Evaluation of long term administration effect of synthetic progesterone (Cidolut depot) on ovariectomized female albino rats

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Introduction

- For over 30 years various synthetic estrogens and progestins combinations had been used in human contraceptives (Maier and Herman, 2001).

- When anestrous cattle and buffaloes were injected three hydroxyprogesterone caproate HPC (Cidolut) injection (750 mg, i.m) 72-hr intervals followed by injection equine chorionic gonadotropin (750 I.U., i.m.) 72hr, their conception rate was increased. (Honparkhe, 2008).
Hydroxyprogesterone caproate (HPC), a natural hormone produced in large quantities during pregnancy, and used in humans in the second trimester as a weekly I/M injected dose of 250 mg to treat preterm labor and preterm rupture of the membrane resulted from inappropriate inflammation. (Mercer et al., 1999).

And when (HPC) administered subcutaneously in rats at a dose of 5 or 10 mg/kg on gestation day 16 significantly reduced the preterm birth rate.
The administration of the hormonal replacement therapy caused a concomitant functional disturbances in liver and kidney (As the liver was the target organ for estrogen and progesterone metabolism and the kidney was organ for their excretion),

Together with alterations in some enzymes serum concentration (Maisaa., 2002).
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

I- Experimental animals:

- A total of sixty (60) adult mature female rats, weighing from 150 - 200 grams, obtained from Ocular Institute of Research in Giza, Cairo University were used.

- Rats were fed on dry commercial standard pallets of rabbits from Tameda company, El-Dakahelia Egypt, contain 16 % protein, and tap water was supplied ad-libitium.

- 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.
Materials and Methods

Experimental design

60

Adult mature female ovariectomized albino rats

Group I
30 Rats

treated group
injected intramuscularly (IM) by 0.45 ml vehicle Csater oil and benzyl alcohol in a ratio 5 : 4

Group II
30 Rats

Treated group
injected I/M with hydroxy progesterone caproate (HPC) at dose 22.5 mg/kg.Bwt

control group
Materials and Methods

**Sampling**

The Experimental period was 9 month

- **Clinical signs and mortality rate** were recorded monthly
- **Blood samples from 5 Rats of each group** were collected at 1.5 month intervals **FOR Clinicopathologic and Hormonal Measurement**
- **Tissue specimens including Lung, Liver, Kidneys and Brain** were collected each 1.5 month **FOR Histopathological Examination**
**Materials and Methods**

**Sampling**

**Blood samples**

The *first* blood sample was anticoagulated used for evaluating hemogram. Total erythrocyte and leukocyte counts, Packed cell volume (PCV%), Hemoglobin concentration, and Differential leukocytic count.

The *second* blood serum sample Used for Measurements of serum levels of hormones (progesterone and Estradiol) using Enzyme Linked Immuno-sorbant Assay (ELISA) + biochemical studies

*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)).
Results and Discussion

Clinical signs

During the whole period of the study rats treated with only progestogenetic preparation (Cidolut depot ampoules) tended to show:

- a slight non-significant decrease in their body weight continued till the end of the study.
2-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 month post-injection,
After (1.5–3) months
Results and Discussion

Clinicopathological changes:

1-Hematological alteration:

• Significant increase in MCVs in rats given Cidolut ampoules injection at 3rd 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 3rd 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.
Results and Discussion

- **Clinicopathological changes:**

- **2- Biochemical-parameters:**
  - The lipid profile showed no changes in such values in Cidolut depot ampoules injected rats.
  - Kidney function tests were recorded as a significant increase in creatinine values in rats received Cidolut depot ampoules at 3rd month post-hormonal administration.
  - Also at that time the uric acid value was significantly increased in all treated rats.
Results and Discussion

- Clinicopathological changes:
- 2- Biochemical-parameters:
  - Liver functions tests:
  - **total bilirubin** value was significantly increased at 3\textsuperscript{rd} month post-injection in Cidolut depot ampoules treated rats. **Direct bilirubin** value was significantly decreased in Cidolut depot ampoules treated rats at 3\textsuperscript{rd} month from the work, and **Significant increased free bilirubin** value was noticed in rats injected Cidolut depot ampoules at 3\textsuperscript{rd} 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 3\textsuperscript{th} month post-injection.
Results and Discussion

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.
Results and Discussion

2-Histopathological changes

- 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)
Results and Discussion

2-Histopathological changes

- 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)
Results and Discussion

- **2-Histopathological changes**

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).
After (4.5–6) months
Clinicopathological changes:

1-Hematological alteration:

- Significant decrease in RBCs counted value.
- 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.
Results and Discussion

- **Clinicopathological changes:**

- 1-Hematological alteration:
  
  - The alterations occurred on differential leucocytes counts showed lasting develop significant neutrophilia in all rats received therapy.
  
