

EVALUATION OF LONG TERM ADMINISTRATION EFFECT OF SYNTHETIC PROGESTERONE (CIDOLUT DEPOT) ON OVARIECTOMIZED FEMALE ALBINO RATS.

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SUMMARY

A total of Sixty (60) adult mature female ovariectomized albino rats were used for evaluating the long term administration effect of synthetic progesterone . The rats were divided equally into two groups; group (I) considered as control group (treated with vehicle (Caster oil & Benzyl alcohol)) of 30 rats , and group (II) considered as treated group with hydroxy progesterone caproate (HPC) of 30 rats. rats of group II were subjected to weekly I/M injection to 9 months with progesterone drugs (Cidolut depot). The study continued for 9 month. Clinical signs and mortality rate were recorded monthly. Moreover, five rats from each group included the control one were taken every 1.5 month till 9th month of the work to study the changes that occurred in the hematological, the biochemical parameters, histopathological findings, and hormonal evaluation. Rats treated with progesterone drugs (Cidolut depot) tended to show significant increase in creatinine, uric acid, bilirubin values and ALT, AST and ALP enzymes activities without changes in body weight gain. Bronchiectasia and bronchitis were examined in lung. Liver had fatty infiltration and vacuolation. Kidney showed congestion and degenerated renal tubules. Spongiosis or focal gliosis were observed in brain.

INTRODUCTION

For over 30 years various synthetic estrogens and progestins combinations had been used in human contraceptives (*Maier and Herman, 2001*). In animals Progesterone (P4) enhanced the ovarian vasculature sensitivity to vasodilator effects of estradiol, so use estradiol subsequent to (P4) in anoestrous ewes significantly increased relative capillary blood flow (*Brown and Mattner, 1984*). Also plasma P(4) profile could be used to differentiate true anestrous from sub-estrus animals and to determine the needed for hormonal therapy , and when anestrous cattle and buffaloes were injected three hydroxyprogesterone caproate injection (750 mg, i.m) 72-hr intervals followed by injection equine chorionic gonadotropin

(750I.U., i.m.) 72hr, their conception rate was increased. (*Honparkhe, 2008*) . Follicular cyst, major reproductive problem in lactating dairy cows was due to failure of hypothalamus to trigger preovulatory surge of luteinizing hormone (LH) in response to estradiol may be progesterone deficiency (*Silvia et al., 2002*). Addition progesterone following ovulation in animals with follicular cysts resumed the normal cyclicity and normal hypothalamic responsiveness to (E2) due to rise (P4) level (*Ahmet and Milo, 2005*). Hydroxyprogesterone caproate (HPC) , natural hormone produced in large quantities during pregnancy, and used in human in second trimester as a weekly I/M injected dose 250 mg to treat preterm labor and preterm rupture of membrane resulted from inappropriate inflammation. (*Mercer et al., 1999*). And when (HPC) administered subcutaneously in rats at dose 5 or 10 mg/kg on gestation day 16 significantly reduced the preterm birth rate. (*Tiboni et al., 2008*). *El-Allawy et al. (1982)* proved that the combined progesterone/ estrogen in contraceptives could produce an electrolytes imbalance and elevated serum uric acid level. The administration of the hormonal replacement therapy caused a concomitant functional disturbances in liver and kidney, together with alterations in some enzymes serum concentration (*Maisaa., 2002*). And added that the enzymatic activities following hormonal treatment exhibited to significant reduction of ALP activity in serum of estrogen-treated rats. Moreover, significant increase in AST and ALT activities in the serum of estrogen and estrogen/progesterone treated rats groups.

As the liver was the target organ for estrogen and progesterone metabolism and the kidney was organ for their excretion, excess estrogen or/and progesterone produced in hepatic ; morphological, biochemical and carcinogenic changes, and in kidney; congestion, lymphocytic aggregation ,local hemorrhage and cystic dilation of renal tubules. (*Fakhry et al.,1988*). The present study aimed to declare long term administration effect of synthetic progesterone on the hematological and biochemical parameters of female rats, Study the histopathological alterations in lung, liver, kidney, and in brain of female rats, and Demonstration the hormonal homeostasis of female rats after the administration of such hormones for long term.

MATERIALS AND METHODS

Experimental Animals

A total of sixty (60) adult mature female, weighing from 150 - 200 grams, obtained from Ocular Institute of Research in Giza, Cairo University were used. Animals were fed on dry commercial standard pallets of rabbits from Tameda company, El-Dakahelia Egypt, contain 16 % protein, and tap water was supplied ad-libitum. Rats were separated as five rats /cage, and subjected to 12 hours light and 12 hours darkness, kept under observation for two weeks before being used to assure the healthy state of the animals.

