MEDICAL CHANNEL

Original Article

EFFECT OF NIGELLA SATIVA ON HISTOMORPHOMETRIC CHANGES TREATED WITH DOXORUBICIN IN TESTIS OF ALBINO RATS

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ABSTRACT:

Objective: To find out the protective effect of Nigella Sativa (NS) on Doxorubicin (DOX) induced histomorphometric changes in male albino rat. **Methodology:** Forty Albino Rats (221 to 238 g), grouped into four. Group A = Control, Group B= Dox (3 mg/ kg bw/week, intraperitonealy) for 5 weeks, Group C= NS (1000mg/ kg bw, orally) daily and doxorubicin (3 mg/kg bw/week, intraperitonealy) for 5 weeks and Group D= NS (1000mg/kg bw, orally) throughout study. **Results:** Mean thickness of germinal epithelium (TGE) in group A was **63.289+2.234 µm.** Mean diameter (um) of seminiferous tubules (DST) in group A was **240.349+6.294 µm.** Mean (TGE) of group B was **33.641+2.561** and mean (DST) of group B was **175.759+5.518.** There were highly significant decreases in ($p < 0.001$) ($p < 0.0001$) (TGE) and (DST) respectively of group B when compared with group A. Mean (TGE) of group C was **59.067+1.890** and mean (DST) of group C was **228.083+4.576.** There were insignificant decreases in ($p < 0.1663$) ($p < 0.252$) (TGE) and (DST) respectively of group C when compared with group A. The data also showed highly significant increases in ($p < 0.007$) ($p < 0.001$) (TGE) and (DST) respectively when compared with group B. Mean (TGE) of group D was **73.411+1.549,** mean (DST) of group D was **266.951+6.883.** There were highly significant increases in ($p < 0.002$) and ($p < 0.048$) (TGE) and (DST) respectively of group D when compared with group A. There were highly significant increases in ($p < 0.0001$) and ($p < 0.0001$) (TGE) and (DST) respectively of group D when compared with the group B. **Conclusion:** NS increases DST and TGE of the Dox toxicated and normal rat.

Key words: Nigella sativa, Doxorubicin, Germinal epithelium, Semniferous tubules and Histomorphometric.

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INTRODUCTION

Nigella sativa seed are utilized broadly in conventional medicine of many countries. Many diseases are treated by various forms of Nigella sativa

because of its reported anti-viral, anti-inflammatory, anti schistosomiasis and immuno modulatory activities. It was found that Nigella Sativa extract not only had anti-tumor properties but also mitigates toxic side effects being caused by numerous chemotherapeutic drugs. (1) Nigella sativa increases the sperm count and motility in diabetic rats. (2) Extract of black seeds increase fertility potential by increasing level of testosterone and other reproductive hormones in albino rat. It also reduces free radicals in seminal plasma. (3)

Scientific Classification of Nigella sativa: Genus: Nigella, Species: sativa Order: Ranunculales, Division: Magnoliophyta, Kingdom: Planate and Family: Ranunculaceae (4). Nigella sativa plant is about 45 cm long. (5) Seeds are most valuable part of plant. (6) It is cultivated on dry soil between November to April and seeds take ten to fifteen days to germinate. Flowering and fruiting occurs during January to April. (7)

Chemical composition of Nigella sativa: During past few decades lot of work is done on phytochemical and pharmacological aspects of Nigella sativa because of its marked biological activities. (8) Eight fatty acid (Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid. linolenic acid and Eicosadienoic acid) are identified from solvent extraction of Nigella sativa representing about 99.5% of total faty acid. Thirty two compounds (consisted of six phenyl propanoid compounds (46.1%), nine monoterpenoid hydrocarbons (26.9%), four monoterpenoid ketones (6%), eight nonterpenoid hydrocarbons (4%), three monoterpenoid alcohols (2.7%) two sequiterpenoid hydrocarbons (1%) are identified from volatile oil of Nigella sativa.(9) Nigella sativa seeds also contains some sterols which include campesterol,stigmasterol and bsitosterol.(10) Powder form of seeds of Nigella sativa on hydro distillation with Clevenger distillation yielded 1.0_1.2% of essential oil with 30_32% of Thymoquinone.(11)

