

Effect of Ketotifen on some reproductive hormones, spermatogenesis and immune parameters in male rats

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Abstract: Mast cells in inflammation, fibrosis and hypersensitivity involved. Ketotifen is a member of allergy medication that reduces inflammation. The purpose of this study was to evaluate the effect of Ketotifen on some reproductive hormones, spermatogenesis, and immune parameters in male rats. A total of 75 male mice of *Balb / C* were selected and randomly divided into 15 groups and kept in the five cages. Mice were exposed for 30 days to the influence of Ketotifen. The experimental groups were consisted of a control group (receiving Ketotifen), treatment 1 (10 mL of Ketotifen), treatment 2 (30 mL of Ketotifen), treatment 3 (50 mL of Ketotifen), respectively. After 30 days, the mice were collected and serum levels of testosterone, free testosterone, FSH, LH, ABP, prolactin, leptin, TNF α , IgE, interleukin-1, cholesterol, TG, HDL, LDL, AST, ALT were investigated. Parallel to that outlines the operations conducted and testes were examined histologically. SAS software for statistical analysis and the comparison between the data of Duncan test was used to compare the means. Ketotifen caused significant increase in the levels of testosterone and free testosterone. Serum concentrations of LH and ABP in treatment 2 were significantly increased compared to controls. Prolactin in treatment 2 was significantly decreased. Concentrations of leptin, TNF α and IL were significant. The results of the testes showed that Ketotifen in the treatment 2 increase the cell's stage of spermatogenesis. Ketotifen in treatment 2 had positive effects on reproduction, sperm and immune parameters in male mice.

Key words: *Ketotifen; Reproduction; Spermatogenesis; Mice*

1. Introduction

Several factors affect the incidence of infertility that is generally divided into two categories of masculine and feminine. 30% of these agents are associated with male factor that often leads to impaired spermatogenesis (Jaffe *et al*; 1991). The most common cause of male infertility is their inability to produce healthy sperm. Several factors are involved in causing infertility that one of them is the increasing number of mast cells in the testis of infertile men and fibrosis formation in the seminiferous tubes (Maseki *et al*; 1981; Meineke *et al*; 2000).

Mast cells are large cells that are found in connective tissues in large numbers that involved in the production and release of chemical mediators of chronic inflammation, fibrosis, and increased sensitivity (Metcalfe *et al*; 1997; Cairns *et al*; 1997). Studies on testes indicate the presence of mast cells in the testis tissue of the organ that has two sub-groups according to their locations: the interstitial mast cells and pre-tubular. Compared to interstitial mast cell, pre-tubular mast cell is closer to seminiferous tubular pipes and their numbers are lower than interstitial mast cells (Meineke *et al*; 2000; Jezek *et al*; 1999; Nagai *et al*; 1992). In the testes of infertile men, a significant increase in the

number of mast cells has been seen in both (Maseki *et al*; 1981; Apa *et al*; 2002), but increasing proportion of pre tubular is higher than the intermediate mast cell (Jezek *et al*; 1999; Nagai *et al*; 1992). This increase is resulted in the induction of chemo taxis of fibroblasts and collagen synthesis, and eventually Pre-tubular fibrosis (Meineke *et al*; 2000; Ruoss *et al*; 1991).

The increased number of mast cells implicated in the testes of men in the path physiology of un explained infertility. Theory of male fertility disorders with mast cell blockers is based on observation of mast cells in the testes (Maseki *et al*; 1981). Schill *et al*; (1986) were reported the positive effects of Ketotifen from drug class of mast cell blocker on semen parameters in men with oligospermia after 3 months of treatment. Also the use of Tranilast drug from drug class of mast cell blockers for 3 months resulted in the creation of sperm in the semen of infertile men (Yamamoto *et al*; 1994). But fexofenadine drug that use as a mast cell blocker had no effect on sperm parameters (Cayan *et al*; 2002). In this study, the effect of Ketotifen on reproduction, spermatogenesis and safety of male mice was evaluated and evaluating its effect was the purpose of this study.

2. Materials and methods

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In this experiment, 75 male mice of *Balb / C* purchased from Tehran Pasteur Institute of Science and Research Branch, Islamic Azad University of Tehran and were kept in the animal house. The rats were randomly divided into 15 groups and were maintained in 5 cages. The experimental group received Ketotifen treatments for 30 days. The control group received Ketotifen, group 1 received 10 micro liters, group 2 received 30 micro liters of Ketotifen, treatment 3 were receiving 50 microliters. Duration of treatments at the time of the experiment depends on the stage of spermatogenesis in mice for 30 days. Ketotifen was given to treatments in this period and then blood was taken. At the end the testes were examined histological.

