Hyperprolactinemia induced histological alterations in mice adrenal glands

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Abstract: The present study is planned to investigate the effect of hyperprolactinemia (HPRL) on the histological changes of the adrenal glands of adult male mice (*Mus musculus*) for different durations by using metoclopramide (MCP). Mice were divided into five groups. Group I: control mice were injected with saline solution i.p. for 10 weeks, groups II, III, IV and V; mice were treated with MCP i.p. at a dose of 2.2 mg/kg/ b.w daily for different durations 2, 4, 7 and 10 weeks, respectively. The results recorded a significant increase in the levels of prolactin hormone of groups IV and V. The hisological structure of the adrenal gland of control mice group stained with H &E demonstrated normal appearance of the three zonal cells of adrenal cortex (ZG, ZF and ZR), adrenal medulla and capsule. HPRL mice groups of 2 & 4 weeks showed approximately no changes in adrenocortical and medullary cells; only congestion of blood sinusoids was seen in the medulla. HPRL mice groups of 7 & 10 weeks showed extension of ZF layer and hyperactivity of their cells that appeared with vacuolation in cytoplasm. Inflammatory cells were also noticed in ZR layer and medulla. By using azan stain, normal delicate distribution of collagen fibers in adrenal of control mice was seen in trabeculae periphery to the three adrenocortical cells, around chromaffin clusters of the medullary cells and blood sinusoids. HPRL mice groups of 2, 4, 7 and 10 weeks exhibited increment of the collagen fibers and was time -dependent comparable to control group.

Key words: Hyperprolactinemia, Metoclopramide, Adrenal gland, Histology, Mice.

Introduction

Prolactin (PRL) is a peptide hormone; it is one of several hormones that are produced by the pituitary gland, also known as luteotropic hormone or luteotropin. PRL is encoded by the PRL gene. Although often associated with human milk production, PRL plays a wide range of other roles in both humans and other vertebrates [1].

Pituitary PRL secretion is regulated by endocrine neurons in the hypothalamus, the most important ones being the neurosecretory tubero-infundibulum (TIDA) neurons of the arcuate nucleus, which secrete dopamine (aka PRL inhibitory hormone) to act on the D_2 receptors of lactotrophs, causing inhibition of PRL secretion [2].

The expression of the PRL gene, as well as its receptor, has been registered in many other sites besides the pituitary gland, such as the brain, myometrium, lachrymal gland, thymus, spleen, mammary epithelial cells, fibroblasts, circulating lymphocytes and lymphoid cells of the bone marrow, among others. PRL can also be found in diverse fluid compartments other than blood, such as liquor, breast milk, sweat and amniotic fluid [3].

When PRL levels are elevated in the blood, the condition is referred to hyperprolactinemia (HPRL) [4]. HPRL is the most common endocrine disorder of the hypothalamic-pituitary axis. HPRL can be a part of normal body changes during pregnancy and breast feeding. HPRL can also be caused by diseases affecting the hypothalamus and pituitary gland or by disruption of the normal regulation of PRL levels by drugs, medicinal herbs and heavy metals. It may also be the result of disease of other organs such as the liver, kidneys, ovaries and thyroid [5].

Adrenal hormones, such as cortisol and aldosterone play key roles in the functioning of the human body, such as regulating blood pressure; metabolism, the way that body uses digested food for energy; and the body's response to stress. In addition, the body uses the adrenal hormone dehydroepiandrosterone (DHEA) to make androgens and estrogens, the male and female sex hormones. The amount of cortisol produced by the adrenal glands is precisely balanced. Like many

other hormones, cortisol is regulated by the hypothalamus, which is a part of the brain, and the pituitary gland [6].

PRL can stimulate corticosterone secretion *in vitro* by diminishing adrenal-reductase activity [7 & 8]. A synergistic effect was also found for PRL and ACTH [9 & 10]. In HPRL animals, PRL-induced adrenocortical celsl hypertrophy by increasing ACTH that increased the stimulation of the adrenal cortex with enlargement of zona fasciculata (ZF) and zona reticularis (ZR), this is probably a combination of hypertrophy [11 &12].

Metoclopramide (MCP) is a dopamine antagonist. It is a medication used to treat certain conditions of the stomach and intestines. MCP is also used in diabetic patients who have poor emptying of their stomachs (gastro-paresis). Treating gastro-paresis can decrease symptoms of nausea, vomiting, and stomach/abdominal fullness. MCP works by blocking a natural substance (dopamine). It speeds up stomach emptying and movement of the upper intestines. It is also used to treat migraine headaches [13].

PRL can stimulate pituitary gland to secrete PRL causing HPRL [4].Common side effects of MCP include: feeling tired, 3Tdiarrhea3T, and feeling restless. More serious side effects include HPRL and depression. In 2014, MCP was one of the top 100 most prescribed medications in the United States [14]. Therefore, the present study aims to evaluate the effect of hypersecretion of PRL (HPRL) experimentally-induced in adult male albino mice (*Mus musculus*) by MCP on the histological alterations of adrenal gland.

