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Effect of High Dose Injection of Methyl Prednisolone Acetate on some Physiological Parameters in Female Rats.

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1.Abstract

This study was conduct on 20 healthy female rats. Ten rats were injected intramuscularly with highly single dose (30mg /kg B.W) of methyl prednisolone acetate (MPA) daily for 60 days. While the other ten injected intramuscularly with normal saline (1ml/kg B.W) and considered as a control. Blood samples were collected from treated and control rats as well. Results revealed that there was significant ($p<0.05$) decrease in, total serum protein , lymphocytic count ,esinophilic and basophilic counts . There were significant ($p< 0.05$) increase in estrogen, progesterone, tri-iodthyronine(T3) , thyroxine(T4) hormones , total serum cholesterol , triglyceride ,total WBC count, neutrophilic and monocytiz counts .

Key words: Methyl predinsolone acetate, female rats, hormones, biochemical parameters, WBC.

2.Introduction

Methyl prednisolone (MP) is one of the exogenous synthetic glucocorticiod steroids that is an anti-inflammatory steroid (AIS) used in the treatment of inflammatory diseases and immune disorder including transplant rejection[1,2]. Methyl prednisolone acetate is hydrolysed its active form by serum cholinesteras which its plasma half- life is approximately 2.5 hour while its biological half- life is 12 - 36 hours [3]. Methyl prednisolone is about 40% to 60% bound to the plasma proteins, which means that this fraction of the drug in plasma is not able to interact with cellular steroids receptors a nor crosses cellular membrane or the blood-brain barrier for most part restricts protein passage [3]. Because of its physiological effects on systemic energy metabolism, long-term therapy causes adverse effects leading to metabolic disorder including steroid induced diabetes, dyslipidemia, protein catabolism, muscle atrophy and metabolic syndrome limiting their therapeutic usefulness [4, 5] .

Other studies show that corticosteroids regularly raise plasma lipid levels in rabbits and chickens[6] ,in contrast [7]showed that corticosteroids increase plasma lipids in rat and mice. One of the main limitations of MPA therapy is negative feed back mechanism on hypothalamus and anterior pituitary gland[8], maintained by the hypothalamic pituitary- adrenal (HPA) axis. The hypothalamus produces corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to synthesize adrenocorticotropic hormone (ACTH) to signal production of cortisol.The main endogenous glucocorticoid

[9,10].Therefore the administration of exogenous glucocorticoids suppresses the release of ACTH from the pituitary. Thus the adrenal cortex ceases secretion of endogenous corticosteroids, producing a secondary adrenocortical insufficiency [11,12] . While it's specific anti-inflammatory effects that have been demonstrated include decreased availability of inflammatory cells, interference in inflammatory cell function [13,14] and suppression of noncellular, inflammatory reaction [15]. On other hand numerous hematological studies find that high dose of MP which induced differentiation and apoptosis of myeloid leukemia cells in children [16,17]. Other studies revealed that high dose of it decrease production of blood in children [18].

The aim of this study is to investigate the effect of single high dose of methyl prednisolone acetate on some hormones (progesterone, estrogen, T3 and T4) and some biochemical parameters (total serum protein, total serum cholesterol, triglyceride)in addition to some hematological parameters (WBC count and differential WBC count) in healthy female rats.

3. Materials and Methods

Twenty healthy female rats, their mean body weight was 200gm were housed at the animal house of the College of Veterinary Medicine during the period from first of October till the end of November 2011 at a similar conditions to room temperature as each three rats were kept in one cage under normal periods of light/dark with free access of food and water. They were randomly divided into two experimental groups (ten/group) each. First group was used as controls which injected intramuscularly with physiological normal saline (1ml /kg), while the other group was intramuscularly treated with high single dose (30 mg/kg)of methyl prednisolone acetate once daily for 60 days. Treated and control rats were scarified immediately after last dose. Blood samples (0.5 ml)were collected directly from rats heart by using disposable syringe in vials containing EDTA for hematological investigation ,another blood samples (1.5)ml were collected in vial without any coagulant agents and immediately centrifuged to get serum which was frozen at -20 C° for hormonal and biochemical analysis.

3.1.Hormonal Analysis

T3, T4 enzyme immunoassay test kit was used Catalogs number:
125-300 Total tri-iodothyronine product code:
225-300 Total thyroxin product code:
Both from monobind inc. Lake forest, CA92630, USA
Enzyme Immunoassay Test KIT Catalog Number: BC-11 for Estrogen (E2), and BC-1111 for Progesterone were used .