2.1. Blood sampling method

The blood samples were taken from the heart. After bloodletting, blood serum was separated by centrifugation at 25000 rpm for 15min and stored at 20° C. The serum concentrations of testosterone, free testosterone, FSH, LH, ABP, prolactin and other factors, paracrine (leptin, TNF α , IgE, IL-1) were measured by using company of DRG Germany kits, cholesterol, triglycerides, HDL, LDL, enzymes of liver were measured by using Iranian Pars test kits. Hormone and cytokine were analyzed by ELISA (Stat fax 303 Model Making America) and biochemical and metabolic factors were measured by auto analyzer.

2.2. Anatomy and Histology of Operation

After taking blood from groups, from any group, 5 mice selected for histological study, the testes were removed. The testes were kept separately in 10% formalin.

2.2. Statistical Analysis

Data were analyzed by using the software SAS 98. For comparison, between the data the Duncan test method was used and considerably less likely than 0.05 was considered significant ($P < 0.05$).

3. Results and discussion

Today, several therapies are used to treat infertility treatments including ART method such as IVF and an ICSI. Given the constraints and the high cost of success in case of successful treatment cycle, patients become frustrated and give up treatment. Drug treatment of male infertility is a non-invasive therapies including medication, mast cell blockers (Youn; 1997).

3.1. The results of the analysis of hormonal variables

Ketotifen effect on reproductive hormones: Analysis of test results showed that the effect of Ketotifen on testosterone concentration was highly

significant with regard to comparison between the mean of treatment 2 (30 micro liter), had the highest concentration compared to the control group (Table 1). The results showed that the effect of Ketotifen on the free testosterone, was highly significant and according to the comparison between the mean of treatment 2 (30 micro liter) and there was a significant increase compared to the control groups (Table 1). LH analysis showed that effect of Ketotifen on serum LH was significant ($P < 0.05$) and the comparisons between means and treatment 2 (30 micro liter) were significantly different than the control group (Table 1). The analysis results showed that the concentrations of FSH and the effect of Ketotifen on FSH concentrations were not significant ($P > 0.05$) and considering the comparisons between treatments in terms of mean FSH concentrations were not significantly different than the control group (Table 1). Experiments have shown that Ketotifen caused significant increase in serum testosterone and free testosterone. Testosterone is the male sex hormone, which is the site of steroid hormones in the testes. In the present experiment, testosterone and free testosterone increased. In Research (Matsuki *et al*; 2000) Tranilast effect on fertile and infertile men were studied and had no effect on testosterone concentration that was not match with the current study. Also in Research (Hibi *et al*; 2002) Tranilast consumption for 3 months resulted in a significant increase in sperm count and testosterone levels and had no effect on level of testosterone serum that was not match with current study. FSH and LH gonadotropins are made in gonadotropin pituitary adenoma. GnRH adjusts gonadal steroids and gonadotropins. FSH in male effects on germ cells that are in the seminiferous tubules and causes spermatogenesis. Experimental results have demonstrated that the gonadotropins LH concentrations in treatment 2 (30 micro liter) was significantly increased compared to controls, indicating a positive effect of this medicine. FSH levels in treatment 2 (30 micro liter) decreased the obtained results that was not match with the results (Hibi *et al*; 2002).

3.1.1. Ketotifen effect on ABP and prolactin

Analysis of test results showed that the effect of Ketotifen on ABP were significant ($P < 0.05$) and comparisons between means with regard to treatment 2 (30 micro liter) were significantly increased compared to the other treatments (Table 2). The analysis results showed that the effect of prolactin was meaningful and based on the comparison between treatment the mean of treatment 2 (30 micro liter) were significantly reduced compared to the other treatments (Table 2). Sertoli cells and germ cells during tubules control secretion of cyclic protein of each other. Number of proteins in the bloodstream can enter the basement membrane and Sertoli cells, including ABP, IGF, and transferrin. In this study, the concentration of ABP in group 2 (30 micro liters) increased compared to the

control, no articles found in this case. Prolactin and growth hormone secretion from the pituitary are chemically similar to each other. Dopamine is a prolactin inhibitor. In Research (Dolecek *et al*; 1984) Ketotifen effect on the growth hormone in children with diabetes were examined and showed no change in prolactin levels and the present experiment ran.

3.1.2. Ketotifen effect on paracrine factors

Analysis of the present experiments showed that Ketotifen effect on leptin was highly significant and with regard to the comparison between the mean, treatment 2 (30 micro liter) were significantly increased compared to controls (Table 3). Analysis

of TNF α showed that, Ketotifen had a significant effect ($P < 0.05$) and comparisons of means with regard to treatment 2 (30 micro liter) was significantly decreased compared to the control group (Table 3). Analysis of IgE showed no significant effect of Ketotifen on it ($P > 0.05$) and according to numerical comparisons between the means; treatment 1 was significantly increased compared to controls (Table 3). Analysis showed that the IL effect on IL Ketotifen is very meaningful and according to the comparison between the mean of treatment 1 (10 ml) were significantly increased compared with controls (Table 3).