Materials and Methods

I- Animal selection and care:

Fifty adult male albino mice *(Mus musculus)* (aged 6-8 weeks) weighing 25±2g was obtained from Vacsera, Cairo. Animals were housed in plastic cage (10 per cage) for one week acclimatization under the same condition of temperature and natural dark- light cycle. Food and tap water were freely available to the animals throughout the experiment. All protocols and procedures adopted for the present investigation were in accordance with the approval of the Institutional Animal Ethics Committee of National Research Center and in accordance with recommendation of the proper care and use of laboratory animals, regulated by Faculty of Science, Tanta University.

II- Induction of hyperprolactinemia:

HPRL was induced in mice by intraperitoneal (i.p.) injection of MCP obtained from "Sigma Chemicals Co., St. Louis, Mo., USA" [10&15].

III- Experimental design:

The mice were divided into 5 equal groups (10 mice / each group). Group I, normal control mice were injected with saline solution i.p. daily for 10 weeks. Groups II, III, IV and V, the mice were treated with MCP i.p. in a dose of 2.2 mg/kg/ b.w daily for different durations as 2, 4, 7 and 10 weeks, respectively.

IV- Sample collection and serum separation:

At the end of each period of the experiment, the animals were anaesthetized using diethyl ether, and then sacrificed. Blood samples were collected from all studied groups and allowed to clot at room temperature for 30 minutes before centrifugation at 1000 revolutions per minute for 20 minutes and stored at -20°C till measure serum prolactin level [16].

V- Calculation of the results:

The mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density were calculated. The standard curve was plotted on log-log graph paper, with standard concentration on the X-axis and absorbance on the Y-axis. The best-fit straight line through the standard points was drawn. The adrenal glands were removed and processed for light microscopic studies.

VI- Histological study:

Pieces of mice adrenal glands were fixed in 10% neutral buffered formalin for 24 hrs. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene, embedded in paraffin wax and sectioned at 5µ thicknesses. Paraffin sections were used for the histological study (H & E and azan stains) [17].

Results

I) Effect of MCP on PRL levels:-

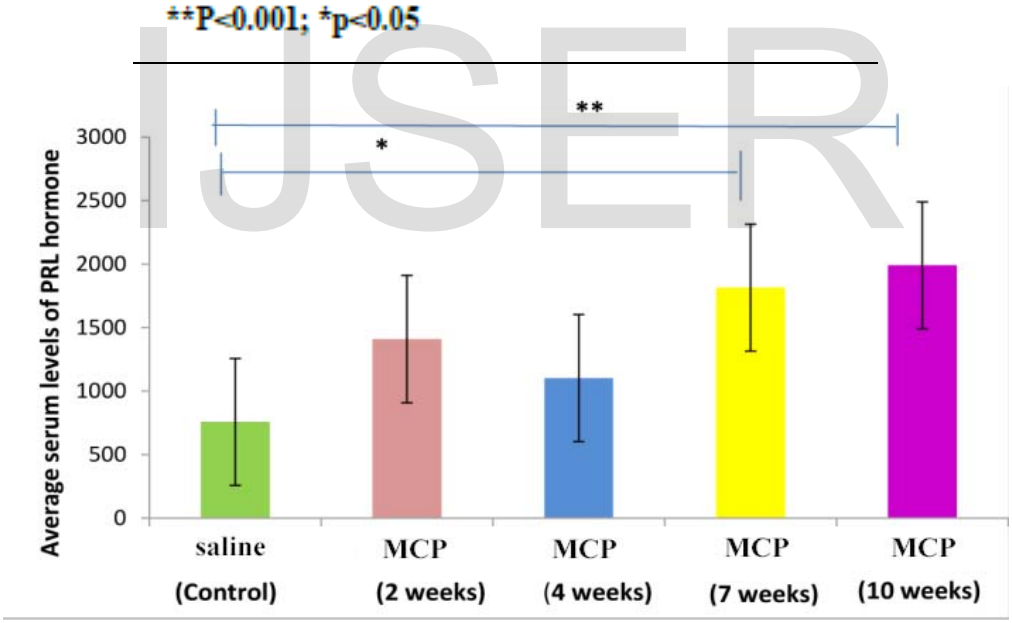
Table 1 illustrated serum PRL levels in control group and HPRL groups received 2.2 mg/kg/ b.w of MCP intraperitoneally for 2, 4, 7 and 10 weeks. These values were 757.86 ± 426.73, 1409.85 ± 1332.23, 1103.71 ± 797.83, 1813.84 ± 719.62 and 1990.67 ± 508.91 [18].

Analysis of variance (ANOVA) test showed non-significant increase in serum

PRL levels in of groups II and III as compared to group >0.05); a significant increase in serum PRL levels of group IV as compared to group I (*p<0.05) and highly significant increase in serum PRL levels of group V as compared to group I (**P<0.001).

Table (1):- levels of mice serum PRL hormone (pg/L) in control and HPRL groups received 2.2 mg/kg/ b.w of MCP intraperitoneally for 2, 4, 7 and 10 weeks.

