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RESEARCH ARTICLE

Immunohistochemical expression of pituitary Luteinizing hormone and Follicle stimulating hormone in male Rats treatment with thymoquinone.

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Abstract

To examine the effect of thymoquinone on the expression level of pituitary luteinizing hormone and follicle stimulating hormone in pubertal male rats , a total of 48 male rats aged 60 days were assigned to two equal groups. First group was drenched distilled water and served as control while second group was drenched with thymoquinone suspension (50 mg/kg) daily for six weeks. Two weeks internal , eight males were sacrificed and pituitaries were dissected for immunohistochemical examination . the results revealed higher expression of luteinizing hormone and follicle stimulating hormone in thymoquinone treated males with gradual increment as treatment periods progressed . it can be concluded that thymoquinone have potent role in elevation of pituitary hormone related to reproduction and fertility .

INTRODUCTION

The cellular localization of gonadotropins has been determined by immunohistochemical IHC techniques in a variety of species (Rahmanian *etal*.,1998). In all species so far examined including cows, both LH and FSH are present in the same gonadotrophs, and cells expressing only FSH or only LH are rare . LH is stored in the gonadotrophs and, therefore, is easily detected by immunocytochemistry, whereas FSH is released mainly through a constitutive pathway, and there may be little storage. Therefore, it is likely that in most instances in which LH only cells are observed, these cells are bi-hormonal but the production of FSH is greatly diminished and FSH cannot be detected in these cells(McNeillyetaal.,2003).

LH and FSH are produced by the anterior pituitary. The production of these two hormones is stimulated by gonadotropin releasing hormone GnRH made by the hypothalamus. It is largely known how LH, FSH and testosterone successful completion of the spermatogenesis process without it, conversion of round spermatids to spermatozoa during spermatogenesis is impaired It should be noted that follicle-stimulating hormone FSH plays important role in this conversion, as well as differentiation of spermatogonia into spermatocytes Since the germ cells have no receptors for testosterone and FSH, these hormones must act through the Sertoli cells, which are responsible for nurturing the germ cells. Testosterone is produced by the Leydig cells after receiving the signal from luteinizing hormone LH for its synthesis. This pathway is collectively known as the hypothalamus-pituitary-gonadal axis HPG axis (Singh *etal*.,2011). The pituitary gonadotropins GTHs, follicle stimulating hormone FSH and luteinizing hormone LH, are the key hormones in the control of reproduction in vertebrates, regulating gonadal gametogenesis and steroidogenesis. The FSH is involved in the initiation of gametogenesis and regulation of gonadal growth, whereas LH mainly regulates gonadal maturation and spermiation / ovulation (Mateos *et al*.,2002).

Successful male fertility requires an adequate sperm count , adequate sperm motility, the appropriate functioning of accessory sex organs(to produce and concentrate semen and to activate and capacitate sperm) , and appropriate sexual behavior (i.e., mounting, intromission ,ejaculation) . The production of viable sperm (spermatogenesis) in

the testes is under genetic control (a male-determining gene on the Y chromosome) and neuroendocrine regulation initiated in the brain by the hypothalamic-pituitary-gonadal (HPG) axis (Lucio *et al.*, 2005; Tyl , 2001) .
Thymoquinone (TQ) is the main bioactive constituent of an oil extract of *Nigella sativa*. The last fifteen years have witnessed hundreds of research reports regarding its therapeutic biological effects as an anti-inflammatory, analgesic, anti-diabetic, antihistaminic and anticancer agent (Woo *et al*., 2012) . Thymoquinone (TQ) (nearly about 50%). Among many other components of the essential oil are cymene (7.1% – 15.5%) , caryacrol (5.8 % – 11.6%), t-anethole (0.25% – 2.3%) , 4-terpineol (2.0% – 6.6%) and longifoline (1.0% – 8.0%) (Gali – Muhtasib,2004; Watanabe *etal*.,2001).