Experimental-Design

Sixty (60) adult mature female ovariectomized albino rats were used, randomly divided into two groups; group (I) considered as control group (treated with vehicle) of 30 rats , and group (II) considered as treated group with hydroxy progesterone caproate (HPC) of 30 rats. The animals of group (2) were then subjected to treatment (**Table1**). Rat's doses were calculated according to the paget represented in the Fundamental of Experimental Pharmacology (*Ghosh 1971*). Clinical signs and mortality rate were recorded monthly. Moreover, five rats from each group included the control one were taken every 1.5 month till 9th month of the work to study the changes that occurred in the hematological, the biochemical parameters, histopathological findings, and hormonal evaluation.

Drugs used: Cidolut Depot 250 mg ampoules

It is composed of an active principle hydroxy progesterone caproate (HPC) 250 mg /ampoule 1 ml. is in oily solution composed of castor oil and benzyl alcohol in a ratio 5: 4, manufactured by Chemical Industrial Development (CID) for drugs – Egypt.

Table (1): Drugs used, treatment period and injectable doses.

Group	Drugs used	Treatment period and injected dose
I	Vehicle (Caster oil & Benzyl alcohol)	Cater oil and benzyl alcohol in a ratio 5 : 4 as vehicle injected intramuscularly (IM) by 0.45 ml and remained as control.
II	Cidolut Depot 250 mg Ampoule	hydroxy progesterone caproate (HPC) injected I/M at dose 22.5 mg/kg.Bwt that represented in 0.45ml from 25ml solution contains 1 ml (HPC) 250 mg dissolved in vehicle to be injected once weekly for 10.5 th m .

Blood-Sampling

After 1.5, 3, 4.5, 6, 7.5, and 9 months respectively from treatment blood samples were collected from five rats from each group included the control one by puncturing the veins in retro-orbital plexus according to the method described by *Van Herck et al.(1998)*. Two types of blood samples were collected at each time, a small amount about (0.5ml) in EDTA anticoagulant for hematological studies, and large amount (3 ml) without anticoagulant for measuring the changes in the biochemical parameters.

Hematological-Studies

Individual Blood sample (0.5 ml) was collected in a dry clean well stopper glass vials contained (0.2 mg EDTA powder / 1.0 ml blood) as anti-coagulant, for estimating:- Packed Cell Volume (PCV), Hemoglobin (Hb) as described by *Van Kampen and Zijstra (1961)*. Total RBCs and WBCs counts, as well as differential leucocytic count were performed on the stained blood smear according to the standard technique described by *Fieldman et al . (2000)*

Biochemical-Parameter-studies

Individual Blood samples (3.0 ml) blood without anticoagulant centrifuged at high speed 3000 r.p.m. for 5 minutes to obtain serum, partly stored at – 20 ° C until assayed for hormonal measurements, and the other part was

collected to be used in the following measurement tests; **Serum Glucose** , **Serum lipid Parameters** (*Serum Triglycerides, Serum Cholesterol, High density lipoprotein (HDL) – Cholesterol, and Low density lipoprotein (LDL) – Cholesterol*), **Kidney Function Tests**(*Serum Creatinine, Blood Urea Nitrogen {BUN},and Serum Uric acid*), **and Liver Function Tests**(*Alanine aminotransferase (ALT) and Aspartate aminotrans- ferase (AST), Bilirubin (Total and Direct Bilirubin), and Alkaline Phosphatase (ALP)).*

Histopathological-Studies

Specimens from lung, liver, kidney, and brain were collected at 1.5 months intervals and fixed in 10% neutral buffered formalin for preparing paraffin tissue sections at 4-6 μ thickness. These sections were stained with hematoxylin and eosin (**Bancfort and Stevens, 1996**).

Measurements of serum levels of hormones using Enzyme Linked Immuno-sorbant Assay (ELISA): Measurements of serum progesterone according to **Radwanska et 1978**. and Measurements of serum Estradiol according to **Ratcliffe et al 1988**.

Statistical-Analysis

Values were expressed as mean \pm S.E. statistical comparisons between the means of different experimental groups in different months were made with completely randomized two ways ANOVA" Student- Newman Kevls test" by COSTAT program version one at probability "P" value ≤ 0.05 was assumed for statistical significance.

