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Effect of *Tribulus terrestris* Extract on Ovarian Activity in Immature Wistar Rat: A Histological Evaluation

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Abstract: *Tribulus terrestris*, puncture vine has long been used in traditional medicine to treat impotency and improve sexual functions in man although, there are little information about effect of *Tribulus terrestris* on female reproduction. The objective of the present study was to investigate the effect of *Tribulus trestris* extract on ovarian activity of immature wistar rat. About 20 immature female wistar rats, aged 21 days and with an average weight of 50 g were used in the study. The rats randomly divided into treatment and control groups. Rats in treatment group received 10 mg of the extract per rat. The period of the study divided into 7 and 14 days period. Half of the rats in each group (n = 5) were euthanized at final day of each period and the ovaries removed for preparating of histology sections. Number and diameter of corpus luteum, thickness of theca interna layer and number of all follicles were evaluated in each left and right ovaries. Results showed that number of corpus luteum and diameter of theca interna increased in treatment group as compared with control group. Number of secondry and graafian follicles after 14 days treatment were be higher than control and 7 days treatment. The present results indicated that *Tribulus terrestris* induces corpus luteum formation and growth and therefore beginning of puberty with its LH-like activity.

Key words: *Tribulus terrestris*, puncturevine, ovary, rat, extract, corpus luteum

INTRODUCTION

Female reproduction directly relates to gonadotropic hormones such as Luteinizing Hormone (LH) and Follicle Stimulating Hormones (FSH). There are a lot of reproductive disorders which need to treated with gonadotropic hormones and other hormones such as human Chorionic Gonadotropin (hCG) that have similar activities. Plant-derived and herbal remedies continue to provide a popular alternative for treating reproductive disorders in men or women, although there are effective conventional medical treatments (Neychev and Mitev, 2005).

Tribulus terrestris L. (Zygophllaceae) (TT) is a medicinal plant which has been commonly used in folk medicine as an aphrodisiac and treatment of urogenital illness (Kianbakht and Jahaniani, 2003; Neychev and Mitev, 2005). Although, experimental and clinical studies on animals (Gauthaman *et al*., 2002, 2003; Arcasoy *et al*., 1998; El-Tantawy *et al*., 2007; Gauthaman and Ganesan, 2008) and men (Brown *et al*., 2000, 2001; Kohut *et al*., 2003; Antonio *et al*., 2000; Neychev and Mitev; 2005) have been evaluated the effects of TT on male reproduction, there is limited information about its effects on female reproductive system. The aim of the current study is to investigate the influence of *Tribulus terrestris* extract on ovarian activity of immature rats.

MATERIALS AND METHODS

Pure *Tribulus terrestris* extract was prepared from Goldaru Pharmaceutical Co. (Isfahan, Iran).

Animals and housing conditions: The laboratory animals were treated in compliance with the Guide to the care and use of experimental animals (Olfert *et al*., 1993). The experiment was conducted in 2009-2010 at Islamic Azad University, Kazerun Branch. About 20 immature female wistar rats with 21 days age and an average weight of 50 g were prepared from animal house of Research Centre of Islamic Azad University, Kazerun Branch. They housed (5 rats cage^{-1}) in a room with controlled light cycle (12 L:12 D) and temperature (22-24°C) and with free access to water and standard food (regular rat chow, Pars Animal Feed Co., Iran). The rats randomly divided into treatment and control groups. The study was conducted

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in 7 and 14 days period. Pure *Tribulus terrestris* extract was resolved in distilled water. The rats in treatment group orally received 10 mg of the extract per rat. Half of the rats in each group (n = 5) were euthanized at final day of each period and the ovaries removed for preparating of histologicl sections. The ovarian samples were fixed in 10% buffered formalin, processed routinely and stained with haematoxylin and eosin. One histological section was prepared from middle of the ovaries. The sections were examined with a light microscope and Dino-Eye pieces camera AM423. The number of corpus luteum, diameter of corpus luteum, thickness of theca interna and number of all follicles were examined in each left and right ovaries.

Statistical analysis: Statistical analysis was done using SPSS version 16. Results are presented as Mean±SE. Statistical analysis was carried out using one-way ANOVA followed by Least Significant Difference (LSD) procedure. A probability value >0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Histological analysis of the ovaries

The 7 days control group: There was not observed any corpus luteum in the ovaries of control group during 7 days period (Fig. 1). Mean number of primary, secondry and graffian follicles in 7 days control group were 9.7±1.7, 15.5±1.3, 2.75±0.95, respectively (Table 1).

The 14 days control group: Size of the ovaries in 14 days control group was >7 days control group. Corpus luteum was observed in the ovaries of 14 days control group (Fig. 2). Mean number and diameter of corpus luteum in this group was 1.25±0.95 and 380.2±8.36 micron, respectively (Table 2). Mean number of primary, secondry and graffian follicles in 14 days control group were 11.25±1.5, 17±0.8 and 4±0.8, respectively (Table 1).

