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Epididymal semen analysis in testicular toxicity of doxorubicin in male albino wistar rats and its amelioration with quercetin

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Abstract
A total of 48 male albino Wistar rats were randomly divided into 4 groups consisting of 12 in each group. Group 1-Control, Group 2-Doxorubicin treated @ 4mg/kg b.wt intraperitoneally weekly once. Group 3-treated with quercetin @ 80mg/kg b.wt, orally daily. Group 4-treated with doxorubicin @ 4 mg/kg b.wt, intraperitoneally weekly once and quercetin @ 80mg/kg b.wt, orally daily. Six rats from each group were sacrificed by cervical dislocation at fortnight intervals of experimental study and the abdominal cavity was opened through a midline abdominal incision to expose the reproductive organs. The cauda epididymis of one testis was excised to obtain the epididymal contents and semen analysis was done. There was significant increase in abnormal sperm count and significant decrease in sperm motility and sperm count in Group 2 animals as compared to other groups. Further sperm count was increased in Group 3 as compared to control Group 1 showing quercetin improves fertility in male animals.

Keywords: doxorubicin, quercetin, wistar rats, testis, semen analysis

Introduction
Doxorubicin is considered a very potent and efficient chemotherapeutic drug, it also kills healthy cells, especially those under rapid and constant proliferation. Therefore, DNA of rapidly dividing cells such as the testicular germ cells can be the preferential target of doxorubicin resulting in reproductive toxicity. Oxygen radical-induced damage of lipids inmembrane is the key factor for DOX-induced toxicity. The sperm DNA is exceptionally prone to damage from reactive oxygen species that may attack the integrity of DNA and accelerate the process of germ cell apoptosis [1].
(Agarwal *et al.*, 2003). Therefore, DNA damage may be liable for the increased level of abnormal spermatozoa. Besides, the anti-oxidant defence system in testicular tissue is inherently deficient in a potent component of the SOD (superoxide dismutase) family, Mn-SOD (SOD2) [2]. (Gu and Hecht 1997). Mammalian spermatozoon is particularly vulnerable to lipid peroxidation because of the molecular anatomy of its plasma membrane, the mammalian sperm cellspresent highly specific lipidic composition with high content of polyunsaturated fattyacids (PUFA), plasmalogenes and sphingomyelins [3] (Rao *et al.*, 1989). Free radicals in the testicular compartment generate oxidative stress leading tosperm DNA damage culminating in chromosome aberrations; sperm head abnormalitiesand germ cell depletion. On the other hand, in the presence of quercetin, the freeradicals are effectively scavenged and DNA damage is ameliorated. Quercetin has also been reported to show *in vivo* inhibitory effect against tertbutylhydroperoxideinduced lipid peroxidation in human sperm cells [4] (Moretti *et al.*, 2012).
This paper describes the changes induced due to DOX and it amelioration by quercetin.

Materials and methods
A total of 48 male albino Wistar rats were randomly divided into 4 groups consisting of 12 in each group. Group 1-Control, Group 2-Doxorubicin treated @ 4mg/kg b. wtintraperitoneally weekly once. Group 3-treated with quercetin @ 80mg/kg b.wt, orally daily. Group 4-treated with doxorubicin @ 4 mg/kg b.wt, intraperitoneally weekly once and quercetin @ 80mg/kg b.wt, orally daily. All the rats were provided with standard pellet diet and deionized water *ad libitum* throughout the experimental period. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (IAEC). Six rats from each group were sacrificed by cervical dislocation at fortnight intervals of experimental

study. The abdominal cavity was opened through a midline abdominal incision to expose the reproductive organs.

Epididymal Semen Analysis
The cauda epididymis of one testis was excised and placed in a sterilised petri dish containing 2 mL warm normal saline of 37 °C, and then, it was macerated by sterilised scissor to obtain the epididymal contents in a suspension that was handled exactly as the semen [5] (Hafez, 1970).

Sperm motility assay
A drop of the suspension was covered by pre-warmed glass cover slide at 37 °C and examined under high power 40x of light microscope for evaluating the individual motility of spermatozoa. Several microscopical fields were examined to evaluate the percentage of motile spermatozoa [6] (Slott *et al*., 1991).

Sperm count
An aliquot of the epididymal sperm suspension was used for spermatozoa count using Neubauerhaemocytometer, with WBC pipette the epididymal fluid was drawn up to '0.5' mark and semen diluting fluid (Sodium bicarbonate 5g, formalin 1 ml and 99 mldistilled water) was drawn up to '11' mark, mixed well. Charged the haemocytometer chamber and the sperms were allowed to settle. The sperm count was done under light microscope at 100x. The number of sperms in 1 ml of the fluid was obtained using the following formula;

$$\text{Sperm count} = \frac{\text{No. of sperms counted} \times \text{dilution factor} \times \text{depth factor}}{\text{No. of areas counted}}$$

Sperm morphology assay
Sperm-shaped abnormalities were observed in Rose Bengal stained smears; place 1-2 drops of stain and a very small drop epididymal sperm suspension (approx. 1/5th of the stain) on a microscopic slide mixed and this mixture was smeared out on slides and examined under oil immersion lens after drying. One hundred spermatozoa were randomly examined in different fields, to measure the percentage of abnormal spermatozoa.

Results
Epididymal Sperm Analysis
Sperm motility (%)
The mean values of sperm motility are summarized in the Table on 15th day the sperm motility was significantly ($P<0.05$) lower in Group 2 (47.50) when compared with Group 1 (86.67), Group 3 (81.67) and Group 4 (78.33). There was no significant difference in mean values between Group 1 (86.67),Group 3 (81.67) and Group 4 (78.33).
On 29th day the sperm motility was significantly ($P<0.05$) reduced in Group 2 (16.67) when compared with Group 1 (83.3) and Group 3 (79.17). Though there was no significant difference, the mean value of Group 2 (16.67) was lower compared with Group 4 (26.67).