3.2.Biochemical Analysis

The biochemical tests (triglyceride ,total cholesterol and total protein) were done by using Chemistry auto analyzer made in Germany by Human Star Company serial no.20628.

3.3.Hematological Analysis

Total White Blood Cell counts (WBC) were obtained according to [19].
Differential WBC count the slide was stained with leishman's stain according to [20].

4.Statistical Analysis

Data were reported as means ±SEM.Statistical significance of the difference between mean was assessed by students t-test according to [21].

5.Results and Discussion

Injected healthy female rats intramuscularly with high single dose of methyl prednisolone acetate (30mg) caused a significant decrease at ($p<0.05$) in total serum protein , lymphocytes,esinophile and basophile counts (table 1) as well as a significant ($p<0.05$) increase in estrogen, progesterone ,tri-iodthyronine(T3) , thyroxine(T4) hormones and also total serum cholesterol , triglyceride ,total WBC count, neutrophil count and monocyte count comparing with control.

Table (1) shows treatment group caused increased significantly in estrogen hormone level, which agree with the results of [22,23] who investigated that the increased estrogen may come from ACTH-stimulated androgens which produced in the inner layer of the adrenal cortex .Also progesterone hormone recorded as significantly increased in rat treated with MPA ,those data contracted with [24].

In relation between the effect of MPA and thyroid hormones (T3and T4), this study found significant increase in these parameters, which is regarded as a same trend of [25,26] ,whose mentioned that the administration of exogenous glucocorticoids can suppress the synthesis and secretion of thyroid stimulating hormone because of its feedback loops on hypothalamus and anterior pituitary glands .

The effect of the injection of methyl predinsolone acetate in this study caused significant increase in cholesterol parameter which is agreed with what is found by [27] in cats .Also Triglyceride recorded highly significant increase in treated rats ,these data are the same of results found by[28] in rabbit and [29] in rat.

The present data showed that treated group significant decrease in the plasmatic total protein level, this result is not in accordance with the findings of [30] in dogs.

The significant increases in hematological parameters in this study was in WBC count which is disagreed with [31] who showed no statistical significant difference in WBC count between treated and untreated dogs. The relative count of neutrophil cells were significantly ($p<0.05$)increased in rat after treatment , those data are the same as the work of [32] ,who indicated that an increase in number of neutrophils probably caused by cortisone level . On the other hand monocyte cells also recorded high significant increase in treated group, which is similar to [33] in cats ,but opposite to[34] in human .

In this study lymphocytic count showed significant decrease compared with above parameters, it is a same as finding by [35] who had shown that cortisol depresses the lymphocyte number in human. Esinophils and basophils showed significant decrease in treated ones which is contracted with [36] in rat.

6.Conclusions

In this study the intramuscular injection of high single dose of MPA in healthy female rats caused a significant changes in the proportion and concentration of some biochemical parameters(total serum protein, cholesterol, triglyceride), levels of sex hormone in blood (estrogen , progesterone), and levels of thyroid hormones(T3,T4) in addition to its effect on blood cells especially leukocytes cells.

Table 1. Effect of methyl prednisolone acetate on some hormones, biochemical and hematological parameters in healthy female rats(means±SE) .

Parameters	**Control ± SE**	**Treated± SE**
Estrogen (pg/ml)	16.78±1.602 b	22.83±0.706 a
Progesterone (ng/ml)	7.33±0.017 b	30.40±0.258 a
T3 (ng/ml	0.84±0.0037 b	0.97±0.0152 a
T4 (µg/dl)	4.78±0.59 b	11.77±0.127 a
Cholesterol (mg/dl)	0.713±0.149 b	46.37±2.0953 a
Triglyceride (mg/dl)	151.50±0.477 b	178.00±1.341 a
Protein (g/dl)	9.72±0.1267 a	1.17±0.0291 b
WBCs cell/ mm3	3474.50±74.988 b	3700.62±72.397 a
Neutropil %	35.00±0.394 b	55.00±1.154 a
Monocyte %	4.90±0.314 b	7.90±0.276 a
Lymphocyte %	56.60±0.498 a	34.20±2.149 b
Esinophil %	3.10±0.314 a	2.10±0.276 b
Basophil %	2.00±0.298 a	0.00 b

Means within a rows differed at ($p \leq 0.05$).

7.References

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