

# Phagocytic activity of macrophages and proliferation of testicular germ cells in hyperprolactinemia induced in male albino rats

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**Abstract:** The present investigation is planned to demonstrate the effect of elevated prolactin hormone secretion in the testicular phagocytic cells & proliferation of the spermatogenic cells of adult male rats for different durations by using metoclopramide (MCP). Rats were divided into four groups. Group I: control rats group that injected with saline solution i.p. for 10 weeks, (groups II, III, and IV) rats were treated with MCP i.p. in a dose of 2.2 mg/kg/ b.w daily for different durations 4, 7 and 10 weeks, respectively. The physiological results recorded a significant increase in the levels of PRL & acid phosphatase of rats injected with MCP i.p. in a dose of 2.2 mg/kg/ b.w daily for different durations 7 & 10 weeks (groups III and IV). Immunohistochemical study demonstrated intense immunoreactivity to CD68 in interstitial tissue and Leydig cells of HPRL rat groups for 4, 7 & 10 weeks in comparable to control group. Intense immune-positive reaction to PCNA in the nuclei of most of the basal spermatogenic cells in the seminiferous tubules of control rats was expressed. In HPRL rats II for 4 weeks, a little reduction in the immunoreactivity to PCNA in the nuclei of the basal spermatogenic cells was expressed. In HPRL groups III & IV for 7, 10 weeks, an obvious decrement in the immunoreactivity to PCNA in nuclei of the basal spermatogenic cells in the seminiferous tubules was expressed. In conclusion, MCP significantly increased PRL and acid phosphatase enzyme in the testes and was time-dependent and subsequently increases of macrophages by using CD68, and decreases the proliferation of the germ cells by using PCNA that led to spermatogenesis arrest. MCP should not be used for long duration, and must be used with caution as a therapy.

**Key words:** Hyperprolactinemia, Metoclopramide, PRL, acid phosphatase, CD68, PCNA, rats.



## Introduction

PRL hormone is one of the anterior pituitary gland hormones that synthesized and secreted from specialized cells called lactotrophs or mamotrophs, its number depending on gender and physiological status of animal [1].

However, PRL has over 300 separate biological activities not represented by its name. Indeed, not only does PRL subserve multiple roles in reproduction other than lactation, but it also plays multiple homeostatic roles in the organism. Furthermore, the synthesis and secretion of PRL is not restricted to the anterior pituitary gland, but other organs and tissues in the

body have this capability. PRL has no known target organ or defined role in male reproduction, yet expression of PRL receptors on choroid plexuses and hypothalamus presupposes a latent role for this hormone in the regulation of male fertility [2].

PRL is secreted under control of hypothalamus by produces releasing and inhibiting hormones, which stop and start PRL secretion such as PRL releasing hormone that stimulate PRL secretion and dopamine that inhibit PRL secretion . Dopamine is a chemical substance secreted into portal blood by hypothalamic neurons, binds to receptors on lactotrophs, and inhibits both the synthesis and secretion of PRL[3]. Some drugs discordance with dopamine secretion or receptor binding and lead to enhance secretion of PRL [4&5].

Normal level of PRL in males stimulates spermatogenesis process by enhancing luteinizing hormone receptors in Leydig cells which leads to testosterone secretion. Testosterone is the most important intra-testicular factor regulating spermatogenesis [2&5].

Hyperprolactinemia (HPRL) is the most frequent abnormality of the anterior pituitary tumors, termed prolactinomas[6]. In men, the most common symptoms of HPRL are loss of libido, sexual dysfunction , erectile dysfunction, infertility, and gynecomastia[7]. HPRL is a well-established cause of male infertility. Elevated PRL may impact reproduction through an action on the gonadotropin releasing hormone (GnRH), neurons of the hypothalamus and/or on the pituitary gland to affect secretion of the gonadotropins, luteinizing hormone (LH), and follicle stimulating hormone (FSH) [8&9].

The regulated release of the hypothalamic GnRH ensures normal functioning of the hypothalamo-hypophysio-gonadal axis, through secretion of gonadotropins and testosterone in systemic circulation, that necessary for spermatogenesis, maturation of spermatozoa and reproductive behavior [10-12].