Material and method

Eight fourty adult male of Whistar strain weighing 130 ±1.6 grams were selected for this study. The animals were divided randomly into two groups, First group control and second group treatment T. Each group was comprised of four twenty animals and was further divided into three sub groups, based on period of treatment *i.e* two, four and six week .
Group-C: C_1, C_2, C_3

Group-T: T_1, T_2, T_3
Animals were put under observation for assessment to see their health. General condition on the basis of weight gain or loss. Group C animals were normal control, received 1c.c normal saline administration orally daily for their respective period of time. Group B rats received dose of 50mg\kg body weight daily for two, four and six weeks. Animals were weighed at the start and continue at the end of treatment .When the treatment was completed, animals were anaesthetized with ketamine and xylene. The animals were decapitated pituitary gland were dissected out, examined.

Preparation of TQ suspension

TQ suspension at a dose of 50 mg/kg bw were prepared by dissolving 5 mg of TQ powder (from Sigma Chemical Co.) in 1 ml of drinking water to be used as 5 mg / 100 g bw, so that each 100 g bw will need drenching 1 ml of TQ suspension to be contain 5 mg (TQ50) (Kanter,2009).

Immunohistochemical for pituitary gland

The tissue sections were first deparaffinized with xylene and hydrated through decreasing concentrations of ethanol. They were incubated for 20 min in a solution of 0.3% H2O2 in water to inhibit endogenous peroxidase activity. Then they were rinsed with distilled water and phosphate-buffered saline (PBS, 0.01 M, pH 7.4).incubate the slide with1% normal serumfor30 min at RT. sections requires boiling tissue sections in 10mM citrate buffer , pH6.0 for 10-20 min followed by cooling at RT for 20 min . Sections were then incubated overnight in a humidified chamber at RT with the mouse monoclonal antibody against pituitary FSH and LH (US Biological). After they were rinsed three time with PBS for 5 min. sections were incubated for 30 min with diluted biotinylated anti-mouse IgG, and after being washed in PBS, add the detection solution, incubate for 30 min and washed in PBS and add development solution for 15min and finally stop reaction by soaking the tissue in water . The sections were counterstained with hematoxylin for 1 min, dehydrated, and mounted .

Statistical Analysis

The data was entered and analyzed on SPSS version 16.0. Mean ± standard deviation of mean were computed for the quantitative variables like weight of animals, weight of pituitary glands and weight testes Two sample t-tests were used to calculate P-values in comparison of population means.

Results

Observation on Body Weight

Animals were studied before the start of the treatment and at the time of sacrifice i.e at the end of second, fourth and sixth week . The changes in animal's weights in both groups at different time periods were recorded which were found different time periods were recorded which were found different time periods were recorded which were found significantly increased in treated group than in the control group.

Body weight gain

The results showed a significant increase ($P<0.05$) in body weight gain (g) inT_1,T_2, T_3 compared with Control groups . It also showed a significant increase ($P<0.05$) in relative weight testis and pituitary weight of T1,T2 and T3 compared with control group (table–1) .

Table (1) : Effect of thymoquinone on the body weight gain, relative weight testis and pituitary weight .

Group	Control			Treatment		
Measurement	2wnd	4wth	6wth	2wnd	4wth	6wth
Body weight gain g/100bw	17.37± 0.73 Aa	34± 1.0 Ab	49± 0.08 Ac	44.89± 1.39 Ba	62.25± 1.08 Bb	78.25± 3.55 Bc
Testes weight g/100g bw	0.638± 0.007 Aa	0.641± 0.002 Ab	0.699± 0.014 Ac	0.705± 0.01 Ba	0.859± 0.014 Bb	0.868± 0.004 Bc
Pituitary weight mg/100g bw	2.7± 0.14 Aa	3.15± 0. 02 Ab	3.47± 0. 02 Ac	3.86± 0.04 Ba	4. 71± 0.02 Bb	4. 81± 0.02 Bc

*Different letters indicate significant differences(P<0.05).

