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RESEARCH ARTICLE

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THE PROTECTIVE ROLE OF SPIRULINA ON DOXORUBICIN INDUCED GENOTOXICITY IN GERM CELLS OF RATS

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ABSTRACT

Doxorubicin is a potent antitumor agent used against many cancers. It has genotoxic effect on human non-tumor (normal) cells. Hence, it is very important to reduce its toxicity to the normal cells. This goal can be achieved by concurrent administration of free radical scavenging agents, like antioxidants. In the present study, adult male albino rats of Wistar strain are administered with Doxorubicin (3 mg/kg body weight, intraperitonially), once in a week for 35 days and Spirulina (250 mg/kg body weight, oral) daily prior to Doxorubicin administration. Doxorubicin treated rats showed a significant decrease in sperm count and increase in the sperm head abnormalities. However, the combined treatment of Spirulina with doxorubicin restores the sperm count and decreases the incidence of sperms with head abnormalities. This study indicates that administration of Spirulina prior to Doxorubicin might prevent the germ cell toxicity in male rats.

KEYWORDS

Doxorubicin, Spirulina, Germ cell toxicity, Genotoxicity, Antioxidants

INTRODUCTION

Genotoxicity refers to the capability of substances to damage DNA and/or cellular components regulating the fidelity of the genome [1]. The main problem posed by anticancer drugs is that they target not only the tumour, but also other cells, thus causing the same damage to both abnormal and normal cells [2].

Doxorubicin, an anthracycline antibiotic, is one of the most widely used anticancer drugs. The main anticancer action of Doxorubicin is believed to involve DNA damage through inhibition of topoisomerase II [3,4]. Doxorubicin causes the generation of free radicals and the induction of oxidative stress, associated with cellular injury [5]. The free radical generation by Doxorubicin may participate as cardiotoxicity and genotoxicity in normal human cells [3,4]. Studies show that Doxorubicin induces chromosome aberrations in murine bone marrow cells and in spermatocytes. Chromosome aberrations sustain in the surviving spermatogonial cells. Doxorubicin also increases the frequency of meiotic micronuclei in male rats, which showed impaired fertility after being treated [6].

Genotoxicity of doxorubicin will lead to genetic damage in germ cells. Generally male germ cells were more sensitive than female germ cells due to chemical exposure. Genotoxicity studies on rats showed decrease in weight of the genital organs, less number of sperms, low sperm motility, low implantation rate and decrease in number of live fetuses. Infertility is a common side effect of chemotherapy that bears a substantial impairment on the quality of life for young survivors of cancer [3].

Spirulina is 50-70% protein by weight and contains a rich source of vitamins especially vitamin B_{12}, β-carotene (provitamin A), vitamin E. It also contains carbohydrates like rhamnose, fructose, ribose, mannose and some minerals like copper, magnesium, zinc, potassium and iron. Beside γ-linolenic acid, it also contains a host of other phytochemicals that have potential health benefits. Spirulina contains phycocyanin (7% dry weight basis) and polysaccharides, both of them have antioxidant properties [7,8].
Previous studies have demonstrated that Spirulina exhibits antioxidant property in various oxidative conditions that cause tissue injury [9,10]. Spirulina treatment reduced the Cyclophosphamide induced testicular spermatogenic cell damage due to genotoxic effect, thereby showing its protective effect of germ cells [11]

The aim of this present study was to investigate the genotoxic effect of Doxorubicin and genotoxic protective effect of Spirullina on germ cells of male rats.

MATERIALS AND METHODS

Animals:

Male albino rats weighing between 170 and 220 g were used for the study. The animals were fed *ad libitum* with standard pellet diet and had free access to water.

Drugs and chemicals used:

Doxorubicin were purchased from V.S.Biotech, Chennai. Spirulina was obtained as gift sample from Antenna Nutritech. All other chemicals used were of analytical grade.

Experimental design:

Animals were randomly selected and divided into four groups with five animals in

each group. Doxorubicin (3mg/kg) was reconstituted with normal saline and administered intraperitonially. Spirulina (250mg/kg) was dissolved in normal saline and administered through oral route [12]. The volume of administration to each animal was 10ml/kg in all the cases. Animals were sacrificed by cervical dislocation 35 days after the last injection, following which the testes and epididymides were isolated.

