

Original Research Article

Effect of Nigella Sativa seed and Thymoquinone on Reproductive Parameters in Streptozotocine Induced Diabetic and Normal Male Albino Rats

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ABSTRACT

Introduction: In diabetes an increase in free radicals, DNA damage and lipid peroxidation in human sperm may occur. Antioxidant components in medicinal herbs such as Nigella sativa have been indicated to improve spermatogenesis and steroidogenesis. Thymoquinone 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 Nigella sativa seed and Thymoquinone on testosterone and LH levels in streptozotocine induced diabetic and normal male albino rats.

Materials and Methods: 36 rats were selected for this study and divided in to 6 groups each contains 6 rats, one group served as normal control, one group served as normal rats received the nigella sativa seed powder(300mg/Kg BW), one groups served as normal rats received the Thymoquinone(4mg/kg BW), one group served as Streptozotocine(50mg/kg BW) induced diabetic control rats, one group served as diabetic rats received the nigella sativa seed powder(300mg/Kg BW) and one groups served as diabetic rats received the Thymoquinone(4mg/kg BW). Testosterone and LH were estimated by Chemiluminosis(CLIA).

Results: There is no change in Testosterone and LH levels in normal rats treated with nigella sativa seed powder and thymoquinone. The levels testosterone and LH are decreased significantly in diabetic rats compared with normal rats, when the diabetic groups treated with nigella sativa seed powder and thymoquinone the Testosterone levels are normalised significantly(p<0.05).

Conclusion: Compared with normal rats the level of testosterone was decreased in diabetic rats, when it is treated with Nigella sativa seed and 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 Nigella sativa seed and Thymoquinone the levels of LH not significant with other groups.

KEY WORDS: Testosterone, LH, STZ, Diabetes, Thymoquinone, Nigella Sativa seed.

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INTRODUCTION

Seeds of *Nigella sativa* have been employed for thousands of years as a spice and food preservative. The oil and the seed constituents, in particular thymoquinone, have shown potential medicinal properties in traditional medicine. The *nigella sativa* seed used as a laxative, diuretic, treating infectious fever and local treatment for skin infections and wounds. *Nigella sativa* seed also used for hair full treatment, headache, ears pain, parturition diseases, toothache, digestive system disturbances, glands diseases, fraction healing, liver, spleen, and eye diseases [1-3]. The insufficient vitamins intake can cause deleterious effects on spermatogenesis and production of normal sperm [4], the sufficient consumption of vitamins and natural antioxidants can protect sperm DNA from oxidative stress and improve male fertility [5].

In diabetes the production of ROS is more common, Reactive oxygen species (ROS) 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; however, 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, this is the reason sperm plasma membrane is more liable to peroxidative damage. Lipid peroxidation could lead to the damage of lipid matrix structure in spermatozoal membranes, and this damage may lead to 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. ROS scavenger's application is likely to improve sperm quality [6,7].

Many cellular metabolites can cause an increase of the concentration of electrophilic radicals, which can react with oxygen giving rise to ROS. The main sources of free radicals include singlet-oxygen (1O_2), hydroxyl radical (OH) and H_2O_2 . ROS are normally generated in many vital metabolic processes for living cells including the

spermatozoa; however, marked generation of ROS produced by spermatozoa [7] or by the combinations of xenobiotics and immunosuppressive agents can induce the production of toxic lipid peroxides [8,9].

Cells exhibit defensive pathways using various antioxidants. The main detoxifying systems for peroxides are GSH and CAT. CAT is an antioxidant enzyme that destroys H_2O_2 which can synthesize a highly reactive OH. On participation of the glutathione redox cycle, GSH together with GSH-Px converts H_2O_2 and lipid peroxides to non-harmful products [9]. Phenolic compounds derived from pomegranate [10]. ROS are highly reactive molecules that can react with many intra-cellular structures, mainly unsaturated fatty acids and trans-membrane proteins. The oxidation of these molecules can produce disturbance in cellular membrane permeability. Spermatozoa are highly susceptible to peroxidative damage due to existence of high concentration poly-unsaturated fatty acids, which are responsible for regulation of sperm maturation, capacitation, acrosome reaction, spermatogenesis and membrane fusion, with low antioxidant capacity. Moreover, sperm lipid peroxidation could destroy the structure of the lipid matrix in spermatozoal membranes, accompanied with a rapid decrease of intracellular ATP that leads to decreased sperm viability, axonemal damage and increased mid-piece morphological defects, and it could dramatically finally inhibit spermatogenesis [11].