Groups	Mean ± SE	P
Group I (Control)	757.86 ± 426.73	
Group II (2 weeks)	1409.85 ± 1332.23	
Group III (4 weeks)	1103.71 ± 797.83	
Group IV (7 weeks)	1813.84 ± 719.62	*
Group V (10 weeks)	1990.67 ± 508.91	**



Graph 1:- PRL serum concentrations (pg/L) in control mice and HPRL groups received 2.2 mg/kg/ b.w of MCP for 2, 4, 7 and 10 weeks.

II) Histological observations:-

a- Haematoxylin&Eosin (H&E):-

Control group (Group I):

The mice adrenal glands stained with H & E consist of two distinct parts (cortex and medulla). The cortex is surrounded by connective tissue capsule.

The cortex consists of three zones; the outer zone beneath the capsule is called zona glomerulosa (ZG), the middle zone is called zona fasciculata (ZF), and the inner zone is called zona reticularis (ZR). ZG is a narrow zone; its epithelial cells are arranged in rounded groups. They are tending to be columnar in shape with basal spherical nuclei. ZF is the broadest; its epithelial cells are cuboidal or polyhedral in shape with rounded nuclei. They are arranged into straight cords, the cytoplasm is full of lipid droplets and vacuolated in H & E sections. The cells of ZR are polyhedral or cuboidal in shape, and arranged in irregular cords, the cells are smaller than the cells of ZF. The adrenal medulla contains medullary cells that are arranged in rounded clusters. The chromaffin cells of medulla are basophils and columnar in shape. The interstitium presents large veins and an extensive capillary network (Fig. 1).

HPRL mice groups treated with MCP for 2 & 4 weeks (Groups II & III):-

The mice groups treated with MCP for 2 & 4 weeks demonstrated normal appearance of the three zonal cells of the adrenal cortex while the adrenal medulla showed congested and dilated blood sinusoids. Thick capsule could also be seen in these two periods (Figs. 2 - 4).

HPRL mice groups treated with MCP for 7 & 10 weeks (Groups IV & V):-

These two group demonstrated vacuolation of the cytoplasm of adrenal cortical cells, hyperactivity and extension with elongation of ZF layer with the increase of vacuolation of its cells. Increment of inflammatory cells in ZR layer and medulla accompanied with congestion of the blood sinusoids was also seen (Figs. 5 - 8).

