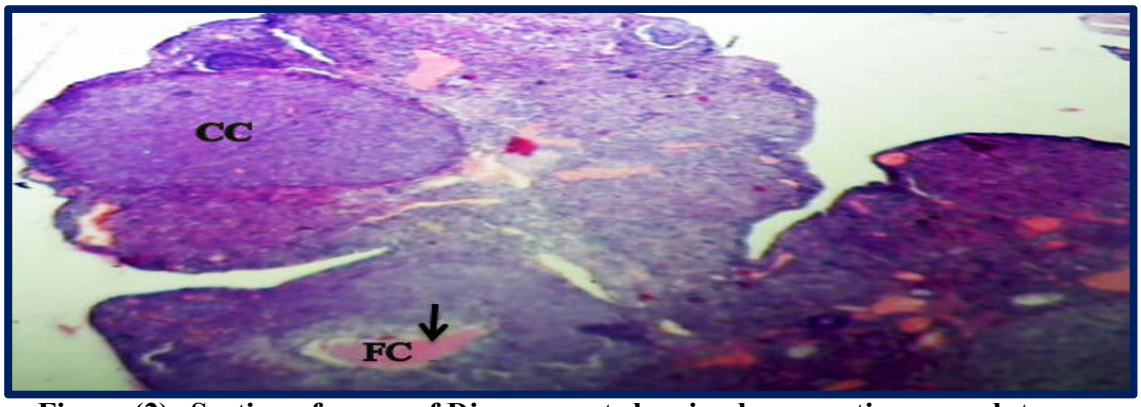


Figure (2):-Section of ovary of Di group rat showing large cystic corpus luteum (CC.L.), clear follicular cyst(FC). (H and E stain) 400X.

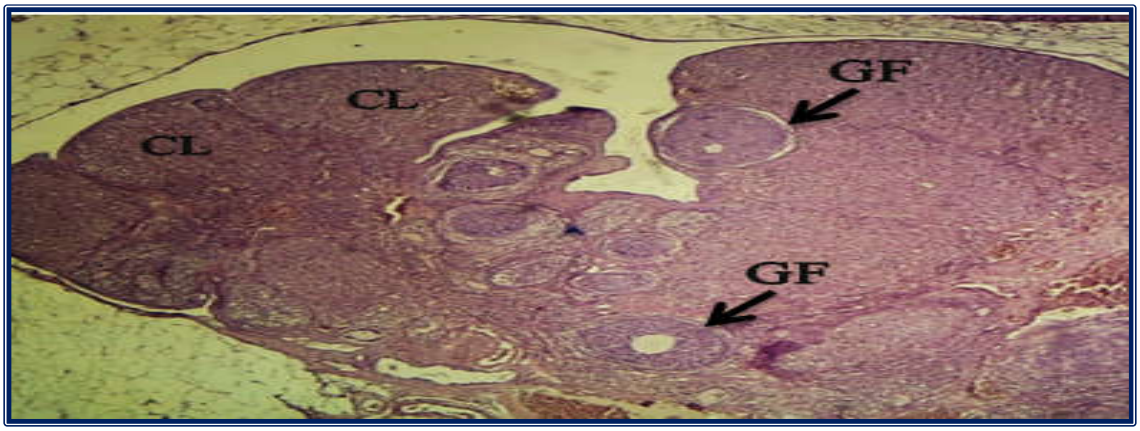


Figure (3):-Section of ovary of (T) group rats shows normal Graafian follicles ((GF), and different follicles in developmental stages (F), as well as persistence corpus luteum (CL). (H and E stain) 400X