In previous studies 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 [12-16]. The present study is conducted to see the effect of *Nigella Sativa* seed powder and thymoquinone on testosterone and LH levels in diabetic induced male albino rats. The aim of present study is to observe the effect of *Nigella Sativa* seed and Thymoquinone effect on reproductive hormones in normal and streptozotocine induced diabetic rats.

MATERIALS AND METHODS

Study design: This work is conducted as part of Ph.D work under Department of Anatomy, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Institution Animal Ethical committee are approved the work according to CPCSEA Rules (BLDEU/Dept of pharmacology 603/13). The 36 rats were selected for this study and divided into 6 groups each contains 6 rats, 3 groups are normal rats out of that one group served as normal control rats, one group was treated with nigella sativa seed powder, one group was treated with thymoquinone. Other three groups were induced diabetic by streptozotocine out of that one group served as diabetic control, one group as diabetic treated with nigella sativa and one group served as diabetic rats treated with thymoquinone, at the end of 45th day blood was collated and measured serum testosterone and LH by manual method.

Plant material: Nigella sativa seeds were purchased from Safa honey & Co, Bangalore and grinded into fine powder [16] with piston and mortar with help of Bapuji pharmacy college, Davangere. Nigella sativa powder administered orally according to study of M. Murugesan [17].

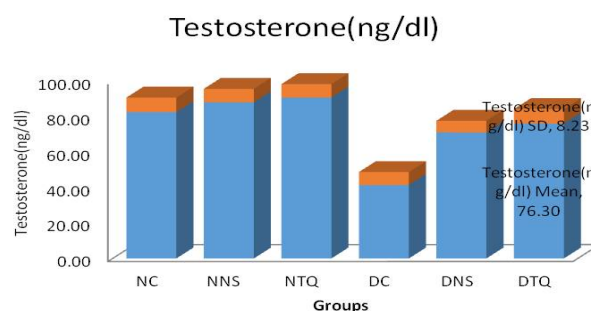
Thymoquinone: Thymoquinone purchased from Sigma-Aldrich, Bangalore and administered to rats through intraperitoneal injections (4mg/body Kg weight).

Streptozotocine – Induced diabetes: The rats were given Streptozotocine intraperitoneal injection 50mg/BW, Streptozotocine dissolved in icecold 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 every day morning.

RESULTS

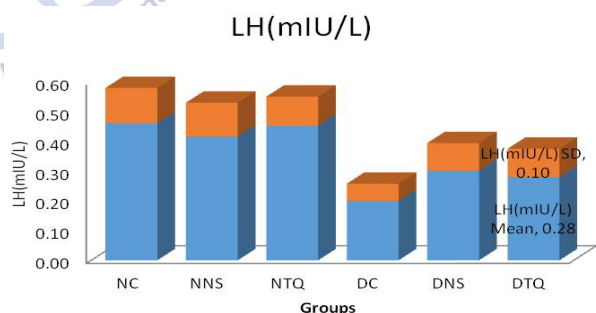
Testosterone (ng/dl) level of Normal Control rats was 82.78 ± 8.26 , normal rat treated with Thymoquinone rats was 91.08 ± 7.51 , diabetic rat treated with nigella sativa rats was 71.34 ± 6.58 . Diabetic control rats was 41.62 ± 7.28 , diabetic rat treated with Thymoquinone rats was 76.30 ± 8.23 , diabetic rat treated with nigella sativa rats was 71.34 ± 6.58 . LH(mIU/L) level of Normal Control rats was 0.46 ± 0.12 , normal rat treated with Thymoquinone rats was 0.45 ± 0.10 , diabetic rat treated with nigella sativa rats was 0.30 ± 0.09 . Diabetic control rats was 0.20 ± 0.06 , diabetic rat treated with Thymoquinone rats was 0.28 ± 0.10 , diabetic rat treated with nigella sativa rats was 0.42 ± 0.11 (Table 1).