Testicular macrophages play a vital role in testis development and adult function, as the absence or increment of macrophages in the testis leads to disordered testicular

development. Macrophages are necessary to remove cellular remnant like apoptotic material or cell debris in inflammable tissue. Testicular macrophages constitute the principal population of immune cells in the testis of most species [13&14].

CD68 is a glycoprotein which binds to low density lipoprotein. It is expressed on monocytes/macrophages. Immunohistochemistry can be used to identify the presence of CD68. It is particularly useful as a marker for the various cells of the macrophage lineage, including monocytes, histiocytes, giant cells, Kupffer cells, and osteoclasts. It has been shown that the expression of this antigen in cells increases during phagocytic activity [15].

Acid phosphatase is specific enzymes which react on compounds containing monophosphate or diphosphate group. These enzymes are found in a wide variety of animal tissues. Its activity is enhanced in certain activated macrophages [16].

Metoclopramide (MCP) is a medication used mostly for stomach and esophageal problems. It is

commonly used to treat nausea and vomiting, following surgery and to help with gastro-esophageal reflux disease. It is also used to treat migraine headaches [17]. In the present study, MCP is used as a hyperprolactinemic drug to study its effect on acid phosphatase, macrophages and on the proliferation of rat's testicular germ cells.

### Materials and Methods

**I- Animal selection and care:** Forty adult male albino rats weighing  $160 \pm 10$  g were obtained from the Animal House, Faculty of Science, Cairo University, Egypt and housed in environmentally controlled optimal conditions for one week. Diet and water were allowed *ad libitum*. All care 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.

### **II- Induction of HPRL:**

MCP obtained from Sigma Chemicals Co., St. Louis, Mo., USA according to

**Sluczanowska-Glabowska *et al.* [18]**, and it was used to induce HPRL by intraperitoneal injection (i.p.).

### III- Experimental design:

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

### IV- Sample collection:

Blood samples were collected from all groups and centrifuged at 3000 r.p.m for 10 min, and the sera samples were collected then kept in clean stopper plastic vial at - 20°C to measure the concentration of prolactin hormone (PRL) and acid phosphatase enzyme.

### V- Immunohistochemical studies of CD68 & PCNA:

Immediately after dissecting of the animals, the testes were removed from all groups, and fixed in 10% neutral buffered formalin for 24 hrs, then specimens were dehydrated in an ascending series of

alcohol, cleared in two changes of xylene and embedded in molten paraffin wax. Sections of 5  $\mu$  thicknesses were cut using rotary microtome and mounted on clean slides. Avidin- biotin method was used to express CD68 and PCNA [19].

The sections of testes from each group were deparaffinized in xylene, washed in phosphate buffered saline (PBS) with pH 7.4, incubated with 3% H<sub>2</sub>O<sub>2</sub> for 20 minute at room temperature, then they incubated for 1 hour at room temperature with primary monoclonal antibody mouse anti-rat CD 68 (Serotec, Niederaula, Germany; dilution: 1:500) to identify the testicular macrophages immunohistochemically, or primary antiserum to PCNA (Clone PC 10, DAKO A/S Denmark; dilution: 1:50) to identify proliferating cell nuclear antigen. Then the slides were washed 3 times in PBS for 5 minutes per each. Sections were incubated with the appropriate secondary antibody (anti-rabbit peroxidase) for 30 minutes at room temperature. Diaminobenzidine (DAB) was the final chromogen, and the hematoxylin was used for nuclear counterstaining. All samples were processed under the same conditions, and

the staining sections were examined by light microscopy.

**Statistical analysis:-**

The obtained data were expressed as (mean ± SE) to determine significance among all treatments, data were statistically analyzed using computerized T-test. Different changes between mean values were at least considered significant when  $p \leq 0.001$ .

