ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Effect of Thymoquinone on Testosterone and LH levels in Sterptozotocine Induced Diabetic male Albino Rats.

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Abstract

Introduction - Thymoquinone (TQ) and unsaturated fatty acids are the main antioxidant components of Nigella Sativa. Nigella Sativa seeds has been used in traditional Iranian medicine as a natural remedy for promotes females menstruation, galactagogue, carminative, laxative and anti-parasitic properties. The present study is conducted to find out effect of Thymoquinone on testosterone and LH levels in streptozotocine induced diabetic male albino rats.

Materials and Methods -This work is conducted as part of Ph.D work under Department of Physiology, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Inistitution Animal Ethical committee are approved the work according to CPCSEA Rules. 18 rats were selected for this study and divided in to 3 groups each contains 6 rats, one group served as normal control, one group served as Diabetic control and one groups served as Treatment group with Thymoquinone(4mg/kg BW).

Results -Testosterone(ng/dl) level of Normal Control rats was 82.78±8.26, Diabetic rats was 41.62±7.28 and treated with Nigella Sativa rats was 76.30±8.23. LH(mIU/L) level of Normal Control rats was 0.46±0.12, Diabetic rats was 0.20±0.06 and treated with nigella sativa rats was 0.28±0.10.

Conclusion -Compared with normal rats the level of testosterone was decreased in diabetic rats, when it is treated with Thymoquinone the levels of testosterone increased significantly. Compared with normal rats the level of LH was decreased significantly in diabetic rats, when it is treated with Thymoquinone the levels of LH not significant with other groups.

Key Words – Testosterone, LH, STZ, Diabetes, Thymoquinone.

INTRODUCTION

Following an increase in free radicals, DNA damage and lipid peroxidation in human sperm may Occur in STZ induced diabetic rats. The insufficient vitamins intake can cause eleterious effects on spermatogenesis and production of normal sperm[1], the sufficient consumption of vitamins and natural antioxidants can protect sperm DNA from oxidative stress and improve male fertility[2]. Thymoquinone and unsaturated fatty acids are the main antioxidant components of Nigella sativa. Thymoquinone derived from Nigella Sativa can improve male fertility parameters through promoting antioxidant defence.

The sperm cell membrane includes a large amount of polyunsaturated fatty acids and phospholipids which are vulnerable to oxidative stress[3]. Oxidative stress has deleterious effects on the structure, function, motility and survival of sperm. Smoking, alcohol ingestion, infection, exposure to environmental toxins or radiation can trigger rising mitochondrial production of reactive oxygen species (ROS) and oxidative stress. Following a rise in free radicals, DNA damage, lipid peroxidation, protein and biomembrane damage in sperm may occur[4]. Ingredients with antioxidant properties can transfer electrons to oxidizing agents and inhibit free radical production and sperm damage[5]. Antioxidant components have been indicated to improve spermatogenesis and steroidogenesis[6].

Reactive oxygen species that belong to the class of free radicals are highly reactive oxidizing agents. Production of ROS in various tissues like testis is a common event, the abnormal increase in its synthesis could stimulate the DNA damage and oxidation of many cells. The sperm plasma membrane contains a high level of unsaturated fatty acids. Lipid peroxidation could lead to the damage of lipid matrix structure in spermatozoal membranes, and could be associated with impaired motility. Antioxidants are compounds which scavenge and decrease the synthesis of ROS and lipid peroxidation. Biological antioxidants include glutathione (GSH), catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide-dismutase (SOD) that have a very crucial role in scavenging of free radicals.

Therefore, ROS scavenger's application is likely to improve sperm quality[7,8].

Nigella sativa is a plant of the Ranunculaceae family. It grows widely in many Middle Eastern countries. Its seed is black in colour and bitter in taste. Nigella sativa has many different chemical ingredients including thymoquinones(30-48%), flavonoids, anthocyanins, alkaloids and essential fatty acids, particularly linoleic and oleic acid. It has been traditionally used for the treatment of different diseases such as respiratory and digestive disorders, kidney and liver dysfunction and rheumatism in different forms[9,10]. Thymoquinone has demonstrated some protective roles in relation to oxidative status, such as superoxide anion scavenger, direct cytoprotective effects and indirect antioxidant and androgen activities, it may protect sperm and semen against a testicular toxin[11]. The present study is conduted to see the effect of thymoquinone on testosterone and LH levels in diabetic induced male albino rats.

