Original Article

Impact of Nigella Sativa on Weight of Testis & Body Weight in Doxorubicin Treated Albino Rats

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ABSTRACT

Objective: To measure impact of nigella sativa (NS) on weight of testis & body weight and doxorubicin treated albino rats.

Study Design: Experimental work

Place and Duration of Study: This study was conducted at the BMSI animal House, Karachi from 1st March 2017 to 4 April 2017 (total 35 days).

Materials and Methods: Experimental work was take place on 40 animals of 16 weeks old in the animal house for 35 days. Animals were separated into 4 sets, A1, B2, C3 & D4. A1 served as control, B2 receive Doxorubicin 3 mg/kg /7 days intraperitonealy, C3 receive Nigella sativa 1000mg/kg everyday orally along with Doxorubicin 3 mg / kg /7 days intraperitonealy and D4 receive extract of Nigella sativa 1000mg/kg everyday orally. After completion of experiment, animals were sacrifice and tissue material were well-preserved for staining.

Results: In B2 body weight was noticeably reduced, bute amended in C3 which were given Nigella sativa along with Doxorubicin.

Conclusion: This study reveals that Nigella sativa amended the weight reduction.

Key Words: NS(nigella sativa), TQ (thymoquinone), doxorubicin (Dox), folkoric, KU (Karachi university)

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INTRODUCTION

Doxorubicin is an anthracycline antibiotic derivative came in knowledge as a anticancerous agent in 1969 for the management of numerous cancerous tumours like breast carcinoma, osteosarcomas, soft-tissue sarcomas, lymphomas, alopecia and ovary carcinoma but usage is limited because it can destroy both healthy as well as cancerous cells thus causing numerous side effects like cardiac, renal and testicular injuries by stimulating production of free radicals and nitrogen varieties.[1-4]

DXR was initially filtered from Streptomyces species, extremely hydrophilic, associated with Nausea, vomiting, and heart arrhythmias.[5] Cancer is the topmost reason of death in the world after cardiovascular diseases.

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It also causes male infertility by gonadal impairment due to antineoplastic drugs. Testicular weight became reduced due to decreased sperm cell count and motility. First indication of genotoxic impairment is increased apoptosis of spermatogonia and spermatocytes due to oxidative stress to testis by destruction of lipids in cell membrane.[6-10]

It can also stimulates oxidative damage to mitochondria which indicates manifestation of transcription factor.[11] Management for cancer is surgical procedure, chemo, radioactivity, hormone remedy, immunotherapy, targeted remedy, and marrow replacement.[12] Local procedures of treatment like; surgery and radiation are more successful when cancer cells are not metastasized, however systemic approach (chemotherapy) is required along with local procedures when early signs of micro metastasis are appeared.[13]

Archaeologically, medicinal plants were used to obtain food and herbal medicines. Nigella sativa is derived from Latin word, nigellus. It is a member of Ranunculaceae family, used globally for the treatment of various ailments. It is strongly recommended in Tibb-e-Nabwi as a healing medicine for several illnesses such as upper respiratory disorders like bronchitis, asthma. It is commonly taken as liver tonic, digestive, anti-diarrheal, hunger stimulant and boost up immunity.[14,15] It is cytoprotective, inhibits cellular membrane oxidation, lipid peroxidation, free radicals neutralization, ROS suppression so it reduces serum lipids and increases body weight.[16] NS improve sperm counts, sperm

viability & motility, weight of testis and reduces sperm abnormalities.[17-19] It is helpful in hypotension, digestive friendly, nephrocurative, antioxidant, hypoglycemia, hypolipidemia &liver friendly.[20-22]

MATERIALS AND METHODS

The study was conducted in BMSI on 40 albino rats which were 16 weeks old originally obtained from Brooklyn Laboratories, USA, hybridize at animal house B.M.S.I. for assessment of their health they were retained under observation for 1 week. Body weights were noted earlier and after completion of study. The standard food & water were given in libitum. Rats were alienated into four sets, each comprised of ten rats. Nigella sativa seeds extract obtained from KU and injectable Doxorubicin 50mg/25ml was obtained from Pfizer.

A1 = Control.

B2= Doxorubicin injection 3 mg/1000g/7days intraperitonealy for 35 days

C3= crushed Nigella sativa (1g/1000g everyday orally) & injection Doxorubicin 3 mg / 1000g /7 days intraperitonealy) for 35 days.