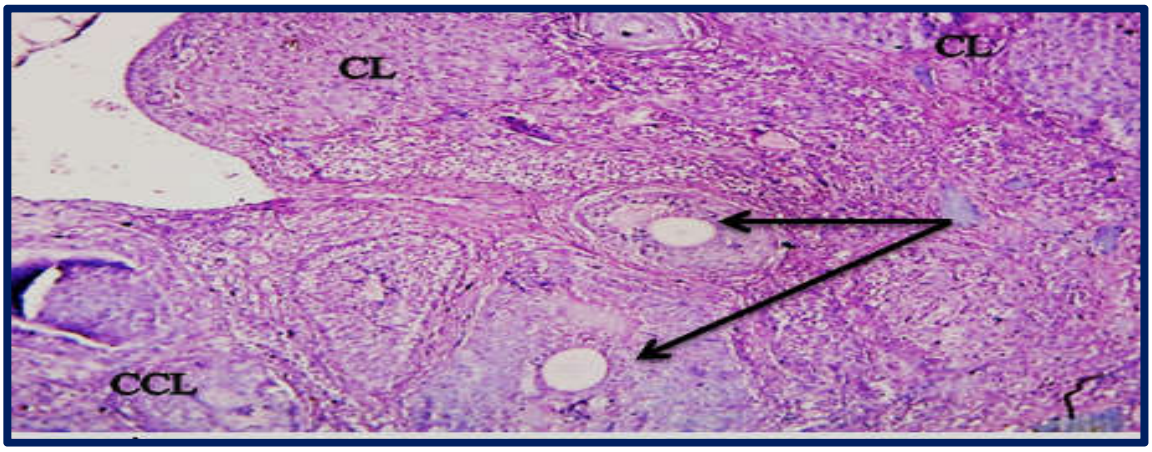


Figure (4) Section of ovary of T&Di female rats relieves improvement of some Graafian follicles (arrows), cystic corpus luteum (CCL), and clear copora lutea(CL). (H and E stain) 400X

DISCUSSION

Number of birth

The estrous cycle in rats is short, lasting 4-5 days. It occurs throughout the year. The first estrous cycle occurs generally between 33 to 42 days after birth. The length of the cycle prolonged slightly with age, about 6 days at the end of the reproductive life span [9].

Evidence for Se role in ovarian function comes from that maternal Se nutritional intake, which involved in the early folliculo-genesis regulation, and cellular proliferation of the follicles, and stromal tissues of fetal ovaries in sheep[10]. Se levels were found to be significantly decreased in infertile with endometriosis than in women with tubal infertility [11]. Study by [12]found that there was no significant difference in numbers of newborns of first-parity gilts in control g(14.3±2.1), inorganic Se treated group(14.3±2.1), and organic Se treated group (15.2±2.1). A study reported that Di treated pregnant rat group from day 5th-15th of pregnancy showed decrease the number of live born due to reabsorption and stillborn[13]. Also [14] reported a case of abortion and acute renal failure following the intake of an overdose of 11.5 g metamizole in a 14-year old girl, the abortion might be due to direct placental toxic effects 14-year old girl. The results reported by [15] that dipyrone had a block the division of placental cell which occurs chiefly in the initial steps of placental development.

Body weight of new born

The results obtained from the preliminary analysis of the differences of the body weight of the new born at the first day. The most obvious finding to emerge from this study is that Di group has no birth during study period, according to the work plan in the current study. The body weight of the new born was statistically not significant among T, and T&Di groups compare to DW group. Organic selenium supplementation would be serious for an efficient development in early gestation. The author also reported that; length, weight, and protein content of embryos at day 30 of gestation were greater in organic Se treated group than in inorganic Se gilts ($P < 0.05$) [12].

Pregnancy percentage

The results of the current study indicate that all rats in group T, and DW were become pregnant, whereas in T&Di group only 4 females from the total treated rats group (10 rats). Also the results illustrate that there was no pregnancies reported in Di treated female rats within the limiting time, this result has not previously been described. To date there has been little agreement on the effects of selenium or selenadiazole on female infertility which refers to Se role in female and male fertility and low Se plasma level in the early stage of pregnancy has been proved to be a reliable predictor of low birth weight of a newborn[16].