The 7 days treatment group: Hyperemia was partly observed in ovarian medulla of 7 days treatment group. Corpus luteum was observed in the ovaries of 7 days treatment group (Fig. 3). Mean number and diameter of corpus luteum in this group was 4.5±1.29 and 407.2±7.22 micron, respectively (Table 1 and 2). Mean number of primary, secondry and graffian follicles in 7 days treatment group were 10.75±1.7, 95±0.8, 4.7±0.16, respectively (Table 1).

The 14 days treatment group: Corpus luteum was observed in the ovaries of 14 days treatment group (Fig. 4 and 5). Mean number and diameter of corpus luteum in his group was 5.25±1.7 and 605.2±10.39 micron, respectively (Table 1 and 2). Mean number of primary, secondry and graffian follicles in 14 days treatment group

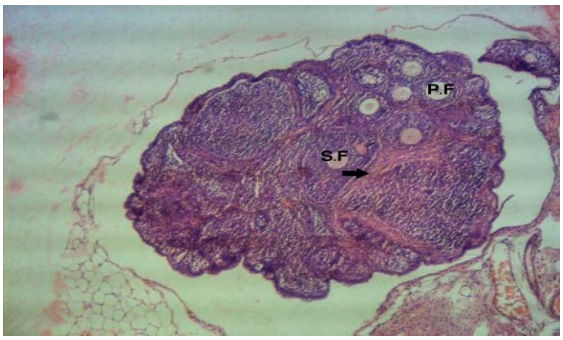

Fig. 1: Light micrograph of ovary of the 7 days immature control group. Theca folliculi (arrow), Primary Follicle (PF) and Secondary Follicle (SF) were seen. Corpus luteum was not seen (×92)

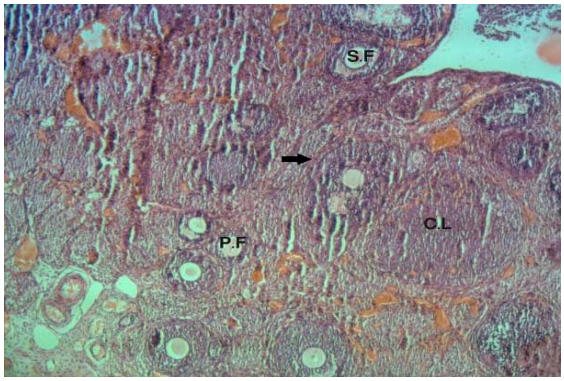

Fig. 2: Light micrograph of ovary of the 14 days immature control group. Theca folliculi (arrow), Corpus Luteum (CL), Primary Follicle(PF) and Secondary Follicle (SF) were seen (×230)

Table 1: Effects of *Tribulus terrestris* on number of follicles and corpus luteum in different experimental groups

Groups	Primary follicle	Secondry follicle	Graffian follicle	Corpus luteum
7 days control	9.7±1.7	15.5±0.29	3.75±0.95	0.0
14 days control	11.25±1.5	17±0.8	4±8	1.25±0.95
7 days treatment	10.75±1.7	16±0.8	4.7±0.95	4.5±1.29
14 days treatment	12.25±1.25	18±0.8	5.5±1.29	5.25±1.7

Table 2: Effects of *Tribulus terrestris* on thickness of theca interna layers and diameter of corpus luteum in different experimental groups

Groups	Thickness of theca interna layers (micron)	Diammeter of corpus luteum (micron)
7 days control	12.12±0.17	0
14 days control	17.5±0.550	380.2±8.36
7 days treatment	29.65±0.43	407.2±7.22
14 days treatment	70.83±0.59	605.2±10.39

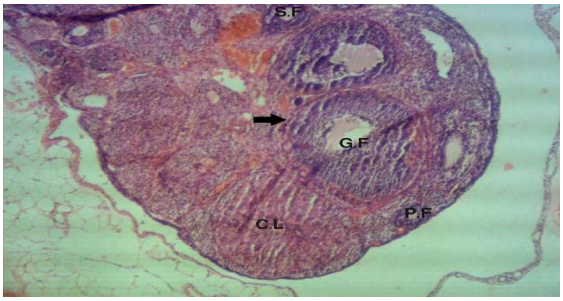

Fig. 3: Light micrograph of ovary of the 7 days immature treatment group. Theca folliculi (arrow), Corpus Luteums (CL), Primary Follicle (PF), Secondary Follicle (SF) and Graffian Follicle (GF) were seen (×92)