D4= crushed Nigella sativa 1g/1000g everyday orally for 35 days.

Animals were observed daily for their wellbeing. After completion of research rats were sacrificed after taking final weight with the help of Sartorius balance. Rats were cut in midline from thoracic region to scrotum. The testes were visualized and examined carefully for any change in contour, color, hemorrhage. Testes were detached and weights were noted.

Relative weights of testes were calculated

$$\text{Comparative weight of testis} = \frac{\text{Mean weight of testis (mg)} \times 100}{\text{Ultimate weight of animal (gm)}}$$

RESULTS

Body Weight

A1: Group A1 animals mean initial weight was 221.30±11.56 & mean final weight was 267.76±13.45. There was substantial raise in the mean final body weight in A1 ($p<0.0174$) as compared to its initial body weight (Table 1).

B2: Group B2 animals mean initial weight was 238.10±11.68 & Mean final body weight was 200.75±4.51.There was highly substantial reduction in the mean final body weight of B2 ($p<0.008$) as compared to its initial body weight(Table1). There was highly substantial reduction in the mean final body weight in B2 ($p<0.0036$) as compared to A1 (Table 2).

C3: Group C3 animals mean initial weight was 231.80±10.93 & mean final body weight was 215.70±11.56. There was inconsequential reduction in the mean final weight ($p<0.324$) as compared to its initial weight (Table1). There was inconsequential reduction in the mean final body weight ($p< 0.1006$) as compared to A1. There was substantial raise ($p<0.0264$) in mean value of final body weight in C3 when compared with B2 (Table 2).

D4: Group D4 animals mean initial weight was 237.90±11.24 gm & mean final body weight was 281.43±12.56 gm. There was substantial raise in the mean final body weight ($p<0.0188$) as compared to its mean value of initial body weight (Table 1). There was inconsequential raise in the mean final body weight ($p<0.2789$) as compared to A1. There was highly substantial raise in the mean final body weight ($p<0.0001$) as compared to B2. There was substantial raise in the mean final body weight ($p<0.0012$) as compared toC3 (Table 2).

Absolute Testicular Weight

A1: In A1 animals mean absolute testicular weight was 1.653±0.098(Table 3).

B2: In B2 animals mean absolute testicular weight was 0.972±0.070. There was highly substantial reduction in the mean absolute testicular weight ($p< 0.0001$) in B2 as compared to mean absolute testicular weight in A1. (Table 3).

C3: In C3 animals mean absolute testicular weight was 1.423±0.021.There was inconsequential reduction in the mean absolute testicular weight in C3 ($p<0.0340$) as compared to the mean absolute testicular weight in A1 (Table 3). There was highly substantial raise in the mean absolute testicular weight in C3 ($p< 0.0001$) as compared to the mean absolute testicular weight in B2 (Table 3).

D4: In D4 animals mean absolute testicular weight was 1.907±0.041(Table 3). There was substantial raise in the mean absolute testicular weight in D4 ($p< 0.0279$) as compared to mean absolute testicular weight in A1. There was highly substantial surge in the mean absolute testicular weight in D4 ($p< 0.0001$) as compared to mean absolute testicular weight in B2 while highly substantial raise was also showed in the mean absolute testicular weight in D4 ($p<0.0001$) as compared to the mean absolute testicular weight in C3. (Table 3)

Mean Relative Testicular Weight

A1: In A1 animals, mean relative testicular weight was 617.344±29.96 (Table 4).

B2: In B2 animals, mean testicular relative weight was 484.184±20.44 (Table 4). There was highly substantial reduction in the mean testicular relative weight in B2 ($p<0.0017$) as compared to the mean testicular relative weight in A1. (Table 4).

C3: In C3 animals, mean testicular relative weight was 659.712±35.68. There was inconsequential raise in the mean testicular relative weight in C3 ($p<0.375$) as

compared to the mean testicular relative weight in A1. There was highly substantial raise in the mean testicular relative weight in C3 ($p<0.0005$) as compared to the mean testicular relative weight in B2 (Table 4).