RESULTS AND DISCUSSION

Clinical signs

During the whole period of the study rats treated with only progestogenic preparation (Cidolut depot ampoules) tended to show a slight non-significant decrease in their body weight continued till the end of the study (**Table 2**). Unlike **Wade et al (1989)** and **Brichmeiere (1969)** who mentioned that the female sex hormones used to stimulate adrenal cortex to subsequent release of cortisone hormone that caused an increase in body weight gained.

Table 2: Mean values \pm SE of weight changes in grams in control and treated rats groups.

Time/G	Control	C group.
• Time	162.7 \pm 4.00 ^a	158.9 \pm 4.07 ^a
1 st m	181.5 \pm 6.24 ^a	185.7 \pm 5.39 ^a
2 nd m	207.2 \pm 8.25 ^a	207.5 \pm 7.02 ^a
3 rd m	228.1 \pm 12.06 ^a	208.6 \pm 8.70 ^a
4 th m	238.1 \pm 6.39 ^a	211.8 \pm 8.67 ^a
5 th m	260.0 \pm 6.81 ^a	230.4 \pm 7.94 ^a
6 th m	264.5 \pm 7.44 ^a	240.6 \pm 8.52 ^a
7 th m	270.2 \pm 9.62 ^a	251.5 \pm 6.69 ^a
8 th m	267.1 \pm 10.11 ^a	256.8 \pm 10.11 ^a
9 th m	272.0 \pm 12.71 ^a	257.0 \pm 8.57 ^a
LSD	31.6	

C group: Rats received Cidolut depot.

LSD: Least significant differences between groups at probability ≤ 0.05 .

a : means different superscript within a raw are significantly different at $p \leq 0.05$

Mortality Rate

In rats received Cidolut depot ampoules during the whole period of the study the mortality rate was near but slightly higher than that existed in control rats group, its increase occurred only at 9th post-injection, as such rate in those rats was 4.35%, 4.76%, 5.26, 12.50% and 13.33% at 2,4,6,8 and 9 months from the study respectively compared to that in control rats was 4.16%, 0%,4.76%,5.26% and 11.11 at the same study time respectively. Moreover total rats death % was 12%, 20%, 28%, 40%

and 48% in those rats while was 8%, 12, 20%, 28% and 36% in control rats at the same time of comparison respectively. The only sign could be noticed in those rats before death was shallow rapid respiration. (**Table3**).

Table3: Mortality rate in control and treated rats group.

Time	control			C group		
	dead	%	Total death%	dead	%	Total death%
1 st m	0	0	0	0	0	0
2 nd m	1	4.16	8	1	4.35	12
3 th m	1	4.35	12	1	4.45	16
4 th m	0	0	12	1	4.76	20
5 th m	1	4.45	16	1	5.00	24
6 th m	1	4.76	20	1	5.26	28
7 th m	1	5.00	24	1	5.88	32
8 th m	1	5.26	28	2	12.50	40
9 th m	2	11.11	36	2	13.33	48

After 1.5-3 months

Clinicopathological changes

1-Hematological alteration

Table 4 explained the values of blood indices as significant increase in MCV s in rats given Cidolut ampoules injection at 3th month post-study with no apparent changes were observed in MCH and MCHC values reflected develop macrocytic normochromic (megaloblastic) anemia in all treated rats at 3th month post-hormonal drugs administration. Moreover monocytes counted value showed its significant increase in Cidolut depot ampoules injected rats at 3rd month from the study.

2-Changes-in-Biochemical-parameters

Concerning to biochemical parameters changes found no changes were observed in Cidolut depot ampoules treated rats. The lipid profile showed no changes in such values in Cidolut depot ampoules injected rats. The changes that occurred in kidney function tests were recorded as a significant increase in creatinine values in rats received Cidolut depot ampoules at 3th month post-hormonal administration. Also at that time the uric acid value was significantly increased in all treated rats.

Regarding to liver functions found total bilirubin value was significantly increased at 3th month post-injection in Cidolut depot ampoules treated rats. Direct bilirubin value was significantly decreased in Cidolut depot ampoules treated rats at 3rd month from the work, and Significant increased free bilirubin value was noticed in rats injected Cidolut depot ampoules at 3th month post-therapy. Activity of transferase enzymes ALT and AST showed its significant increase in all treated rats, Moreover the ALP activity in rats received Cidolut depot ampoules was significantly increased at 3th month post-injection (**Table 5**).