  - Significant increase esinophils counts in rats injected Cidolut depot ampoules at 6th month post-drug administration.
  
  - Monocytosis was recorded in Cidolut depot ampoules treated rats.
Results and Discussion

- Clinicopathological changes:

- 2- Biochemical-parameters:

  • Significant decreases of serum glucose values in an exaggerated manner in rats received Cidolut depot ampoules only at 6th month post-injection.
Results and Discussion

- **Clinicopathological changes:**
- 2- **Biochemical-parameters:**

  - Regarding to changes in lipid profile registered no apparent recorded changes in rats received Cidolut depot ampoules.
Results and Discussion

- Clinicopathological changes:
- 2- Biochemical-parameters:
  - kidney functions noticed significant increased serum creatinine and uric acids values in rats received Cidolut depot
  - In respect to bilirubin found that the total, direct and free bilirubin values were significantly increased in Cidolut depot ampoules treated rats.
Results and Discussion

- **Clinicopathological changes:**
- **2- Biochemical-parameters:**
  - Kidney functions noticed significant increased serum creatinine and uric acids values in rats received Cidolut depot.
  - In respect to bilirubin found that the total, direct and free bilirubin values were significantly increased in Cidolut depot ampoules treated rats.
Results and Discussion

- **Clinicopathological changes:**
- **2- Biochemical-parameters:**

  - Liver function test noticed 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
Results and Discussion

Pathological alterations:

- 1-Post-Mortem Findings:

- **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 of rats treated with Cidolut 4.5 -6 month showing severe bronchoectasia with massive mononuclear cells infiltration peribronchial, perivascular, and perialveolar, (H&EX200).
**Results and Discussion**

- **2-Histopathological changes**

- **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)
Results and Discussion

2-Histopathological changes

- 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).
After (7.5–9) months
Results and Discussion

- **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.
Results and Discussion

Clinicopathological changes:

1-Hematological alteration:

- **Total leucocytes** revealed no apparent changes in value in Cidolut depot ampoules received rats.
- 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
Clinicopathological changes:

2- Biochemical parameters:

• Continuous significant decrease in glucose level in Cidolut depot ampoules injected rats at 9th month from the study.
Clinicopathological changes:

2- Biochemical-parameters:

- 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.
Clinicopathological changes:

2- Biochemical-parameters:

• Serum creatinine and uric acid values as kidney functions were continuously showing significant increased values in treated rats.
Results and Discussion

Clinicopathological changes:

2- Biochemical parameters:

- 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.
Results and Discussion

Pathological alterations: -
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 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).
Results and Discussion

2-Histopathological changes

Liver of rats treated with Cidolut 7.5-9 month showing widening of sinusoidal space and hyperplasia of kupffer's cells Togethrt with multiple focal areas of degenerated hepatocytes replaced by mononuclear cells infiltration (H&EX200).
Results and Discussion

2-Histopathological changes

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)
Results and Discussion

2-Histopathological changes

Brain of rats treated with Cidolut 7.5-9 month showing status spongiosis with enlargement of extracellular space and congested blood vessels (H&E X 200).
Results and Discussion

Measurement of hormones

2- Measurement of serum Estradiol 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.

- And showed a significant decrease at 9th months from the study
Results and Discussion

- **Measurement of hormones**

  1. **Measurement of serum progesterone hormone:**

  - **Serum estradiol** levels in different rats groups received hormonal drugs when compared to that in control rats.

  - Showed a **significant increased values** in rats received Cidolut depot at 4.5\textsuperscript{th} month from the study.
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 an adverse effect on the hematological and biochemical parameters reflected on the clinicopathological results and confirmed histopathologically.
Thank You