**Results**

**I) Effect of MCP on PRL levels:**

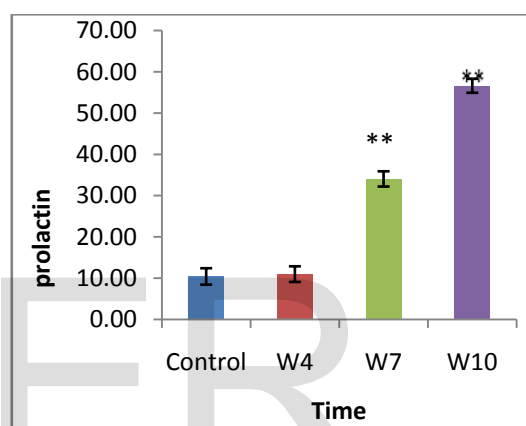
Table 1 & graph 1 illustrated mean values of PRL level in control and HPRL groups received 2.2 mg/kg/b.w of MCP i.p. for 4, 7 and 10 weeks. These values were  $10.42 \pm 0.81$ ,  $10.98 \pm 0.85$ ,  $34.05 \pm 0.75$  and  $56.62 \pm 0.75$ , respectively.

Analysis of the variance showed non-significant increase in PRL level of group II compared to group I ( $P > 0.05$ ), a significant increase in PRL level of group III and group IV compared to group I ( $p^{***} < 0.001$ ).

**Table 1:-** PRL levels of normal control & treated rat groups with MCP (2.2 mg/kg/b.w) for different durations.

Groups	Mean + SE	P
Group I ( Control)	$10.42 \pm 0.81$	
Group II (4 Weeks)	$10.98 \pm 0.85$	
Group III(7 Weeks)	$34.05 \pm 0.75$	**
Group IV (10Weeks)	$56.62 \pm 0.75$	**

durations.  
**\*\*P<0.001**



**Graph 1:-** Mean PRL levels of control and HPRL rat groups received 2.2 mg/kg/ b.w of MCP for 4, 7 and 10 weeks.

**II-Effect of HPRL on acid phosphatase**

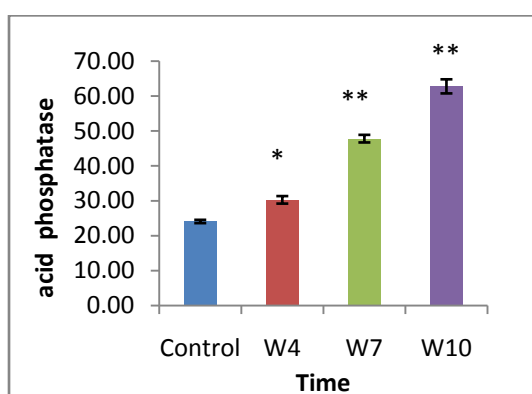
Table 2 & graph 2 illustrated mean values of acid Phosphatase concentration in control and HPRL rat groups received 2.2 mg/kg/b.w of MCP intraperitoneally for 4, 7 and 10 weeks. These values were  $24.07 \pm 0.47$ ,  $30.27 \pm 1.08$ ,  $47.80 \pm 1.09$  and  $62.78 \pm 2.02$ , respectively.

Analysis of variance showed a significant increase in acid Phosphatase concentration of group II compared to group I ( $P^* < 0.05$ ), and highly significant increase in acid Phosphatase concentration of group III and group IV compared to group I ( $p^{**} < 0.001$ ).

**Table 2:-** Acid phosphatase levels of normal control & treated rat groups with MCP (2.2 mg/kg/b.w).

Groups	Mean + SE	P
Group I (control)	24.07+ 0.47	
Group II (4 Weeks)	30.27+ 1.08	*
Group III (7 Weeks)	47.80+ 1.09	**
Group IV (10 Weeks)	62.78+ 2.02	**

\*\* $P < 0.001$ ; \* $p < 0.05$



**Graph 2:-** Mean acid phosphatase levels of control and HPRL rat groups of rat received 2.2 mg/kg/ b.w of MCP for 4, 7 and 10 weeks.