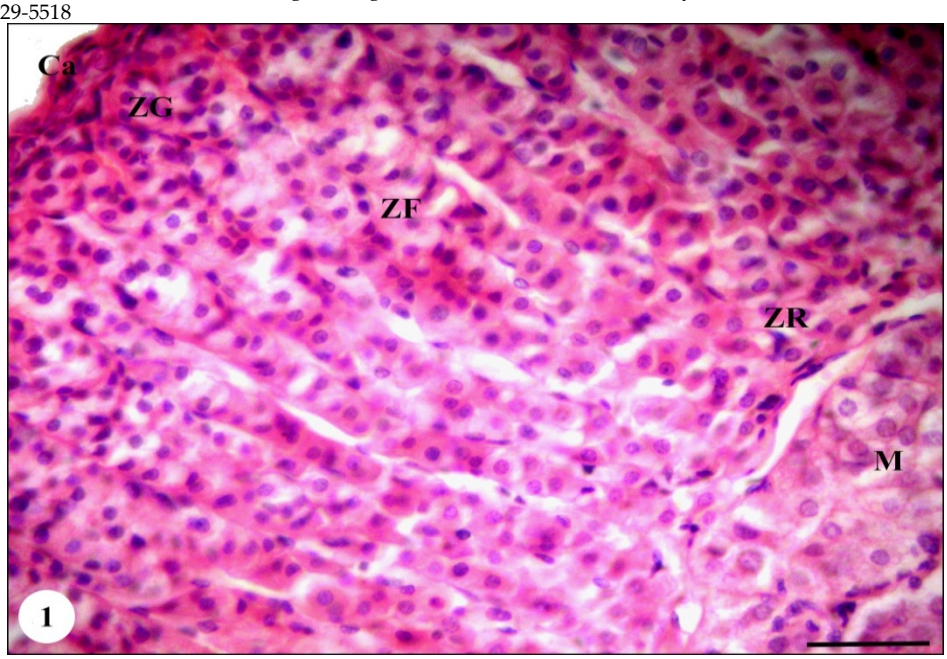
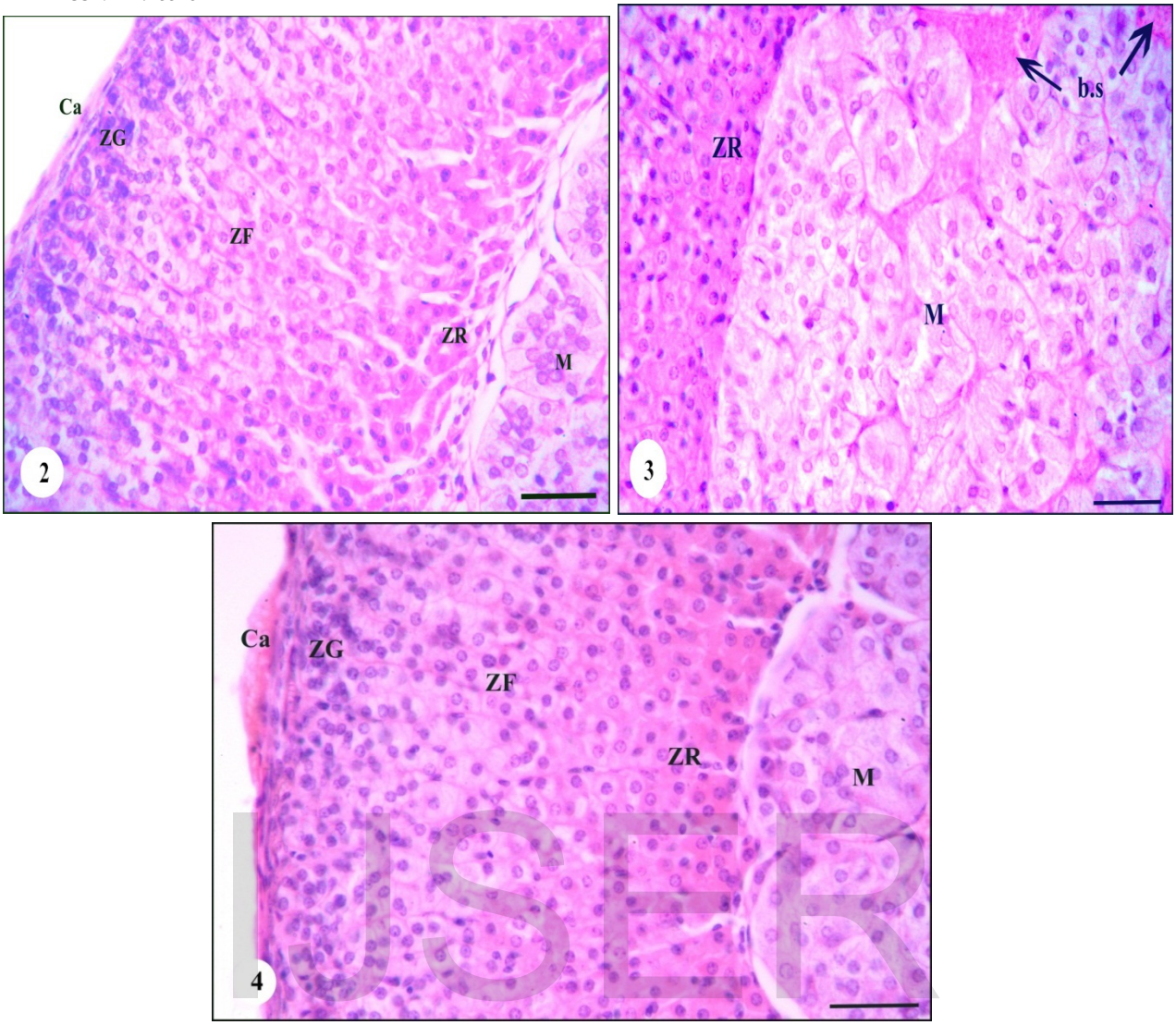
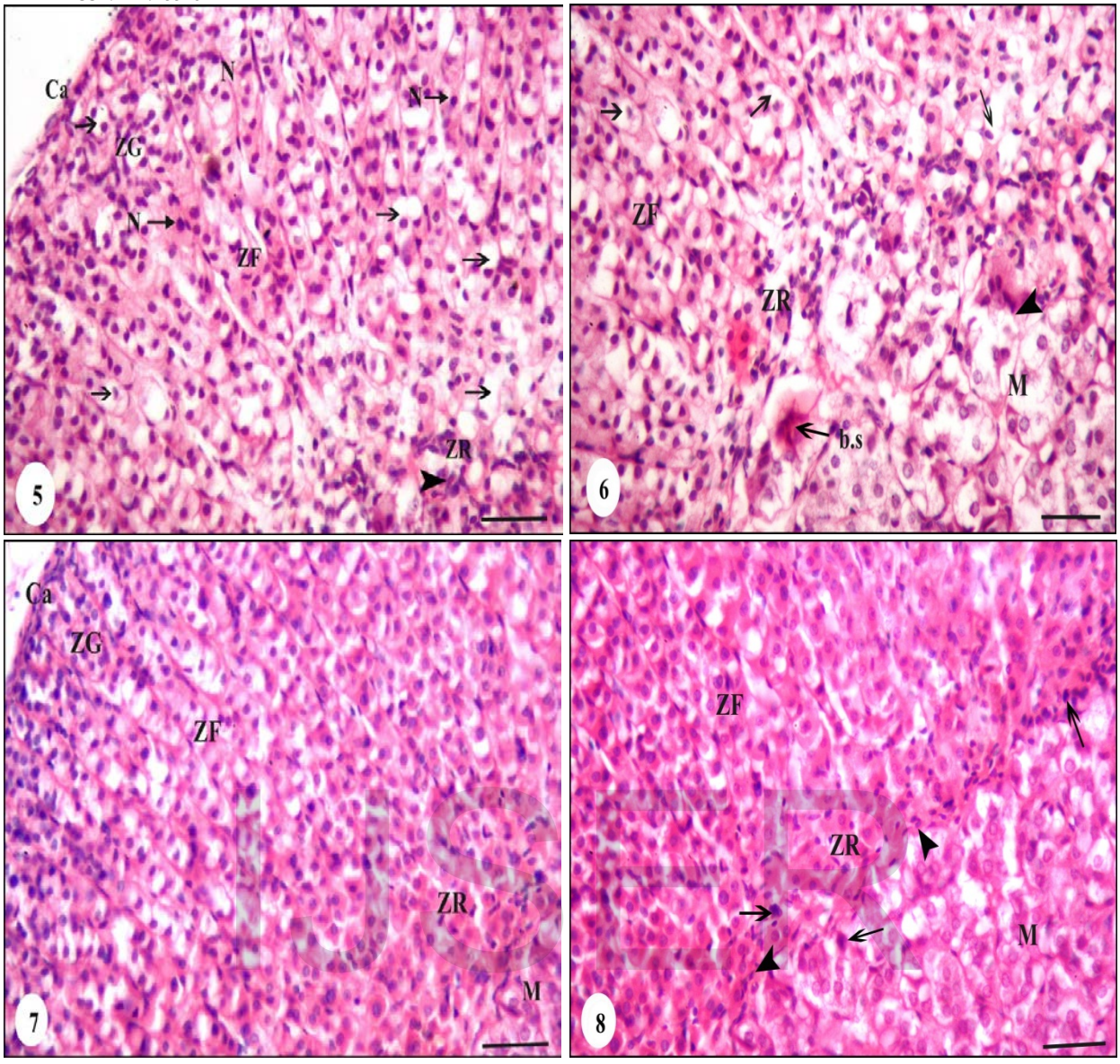