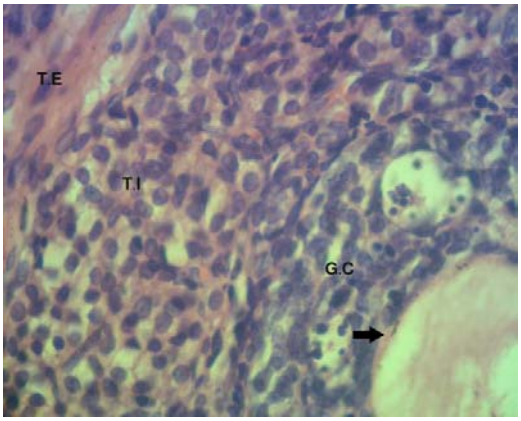

Fig. 4: Light micrograph of ovary of the 14 days immature treatment group. Theca interna (TI), Theca Externa (TE), Granulosa Cell (GC) and zona pellucid (arrow) were seen (×920)

were 12.25±1.25, 29±0.8, 5.5±1.18, respectively (Table 1). Mean thickness of theca interna layer in 7 and 14 days control groups and 7 and 14 days treatment groups were 12.12±0.17, 17.5±0.55, 29.65±0.43, 70.83±0.59 micron, repectively (Table 2).

Statistical analysis showed that thickness of theca interna layer, number of secondry and graffian follicles, number and diameter of corpus luteum in different groups were significantly different ($p<0.05$). Thickness of theca interna layers and diameter of corpus luteums were significantly different between all treatment and control groups (7 and 14 days). These factors were be highest in 14 day treatment group ($p<0.05$). Number of corpus luteums were not different between 7 and 14 days treatment groups, although they had higher numbers than control groups ($p<0.05$). Clinical and experimental studies

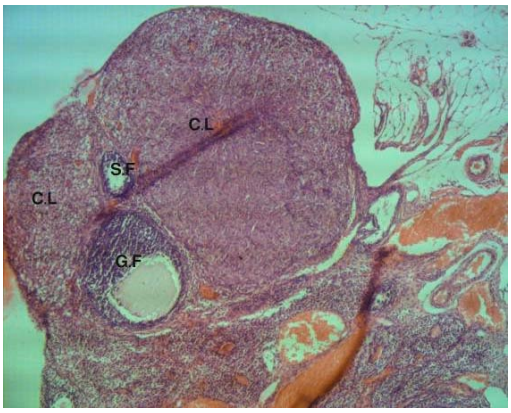

Fig. 5: Light micrograph of ovary of the 14 days immature treatment group. Corpus Luteums (CL), Secondary Follicle (SF) and Graffian Follicle (GF) were seen (×92)

on animals and men indicated that TT can improve some aspects of male sexual performance, spermatogenesis and sperm quantity and quality (Arsyad, 1996; Gauthaman *et al*., 2002, 2003). In addition, there is no consensus on the exact mechanisms underlying these effects and there is still much debate regarding possible mechanisms of action (Martino-Andrade *et al*., 2010).

It is believed that the steroidal saponins present in TT extracts, particularly protodioscin can increase endogenous androgen production by increasing Luteinizing Hormone (LH) release from the pituitary gland (Martino-Andrade *et al*., 2010). Alternatively, it has been proposed that TT active components might be enzymatically converted into weak androgens like Dehydroepiandrosterone (DHEA) which could in turn be converted into more potent androgens like testosterone in the gonads and peripheral tissues (Adailkan and Gauthaman, 2001).

However, changes in endogenous hormone levels following TT administration are still controversial (Neychev and Mitev, 2005; Martino-Andrade *et al*., 2010). The role of luteinizing hormone during follicle development and ovulation is well known. Prepubertal follicles recurrently develop into preovulatory size and regress if surge of LH does not occurs. Thereafter, next follicular wave recruits again. After 1st prepubertal LH surge, ovulation occurs, first corpus luteum develops and puberty begins. Results of the present study indicated that TT can develop corpus luteum in prepubertal rats. Corpus luteum observed in all the rats in treatment group and some 14 days control group, although there was no corpus luteum in 7 days control group. Puberty in Wistar rats begin about 32 days after birth (Laws *et al*.,

2000). Age of the rats was 35 days at 14 days after beginning of the study and it seems that some of the rats in 14 days control group naturally start their puberty. As the results presented thickness of theca interna layers and diameter and number of corpus luteums in TT treated groups were significantly higher than the untreated groups. It seems that LH-like activity of TT extract induces thickening of theca interna layers and corpus luteum formation and growth.

CONCLUSION

In this study, the presented results indicate that *Tribulus terrestris* has a LH-like activity which induces corpus luteum formation and therefore beginning of puberty. In addition, observed increase in corpus luteum diameter may relate to increase in progesterone production that needs further researches.

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