MATERIALS AND METHODS

Study design - This work is conducted as part of Ph.D work under Department of Physiology, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Inistitution Animal Ethical committee are approved the work according to CPCSEA Rules. 18 rats were selected for this study and divided in to 3 groups each contains 6 rats, one group served as negative control, one group served as Diabetic control and one groups served as Treatment group with Thymoquinone(4mg/kg BW), at the end of 45th day blood was collated and measured serum Testosterone and LH by kit method.

Thymoquinone – Thymoquinone purchaged from Sigma-Aldrich, Bangalore and administrated to rats through intraperitoneal injections(4mg/body Kg weight).

Streptozotocine – Induced diabetes -The rats were given Streptozotocine intraperitoneal injection 50mg/BW, Streptozotocine dissolved in ice-cold citrate buffer(PH 4.5). The diabetes was confirmed by measuring glucose by Code free Glucometer, the glucose level above 250mg/dl considered as diabetes, glucose levels were checked at regular periodical periods.

Results -Testosterone(ng/dl) level of Normal Control rats was 82.78±8.26, Diabetic rats was 41.62±7.28 and treated with Thymoquinone(4mg/KgBW) rats was 76.30±8.23. LH(mIU/L) level of Normal Control rats was 0.46±0.12, Diabetic rats was 0.20±0.06 and treated with Thymoquinone(4mg/KgBW) rats was 0.28±0.10(Table 1).

DISCUSSION

The testes, epididymis and other reproductive organs are structurally and physiologically dependent upon the testosterone and other androgens. Testosterone stimulates growth and secretary activity of the reproductive organs so a significant increase of these hormones could increase the number and function of somatic and germinal cells of testis and in results increase the testis and epididymis weight[12]. Thymoquinone is the major active component derived from Nigella sativa and many of the pharmacodynamic effects reported above for N. sativa are due to Thymoquinone [13]. Gokce et al [14] has been confirmed that Thymoquinone treatment has protective effects on testicular parameters. LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and indirectly stimulates spermatogenesis via testosterone. In our study the testosterone was decreased in diabetes induced rats and at the same time LH was also decreased, in thymoquinone treated rats the testosterone levels are increased significantly but LH levels were not significant change. Our results are in agreement with studies of Mukhalad AM et, al.[15], Gokçe A et.al.[16], Rahmatollah Parandin[17]. Thymoquinone, the major active constituent of Nigella sativa, could lead to decreased total antioxidant capacity and could prevent the increase in the myeloperoxidase Activity[14].

According to singh etal Thymoquinone can improve dyslipidaemia and antioxidant defence[18]. Wafai et al.'s study reported that Thymoquinone could suppress cyclooxygenase-2 enzyme expression and lipid peroxidation, and raise SOD levels in diabetic rats [19]. Zohra et al treated diabetic rats by adding 2% NS seeds to their diet for 30 days. This addition improved testosterone levels and testis tissue, semen quantity and mobility, and it reduced blood glucose and oxidative stress parameters[20]. According to studies of Sultan et al and Ahmad, reported that the antioxidant characteristics of Thymoquinone led to an improved antioxidant status in diabetic rats[21,22].

Nigella sativa and its main constituent, thymoquinone can improve sperm parameters, semen,Leydig cells, reproductive organs and sexual hormones in animal studies. The main potential mechanism is the antioxidant properties of Nigella sativa which play a key role in free radical scavenging. Although the previous studies findings suggest that Nigella sativa and thymoquinone are good candidates for male infertility treatment, to date there is insufficient evidence to make recommendations for its use as an adjunct therapy in infertile men. Based on the positive effects of thymoquinone of our study conclude that further research is to be done on humans for finding beneficial reproductive effect of thymoquinone in diabetic human individuals.

Table 1. One way results of Testosterone(ng/dl)and LH (mIU/L)

Parameter	Group 1 Normal Rats– Control	Group 2 Diabetic Rats– Control	Group 3 Diabetic Rats – Thymoquinone(4mg/KgBW)	F	P
Testosterone (ng/dl)	82.78 ±8.26[a]	41.62±7.28 [b]	76.30±8.23 [a,c]	33.625	.000
LH(mIU/L)	0.46±0.12 [a]	0.20±0.06 [b]	0.28±0.10 [a,b]	7.088	.000

The difference between groups P<0.05 considered as significant.

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