Pathological alterations

1-Post-Mortem Findings

Liver and kidney of rats received Cidolut depot ampoules exhibited to show much sub-capsular peticheal hemorrhage, Moreover liver and lung appeared slightly enlarged in size, and no apparent macroscopic changes were seen in brain in such rats group.

2-Histopathological changes

Figure(1) denoted that rat's lung due to administration of Cidolut depot ampoules showed hyperplasia of the bronchial epithelium and peribronchial lymphoid follicles. Moreover, blood vessel revealed vasculitis. Figure(2) showed that **liver** of rat received cidolut ampoules had vacuolated and degenerated hepatocytes which had been interpreted by many authors *Gray et al. (1981)* who reported that due to ovarian hormones therapy fatty changes occurred and included vacuolation ,

degeneration and fatty infiltration in different organs due to the direct effect on adipose tissue and resulted in increase in body weight gain and fatty deposition., the nuclei were vesiculated with increased number of binucleated hepatocytes. In addition central vein and sinusoidal dilatation together with Kupffer's cells hyperplasia were also noticed. **Kidney** in Cidolut depot ampoules treated rat exhibited to show congestion of the interstitial blood vessels with vasculitis. Also there were peri-glomerular mononuclear cellular infiltration. **(Figure 3)**. Microscopic examination of **brain** in rats treated with Cidolut depot ampoules showed no significant histopathological alterations.

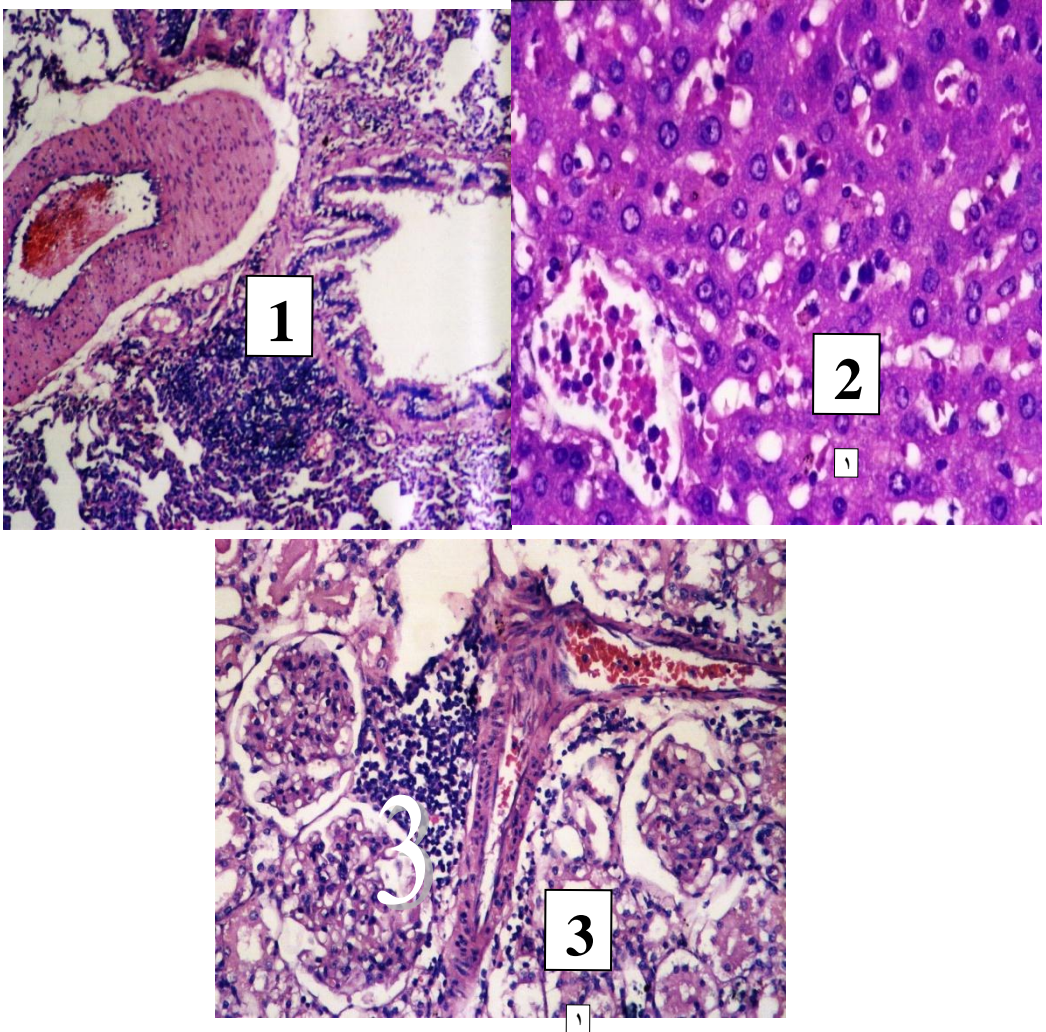


Figure 1: lung of rats treated with Cidolut 1.5-3 month showing hyperplasia of the bronchial epithelium and peribronchial lymphoid follicles together with vasculitis (H&EX200)