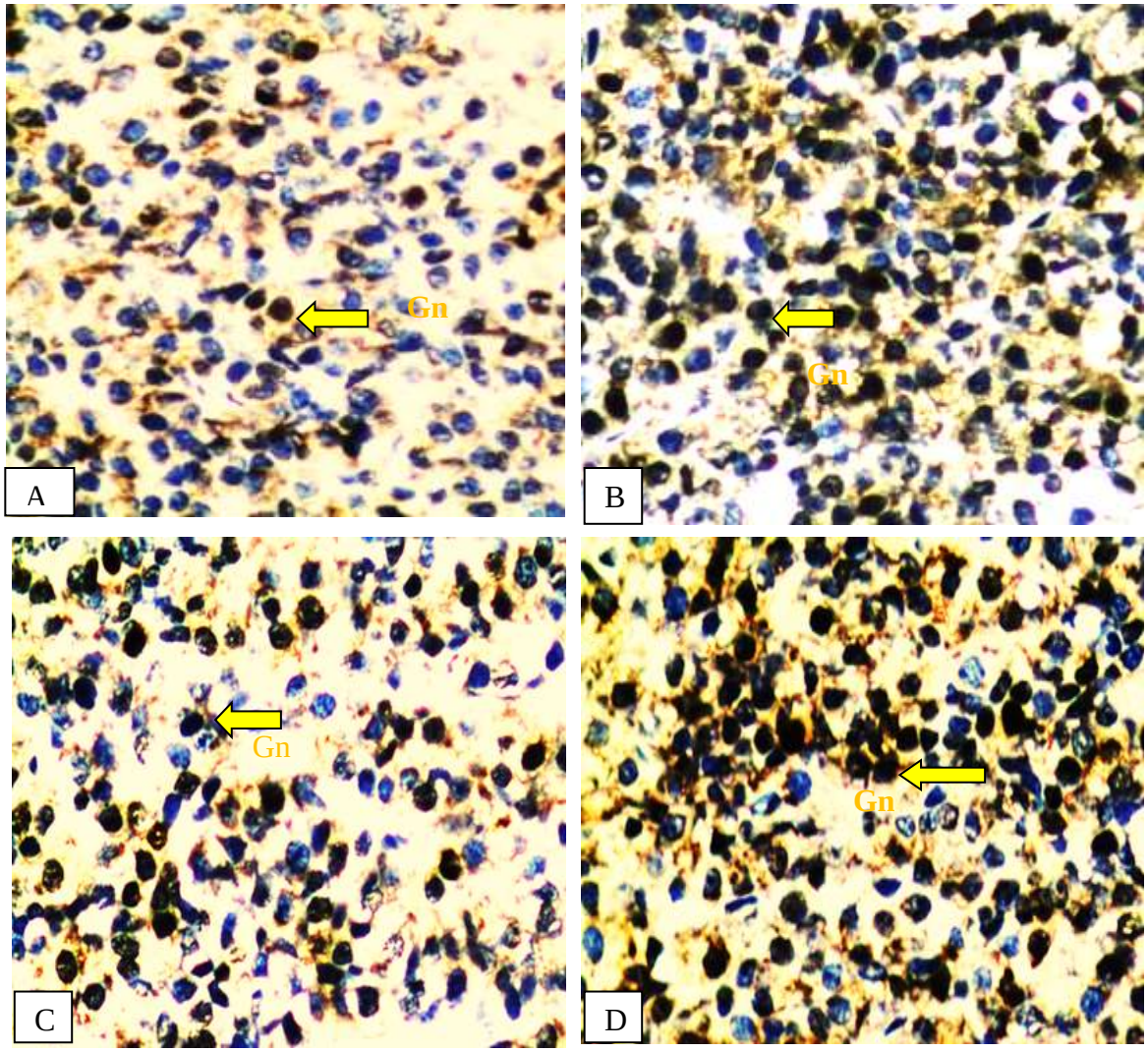

Fig. 1.: Immune interaction for FSH in the tissues of adenohypophysis male rats (A) control group, (B-C-D) Periods (2- 4 - 6) week treatment group shows the presence of immune interaction of follicles stimulating hormone FSH in

separator shall cells feeder cells gonads Gn and the interaction thymoquinone in (D and C) compared with the rest of aggregates (IHC 500X).

