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The Effects of *Vitex agnus castus* Extract and its Interaction with Dopaminergic System on LH and Testosterone in Male Mice

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Abstract: The purpose of this study was to evaluate the probable effects of *Vitex agnus castus* (Vac.) on the male reproductive physiology. It is a well known fact that LH secretion from the anterior pituitary of mammals is controlled by many neurotransmitters such as dopamine. In this experiment, we have studied the effect of Vac. extract on the LH and testosterone hormones and its interaction with the dopaminergic system on male mice. In order to evaluate these effects, we used the hydroalcoholic Vac. extract (for extraction we used percolation technique) injection with the following doses: 65, 165, 265, 365 and 465 mg kg⁻¹, bromocriptine as a dopamine receptor agonist (5, 10, 20 mg kg⁻¹) and haloperidol as a dopamine receptor antagonist (1, 1.5, 2, 2.5, 3 mg kg⁻¹). To study the interaction between Vac. extract and dopaminergic system, we injected the optimum doses of Vac. with bromocriptine or haloperidol at the same time. Intraperitoneal injections were applied in all experiments, once a day for 30 days. The control group remained intact and the sham group received vehicle. After the last injection, we collected the animal blood serums for hormonal assays. LH and testosterone were measured by Radio Immuno Assay (RIA). LH and testosterone, showed significant decrease in bromocriptine group and haloperidol increased these hormones. Vac. extract decreased significantly the LH and testosterone levels. The coadministration of Vac. extract and bromocriptine decreased LH and testosterone. Coadministration of Vac. extract and haloperidol decreased LH and testosterone levels. These results suggest: dopamine regulates the gonadotroph-Leydig cells axis. It appears that Vac. exerts effects through dopaminergic system and other pathways. The findings of this study show we can use Vac. extract for pathological cases of increasing LH and testosterone.

Key words: *Vitex agnus-castus*, bromocriptine, haloperidol, LH, testosterone

INTRODUCTION

Vac. is a member of the Verbenaceae family, native to the Mediterranean and central Asia. The fruit has a spicy pepper like aroma and taste. The dried ripe fruits are used medicinally. It is mentioned by the Greek physician Dioscorides, as a beverage taken to lower libido (Blumenthal *et al.*, 2000; Christie and Walker, 1997; Houghton *et al.*, 1994). It has been used to treat PMS (premenstrual syndrome), menopausal symptoms, hyperprolactinemia. Vac. precise mechanism of action and its active constituents have not been established, yet. (Jarry *et al.*, 2006; Prilepskava *et al.*, 2006; Milewicz and Jedrzejuk, 2006; Loch *et al.*, 2000, Brown *et al.*, 1999). The extract may be useful for the prevention and treatment not only of benign prostatic hyperplasia but also of human prostate cancer (Weisskoof *et al.*, 2005). Diterpenoids,

vitexilactone, 6B, 7B-diacetoxy-13-hydroxy-labda-8-14-diene in Vac. extract which have high binding affinity to dopamine receptors (Kuruuzum *et al.*, 2003; Newall *et al.*, 1996; Hoberg *et al.*, 1999, 2000). Therefore, we conducted some experiments to probe Vac. extract on LH and testosterone. To know that whether Vac. act by dopaminergic system on pituitary-gonadal axis or not? As, Vac. has a number of compounds with dopaminergic properties. They suppress prolactin release (Wuttke *et al.*, 2003).

MATERIALS AND METHODS

Plant material: We collected Vac. fruits from Ghom area (Tehran province, Iran) in the summer. The *Vitex agnus castus* was identified at Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Medical University

of Tehran. The fresh material was dried in air under shade then ground to a fine powder and using percolation technique, the fruit hydroalcoholic extract (80% alchole and 20% water) was obtained. Then, the hydroalcoholic extract was filtered and the solvent was removed under reduced pressure at 30-40°C and extract was dissolved in tween and then water was added. resulting extract was stored in refrigerator for biological studies.

Animals: Adult Balb/C male mice weighting 20-25 g were obtained from Pasteur institute, Tehran, Iran. They were maintained in animal room with a controlled temperature (23±2°C) on a 12 L: 12D schedule and allowed to free access to food and water.

