ORIGINAL ARTICLE

EFFECT OF *TRIBULUS TERRESTRIS* ON SERUM LUTEINIZING HORMONE IN SPRAGUE DAWLEY RATS

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Background: *Tribulus terrestris* is a creeping herb that has been used in the past for treating impotence. The extract obtained from *Tribulus* mainly contains the active component protodioscin. Administration of the extract improves libido and spermatogenesis. Protodioscin increases the levels of luteinizing hormone (LH), testosterone, dehydroepiandrosterone (DHEA) and dihydrotestosterone (DHT). The objective of present study was to determine the effect of *Tribulus terrestris* on serum luteinizing hormone in male Sprague Dawley rats. **Methods:** The study was carried on 60 adult male Sprague Dawley rats aged 90–120 days and with average body weight of 200±50 grams. Rats were divided into two groups with 30 rats per group. Group A received 0.5 ml distilled water once daily orally. Group B was administered orally *Tribulus* aqueous extract 6 mg/Kg once daily. All the doses were given to rats for 8 weeks. After 8 weeks, serum luteinizing hormone was measured in both groups. The mean values of LH were compared using SPSS. Alpha value was kept at 0.05. **Results:** The mean LH level was 1.26±0.54 for control group while it was 1.75±0.65 for the experimental group. The difference between control group and experimental group was statistically significant (p<0.001). **Conclusion:** *Tribulus terrestris* increases the levels of LH when administered in appropriate doses.

Keywords: *Tribulus terrestris*, Serum, luteinizing hormone (LH)

Pak J Physiol 2017;13(4):38–40

INTRODUCTION

In the developing countries of the world, about 75 to 80% of the population uses herbal medicines because of the reason of good acceptability by human body and less incidence of side effects.[1] One class of these herbal medicines is termed as aphrodisiac which stimulate the sexual instinct, induces venereal desire and enhances pleasure and performance. These substances are derivative of plants, animals or minerals.[2]

In traditional medicine, asphaltum, *Tribulus terrestris* and sidacordifolia have been accounted to have aphrodisiac activity and were among the oldest modalities for stimulating sexual function in humans.[3] However, Ayurveda knowledge should be paralleled with modern medicine and more of the scientific research may be carried out for the verification of efficacy and safety profile of these drugs.[1]

Tribulus is a natural plant used for the remedy of different ailments.[4] *Tribulus terrestris* (family: Zygophyllaceae) is a plant with flowering character, found mainly in the temperate and tropical parts of Australia, Southern Asia and Africa. It finds important position in traditional Ayurvedic medicine for the cure of sexual dysfunction in males.[5] Stem, roots, leaves and fruits of this plant are used in herbal medicines, for which *Tribulus* can amplify and increase sexual drive and cure urolithiasis, menorrhagia, impotence, joint pains, and premature ejaculation.[6]

The *Tribulus* extract chemically consists of steroids, saponins, flavonoids, alkaloids and glutamic acid.[7] The wide variety of notable compounds present in various types of *Tribulus* extract are protodioscin (PTN), prototribestin, pseudoprotodioscin, dioscin, tribestin, tribulosin and the flavonoid rutin.[8] Fruit of *Tribulus terrestris* which is generally the most acknowledged part of plant in account of its medicinal and dietary application, particularly contains two steroidal saponins, PTN and prototribestinin in its extract.[9]

The Protodioscin has been stated to increase the levels of serum testosterone, dehydroepi-androsterone and dihydro-testosterone.[5] Another compound called as dioscin present in the extract enhances male sexual attribute by uplifting the level of free testosterone and balancing the levels of estrogen, progesterone and pregnenolone in body. Since this plant contains steroidal saponins which potentiate levels of luteinizing hormone (LH) and testosterone[7], administration of this extract to humans and animals improves libido and spermatogenesis[10,11].

There are multiple mechanisms proposed for the action of *Tribulus*. The extract of *Tribulus* is reported to increase serum testosterone, one of the suggested mechanisms is that the level of testosterone is augmented by increasing LH and the gonadotropin releasing hormone[12] as *Tribulus terrestris* has gonadotropin-like activity[13]. The objective of this study was to determine the effects of *Tribulus terrestris* on serum luteinizing hormone in male Sprague Dawley rats.

MATERIAL AND METHODS

This quasi experimental study was carried out from Oct 2012 to Apr 2014, at research laboratory of Shifa College of Medicine, Islamabad in collaboration with National Institute of Health (NIH), Islamabad.

Sixty adult male Sprague Dawley rats weighing 200±50 gm, bought from National Institute of Health, Islamabad, were kept under standard laboratory environmental conditions at 23±2 °C with constant light-dark cycle, with standard rat diet and water *ad libitum*. Rats were divided into two groups, Group A (control) was administered 0.5 ml distilled water/rat/day orally and group B (experimental) was given *Tribulus terrestris* aqueous extract 6 mg/Kg/rat once daily orally with gavage needle for 8 weeks.