Figure 2: Liver of rats treated with Cidolut 1.5-3 month showing vacuolated and degenerated hepatocytes. In addition central vein and sinusoidal dilatation (H&E X 400)

Figure 3: Kidneys of rats treated with Cidolut 1.5-3 month showing , congestion of the interstitial blood vessels with vasculitis. Also there were peri-glomerular mononuclear cellular infiltration. (H&E X 200).

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After 4.5-6 months

Clinicopathological change

1-Hematological alteration

Rats injected Cidolut depot ampoules tended to show significant decrease in RBCs counted value with no apparent changes in other mentioned values. Significant increase of MCV values in all treated rats with no changes in MCH and MCHC values in all treated rats, indicated still existence of megaloblastic anemia in treated rats. These findings came in agreement with **Holmes (1970)**.

The alterations occurred on differential leucocytes counts showed lasting development of significant neutrophilia in all rats received therapy. Significant increase in eosinophils counts in rats injected Cidolut depot ampoules at 6th month post-drug administration. Monocytosis was recorded in Cidolut depot ampoules treated rats (Table 6).

2-Changes-in-Biochemical-parameters

The alteration in biochemical parameters could be summarized in Table 7 as significant decreases of serum glucose values in an exaggerated manner in rats received Cidolut depot ampoules only at 6th month post-injection. Regarding to changes in lipid profile, registered no apparent recorded changes in rats received Cidolut depot ampoules. Concerning to kidney functions, noticed significant increased serum creatinine and uric acid values in rats received Cidolut depot. These results were explained by the opinion of **Antus et al. (2005)** who stated that progesterone increased urinary protein excretion, glomerulosclerosis and mononuclear cell infiltration with development of chronic allograft nephropathy (CAN) characterized by progressive deterioration of renal functions with tubular atrophy. In addition, no apparent changes in BUN values observed in all treated rats.

In respect to bilirubin, found that the total, direct and free bilirubin values were significantly increased in Cidolut depot ampoules treated rats. Serum AST and ALP activities were significantly increased in Cidolut depot ampoules received rats, while ALT activity was significantly increased in such rats group at 6th month from the study. **El-Allawy et al.**

(1982) reported that contraceptives induced synergistic effect could be interpreted as disturbance in hepatorenal function ;increased activities of ALT and AST enzymes due to liver and kidney damage resulted in raise their plasma concentration.

Pathological alterations

1-Post-Mortem Findings

Rat's lungs received Cidolut depot ampoules injections appeared reduced in size and showed congestion, peticheal hemorrhage or ecchymotic spots with small emphysematous areas. Moreover kidneys of those rats were exhibited to show mild peticheal or ecchymotic spots in sub-capsular areas. And apparently normal appearance of liver and brain in such rats group at that time.

2-Histopathological changes

lung treated with Cidolut depot ampoules showed severe broncoectasia with massive mononuclear cells infiltration peribronchial, perivascular, and perialveolar. (**Figure 4**).

Regarding **kidneys**, extensive severe glomerular vacuolation, enlargement and swollen glomeruli , lobulated capillary tuft , proliferated capillary endothelium were recorded in kidney of rat received Cidolut depot ampoules. (**Figure 5**).

Brain cerebrum of rats treated with Cidolut depot ampoules showed extra-celluar brain edema, dilated Virchow-Robnin spaces, degenerated myelin sheath with focal areas of mononuclear cells infiltration and atrophy of some nerve cells (**Figure 6**).