Cancer can be treated by many ways like chemotherapy, hormone therapy, surgery and radiation etc. (12) Ideally cancer patients are treated by team who coordinate themselves and give psychosocial, symptomatic support and treat the disease. (13) One third of patients of cancer are treated by local measures like surgery and radiation therapy. Systematic approach (Chemotherapy) along with surgery and radiation will require when there are early signs of micro metastasis. Fifty percent of cancer patients are treated out of which chemotherapy contribute about seventeen percent. (14) The chemotherapeutic agents act on the dividing cells, unfortunately these drugs can not differentiate between the cancerous cells and other normal cells, so damage both cancer cells and rapidly dividing normal cells. (15) Doxorubicin is used for the treatment of many types of cancer and can damage to normal tissues. According to some researchers it may causes apoptosis of germ cells. (16) The occurrence of infertility long after treatment with this anti-cancer drug is serious concern. (17)Toxicity of testis is one of the main toxic effects of combination and single agent chemotherapy, it occurs more commonly in male (90% post pubertal) compared to female.(18) It causes severe degenerative changes in spermatogonial cell layer and decrease diameter of seminiferous tubules.(19) It significantly decreases testis weight, testosterone level and sperm count.(20)

Researcher perceived that through using Doxorubicin the cancer is healed but the patient loses his reproductive ability. Thus; the patient suffers from another highly critical problem of his life even after the treatment. There is immense debate can be found on this matter to cure this side effects of doxorubicin but no one can explore the advantages and benefits of Nigella sativa.

MATERIAL AND METHOD;

This experimental study was conducted in the Anatomy department, in collaboration with the Department of Pathology B.M.S.I, JPMC, Karachi, after the approval of ethical committee J.P.M.C., Karachi. Forty albino rats about (221 to 238 g) 90-120 days were obtained from animal house J.P.M.C Karachi, originally obtained from Charles River Breeding Laboratories Brooklyn, Massachusetts USA, crossbreed at Animal house B.M.S.I JPMC Karachi.

One week before starting the study all albino rats were kept under observation to assess their health. Weights were recorded at the beginning of study. The standard laboratory chow and tap water were available at libitum.

All albino rats were separated into four groups each group consisted of ten animals.

Group –A = Control.

Group –B= Doxorubicin injection, (Pfizer pak) (3 mg/kg bw/week intraperitonealy) for 5 weeks. (21)

Group -C= Aqueous suspension of powdered Nigella sativa (1000mg/per kg bw orally) daily and Doxorubicin injection (3 mg/kg bw/week intraperitonealy) for 5 weeks.

Group -D= Aqueous suspension of powdered Nigella sativa (1000mg/per kg bw orally) daily throughout study.

TISSUE TREATMENT:

Bouin's fluid was selected as a fixative; testis was kept in fixative for whole day. On next day testis was sliced vertically into two equal halves. The tissue was kept in capsule, washed with running water in big beaker for three to four hours to remove excess fixative. For dehydration process, tissue was processed through ascending strength of alcohol (70 to 100 %) and cleared in xylene. The process of Infiltration and embedding carried out in Paraffin wax 4µm thick sections were obtained with the help of rotatory microtome and mounted on glass slides, set on hot plate at 32 to 40 degree centigrade and saved. For routine histological study the sections were stained with Heamatoxylin and Eosin (H&E).

MICROMETRY

Ten slides were selected randomly from each rat. Five fields were observed in each slide and in each field three oval or nearly oval tubules were selected. The stained slides were examined by light microscopy (magnification X10) Nikon eclipse 50 I microscope was connected to DS Camera control unit DS-L2. Digitalized micrometry was done; the dimensions were taken in x-axis and y-axis from basement membrane to basement membrane for diameter of semniferous tubules and from basement membrane to the last cell of spermatogonial layer for thickness of germinal epithelium.

PREPARATION OF NIGELLA SATIVA:

Black seeds (Kalvanji) purchased from local market of Karachi and re- identified from university of Karachi. Nigella Sativa seeds cleaned, freed of dust dried under shade, and crushed in a domestic grinder and prepared extract powder collected, and stored in cool place till use.

Injection **Doxorubicin** (Pfizer Pak) 50mg/25ml was purchased from Kousar medicos Karachi Pakistan.

STUDY DESIGN: This was an experimental Study.

STUDY PERIOD: The duration of study was five weeks

RESULTS

GROUP A (CONTROL)

Mean thickness of germinal epithelium was **63.289+2.234 µm** (Table 1).

Mean diameter (µm) of seminiferous tubules was **240.349+6.294 µm** (Table 3).

GROUP B (DOXORUBICIN TREARTED);

Mean thickness of germinal epithelium of seminiferous tubules was **33.641+2.561(µm)** (Table 1). There was highly significant decrease in thickness of germinal epithelium ($p < 0.001$) of group B when compared with group A.