Fig. (1): Section of the adrenal gland of a control mouse showing normal appearance of the three cortical zones of cortex; zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR); covered with thin capsule (Ca). A part of normal medulla (M) is also seen. H&E, Bar = 6.25 µm.



Figs. (2 - 4): Sections of the adrenal glands of mice treated with MCP for 2 & 4 weeks illustrating normal appearance of the three zones of cortex; zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis (ZR) covered with the capsule (Ca). A part of medulla (M) with congestion of dilated blood sinusoid (b.s) is noticed. H&E, Bar = 6.25 µm



Figs.(5-8): Sections of the adrenal glands of mice treated with MCP for 7 & 10 weeks showing vacuolation of the cytoplasm (arrows) in the cortical cells, extension of ZF layer and increment of inflammatory cells in ZR layer and medulla (arrow heads). Congested blood sinusoid (b.s) is noticed. H&E, Bar = 6.25 µm.

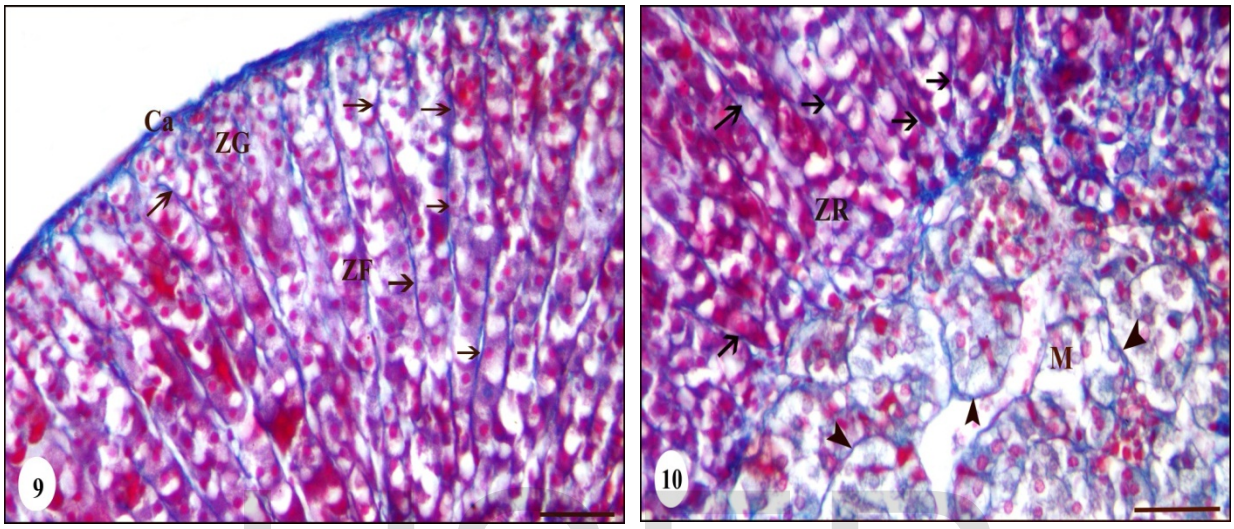
b-Azan stain:-

The collagen fibers are stained with blue colour by using azan stain. In control mice group, the normal delicate collagen fibers are distributed periphery to parenchymal cells of ZG, ZF and ZR as well as in the capsule. The collagen fibers of adrenal medulla are demonstrated around the chromaffin clusters of medullary cells and blood sinusoids (Figs. 9 & 10).