Microscopic examination of **liver** in rats treated with Cidolut depot ampoules showed no significant histopathological

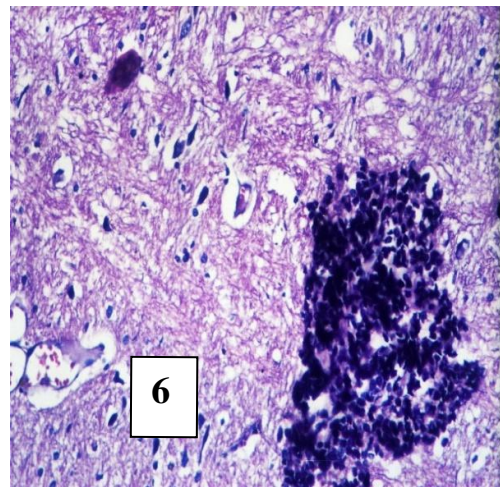
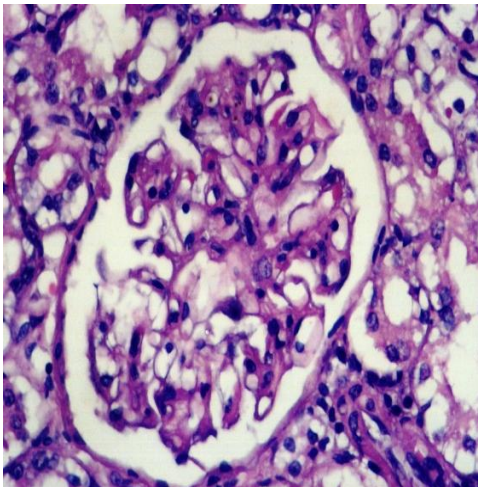


Figure 4: lung of rats treated with Cidolut 4.5 -6 month showing severe bronchoectasia with massive mononuclear cells infiltration peribronchial, perivascular, and perialveolar, (H&EX200).

Figure 5: Kidneys of rats treated with Cidolut 4.5 -6 month showing glomerular vacuolation, enlargement and swollen glomeruli , lobulated capillary tuft , proliferated capillary endothelium (H&E X 400)

Figure 6: Brain of rats treated with Cidolut 4.5 -6 month showing . extra-cellular brain edema, dilated Virchow-Robnin spaces, degenerated myelin sheath with focal areas of mononuclear cells infiltration and atrophy of some nerve cells (H&E X 200).

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After 7.5- 9th month

Clinicopathological changes

1-Hematological alteration

PCV%, Hb concentrations and RBCs counts values in rats received Cidolut depot ampoules showed no changes. In addition a significant increased MCV values in all treated rats, while the MCH and MCHC values remained without apparent changed indicating continuation formed anemia in all treated rats.

Total leucocytes revealed no apparent changes in value in Cidolut depot ampoules received rats with lasting significant neutrophilia in rats injected Cidolut depot ampoules. At 9th month significant esinophilia and monocytosis was demonstrated in Cidolut depot ampoules treated rats, lymphocytes showed no apparent changes in value in rats treated with Cidolut depot ampoules injection *Staples et al. (1983)* . (Table8).

2-Changes-in-Biochemical-parameters

The alterations in biochemical parameters as shown in **Table 9** revealed continuous significant decrease in glucose level in Cidolut depot ampoules injected rats at 9th month from the study. These finding were agreed with *Dahm et al. (1977)* who indicated that progesterone had insulin effects, and increase the capability of glycogen formation due to activation of some enzymes in lipogenesis and gluconeogenesis. Alterations in lipid profile in cholesterol and HDL rats received Cidolut depot ampoules did not show apparent changes except for LDL values that had been showed high significant increased values at 9th month from the work. Similar finding were previously reported by *Enk et al (1992)* who found that long norethisterone (NET) therapy for one year in rats induced a decrease in HDL value by approximately 30% while the LDL value was tended to be increased. Serum creatinine and uric acid values as kidney functions were continuously showing significant increased values in treated rats. These results agreed with with *El-allway et al. (1982)* who proved that contraceptives produced electrolyte imbalance and elevated uric acid value in the serum and with *Atallah et al. (1988)*

who indicated the raised plasma uric acid values with progesterone therapy attributed to decrease uric acid clearance.

Regarding to liver functions, a significant increase in values of all bilirubin components was seen in rats received Cidolut depot ampoules. Significant increased in ALT and AST activities in all treated rats, Moreover ALP activity was significantly increased at 9th month in rats received Cidolut depot ampoules. *Pushpalatha et al. (2006)* found that 17 α hydroxy progesterone caproate significantly increased the activities of aminotransaminases, antioxidant glutathione-S-transferase, and catalase due to disruption of mitochondrial integrity resulted from tissue damage.