Mean diameter (µm) of seminiferous tubules was **175.759+5.518 (µm)** (Table 3). There was highly significant decrease in diameter (µm) of seminiferous tubules (($p < 0.0001$) in group B when compared with group A.

GROUP C (DOXORUBICIN WITH NIGELLA SATIVA GROUP)

Mean thickness of germinal epithelium of seminiferous tubules was **59.067+1.890 (µm)** (Table 1). There was insignificant decrease in thickness of germinal epithelium ($p < 0.1663$) in group C when compared with group A. The data also showed there is a highly significant increase in thickness of germinal epithelium ($p < 0.007$) in group C when compared to with group B.

Mean diameter of seminiferous tubules was **228.083+4.576 (µm)** (Table 3). There was insignificant decrease in mean diameter of seminiferous tubules ($p < 0.252$) in group C when compared with group A. Data showed highly significant increase in diameter of seminiferous tubules ($p < 0.001$) in group C when compared with group B.

GROUP D (NIGELLA SATIVA TREATED)

Mean thickness of germinal epithelium of seminiferous tubules was **73.411+1.549 (µm)** (Table 1). There was highly significant increase in thickness of germinal epithelium ($p < 0.002$) of group D when compared with group A. The data showed highly significant increase ($p < 0.0001$) in group D when compared with group B. The data also showed highly significant increases ($p < 0.0001$) in group D when compared with group C.

Mean diameter of seminiferous tubules was **266.951+6.883(µm)** (Table 3). There was highly significant increase in the diameter of seminiferous tubules ($p < 0.048$) in group D when compared with group A. Data showed highly significant increase ($p <$

FIGURE 1

Comparison of mean thickness of germinal epithelium of seminiferous tubules in different groups of Albino rats

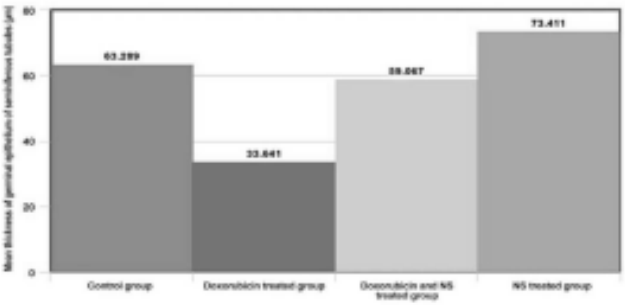


FIGURE 2

Comparison of mean diameter of seminiferous tubules in different groups of Albino rats

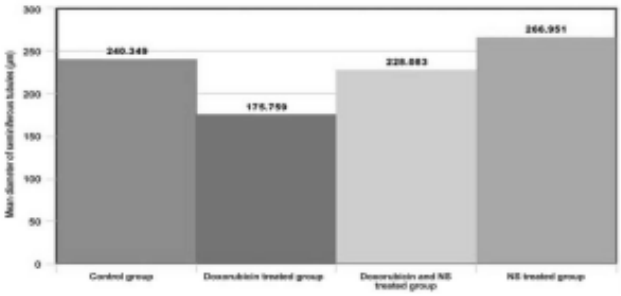
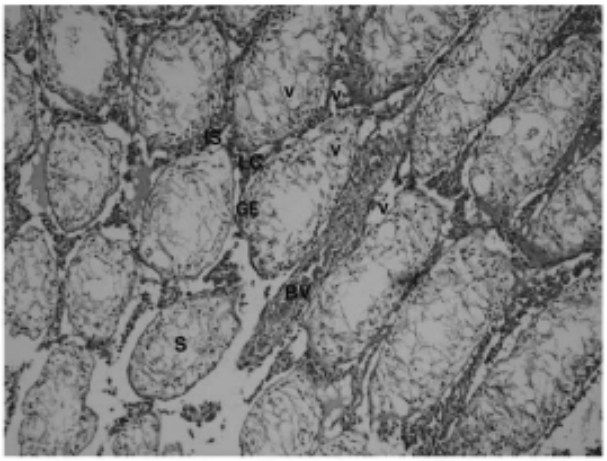
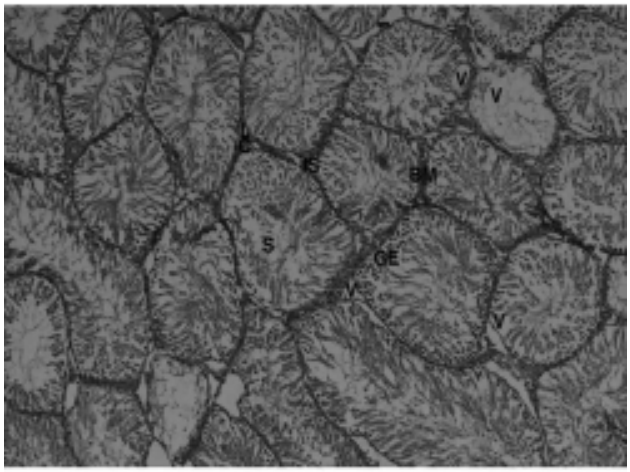