Graph 1: Testosterone(ng/dl).



NC- Normal Control Rats, NNS- Normal rats treated with Nigella Sativa, NTQ- Normal rats Treated with Thymoquinone, DC- Diabetic Control Rats, DNS- Diabetic rats treated with Nigella Sativa, DTQ- Diabetic rats Treated with Thymoquinone.

Graph 2: LH(mIU/L).



NC- Normal Control Rats, NNS- Normal rats treated with Nigella Sativa, NTQ- Normal rats Treated with Thymoquinone, DC- Diabetic Control Rats, DNS- Diabetic rats treated with Nigella Sativa, DTQ- Diabetic rats Treated with Thymoquinone.

Table 1: One Way ANOVA Results of Reproductive Hormonal Profile (Testosterone, Progesterone, Oestrogen, FSH, LH).

Parameter	Groups							F	P
	Group A Normal Rats- Control	Group B Normal Rats - Nigella Sativa Seed Powder	Group C Normal Rats - Thymoquinone	Group D Diabetic Rats - Control	Group E Diabetic Rats - Nigella sativa seed powder	Group F Diabetic - Thymoquinone			
Testosterone (ng/dl)	82.78 ± 8.26^a	88.35 ± 7.66^a	91.08 ± 7.57^a	41.62 ± 7.28^b	0.28 ± 6.58^{ac}	76.30 ± 8.23^{ac}	33.625	0	
LH (mIU/L)	0.46 ± 0.12^a	0.42 ± 0.11^a	0.45 ± 0.10^a	0.20 ± 0.06^b	0.30 ± 0.09^b	0.28 ± 0.10^b	7.088	0	

Values with superscripts in each row among various groups are statistically significant with each other (P<0.05)

DISCUSSION

In our present study the testosterone and LH serum levels in normal rats treated with nigella sativa seed powder and thymoquinone not shown any significant change compared to normal control rats, in diabetic rats the testosterone and LH levels are decreased significantly compared with normal rats, when treated diabetic rats with Nigella sativa seed powder and thymoquinone the testosterone levels are normalised and there is no significant change in LH. In study of Rahmatollah Parandin found that Nigella sativa having enhancing effect on reproductive hormone profile [18]. In study of E.A. Datau, also observed that nigella sativa increased testosterone levels in treated groups [19].

In study of Farooq T found same results [20]. In previous studies showed that Nigella sativa treated rats are not shown any toxicity and shown significant change in body weight [21, 22, 23]. In a previous study shown that Nigella sativa increased the number of Leydig cells and its diameter nuclear in rat testes [24]. Testosterone has direct effect on the growth of testes, epididymis and other reproductive organ, structurally and physiologically dependent upon the testosterone. Testosterone stimulates growth and secretory activity of the reproductive organs it is proved that our study shows that testosterone levels are increased and testicular somatic index, germinal cells of testis and in results increase the testis and epididymis weight.

In previous studies it is well confirmed that, the reproductive hormone parameters in mammals are regulated by the two Gonadotropins, LH and FSH. FSH binds with receptors in the sertoli cells and directly stimulates spermatogenesis. 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 [25-27].

Therefore, a significant decrease in LH hormone concentration in diabetic group lead to decrease in testosterone level. Our study found that the FSH levels remained unaltered in diabetic treated rat. It is possible that testis seminiferous

tubules induced directly by the N. sativa extract or indirectly by testosterone and stimulated sperm counts. The present study shows that Nigella sativa and thymoquinone having the ability to increase number of Leydig cells in testis and increased testosterone levels.

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