Sperm count (10^6 /ml)
The mean values of sperm count are presented in the Table.
The mean values of sperm counts in Group 2 (11.41) was significantly ($P<0.05$) reduced when compared with Group 1 (63.44), Group 3 (51.25) and Group 4 (28.39) on 15th of experiment. On 29th day there was significant ($P<0.05$) reduction in mean value of sperm count in Group 2 (5.99) compared with Group 1 (69.90) and Group 3 (71.67). There was insignificant increase in mean value in Group 4 (15.58) compared with Group 2 (5.99).

Abnormal sperm morphology (%)
The mean values of abnormal sperm morphology are given in the Table.
The abnormal sperm morphology include sperm head bent at cephalocaudal junction (Fig. 1, amorphous head sperm (Fig. 2), sperm without hook (Fig. 3), tailless sperms (Fig. 4), bent (Fig. 5) and folded tail (Fig. 6). On 15th day there was significant ($P<0.05$) increase in mean value of abnormal sperm count in Group 2 (47.50) compared with Group 1 (23.50), Group 3 (17.83) and Group 4 (34.33). On 29th day the abnormal sperm count was increased in Group 2 (52.50) and Group 4 (50) compared with Group 1 (27.67). The mean value of abnormal sperm count was significantly ($P<0.05$) low in Group 3 (18) compared to Group 1 (27.67).

Discussion
Epididymal Sperm Analysis
Sperm motility
The sperm motility was significantly lower in Group 2 compared with control on day 15 and day 29 of experiment. Similar type of results was observed by Atesahin*etal*. [7], Sato *et al*. [8] and Jalali *et al*. [9]. The loss of sperm function is due to the peroxidation of unsaturated fatty acids in sperm plasma membrane, as a consequence of which the latter loses its fluidity and the cells lose their function. Sperm motility is an important functional measurement to predict sperm fertilizing capacity, any negative impact on motility would seriously affect fertilizing ability [10]. There was no significant difference in sperm motility among Group 1, Group 3, and Group 4 on 15th day of experiment. The sperm motility was restored to normal in Group 4 (DOX+QUER).On day 29 of experiment there was improvement in sperm motility in Group 4 compared with Group 2, though the values were not significant. The results were in accordance with the reports given by workers [11, 12] where quercetin was used against cisplatin and fenitrothion respectively. It is possible that the increased epididymal sperm quality might be a result of the antioxidant activity of quercetin on the epididymis [13]. The epididymis serves important functions in the transportation, maturation and storage of sperm cells, during which period the spermatozoa develop motility.

Sperm count
The sperm count was reduced in Group 2 compared with Group 1 on 15th and 29th day of experiment. The observations in Group 2 were similar to the results ofprevious reports [14-18]. The decreased sperm count clearly shows the elimination of sperm cells at different stages of development and points to free radical attack through DOX metabolism [9]. Increase in sperm counts was noticed in Group 4 compared with Group 2 on 15th and 29th day of experiment indicating quercetin administration alleviated the negative effect of DOX in present study.
Experimental Studies conducted by Khaki *et al*. [19], Aldemir*et al*. [11] and Altintas *et al*. [20] using quercetin as ameliorative agent against several xenobiotics revealed improvement in sperm counts.

Abnormal sperm morphology

The abnormal sperm morphology was increased in Group 2 compared with Group 1 throughout the experiment and majority of abnormalities included sperm head bent at cephalocaudal junction, amorphous head sperm, sperm without hook, tail less sperms, with bent and folded tail. The results were in accordance with findings of other workers [9, 15, 17]. The sperm morphological abnormalities possibly result due to the interference of doxorubicin with the DNA integrity and/or the expression of the genetic material indicating its genotoxicity to germ cells [15]. Less number of abnormal sperms were noticed in ameliorative Group (Group 4) compared with Group 2 on day 15 of experiment, these findings were in line with Aldemir *et al.* [11]. There was insignificant decrease in abnormal sperm morphology in Group 4 compared with Group 2 on day 29 of experiment. The abnormal sperm count was significantly low in Group 3 compared with Group 1 throughout the experimental period indicating protective role of quercetin.

Conclusion

In this Study Quercetin was found to have a significant protection against the DOX induced testicular toxicity in rodents. In DOX control group (GII) there was decrease in sperm count, motility and increase in sperm abnormalities whereas in DOX+Quercetin (G IV) the percentage of sperms with normal morphology are significantly very high.

Table 1: Mean sperm motility (%), sperm count and sperm morphology in different groups

Group	Day	Mean sperm motility (%)	Mean sperm count (106/ml)	Mean abnormal sperm morphology (%)
GI	15	86.67 ± 2.11a	63.44 ± 2.66a	23.50 ± 1.12bc
GII	15	47.50±3.59b	11.41 ± 1.45d	47.50 ± 4.44a
GIII	15	81.67±1.05a	51.25± 4.36b	17.83 ± 1.05c
GIV	15	78.33 ±3.33a	28.39 ± 1.01c	34.33 ± 6.07b
GI	29	83.33 ± 4.22a	69.90 ± 6.90a	27.67 ± 2.14b
GII	29	16.67 ± 2.47b	5.99 ± 0.78b	52.50 ± 0.50a
GIII	29	79.17± 5.83a	71.67 ± 7.04a	18.00 ± 4.49c
GIV	29	26.67 ± 1.67b	15.58 ± 2.30b	27.67 ± 2.14b

Values are Mean + SE (n = 6) One way ANOVA Means with different superscripts differ significantly (P<0.05)

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