**III- Immunohistochemical results:-**

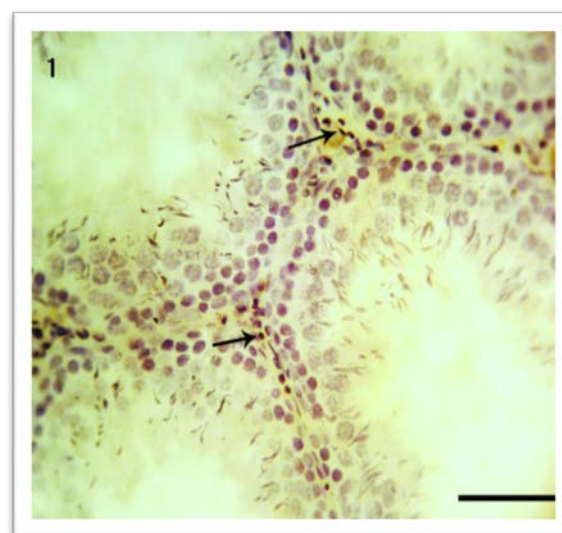
**a- CD68 immunostain observations:-**

**Control group (Group I)**

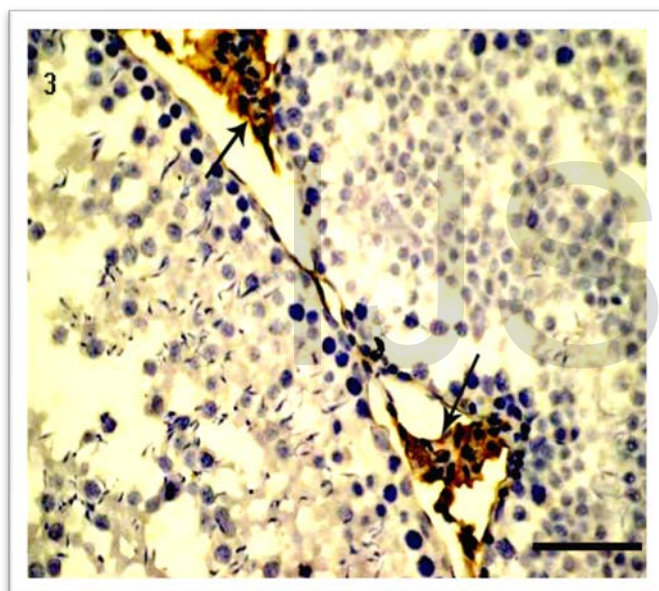
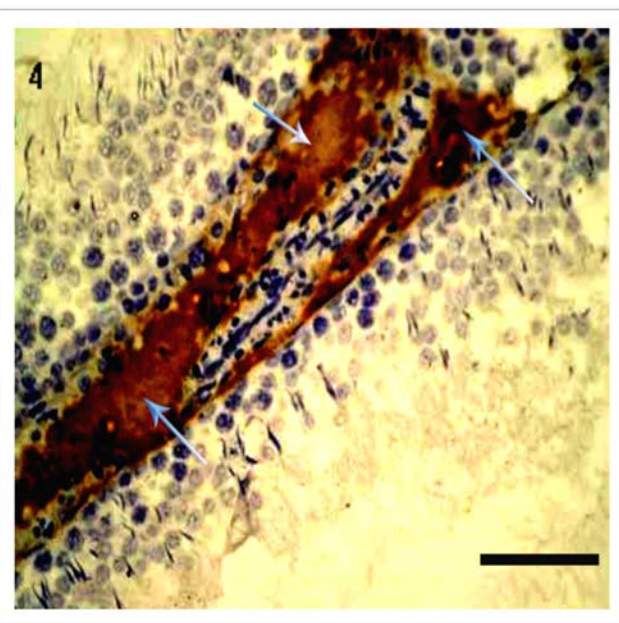
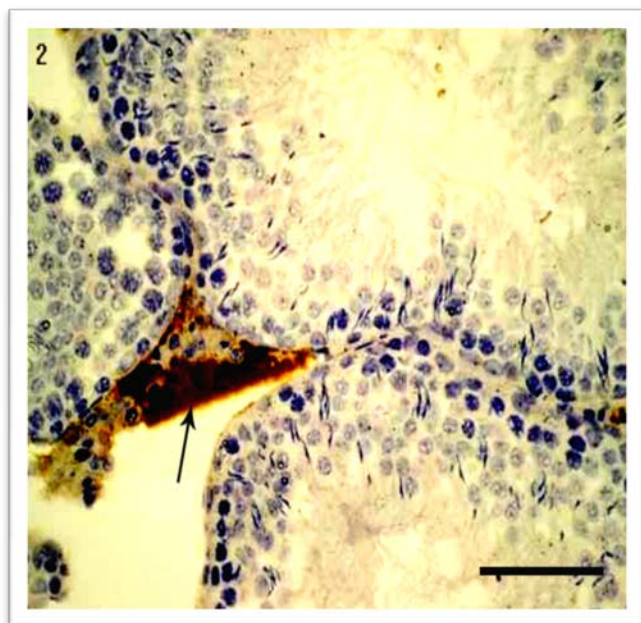
Sections of the testes of control rats group (group I) expressed normal weak immunoreactivity to CD68 in the interstitial tissue and Lydig cells (Fig. 1).