Table 1: Effect of treatments Ketotifen on reproductive hormones

Variable	Control	Treatment1	Treatment2	Treatment3	SE
T	22.7 ^b	22.7 ^b	28.5 ^a	24.5 ^b	2.8
FT	5.7 ^b	5.7 ^b	5.9 ^a	5.7 ^b	0.01
LH	0.75 ^a	0.72 ^a	0.90 ^b	0.78 ^{ab}	0.01
FSH	0.47 ^{ab}	0.38 ^{abc}	0.47 ^{ab}	0.49 ^a	0.01

Common letters in each row indicate no significant difference

Table 2: Effect of treatments Ketotifen on ABP and prolactin

Variable	Control	Treatment1	Treatment2	Treatment3	SE
ABP	1.18 ^b	1.14 ^b	1.28 ^a	1.16 ^b	0.01
Prolactin	15.69 ^a	15.75 ^a	15.10 ^b	15.63 ^a	0.04

Common letters in each row indicate no significant difference

Table 3: Effect of treatments Ketotifen paracrine factors

Variable	Control	Treatment1	Treatment2	Treatment3	SE
Leptin	6.40 ^b	6.26 ^a	6.87 ^a	6.50 ^b	0.03
TNF	4.08 ^a	4.08 ^a	3.99 ^b	4.06 ^a	0.01
IgE	110.6 ^a	114 ^a	112 ^a	114.8 ^a	21
IL	0.20 ^c	0.24 ^a	0.22 ^b	0.20 ^c	0.0001

Common letters in each row indicate no significant difference

Table 4: Effect of treatments Ketotifen on metabolic and biochemical

Variable	Control	Treatment1	Treatment2	Treatment3	SE
cholesterol	2.23 ^a	2.23 ^a	2.25 ^a	2.28 ^a	0.01
TG	2.3 ^a	2.2 ^a	2.2 ^a	2.3 ^a	0.02
HDL	0.19 ^{ab}	0.21 ^a	0.15 ^{ab}	0.17 ^{ab}	0.01
LDL	2.02 ^a	2.02 ^a	2.11 ^a	2.09 ^a	0.01

Common letters in each row indicate no significant difference

Table 5: Effect of treatments Ketotifen on liver enzymes

variable	Control	Treatment1	Treatment2	Treatment3	SE
ALT	83.4 ^a	82.8 ^a	82.0 ^a	82.0 ^a	2.2
AST	42.0 ^a	41.4 ^{ab}	40.2 ^b	41.2 ^{ab}	1.4

Common letters in each row indicate no significant difference

Leptin is produced by adipose tissue in the body in the bloodstream. Leptin is a way of reducing appetite, decreases the production of neuropeptide Y in the hypothalamus. Leptin leads to sexual development in sexes, increases gonadotropin in males, seminal vesicle and testicles weight gain. In this experiment, the amount of the leptin hormone in treatment 2 (30 ml) increased compared to the control group. Inflammatory cytokine TNF α is an inflammatory leukocytes across the endothelium passes. In the present experiment TNF α had a significant reduction in treatment 2 compared to the control group (30 ml) that shows the anti-

inflammatory effect of Ketotifen. Immune globulins are a class of biological molecules that are active in the immune system and the specific antigens and antibodies are secreted. IL, a cytokine made by white blood cells that are often included in other leukocytes. In this study, Ketotifen increased IL which represents increase of the level of safety that no paper was found in this regard.

3.1.3. The effect of Ketotifen on the metabolic and biochemical blood

Analysis showed that the effect of Ketotifen on cholesterol, triglycerides, HDL, LDL was not significant ($P > 0.05$) and according to the comparison between the means in level of cholesterol, treatment 2 (30 ml) and treatment 3 (50 ml) had a numerical increase compared with the controls (Table 4). The mean levels of triglyceride indicated that treatment 1 (10 ml) and treatment 2 (30 micro liters) decreased compared to the control group (Table 4). Ketotifen effect on HDL in group 1 (10 ml) than in the control group had a significant increase but LDL in treatment 2 (30 ml) increased compared to the controls (Table 4). Fats in blood are cholesterol, triglycerides, HDL and LDL. In the present study, cholesterol, HDL and LDL levels were increased in the treatment groups than in the control group. A study that conducted by Caps *et al*; 1991 showed that Ketotifen cause weight gain of asthmatic children that were consistent with the present experiments.