Pathological alteration

1-Post-Mortem Findings

In rats treated with Cidolut depot ampoules their lungs appeared pale in color, reduced size with thick grayish exudates oozed out in cut section. In addition, liver of such rats was friable consistent, reduced in size, congested with several areas of white discoloration. Multiple white discolorations areas were observed in the renal cortex of congested reduced size kidneys. Normal appearance of brain was noticed with exception a slightly reduced size.

2-Histopathological changes

Lung of rats received Cidolut depot ampoules showed marked bronchiectasia and bronchitis with marked bronchial epithelium hyperplasia, leucocytic cells infiltrations, and formation of newly formed brochioles, (**Figure 7**).

Liver of rat received Cidolut depot ampoules shown degenerated hepatocytes, increased number of binucleated cells with appearance of vesiculated nuclei in association with widening of sinusoidal space which were explained by *Attia et al. (1994)* who found after estrogen/ progesterone therapy a leucocytic cells migrated from blood stream to

hepatic vessels particularly sinusoids in female rats that resulted in a cellular leucocytic infiltration in blood sinusoids and portal area. In addition, hyperplasia of kupffer's cells Together with multiple focal areas of degenerated hepatocytes replaced by mononuclear cells infiltration were also detected, (**Figure 8**).

Kidney of rats treated with Cidolut depot ampoules showed glomerular vacuolation , renal tubular degeneration. In addition to vasculitis and perivascularitis, (**Figure9**).*Edresset al.(1991)* reported that oral contraceptives induced many histological alterations in adult female albino rats such as degeneration of renal tubules, renal blood vessels congestion with abundance inflammatory leucocytes infiltration, as such hormones stimulated proliferation of kidney cells.

Regarding **brain** histopathology, status spongiosis with enlargement of extracellular space and congested blood vessels were detected, (**Figure 10**).