We injected intraperitoneally Vac. extract: 65, 165, 265, 365, 465 mg kg⁻¹, bromocriptine: 5, 10, 20 mg kg⁻¹ and Halopridol: 1, 1.5, 2, 2.5, 3 mg kg⁻¹ daily for 30 days (n= 6). After the last injection, under an anesthetic condition we collected the blood samples of animals from portal vein of liver and the serum was stored at -20°C until assayed. Then, LH (Luteneizing Hormone) and testosterone of the serums were measured by Radio Immuno Assay (RIA). Serum hormones were analyzed by Kavoshyar radioimmunoassay kit (They obtained of Kavoshyar Co. Iran). We studied this research out during 2004-2006 at Azad University.

Statistics: Statistical analysis was performed using one-way ANOVA with post Tukey test. p<0.05 was considered significant. All data are expressed as mean±SD.

RESULTS

Halopridol increased LH and testosterone levels comparing to the control and sham groups. The high effective dose of Halopridol was shown to be 2 mg kg⁻¹ (Fig. 1 and 2). bromocriptine inhibited LH and testosterone levels comparing to the control and sham groups. The least level of hormone obtained 10 mg kg⁻¹ (Fig. 3 and 4).

Vac. fruit extract decreased LH and testosterone levels of the male mice serum comparing to the control and sham groups. The more effective dose of the Vac. fruit extract was shown 365 mg kg⁻¹ (Fig. 5 and 6).

Coadministration of Vac. and Halo. decreased LH and testosterone levels (Fig. 7 and 8). Coadministration of Vac. and Br. decreased LH and testosterone levels (Fig. 9 and 10).

DISCUSSION

In the present study, we used a hydroalcoholic extract of Vac., agonist and antagonist of dopamine and interaction between Vac. and each of them.

Agonist of dopamine (Br.) decreased LH and testosterone (Fig. 3 and 4). Antagonist of dopamine (Halo.) increased LH and testosterone (Fig. 1 and 2). These data are according to previous reports (Waeber *et al.*, 1983; Jagannadha and Kotagi, 1989; Pakarinen *et al.*, 1994). Treatment with a dopaminergic antagonist increases density of testicular LH binding sites (Waeber *et al.*, 1983). Therefore this increase persuades

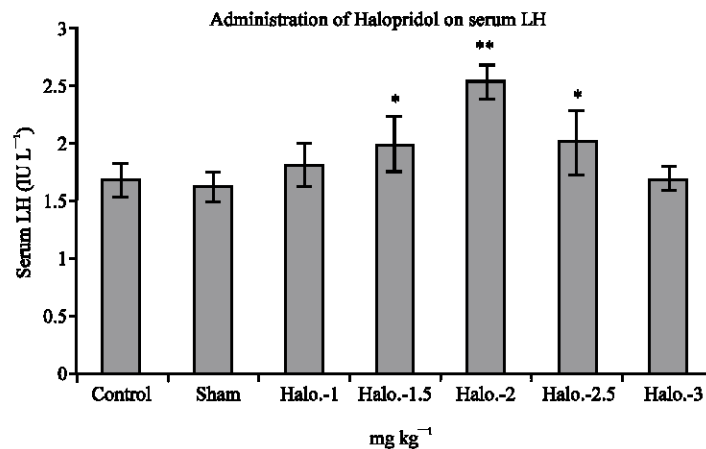


Fig. 1: The effect of different doses of Halopridol (Halo.) or vehicle on serum level of LH in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. *p<0.05, **p<0.01 when the data obtained from the treated animals are compared with sham animals