3.1.4. The effect of Ketotifen on liver enzymes

Analysis of tests showed that the effect of Ketotifen on ALT and AST enzymes did not make sense ($P > 0.05$) and according to the comparison between the mean, the levels of liver enzyme compared to the control group numerically found (Table 5). The liver is the largest gland in the body and detoxification is the metabolic functions of the body such as proteins. Liver enzymes are alanine aminotransferase and aspartate aminotransferase enzymes. These enzymes catalyze chemical reactions in the cell. These enzymes are used in the diagnosis of liver diseases. Ketotifen in the treatment groups was not significant in the present experiments, in which the article is not found.

3.2. Histological examination of the testis

After preparing and staining tissue sections by using a light microscope, photo of the epididymal sections were taken and images were evaluated.

Images of testicular tissue showed that cell count of Spermatogonium in treatment 2 (30 ml) than in the control group increased (Fig. 1 and Fig. 2) and the number of sperm cells in group 2 (30 ml) than in the control group increased (Fig. 3 and Fig. 4).

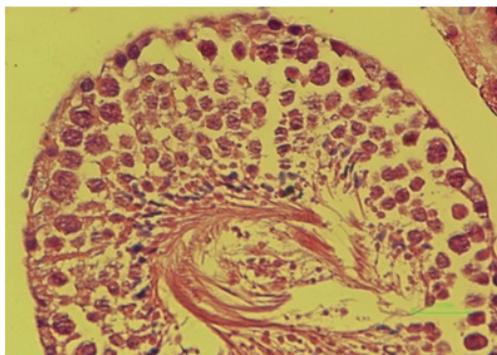


Fig. 1: Number of cells in the control group, stage spermatogonium

Spermatozoa arise in the seminiferous tubules. These pipes are generating a growing collection of cells in the male gamete that eventually make up. Research shows that mast cells in the testis and epididymis are normal and fibrotic disorders involved in inflammation (Cinik *et al*; 2003). The researchers also found that sperm motility in patients who have an increased number of mast cells in the testicles, reduced sperm motility, leading to an increase (Cinik *et al*; 2003). In the testing phase sprmatogonium cell count and sperm in treatment 2 (30 micro liter) than in the control group was shown Ketotifen had a positive effect on epididymal tissue and cells Spermatozoa. In the study (Cayan *et al*; 2002) showed that use of fexofenadine drug from the drug class of mast blockers had no effect on sperm parameters that did not match with the present experiments.

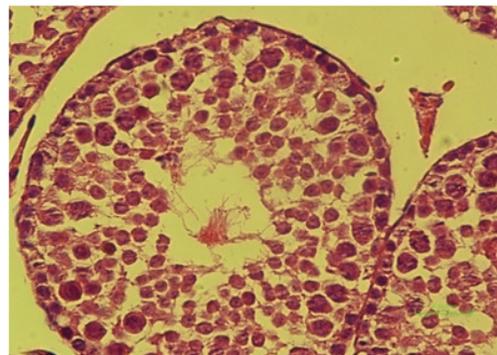


Fig. 2: Number of cells, treatment 2 spermatogonium stage (30 ml)

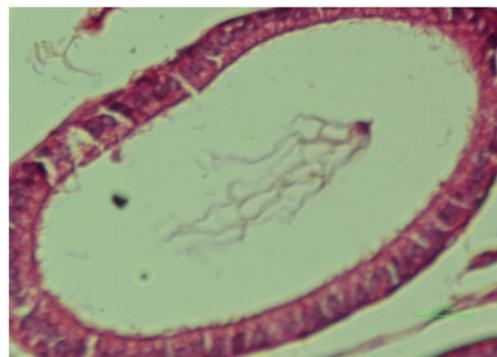


Fig. 3: Epididymal tissue controls

4. Conclusions

According to the results of this study, treatment with Ketotifen and immune parameters in adult male rats, the long-term use of the drug may be increased chances of improving semen parameters and fertility.

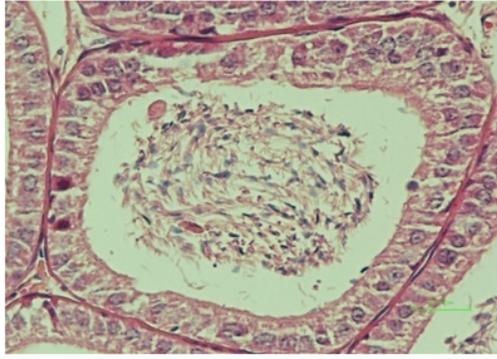


Fig. 4: Epididymal tissue treatment 2 (30 ml)

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