Research on the Di has been mostly restricted to limit comparisons of infertility effects, most research's emphases on the effects of Di on pregnancy and fetal malformations, to the best of our knowledge quite a few studies on infertility effects of Di[17]. Reported data on pregnant women, may suggest that short term exposure to dipyrone in the first trimester of pregnancy is possibly not related with a significantly increased malformations or spontaneous abortions. Also[18] revealed that during pregnancy, dipyrone is not related to increased side effects or malformations. In addition a study illustrated that Di administration to Sprague-Dawley, and Wister rats, in addition to White rabbits, increased incidence of resorptions with decreases in the number of fetuses and the numbers of foetuses per dam, without evidence of teratogenicity[13].

Reproductive Hormones

Gonadotropins hormone

Our finding revealed that T and T&Di treated groups caused a significant enhancement ($p<0.05$) in FSH level than DW and Di groups FSH level had no important alteration compared to DW group value. The finding is consistent with findings of previous studies by [19], point towards increments of FSH in Se supplements arsenic treated group toward the control level. Similarly [12], found that FSH levels increased in Se treated groups than in control group. A study carried out by [20] reported that

enhancement of serum FSH levels associated with significant increase in GPx activity ($P < 0.005$) lead to suppression of ROS production and stimulation of vitamin E activity. The above findings contradict the study by [21]. There were no significant differences in Plasma FSH and estrogen among rats received combination of doxorubicin and Se treatments for concentrations in control group.

Finding of the current study revealed that there was numerical increased in LH levels in (T) group female rats; but was statistically not-significant alteration with LH levels of T&Di and DW groups. LH level of Di group (0.86±0.48) was significantly decreased ($p<0.05$) than other experimental groups. Unfortunately, few studies have been carried out on Se or Di effects on gonadotropic hormones. Previous studies like a research by [17] reported that LH value of rats received combination of doxorubicin and Se nanoparticles have great levels than in those received doxorubicin alone. Also a study [19] reported that Selenium was able to increase the plasma levels of LH, FSH, and estradiol toward the control level. Furthermore a study on effects on male rats LH value, in which there was no significant differences between Se selenite treated group and control group[22]. This rather contradictory result may be due to inadequate sample size, or may be as explained by [23] they suggested that increase of LH level over the physiological limits could be explained by the low level of estradiol and progesterone, which were contract estradiol and progesterone in current study.

Steroid hormones

Our finding revealed that highly significant increase in progesterone values of T, T&Di groups than that in DW group, whereas Di group progesterone value decline significantly. These results are supported by [17] study which revealed that Se increase serum progesterone level in female exposed to doxorubicin. Also [16] reported that the sodium selenite (0.6 mg/100 g body weight/rat/day) for 28 days along with arsenic treatment increased the activities of the ovarian steroidogenic enzymes at the control level.

Based on the results, estradiol values increased significantly in all treated groups in the present study compare to DW estradiol level. Similar to our data [16] concluded that Selenium supplementation increased the plasma levels of LH, FSH, and estradiol

toward the control level. However, interestingly, this is contrary to a study conducted by [17]. There were no important variances among treatments for Plasma FSH and estrogen concentrations.

Ovarian Histopathological examination

Histological investigation of ovary of DW treated female rat's group cross section showed follicles of different developmental stages. Ovarian section of Di group rat reveals large cystic corpus luteum and clear follicular cyst and absence of Graafian Follicles and follicles at different stages of development, this finding is consistent with findings by [24] in which reported that histopathological examination in the ischemia-reperfusion +100 mg/kg Di sodium rats ovarian tissues exhibited hemorrhage, with dilated vessels, marked congestion, edema, migration of neutrophil and degenerative cells, ischemia –reperfusion+200mg/kg I sodium.

Ovarian sections of T group rats showed normal Graafian follicles and different follicles in developmental stages, as well as there were several persistence corpus luteum. Previous study by [16] added that Se had an important action on proliferation of oocytes, and increased stimulating effects of gonadotropins. In addition[25]showed that Se had a part in oocyte maturation. Furthermore [26] concluded that Selenium is effective in inhibiting damage of rat's ovarian tissue induced by ischemia/reperfusion injury. Ovary of T&Di female rats relieved improvement of some Graafian follicles, cystic corpus luteum was observed, furthermore some of clear copora lutea. Also provided that sections of ovary approved that sodium selenite in diet can prevent toxic histological change induced by arsenic [16].