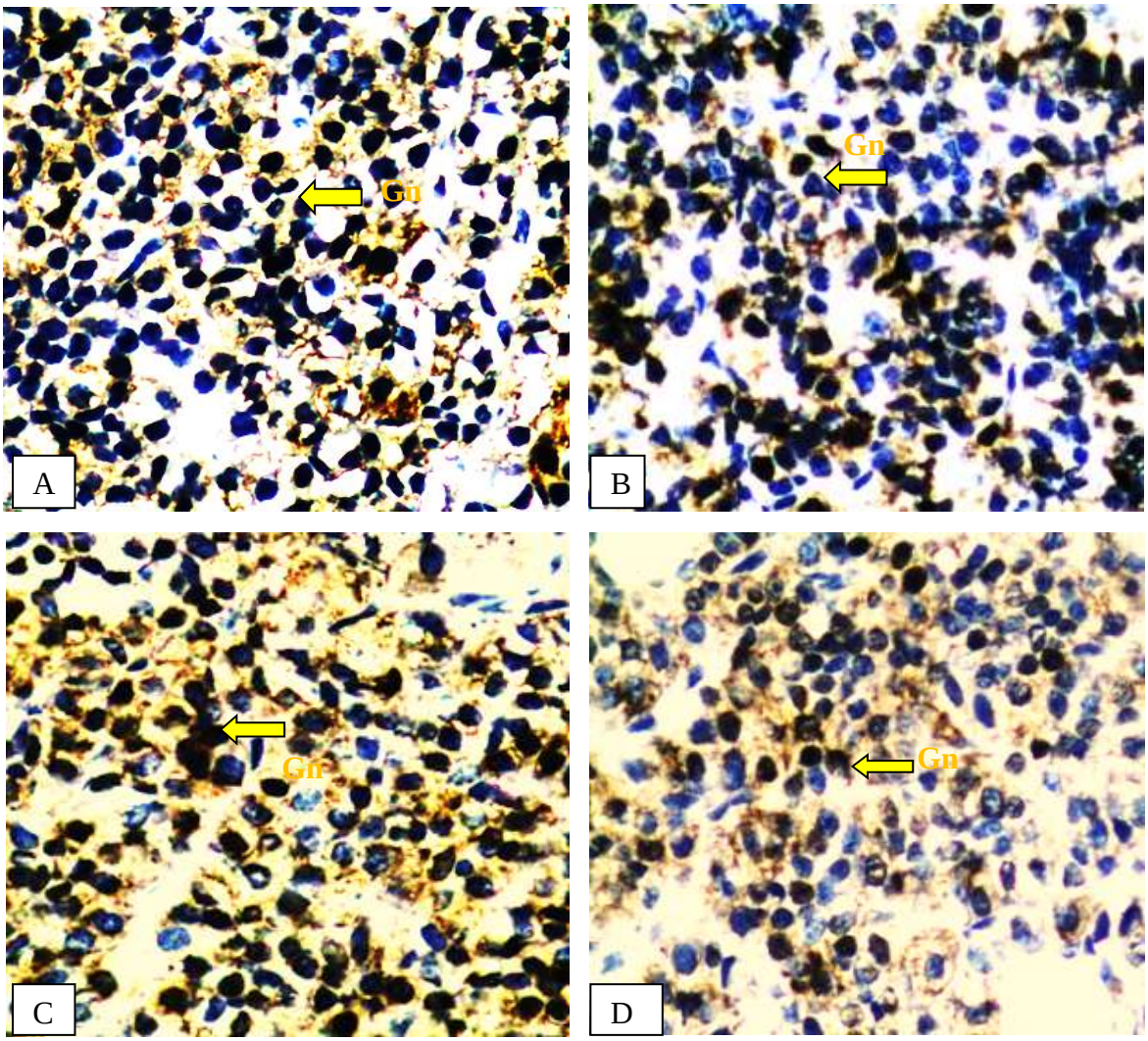

Fig. 2.: Immune interaction for LH in the tissues of adenohypophysis to male rats (A) control group. (B-C-D) periods (2-4 -6) week treatment group shows the presence of immune interaction of the luteinizing hormone LH in the separator shall gonadotrophs Gn and interaction is more intensity in the (C and B) compared with the rest of aggregates (IHC 500X).