**HPRL rat groups for 4, 7 & 10 weeks**

**(Groups II&III):** expressed a marked increment of immunoreactivity to CD68 in the interstitial tissue and Leydig cells and it was time-dependent (Figs. 2-4).







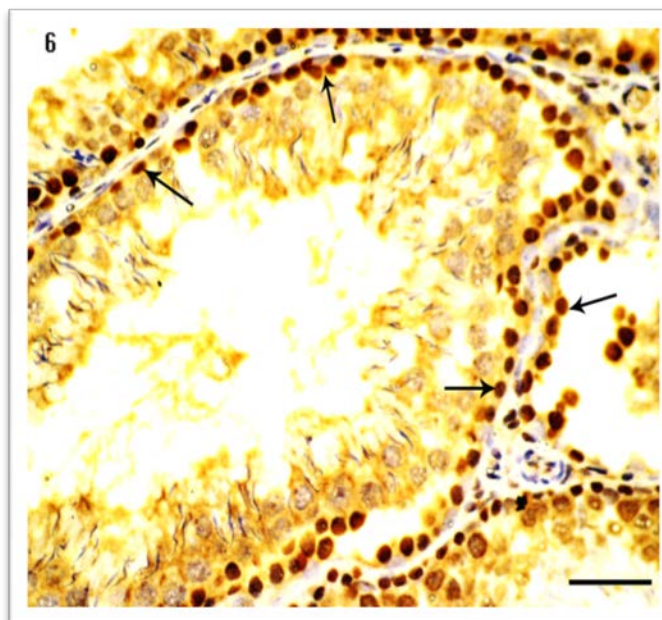
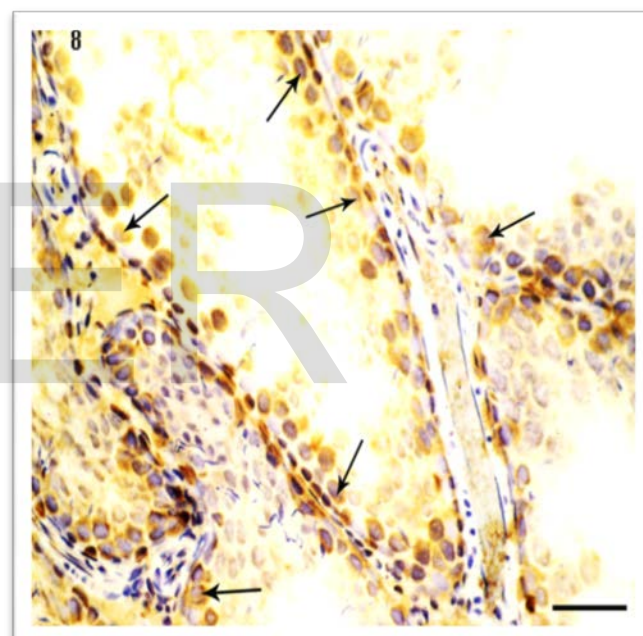
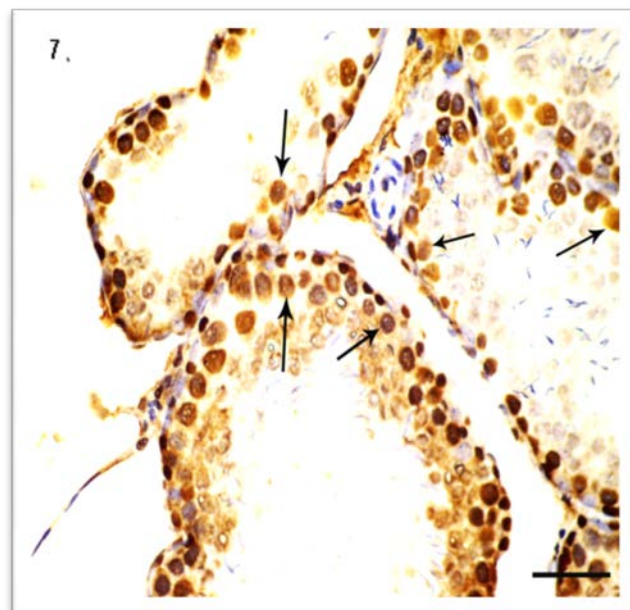
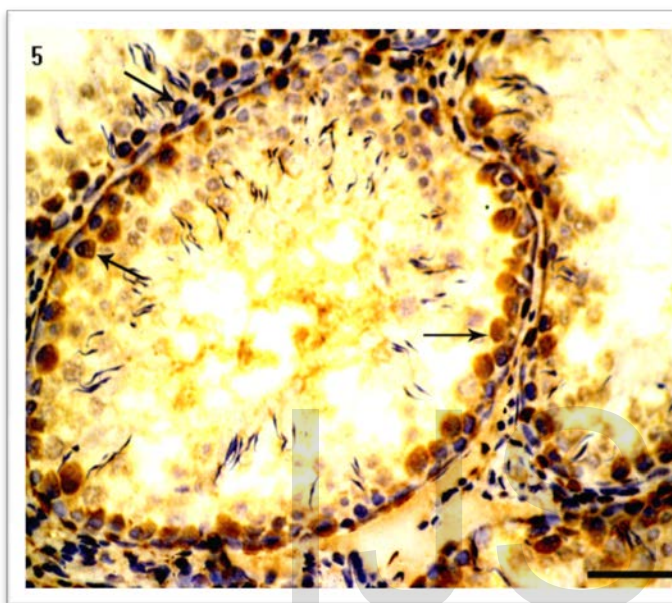
**Figs. 1-4:**Sections of rat testes expressing CD68 immunostain in the interstitial tissue and Leydig cells (arrows): **Fig. (1):** control rat testes with normal weak immunoreactivity **Figs. (2&3):** rat treated with MCP for 4&7 weeks with intense immunoreaction (arrows). **Fig. (4):** rat treated with MCP for 10 weeks with more intense of immunostain. All, CD68 immunostain, Bar = 6.25  $\mu$ m

### **b- PCNA immunostain**

#### **Control group (group I)**

Section of the testes of control rats group (group I) expressed normal intense immuno-positive reaction to nuclei of most of basal spermatogenic cells in the seminiferous tubules (**Fig.5**).

**HPRL group for 4, 7&10 weeks (Groups II, III, IV):** expressed a reduction of the immunoreactivity to nuclei of the basal spermatogenic cells in the seminiferous tubules and the decrement was time dependent (Figs. 6- 8).



**Figs. (5-8):**Sections of rat testes expressing PCNA immunostain to nuclei (brown nuclear reaction) of the basal spermatogenic cells in the seminiferous tubules: **Fig. (5):**



control rat testis with intense immuno-positive reaction to nuclei (arrows). **Fig. (6)**: rat treated with MCP for 4 weeks with a little reduction of the immunoreactivity to nuclei (arrows). **Figs. (7&8)**: rats treated with MCP for 7&10 weeks with an obvious reduction of the immunoreactivity to nuclei. All, PCNA immunostain, Bar = 6.25  $\mu$ m.