تأثير مركب (4,4¯-(4,5,6,7-TETRAHYDRO- [1,2,3-] SELENADIAZOLO [4,5E] PYRIDINE-4,6-DIYL) BIS(BENZENE-1,3-DIOL)) على الخصوبة والهرمونات الإنجابية والتغييرات النسيجية المبيضية مقارنة مع اناث الجرذان المعالجة بالدايبايرون.

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الخلاصة

تهدف الدراسة الحالية لتقييم تأثيرات مركب(T)

[4,4¯-(4,5,6,7-TETRAHYDRO- [1,2,3-] SELENADIAZOLO [4,5E] PYRIDINE-4,6-DIYL) BIS(BENZENE-1,3-DIOL)]

على خصوبة الاناث وهرمونات التكاثر، بالإضافة الى التغييرات النسيجية المرضية في المبايض بالمقارنة مع تاثيرات الميتاميزول او الدايبايرون(Di). اربع مجاميع من اناث الجرذان الناضجة وكل مجموعة تضم عشرمن اناث الجرذان الصحية. ثلاثة مجاميع أعطيت مركب (T) مع/ او (Di) مذاب في ٢ مل من ماء الشرب،المجموعة الرابعة أعطيت ٢مل من ماء الشرب لمدة ٢٠ يوم قبل التزاوج وعشرة أيام بعد الجماع. النتائج تدل على ان هناك زيادة معنوية في عدد المواليد في مجموعة (T) (13.70±3.34) مقارنة مع مجموعة ماء الشرب (9.7±1.06). كذلك انخفاض معنوي في مجموعة T&Di الى (6.91±0.32) ، مع عدم تسجيل حالات حمل في مجموعة المعالجة ب Di. النسبة المئوية للحمل في مجموعة T&Di ٤٠% مقارنة مع مجموعتي T و DW ١٠٠%. مستوى هرمون محفز الجريب (FSH) اظهر ارتفاع معنوي في مجموعتي (T) (5.19 mIU/mL ±0.72) و T&Di (5.12 mIU/mL ±0.78) مقارنة مع مجموعتي DW(1.61mIU/mL±0.52) و Di (1.46 mIU/mL ±0.60). تركيزهرمون الليوتين LH في مجموعتي T(1.88 mIU/mL ±0.48)، و T&Di (1.67 mIU/mL ±0.41) عن تركيز LH في مجموعة DW (1.75 mIU/mL ±0.67).

فقط في مجموعة Di (0.48± 0.88 mIU/mL) مستوى LH انخفض معنويا عن بقية مجاميع الاختبار. مستوى هرمون البروجستيرون في مجموعة (T) (4.50± 17.22 mIU/mL) ، وكذلك في مجموعة T&Di (2.05± 10.11 mIU/mL) احصائيا ازداد مقارنة مجموعة DW (1.41± 6.3 mIU/mL) . نتائج الفحص النسيجي الامراضي أظهرت ان مقاطع المبيض ي مجموعة Di كشفت عن تكيس كبير في الجسم الأصفر مع غياب جريب المبيض و الجريبات في مختلف مراحل التطور. المقاطع في مجموعة (T) تظهر جريبات مبايض طبيعية مع جريبات مختلفة في مراحل مختلفة من التطور، وكذلك هناك مختلف من الجسم الأصفر. المبيض في مجموعة اناث T&Di تظهر اناث الجرذان تطور بعض الجريبات ،مع تكيس الجريب الأصفر. مع وجود جريبات صفر. يمكن الاستنتاج ان مركب(T) يمتلك تاثير جيد على مستوى هرمونات التكاثر و زيادة الخصوبة في اناث الجرذان. بينما فترة المعالجة طويلة الأمد ب Di تظهر تاثير رديء على خصوبة الاناث، بالتاثير على مستويات هرمونات التكاثر والتغييرات النسيجية في المبايض.

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