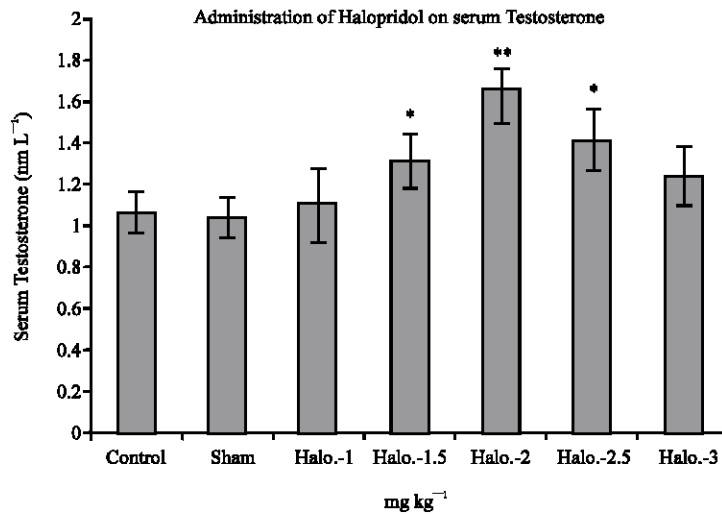


Fig. 2: The effect of different doses of Halopridol (Halo.) or vehicle on serum level of testosterone in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. *p<0.05, **p<0.01 when the data obtained from the treated animals are compared with sham animals

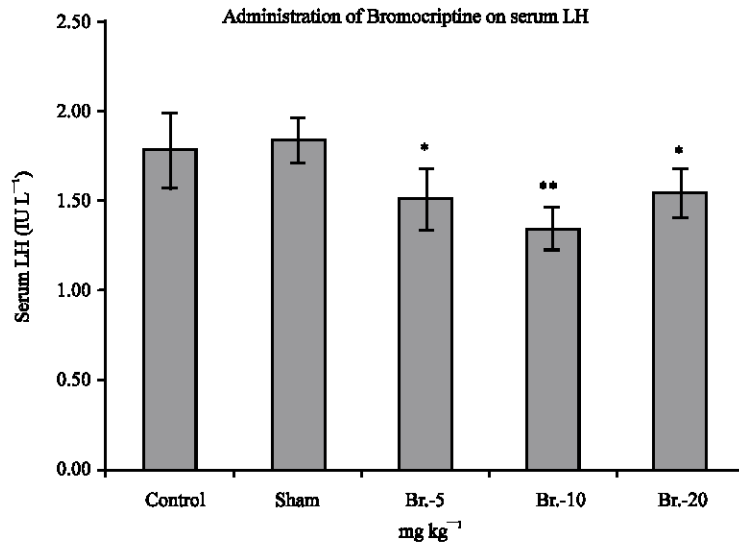


Fig. 3: The effect of different doses of bromocriptine (Br.) or vehicle on serum level of LH in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. *p<0.05, **p<0.01 when the data obtained from the treated animals are compared with sham animals

testosterone secretion by leydig cells. Treatment with a dopaminergic agonist decreases density of testicular LH binding sites (Waeber *et al.*, 1983; Pakarinen *et al.*, 1994). Then, the decrease cause reduction of testosterone secretion by leydig cells.

In the our previous study, Vac. extract inhibited LH, FSH and testosterone in male mice (Nasri *et al.*, 2004). In our studies we have reaffirmed LH and testosterone inhibition by Vac. treatment (Fig. 5 and 6). Vac. in combination with Halo. or Br. Suppresses LH and testosterone levels (Fig. 7-10).

The pre menstrual syndrom is due to the imbalance of estrogen and progesterone hormone levels. The imbalance of these two hormones could be regulated by Vac. fruit extract (Westphal *et al.*, 2004; Milewicz *et al.*, 1996).

The administration of the plant extract has a regulative effect on the level of LH and FSH secreted by adenohipophysis in female (Mills and Bone, 2000; Bone, 1994).

Regarding to the mentioned findings, the site effect of this extract could be the adenohipophysis of male similar to female.