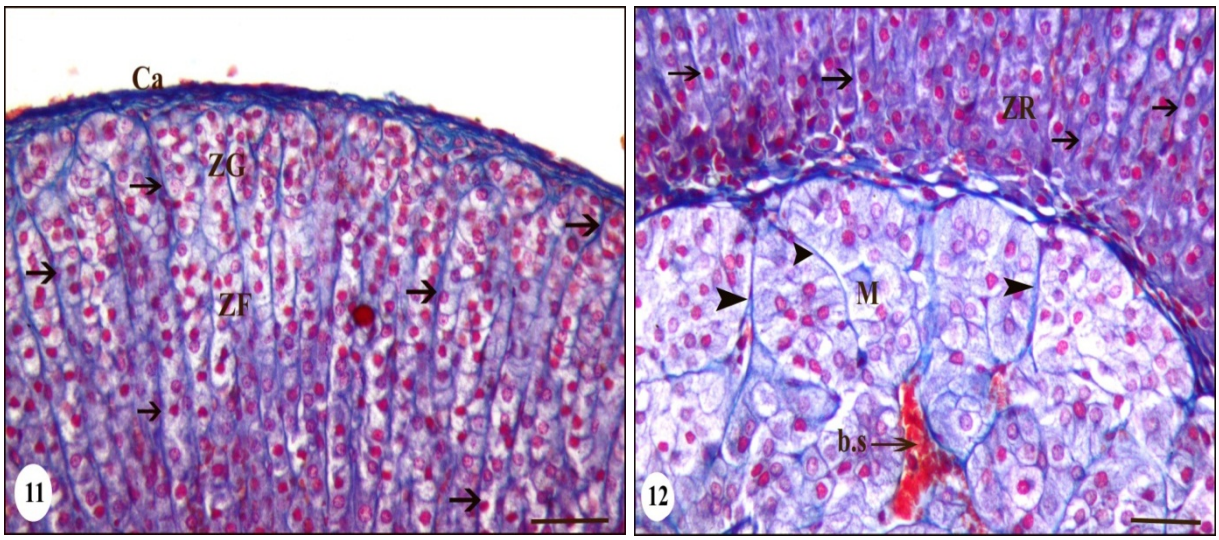
The mice groups treated with MCP for 2 & 4 weeks, exhibited increment of collagen fibers in the trabeculae peripheral to

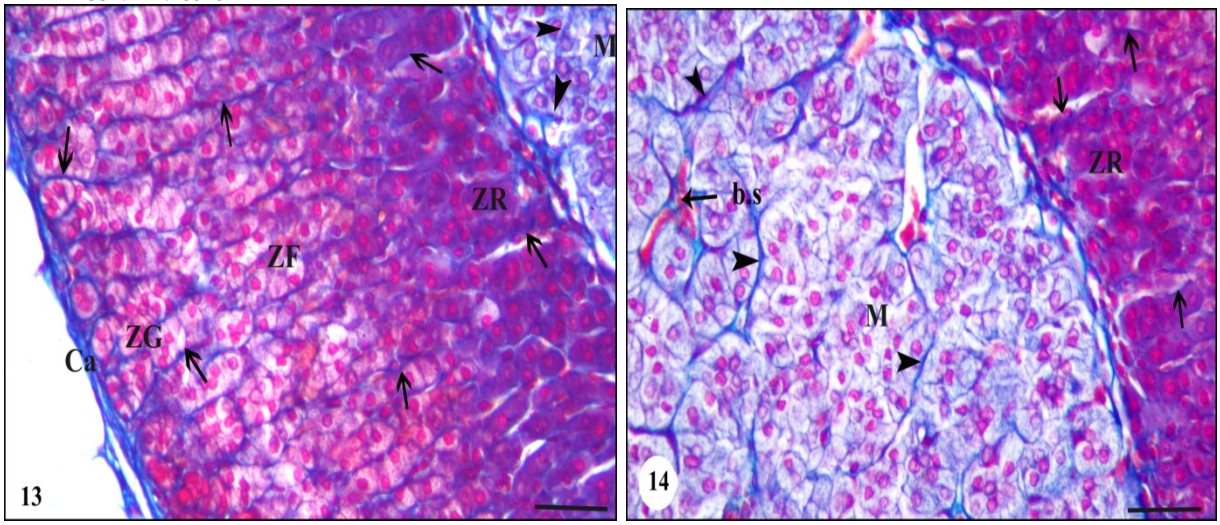
cortical cells of the three zones (ZG, ZF and ZR), around the clusters of medullary cells and around blood sinusoids. Dense collagen fibers are also noticed in the capsule (Figs. 11 -14).

The treatment of mice with MCP for 7 & 10 weeks exhibited an obvious increase in the collagen fibers in the trabeculae peripheral to cortical cells of the three zones, around the clusters of medullary cells and in the capsule (Figs. 15 - 18).