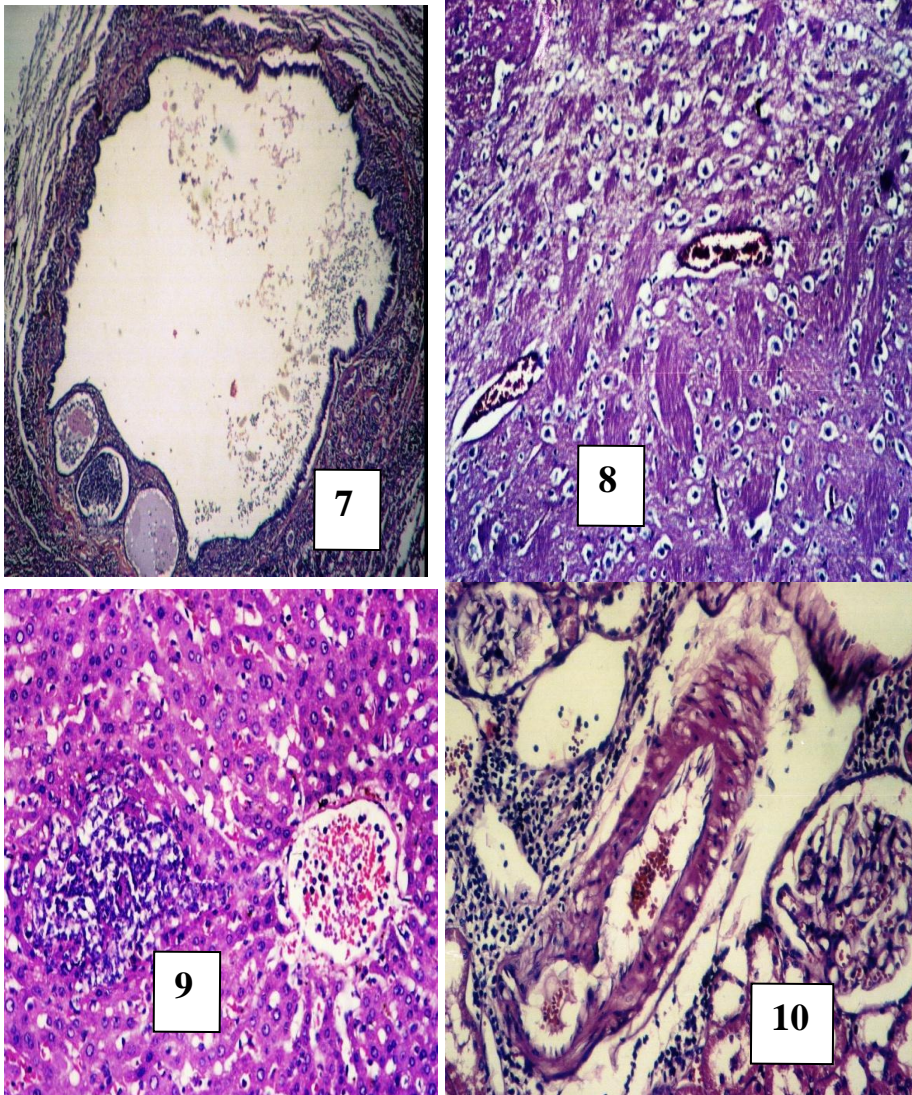


Figure 7: Lung of rats treated with Cidolut 7.5-9 month showing severe bronchoectasia and bronchitis with marked bronchial epithelium hyperplasia, leucocytic cells infiltrations, and formation of newly formed bronchioles (H&EX200). **Figure 8:** Liver of rats treated with Cidolut 7.5-9 month showing widening of sinusoidal space and hyperplasia of Kupffer's cells. Together with multiple focal areas of degenerated hepatocytes replaced by mononuclear cells infiltration (H&EX200). **Figure 9:** Kidneys of rats treated with Cidolut 7.5-9 month showing glomerular vacuolation, renal tubular degeneration. In addition to vasculitis and perivasculitis (H&E X 400). **Figure 10:** Brain of rats treated with Cidolut 7.5-9 month showing status spongiosus with enlargement of extracellular space and congested blood vessels (H&E X 200).

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1. Measurement of serum progesterone hormone

Serum progesterone levels in different rats groups received cidolut depot when compared to that in control rats group was tended to decrease from 3.5 ng/ml to 1.5 ng/ml in rats group received Cidolut depot ampoules that showed a significant decreased at 9th months from the study(**Table 10**). These results could be explained by *Pepe and Rothchild (1973)* who stated that serum progesterone level not differed in ovariectomized rats subjected to progesterone or estrogen/progesterone treatment, directly related to dose of progesterone administered not by duration of treatment, while its change reflected secretion.

2. Measurement of serum Estradiol hormone

Serum estradiol levels in different rats groups received hormonal drugs when compared to that in control rats groups showed a significant increased values in rats received Cidolut depot at 4.5th month from the study (**Table 11**). *Hong et al.(2004)* mentioned that in ovariectomized rats estrogen was also produced from adrenal gland through increase androgen formation and its conversion (aromatization) to estrogen, while repeated estradiol treatment to those rats resulted in significant increase ACTH with consequence elevation of adrenal activity, cortisone and estradiol level.

Table 10: Mean values \pm SE of Progesterone level changes in control and C group

Time	Control	C group
0 Time	4.74 \pm 0.01 ^a	3.58 \pm 0.26 ^a
1.5 th m	4.25 \pm 0.18 ^a	3.01 \pm 0.07 ^a
3 rd m	3.49 \pm 0.61 ^{ab}	2.74 \pm 0.25 ^a
4.5 th m	2.88 \pm 0.26 ^a	2.18 \pm 0.21 ^a
6 th m	2.51 \pm 0.04 ^{ab}	1.98 \pm 0.26 ^a
7.5 th m	2.62 \pm 0.03 ^{ab}	2.11 \pm 0.17 ^a
9 th m	3.23 \pm 0.04 ^a	1.41 \pm 0.31 ^b
LSD	1.34	

Table 11: Mean values \pm SE of Estradiol level changes in control and C group.

Time	Control	C group
0 Time	4.62 ± 0.04^a	4.53 ± 0.16^a
1.5 th m	2.15 ± 0.03^a	2.27 ± 0.01^a
3 rd m	3.42 ± 0.02^b	2.36 ± 0.02^{ab}
4.5 th m	2.47 ± 0.09^{ab}	3.21 ± 0.07^b
6 th m	2.43 ± 0.04^a	2.44 ± 0.25^a
7.5 th m	4.62 ± 0.03^b	2.38 ± 0.11^a
9 th m	2.23 ± 0.04^a	1.43 ± 0.03^a
LSD	1.71	

C group: Rats received Cidolut depot ampoules

LSD : Least significant differences between groups at probability ≤ 0.05 .

a,b,: means different superscript within a raw are significantly different at $p \leq 0.05$.

CONCLUSION

From the present study, it is concluded that, great attention must be taken from the haphazard use of the used synthetic progesterone as possible as it has adverse effect on the hematological and biochemical parameters reflected on the clinicopathological results and confirmed histopathologically.

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