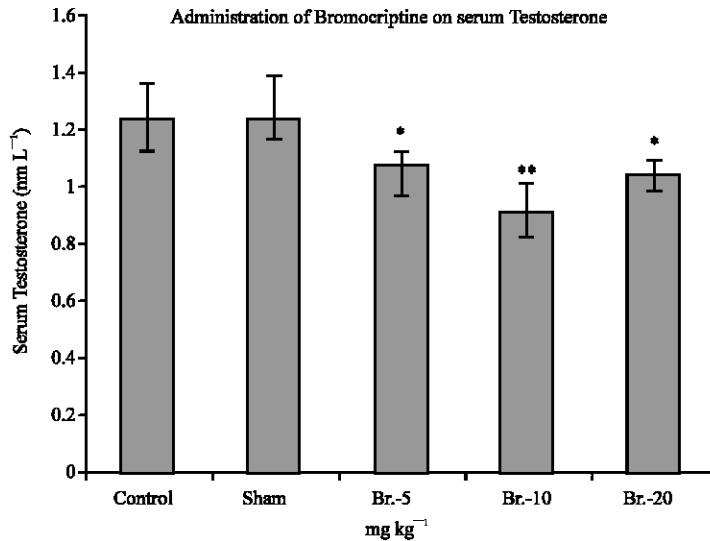


Fig. 4: The effect of different doses of bromocriptine (Br.) or vehicle on serum level of testosterone in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. *p<0.05, ** p<0.01 when the data obtained from the treated animals are compared with sham animals

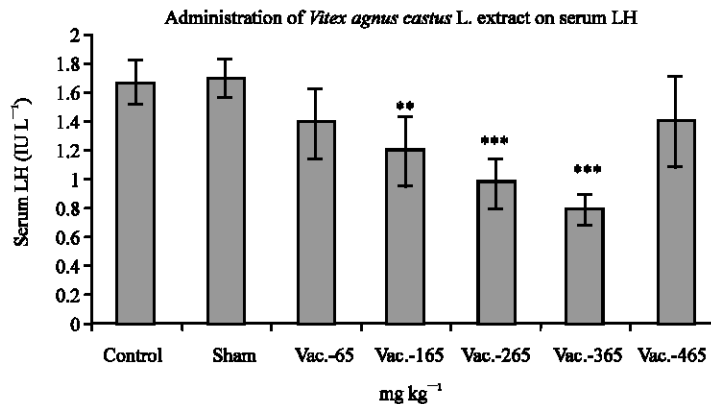


Fig. 5: The effect of different doses of *Vitex agnus castus* (Vac.) or vehicle on serum level of LH in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. **p<0.01, ***p<0.001 when the data obtained from the treated animals are compared with sham animals

In addition, Vac. extract contains phytoestrogens. The flavonoid apigenin has isolated and identified as the most active phytoestrogen in Vac. Other isolated compounds are vitexin and penduletin. These data demonstrate that the phytoestrogens in Vac. are binded to Estrogen reseptor (Jarry *et al.*, 2003). Using plant extracts chronically that have phytoestrogens affect negative feedback of LH. As a result, decrease of LH reduces testosterone level (Struss *et al.*, 1998; Malaivijitnond *et al.*, 2004).

Vac. extract acts as an agonist at the mu-opiate receptor (Webster *et al.*, 2006) and opiate peptides

regulate GnRH and LH in male (Jakson and Kuehl, 2000; Tilbrook and Clarke, 2001).

Moreover, effect of Vac. extract on dopamine receptor is identically like the effect of dopamine agonist receptor named Lisuride (Sliutz *et al.*, 1993).

By using dopamine receptor Binding assay, it was found that Vac. fruit extract has high binding affinity to the dopamine receptors (Jarry *et al.*, 1994; Johnson *et al.*, 2005). So it could be suggested that Vac. acts through a dopaminergic pathway.

Dopamine is one of the neurotransmitter which has inhibitory effect on the Hypothalamic-Pituitary-Gonadal (HPG) axis in male (Kacssoh *et al.*, 2000).

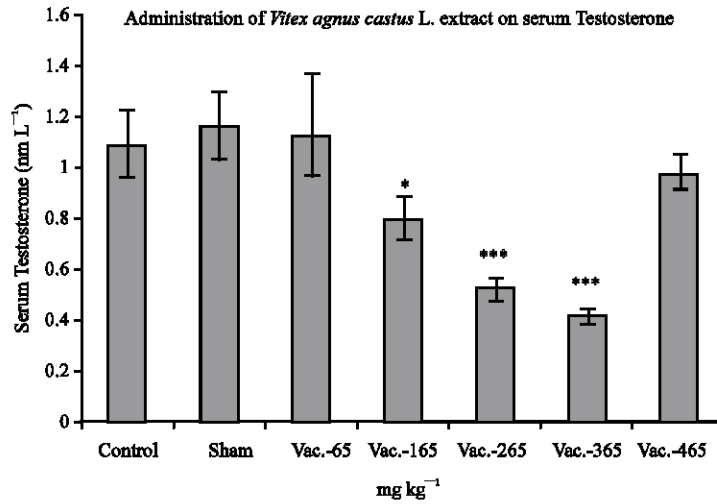


Fig. 6: The effect of different doses of *Vitex agnus castus* (Vac.) or vehicle on serum level of testosterone in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. *p<0.05, ***p<0.001 when the data obtained from the treated animals are compared with sham animals