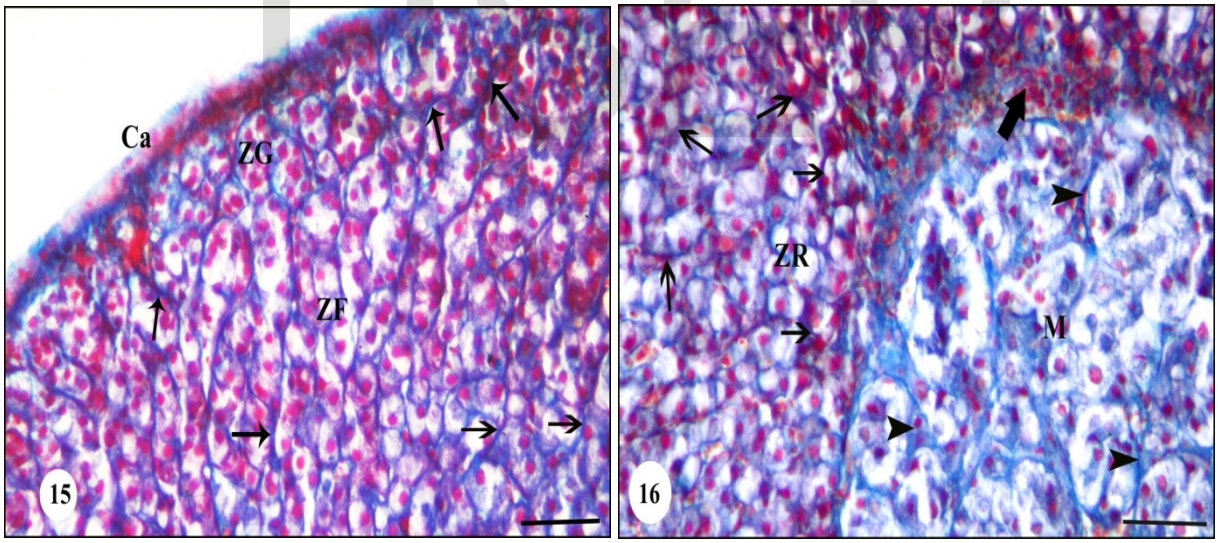


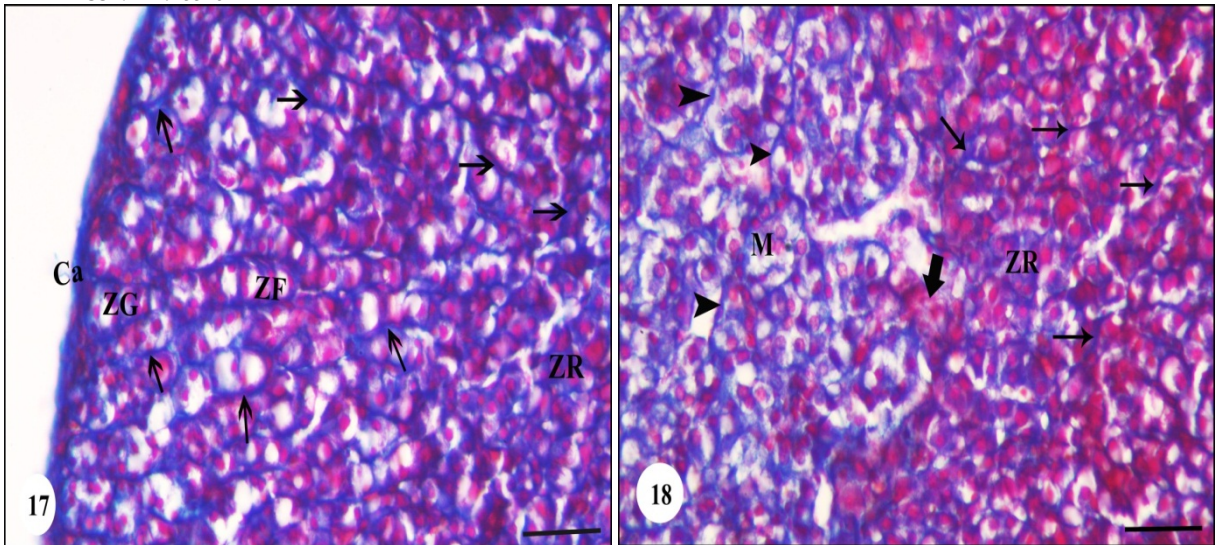
Figs. (9& 10): Sections of the adrenal glands of control mice showing normal delicate distribution of collagen fibers periphery to the parenchymal cells of zona glomerulosa (ZG), zona fasciculata (ZF) and the zona reticularis (ZR) (arrows), around chromaffin clusters of the medullary cells (M) (arrow heads) and in the capsule (Ca).Azan, Bar = 6.25 µm.





Figs. (11 - 14): Sections of the adrenal glands of mice treated with MCP for 2 & 4 weeks showing little increase of collagen fibers in the trabeculae peripheral to the cells of zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) (arrows); peripheral to chromaffin clusters of the medullary cells (M) "arrow heads" and around the congested blood sinusoids (b.s). Increment of collagen fibers is also seen in the capsule (Ca).Azan, Bar = 6.25 µm.