FIGURE 3



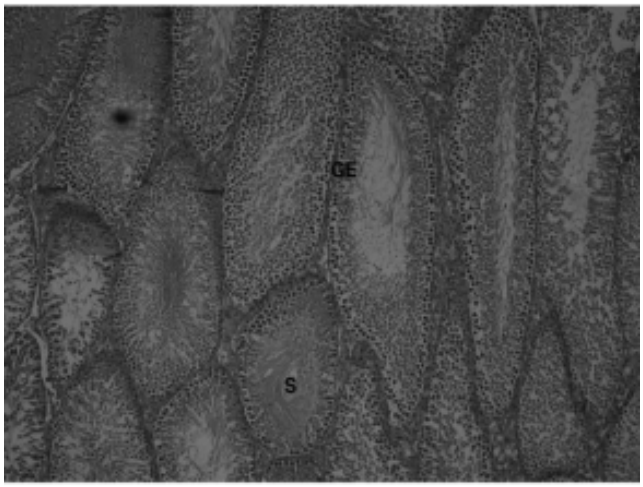
Photomicrograph of an adult rat testis, treated with Doxorubicin showing semniferous tubules (S), nearly devoid of Germinal Epithelium (GE) with vaculation (V) and blood vessels (BV), Leydig Cell (LC) and vaculation in the interstitial spaces (IS) (H&E 10x).

FIGURE 4



Photomicrograph of an adult rat testis, treated with Doxorubicin and Nigella sativa, showing nearly normal semniferous tubules (S) with regeneration of germinal epithelium (GE), completely bounded by basal membrane (BM), triangular interstitial spaces (IS) and leydig cells (LC) some vaculation(V) also seen in germinal epithelium and interstitial space (H&E 10x).

FIGURE 5



Photomicrograph of an adult rat testis, treated Nigella sativa, showing semniferous tubules (S) cut in various planes with enhanced mitotic activity in germinal epithelium (GE) than control (H&E 10x).

0.0001) in group D when compared with group B. The data also showed highly significant increase ($p < 0.002$) in group D when compared with group C.

DISCUSSION

Morphemetric study is very vital tool by which several faint differences those cannot be explored by simple histopathological analysis can be detected. [17]

Doxorubicin is an effective drug commonly used against diverse types of cancer and can cause damage to healthy tissues. According to some researchers this drug causes apoptosis of male germ cells.[16] It causes severe degenerative changes in germinal epithelium, and decreases the diameter of seminiferous tubules and germinal cell thickness.[19] It causes progressive and dose dependent deterioration in

Annexure:

TABLE 1

Comparison of mean thickness of germinal epithelium of seminiferous tubules in different groups of Albino rats

GROUPS (n=40)	**Treatment received**	**Thickness of GE**
A (n=10)	Control	63.289+2.234
B (n=10)	Doxorubicin	33.641+2.561
C (n=10)	Doxorubicin and NS	59.067+1.890
D (n=10)	NS	73.411+1.549

Where n is the number of albino rats
Data is presented as Mean+SEM (Standard Error of Mean)

TABLE 2

Stastical analysis of mean thickness of germinal epithelium of seminiferous tubules between various groups of Albino rats

Groups	**N**	**T**	**P-Value**
A & B	20	11.191	0.001**
A & C	20	1.4428	0.1663
A & D	20	-4.267	0.002**
B & C	20	-3.729	0.007**
D&B	20	13.2876	0.0001**
D&C	20	5.8699	0.0001**

Where n is the number of albino rats
t refers to t-test score
P<0.01 (**) highly significant P<0.05 (*) is s significant

TABLE 3

Comparison of mean diameter of seminiferous tubules in different groups of Albino rats

GROUPS (n=40)	**Treatment received**	**Diameter of ST**
A (n=10)	Control	240.349+6.294
B (n=10)	Doxorubicin	175.759+5.518
C (n=10)	Doxorubicin and NS	228.083+4.576
D (n=10)	NS	266.951+6.883

Where n is the number of albino rats
Data is presented as Mean+SEM (Standard Error of Mean)