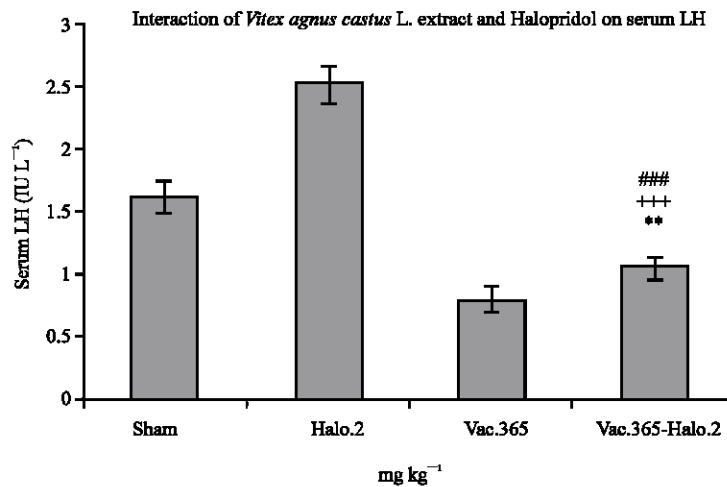


Fig. 7: The effect of coadministration of *Vitex agnus castus* (Vac.) and Halopridol (Halo.) or vehicle on serum level of LH in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. ###p<0.001 when the data obtained from the combination treated animals are compared with Sham operated animals, +++p<0.001 when the data obtained from the combination treated animals are compared with Halopridol injected animals, **p<0.01 when the data obtained from the combination treated animals are compared with experimental animals received only Vac

The results of this study also demonstrate that Vac.extract has inhibitory effects on LH and testosterone secretion. It seems that Vac. fruit extract influences by activating the dopaminergic pathway and inhibits HPG axis and decreases LH and testosterone hormones. It appears the other mechanism by which these inhibitory effects of Vac. extract could be mediated, are phytoestrogens. Hypothalamus is a site of negative

feedback regulation by phytoestrogens. Therefore, the inhibitory effect of Vac. on testosterone level could influence some pathways such as indirect effect on dopamine, opiate, oestrogen receptor and direct effect on LH response. So the effect of interaction of Vac. and Br. are more effective than Br. (agonist of dopamine) alone. The findings of this study show we can use Vac. extract for pathological cases of increasing LH and testosterone.

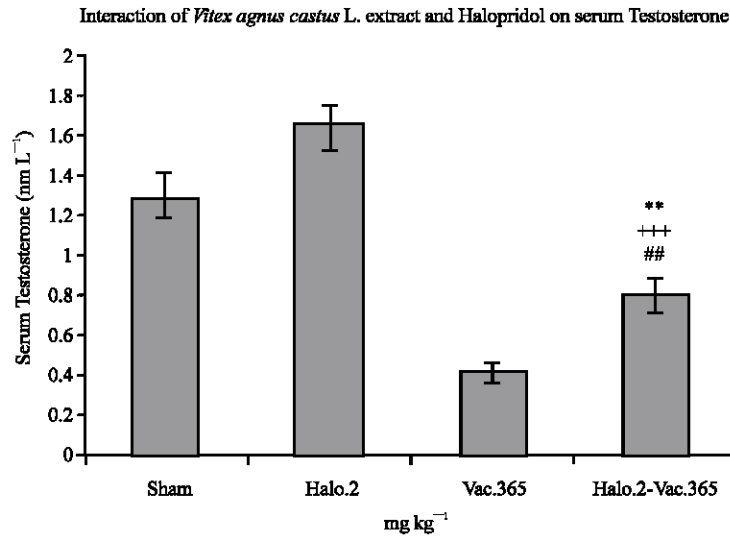


Fig. 8: The effect of coadministration of *Vitex agnus castus* (Vac.) and Halopridol (Halo.) or vehicle on serum level of testosterone in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. ##p<0.01 when the data obtained from the combination treated animals are compared with Sham operated animals, +++p<0.001 when the data obtained from the combination treated animals are compared with Halopridol injected animals, **p<0.01 when the data obtained from the combination treated animals are compared with experimental animals received only Vac