The dry fruits of *Tribulus terrestris* were procured from herbarium of National Agriculture Research Centre after plant identification. The fruit was blended. This macerated pulp was kept soaked in 1 litre of distilled water over the night, filtered and dried using rotary evaporator. From 500 grams of fruit, 25 grams of filtrate was obtained and incubated at room temperature. The dry mass was considered as an aqueous fruit extract of *Tribulus terrestris*.[14] For 250 gm rat 1.5 mg dry extract was dissolved in 1 ml distilled water. After 24 hours of the last dose, the animals were sacrificed. Samples of blood for hormone assay were collected by intra-cardiac sampling under deep ether anaesthesia. Serum separation was carried out by centrifugation, and was stored at -80 °C until assayed.

Enzyme immunoassay of serum LH was done using Luteinizing Hormone EIA kit, Catalogue Number: CSB-E1265. Statistically the data were processed using SPSS-21. The arithmetic mean and standard deviation of observations were computed. Difference in mean among control and treated groups was computed by independent *t*-test. Alpha value was kept at 0.05.

RESULTS

The comparison of mean values of LH levels between two groups at the end of 8 weeks is shown in Table-1. The mean LH level was 1.26±0.54 for control group while it was 1.75±0.65 for *Tribulus terrestris* group. The difference between control group and experimental groups was statistically significant (p<0.001). Mean LH level was significantly improved in experimental with respect to the control group rats.

Table-1: Comparison of mean LH values between control and experimental groups (Mean±SD)

	Luteinizing Hormone		
Variable	Control Group (n=30)	Treated Group (n=30)	*p*
LH IU/L	1.26±0.54	1.75±0.65	<0.001

DISCUSSION

Infertility is the health problem which has worldwide distribution, and male factors contribute about 30%. In more than 90% of male sterility reports, sperm related morbidity is the cause.[15] More than 190 studies have been carried out on the different aspects of aphrodisiac therapy in Asian region, with more than 50 on different sperm factors[12,16] but only few studies have been carried out to estimate the clinical efficacy of a single herb.

Shalaby *et al*[17] carried out research on mature male rats and the results revealed that methanolic extract of fruit of *Tribulus* elevates serum FSH, serum LH and increases testosterone production. Also, positive effects on sperm motility, count and viability along with enhanced activity of testicular anti-oxidant enzymes were observed. These results favour our results though we used aqueous extract and Shalaby *et al,* used alcoholic extracts.[17] The probable reason of similarity might be due to the same active principle compound PTN in both the extracts and same model of experimentation utilized in both the studies.[17]

Dehghan *et al*[13] conducted study on female rats but revealed that *Tribulus* has a luteinizing effect due to its gonadotropin hormone like activity and in maximum heavy doses, the extract has the property of proficiently removing ovarian cysts and continue ovarian functionality. Moghaddam *et al*[18] showed that LH levels increased significantly in *Tribulus* administered group in contrast to that of control male Wister rats. These findings are consistent with results of our study.

It was reported that *Tribulus* contained steroidal saponins, which might augment the level of LH.[19] In some studies, it has been stated that *Tribulus* has GnRH activity and it elevates the levels of LH and FSH.[14] In experimental studies on animals, *Tribulus* is claimed to have a LH-like activity, which can induce testosterone secretion in male rats.[18]

Conflicting results were illustrated by Neychev *et al*[20] whereby they showed no significant increase in serum luteinizing hormone, serum testosterone and androstenedione which were estimated 24 hours before administration, and at 24, 72, 240, 408 and 576 hours from the commencement of the *Tribulus* administration. The above-mentioned facts and findings anticipated that *Tribulus terrestris* steroid saponins own neither direct nor indirect androgen enhancing properties.[20] This variation can be explained on the basis of dose of *Tribulus* and duration of administration of the drug.

In human beings Pokrywka *et al*[21] reported that, *Tribulus* extract when used alone does not amplify the production of androgens. A few research studies have reported that the combination of *Tribulus* extract with other herbal products increases androgen levels. As *Tribulus* has a number of organic compounds,

pharmacological mechanism of action in humans is not entirely explained.[21] The observed difference might be due to several reasons like different experimental model and variation in dose used.

A prospective, randomized, double-blind and placebo-controlled study conducted on males with erectile dysfunction revealed that *Tribulus terrestris* was not more helpful as compared to placebo on improving erectile dysfunction or serum androgenic levels. This study is contrary to our study.[22] Another placebo-controlled double-blind study conducted among men with erectile dysfunction reported great effectiveness of *Tribulus terrestris* preparation 'Tradamixina' composed of *Tribulus*, *Alga eckonia*, D-glucosamine and N-acetylglucosamine as it improved libido. It was not certain in both the studies as which of the compounds was beneficial, and whether *Tribulus terrestris* contributed to the effects. The improvement might be due to increase in LH followed by upsurge of testosterone.[23]

As this effect of *Tribulus terrestris* in humans has not been addressed in literature so far, other studies should be planned to see the same effect and elucidate the mechanisms behind this action.

CONCLUSION

Tribulus terrestris extract enhances the release of Luteinizing Hormone in Sprague Dawley rats when given in appropriate doses.

LIMITATIONS

We could not study the stages of seminiferous epithelium in detail because of time limitations. *In vitro* study to elucidate the cellular mechanism of *Tribulus terrestris* at Leydig cell level was not done due to technical limitations.

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Received: 17 Dec 2017 Reviewed: 21 Dec 2017 Accepted: 22 Dec 2017