TABLE 4

Stastical analysis of mean diameter of seminiferous tubules between various groups of Albino rats

Groups	**N**	**T**	**P-Value**
A & B	20	14.525	0.0001**
A & C	20	1.224	0.252
A & D	20	-2.283	0.048*
B & C	20	-5.411	0.001**
D & B	20	10.3371	0.0001**
D & C	20	4.7025	0.0002**

Where n is the number of albino rats
t refers to t-test score
P<0.05 (*) significant
P<0.01 (**) highly significant

histomorphometric and histological parameter.[17]

The seeds of Nigella sativa are used in conventional medicines since ancient time.[1] It improves spermatogenesis, testosterone and other reproductive hormones.[3] Nigella Sativa increases the germinal cell thickness and diameter of semniferous tubules.[22]

The present study was conducted on albino rats to observe the protective effects of Nigella sativa on doxorubicin treated testicular toxicity and effects of Nigella sativa on reproductive parameters of normal animals. In our study, group B received doxorubicin at the dose of 15mg /kg b/w in 5 divided doses at day 1, 7, 14, 21 and 28. [21], [23] & [24]

Our findings showed that the mean diameter of seminiferous tubules of Doxorubicin treated group was decreased as compared to control group, which is in agreement with the findings of Sallu et al., 2009,[25] who noticed reduction in tubular diameter and cross sectional area of seminiferous tubules of albino rats treated with single dose of Doxorubicin (10mg/kg b/w on different periods); and Yeh et al., 2009,[26] who reported reduced diameter of seminiferous tubules of albino rats treated with Doxorubicin (9mg/kg b/w, in three divided doses). Brilhante et al., 2011,[16] observed alteration of seminiferous tubules, leading to temporary and reversible or permanent infertility in prepubertal rats after using Doxorubicin (5mg/kg b/w and scarifying the animals on day 40, 64 and 127). This damage is supposed to be dose and age related and could be due to damage of sertoli cell and spermatogonia type A. Sastry, M.S. and Gotmare, V.V, 2014,[17] also reported that Doxorubicin decreases the diameter and cross sectional area of seminiferous tubules of wistar rats in dose dependent manner.

Our study also showed decrease in germinal epithelial cell thickness of Doxorubicin treated group (39.498 µm) as compared to control group (63.09 µm).

In group C there was improvement in the diameter and the germinal cell thickness of seminiferous tubules of albino rats, treated by Doxorubicin (3 mg /kg bw/week, intraperitonealy) for 5 weeks and Aqueous suspension of powdered form of Nigella sativa (1000mg/kg/ b/w day, orally). [1]In this group mean diameter of seminiferous tubules of doxorubicin plus Nigella sativa group was increased (Table 3) as compared to group B and germinal cell thickness of group C was also increased as compared to group B (Table 1). These results are in accordance with the study of Mohammad et al., 2009,[22], who established that Nigella Sativa (300mg/kg b/w for 60 days) increases the germinal cell thickness and diameter of semniferous tubules because it may act on pituitary gland and increase hormones of spermatogenesis. Cho et al., 2014,[27] reported improvement in germinal cell thickness and diameter of semniferous tubules of rats after being treated with Nicotine, with Nigella Sativa oil (6.0 uL/100gm bw, orally).

A remarkable aspect observed in this study is that; group D rats, treated only with aqueous suspension of powdered Nigella sativa 1000mg/kg b/w /day orally, showed mean diameter of their seminiferous tubules (**266.951+6.883µm)** (Table 3) higher than control group; and mean thickness of germinal epithelium (**73.411+1.549µm)** (Table 1) higher than control group. These findings are compatible with the findings of Rihab Galib and Mohammad AL- Zuhairy, 2012,[28] (0.3 ml crude oil of Nigella Sativa in albino rats).

CONCLUSION:

Our results suggest that Nigella sativa causes improvement of the doxorubicin mediated decrease in the diameter and germinal cell thickness of seminiferous tubules. Nigella sativa had multiple beneficial functions without any harmful results. Forgoing it is considered in future and advised to be administered in combination with doxorubicin in cancer patients to prevent doxorubicin mediated testicular toxicity. Furthermore according to our results, Nigella sativa improves diameter of seminiferous tubules and increases the germinal cell thickness in normal subjects. So it can be given in normal and infertile subjects for harmless improvement in the reproductivity.

AUTHORS INPUT

AK: Main Author **BS:** Supervise **SA:** Data collection **MA:** Data analysis**.**

CONFLICT OF INTEREST:

Authors declare that they have no competing interests in conducting this experimental study.

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