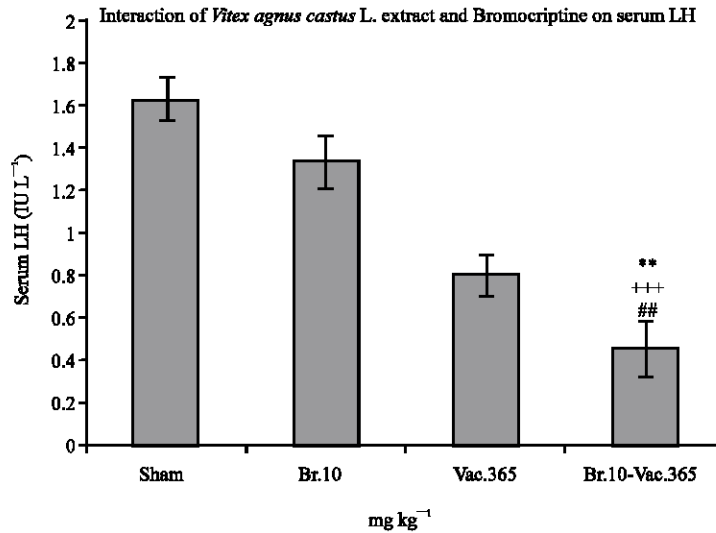


Fig. 9: The effect of coadministration of *Vitex agnus castus* (Vac.) and bromocriptine (Br.) or vehicle on serum level of LH in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. ###p<0.001 when the data obtained from the combination treated animals are compared with Sham operated animals, +++p<0.001 when the data obtained from the combination treated animals are compared with Br. injected animals. **p<0.01 when the data obtained from the combination treated animals are compared with experimental animals received only Vac

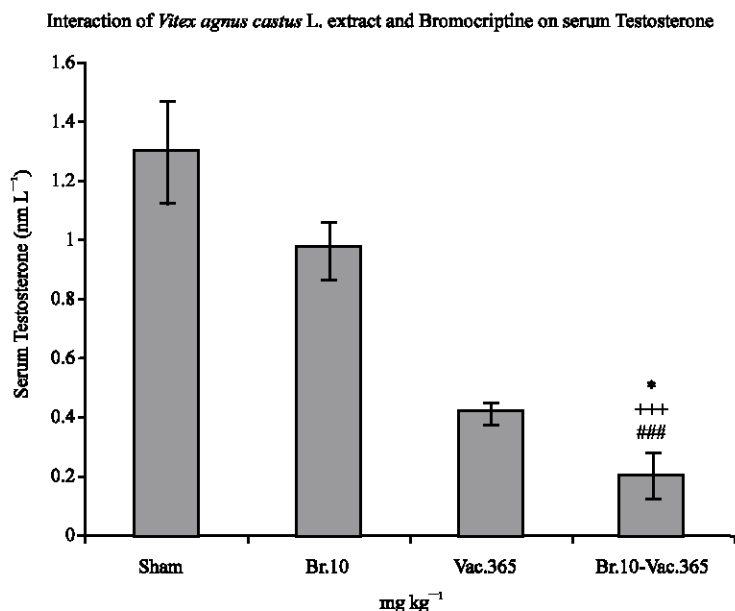


Fig. 10: The effect of coadministration of *Vitex agnus castus* (Vac.) and bromocriptine (Br.) or vehicle on serum level of testosterone in male mice after 30 days of treatment. Each group consisted of 6 animals and the bars depict mean±SD. ###p<0.001 when the data obtained from the combination treated animals are compared with Sham operated animals. +++p<0.001 when the data obtained from the combination treated animals are compared with Br. Injected animals. *p<0.05 when the data obtained from the combination treated animals are compared with experimental animals received only Vac

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