Discussion

The results of the present study was to a significant increase (P <0.05) and clear in body weight gain when treatment TQ for 6 weeks this increase appeared at the end of the second week and continued to the end of the experiment where the third group recorded (6 weeks), the highest increase compared with the control group this increase related to effect thymoquinone on increases the catabolism of glucose and energy production which ultimately lead to improve the growth (Passos & Von Zglinicki, 2006) . The other things can help to improve the body weight, is the thymoquinone antibacterial activities that will lead to consume less energy by immune system. The use of black seed extracted from broiler diets has shown antibacterial, antifungal and improved body weight gain (Saeid & Mohamed, 2013). Thymoquinone enhances the glucose and other nutrients absorption but an inverse function happened by overusing it. Excessive thymoquinone creates pancreatitis and hypocalcaemia which lead to reduction of feed intake and growth performance. Poisoning symptoms and hypocalcaemia was observed by excessive injection of thymoquinone in more than 50 percent of rats (Farah *et al*., 2005). The use of thymoquinone increased spleen weight than control group in this experiment (p<0.05). Thymoquinone causes an increase in the

systemic immune response. There were clearly increased differentiation of spleen cells, macrophage and NK anti-tumor activity while using *Nigella sativa*. The use of thymoquinone reduced the number of neutrophils and increased the number of lymphocytes and monocytes in rat. Lymphocytes migrate to spleen to full maturation and specialization. The cells migrate to spleen called transitional lymphocytes. Thymoquinone effects in increasing the number and proportion of lymphocytes, and their migration to the spleen increase the activity of its tissue and therefore its weight (Khan *et al*.,2012).

As well as results showed immune interaction in the tissues of adenohypophysis the presence of the follicles stimulating hormone FSH in cells producing hormones gonadotrophs, immune interaction of follicles stimulating hormone FSH in the second week, fourth and sixth periods of the experiment compared with the control group which immune reactions appear less frequently intensity show. This is agree with (Hussein & Safwat , 2014) He show that where there was a significant increase in levels of the luteinizing hormone LH and follicles stimulating and testosterone when his study of the role of black seed oil to increase sexual hormones and role in the treatment of inflammation-causing cadmium by causing testicular toxin in rats . Also, the strong of immunostaing gonadotrophs is due to the effect of TQ on prepared where it is believed that TQ has an impact on the level of growth hormone in the pituitary within her anterior lobe. TQ that is one of the factors helping to grow factors and this is agreement with (Hull & Harvey , 2002) when noted that the growth hormone GH his importance in controlling the function of gonadotrophs Gn and improve its work as the growth hormone works as a companion to the hormone co-gonadotropin is a growth hormone and gonadotrophs are important in the process of growth and sexual maturation. The results of the immunohistochemical to increased numbers of adenohypophysis cells of the pituitary by observing the strength of the immune cells nutrients interaction of the gonads Gonadotrophs and this increase is due to the impact of TQ on the gland hypothalamus responsible for the Gonadotrophins- Releasing Hormone GnRH, which controls the gland as the control be it by the neural control and hormonal control of the hypothalamus , is GnRH editor of the hypothalamus is the one who controls the catalyst for the liberation of the follicles stimulate hormone FSH and luteinizing hormone LH of gonadotrophs Gn . This is in line with the study of mature treatment black seed oil rats where the results showed that there was a significant increase in levels LH , follicles stimulating hormone FSH and this increase is due to direct impact of oil on the hypothalamus, which in turn increases the liberated hormones of the gonads, which can stimulate liberated paths for gonads, which begins with changes in the functions of the gonads This is consistent with results of a study that came out (Boukhliq *et al*., ١٩٩٧) .

thymoquinone is role in female by stimulating estrogen and progesterone from the ovaries where it enters the building in cholesterol, have a role in the construction of progesterone and estrogen (Juma & Abdulrahman , 2011) That there is a positive increase in the concentration of progesterone and estrogen in the treatment aggregates black seed oil, and this effect is due to the main active compound thymoquinone TQ. Since the liberation of progesterone and estrogen from the ovaries be under the pituitary gland's control and under hormonal stimulating process and LH therefore believes that the impact be on the liberated cells of the gonads Gn, which in turn stimulates the ovaries to edit hormones mentioned and also TQ enters the paths transfer signal for the production of hormones which affect the enzymes phosphorylation enzyme where phosphorylation proteins and that the activity of these enzymes either be increased or discouraged, as a result of phosphorylation and as a result of the increase and the inhibition of the activity of these enzymes affect the amount of response that occur in the target cells and the hormones that are affected by these enzymes are hormonal follicles stimulating FSH and LH.

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