Figs. (15 - 18): Sections of the adrenal glands of mice treated with MCP for 7 & 10 weeks showing a marked increase of collagen fibers in the trabeculae peripheral to the cells of zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) (arrows), around the clusters of medullary cells (M) (arrow heads) and in the capsule (Ca). See congested blood sinusoids (thick arrow).Azan, Bar = 6.25 µm.

Discussion

The present study illustrated that; there was a significant elevation of PRL levels in mice treated with MCP for long duration 7 and 10 weeks in comparison to control mice group. These results are agreed with many authors [18&19] who recorded that metoclopramide produced a significant increase in prolactin serum concentration.

In the present study, HPRL showed approximately no changes in adrenocortical and medullary cells of mice treated with MCP for 2 and 4 weeks, only congestion of blood sinusoids can be seen in the medulla. In HPRL mice treated with MPC for long durations 7 & 10 weeks showed the activation of ZF layer i.e. it seemed with extension and hyperactivity of their cells that appeared with more vacuolations in cytoplasm, probably due to hypersecretion of the cortisone hormone. Inflammatory cells are also noticed in ZR layer and medulla.

The present results agreed with **Silva *et al.*** [12] who reported that in HPRL animals, PRL-induced the adrenocortical cells hypertropied, by increasing ACTH from pituitary gland that stimulated the adrenal cortex with enlargement of ZF and ZR. **Pudney *et al.*** [20] reported that ZF cells are larger and

contain more lipid than ZG cells after stimulation with ACTH. Stressful stimuli are also able to activate the release of hormones that are independent on hypothalamic pituitary adrenal axis (HPA), such as prolactin, which has been shown to induce ZR hypertrophy [21].

Several reports have suggested that, the PRL plays a significant role in the regulation of adrenal function [12, 22 & 23]. HPRL stimulates the activity of 5α-reductase leading to a decrease in testosterone production in adult male rats and stimulates adrenal growth and corticosterone synthesis [24 & 25]. It is known that glucocorticoids have a synergistic action with PRL. PRL is a stress-related peptide, and HPRL is frequently associated with hypogonadism and amenorrhea in humans [22]. PRL acts directly on the adrenal. PRL stimulates steroidogenesis in the adrenal gland of rats and this effect seems to be synergistic with that of ACTH [26]. Several studies have reported that PRL has a stimulatory effect on human adrenal aldosterone-producing adenomas and on adrenocortical carcinoma [27 &28].

It has been speculated that HPRL has effective roles in stimulating androgenic alopeci. HPRL stimulates androgen production from adrenal, and thyroxin effects on free and total testosterone with effect on thyroid binding globulin (TBG) [29 & 30]. MCP induced HPRL increases PRL expression in the adrenal glands of mice [23]. HPRL following administration of MCP in male rats significantly increased the quantity of androgen receptor and caused morphological changes in the epithelial cells of lateral prostate lobe in spite of decreasing testosterone serum level [15].

There is a relationship between HPRL, stress, anxiety and depression. HPRL may prevent the formation of the homodimers necessary for the physiological function of PRL. HPRL reduces the ability of the tuberoinfundibular neurons to synthesize dopamine [31].

In the current results, the normal delicate distribution of collagen fibers in adrenal of control mice is seen in trabeculae periphery to the three

adrenocortical cells, around chromaffin clusters of the medullary cells and blood sinusoids. The increment of collagen fibers was time-dependent of the treatment of mice with MCP 2, 4, 7 &10 weeks and with the increase of PRL.

Several lines of evidence suggested that stress, which is characterized by increased levels of cortisol, inhibits NK cell activity. In fact, these cells are the most susceptible to the effects of cortisol, and their activity is considered to be a reliable indicator of the cell immunity suppression caused by stress [32]. As well as, in HPRL patients; cortisol level was high. Moreover, since higher levels of cortisol and prolactin are often associated with stress. Stress activates neurons that secrete corticotrophin-releasing hormone (CRH), which results in higher plasma cortisol levels. Prolactin is also released in response to stressor stimuli [33]. **Gabry *et al.*** [34] illustrated that the collagen fibers were increased in the stomach of rats after exposure to restraint stress and they recorded the increment of cortisone level. Moreover, **El-Desouki *et al.*** [35] elucidated that the stressed- rats till 30 days recorded high level of cortisone and illustrated a marked increase in collagen fibers in the the lamina propria of mucosa and muscularis mucosa of colon.

Moreover, **Viswanathan *et al.*** [36] reported that the inflammation affected on the thickness of the collagen fibres. **William and Shiel** [37] founded that in patients with connective tissue disease, it is common for collagen and elastin to become injured by inflammation. Many connective tissue diseases demonstrated abnormal immune system activity with inflammation in tissues as a result of an immune system that is directed against one's own body tissues (autoimmunity).

In conclusion, MCP caused an increase in prolactin levels (HPRL) which in turn led to histological changes in the adrenal glands in HPRL mice groups for long durations, included extension of ZF layer and hyperactivity of their cells that appeared with vacuolation in the cytoplasm, inflammation in ZR layer and medulla. The deposition of collagen fibers was also seen and their increment was time -dependent comparable to control group. Therefore, MCP must be used under medical supervision.

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