D4: In D4 animals, mean testicular relative weight was 677.611±37.33. There was inconsequential raise in the mean testicular relative weight in D4 ($p<0.224$) as compared to the mean testicular relative weight in A1. There was highly substantial raise in the mean testicular relative weight in D4 ($p<0.0003$) as compared to the mean testicular relative weight in B2 and inconsequential raise in the mean testicular relative weight in D4 ($p<0.732$) as compared to the mean testicular relative weight in C3 (Table 4).

Table No.1: Evaluation of mean (Initial and Final) body weight in various Albino rats groups

SETS (n=40)	Management	Initial Weight	Final Weight	P-Value
A1	Control	221.30±11.56	267.76±13.45	0.0174*
B2	Doxorubicin	238.10±11.68	200.75±4.51	0.008**
C3	Doxorubicin and NS	231.80±10.93	215.70±11.56	0.324
D4	NS	237.90±11.24	281.43±12.56	0.0188*

n: number of albino rats Data: Mean±SEM P<0.05: significant
P<0.01: highly statistically significant

Table No.2. Evaluation of mean final body weight between various Albino rat groups

Sets	T	P-Value
A1 & B2	3.4393	0.0036**
A1 & C3	1.7305	0.1006
A1 & D4	1.1165	0.2789
B2 & C3	2.7144	0.0264*
D4 & B2	6.0456	0.0001**
D4 & C3	3.8506	0.0012**

T: t-test score P<0.01 (**) is highly statistically significant

Table No.3: Evaluation of mean absolute weight of testis in various Albino rat groups

Sets (n=40)	Management	Mean absolute weight of testis	Statistical comparison	P-Value
A1	Control	1.653±0.098	A1 & B2	0.0001**
B2	Doxorubicin	0.972±0.070	A1 & C3	0.0340
C3	Doxorubicin and NS	1.423±0.021	A1 & D4	0.0279*
D4	NS	1.907±0.041	B2 & C3	0.0001**
			D4 & B2	0.0001**
			D4 & C3	0.0001**

N: number of albino rats Data: Mean±SEM
P<0.05: statistically significant P<0.01: highly statistically significant

Table No.4: Evaluation of mean relative weight of testis in various Albino rat groups

SETS (n=40)	Management	Relative Weight	Statistical comparison	P-Value
A1	Control	617.344±29.96	A1 & B2	0.0017
B2	Doxorubicin	484.184±20.44	A1 & C3	0.375
C3	Doxorubicin and NS	659.712±35.68	A1 & D4	0.224
D4	NS	677.611±37.33	B2 & C3	0.0005
			D4&B2	0.0003
			D4&C3	0.732

n: number of albino rats Data: Mean±SEM P<0.01:highly statistically significant

DISCUSSION

Management of cancer comprises of surgical procedure, Radio & Chemotherapy. It is used for the management of numerous cancerous tumours but its usage is limited because it can destroy both healthy normal body cells too thus causing numerous side effects like cardiac, renal and testicular injuries by stimulating production of free radicals, reactive oxygen and nitrogen species.[1,2]

Nigella sativa belongs to the Ranunculaceae family and used globally as a therapeutic herb for the treatment of various ailments. It is strongly recommended in Tibb-e-Nabwi as a healing medicine for several illnesses such as upper respiratory disorders like bronchitis, asthma.[14,15]

The body weight of B2 animals in was decreased as compared to all other groups; due to anorexia and vomiting caused by doxorubicin as supported by.[1,2] Decrease in body weight is also reported by. [7] testicular weight was also became decreased as explained by [6]

C3 animals were looking active and healthy. Their appetite was good as compared to group B2 due to ameliorating effects of Nigella sativa. Our observations were supported by [14,15] who reported that Nigella Sativa decreases toxic side effects caused by several chemotherapeutic agents. Increase in testicular weight was also observed by [20-23]

CONCLUSION

Research determined that B2 animals had reduced body weight, absolute & relative wt of testis but in C3 animals we observe raise level of body weight, absolute & relative wt of testis as compared to B2. Therefore our hypothesis from this research work is that avoid the treatment of Doxorubicin and if mandatory don't use it without *nigella*, in order to minimize its harmful effects.

Author's Contribution:

Concept & Design of Study:	Ashok Kumar
Drafting:	Sadia Sundus, Mona Rani
Data Analysis:	Ashok Kumar, Saad Saleem
Revisiting Critically:	Ashok Kumar, Sadia Sundus
Final Approval of version:	Ashok Kumar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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