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## Discussion

The present study illustrated that; there was a significant elevation of PRL levels in rats treated with metoclopramide (MCP) for long duration 7 and 10 weeks in comparison to control rat group. These results are agreed with many authors who recorded that several drugs may determine a significant increase in prolactin serum concentration like MCP [8, 20&21].

The present study was undertaken to investigate the direct relationship between acid phosphatase and male infertility and confirmed by using CD68 as a macrophage marker. The present results demonstrated that acid phosphatase concentration level has a significant increase by increasing the duration of hyperprolactinemia. Acid phosphatase has been used as a specific marker for determining the activity of the

prostate gland. A relationship between semen quality and prostatic secretion has been demonstrated by [22&23]. Jitendra *et al.* [24] said that there is inverse relationship between acid phosphatase concentration level and sperm counts.

There is a growing body of evidence that testicular macrophages play an important role in regulating steroidogenesis of Leydig cells and maintain homeostasis within the testis [25]. Under normal physiological and non-inflammatory conditions, macrophages play an important role in Leydig cell development. If macrophages are absent from the testicular interstitium, Leydig cells do not succeed in developing normally, which suggests that macrophages provide important growth and differentiation factors for Leydig cells. In infertile men with damage of spermatogenesis and/or

chronic orchitis, when macrophages are activated and secrete an array of inflammatory mediators, Leydig cell steroidogenesis is inhibited[26&27].

Macrophages were detected with ED1 (mouse anti-rat CD 68), a monoclonal antibody which recognizes the antigen that is the rat homolog of human CD 68. It has been shown that the expression of this antigen in cells increases during phagocytic activity[28], which leads to the conclusion that MCP, raises phagocytic activity in rat testicular interstitium.

Macrophages are necessary to remove cellular remnant like apoptotic material or destroyed cell components in inflammable tissue. So, an increase in macrophage number suggests that either apoptotic or inflammable processes rise in the rats' testicular interstitium [29]. In the present study, it has been demonstrated that rats treated with MCP for 4 and 7&10 weeks showed intense positive immunoreactivity to CD68, this means that the increment of macrophage numbers in interstitial cells, therefore, phagocytic activity increase. So there was a positive

correlation between the volume density of CD68 positive and vacuolated Leydig cells increased at HPRL induced by MCP.

PCNA is an auxiliary protein to DNA polymerase-d, being involved in nucleotide excision repair mechanisms. In the present work, PCNA was used as a proliferation marker for testicular germ cells by applying immunohistochemical technique. The current results demonstrated the decrement of proliferation of spermatogenic cells in hyperprolactinemic rats and it was time dependent till 10 weeks. Similar results were recorded by **Morales *et al.*[30]**

The regulatory mechanisms of spermatogenesis are considered to result from an interaction between cell proliferations, differentiation and cell death [31]. In the spermatogenic epithelium, a tissue which holds cells that are continuously dividing, and apoptosis is the primary mechanism for regulating sperm numbers and for eliminating aberrant germ cells [32]. Apoptosis may be enhanced after various testicular injuries, including withdrawal of hormonal

support, radiation, toxicity, heat exposure or experimental cryptorchidism [33].

As indicated, homeostasis of the seminiferous epithelium depends on cell death and on the proliferative activity of the epithelium, and any imbalance of the two processes may result in histological changes. The importance of studying these processes simultaneously was described in other models of atrophy of the seminiferous tubules, for example ageing or regression before exposure to short photoperiods in *Mesocricetus auratus* [34].

In conclusion, MCP caused HPRL & subsequently, increased acid phosphatase enzyme as well as the increment of testicular macrophages. Furthermore, MCP is reduced the testicular proliferation process which led to spermatogenic cells disintegration, and led to spermatogenesis arrest. So, MCP that induced hyperprolactinemia can lead to infertility. Therefore, MCP must use under medical supervision.

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