Dosing schedule:

All groups of rats received dosing were follows,

Group 1 (control group) – Normal saline

Group 2-Spirulina (250mg/kg) daily

Group 3 (Positive control group)- Doxorubicin (3mg/kg) once in a week

Group 4- Spirulina (250mg/kg) daily followed by Doxorubicin (3mg/kg) on seventh day.

Epididymal sperm count:

Animals were sacrificed by cervical dislocation. The cauda epididymis was removed and placed in a normal saline. The epididymis was minced into small pieces to allow the sperms to swim out. The sperm suspension thus obtained was centrifuged at 1000 rpm for 5 min. After centrifugation, 1 ml of the supernatant was taken and the epididymal sperm count was determined using Neubauer's hemocytometer. Data were expressed as number of sperms per mg weight of epididymis [13].

Sperm Head Abnormality Test:

Animals were sacrificed by cervical dislocation. The cauda epididymis was removed and placed in normal saline solution. The epididymis was minced into small pieces to allow the sperms to swim out. The sperm suspension thus obtained was stained with 2% eosin solution and kept undisturbed for 1 hour. Smears were prepared using the above solution, air dried and fixed with absolute methanol for 5 min. 200 sperms per animal were examined for sperm head morphological abnormalities at 1000X magnification. Sperm head morphology was scored under the category of normal, sperm without hook, amorphous head, banana head and bent at cephalocaudal junction. Data were shown in terms of percentage of abnormal sperms [13].

Statistical analysis:

Statistical analysis were reported in terms of mean ± SEM. Difference between the groups was statistically determined by Student't' test . The average data generated for the group of rats treated with Doxorubicin (3mg/kg) were compared with our earlier reported respective data on Vehicle control group of rats treated with 0.9% NaCl (normal saline) at the rate of 1ml/100 g body weight. The average data generated from the group of rats treated with both Doxorubicin and Spirulina were compared with our earlier reported respective data on rats treated with Doxorubicin (Positive control) alone [14]. Statistically significant ($P < 0.001$).

RESULTS

Epididymal sperm count:

Doxorubicin (3mg/kg) treated group showed significant decrease in sperm counts as compared to control group ($P < 0.01$). Group that treated with Spirulina (250 mg/kg day) prior to Doxorubicin showed significant restoration in these sperm count as compared to control ($P < 0.02$) (Table 1).

Table 1

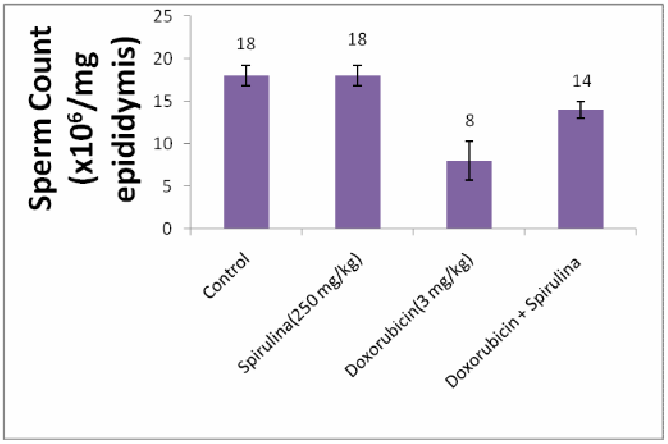
Effect of Doxorubicin alone and along with Spirulina on epididymal sperm count.

Groups	Sperm Count ($x10^6$/mg epididymis)
Control (0.9% saline)	18±1.2
Spirulina (250 mg/kg)	18±1.2
Doxorubicin (3mg/kg)	8±2.3*
Doxorubicin (3mg/kg) + Spirulina (250 mg/kg)	14±1.0**

*Values are expressed as mean ± SEM. Doxorubicin was compared with Control. Doxorubicin + Spirulina was compared with Doxorubicin.*P<0.01, **P <0.02.*

Fig 1

Effect of Spirulina administration on the Epididymal sperm count of Doxorubicin treatment.



Doxorubicin was compared with Control, P<0.01. Doxorubicin +Spirulina was compared with Doxorubicin P <0.02.

Sperm Head Abnormality:

Sperm head abnormalities were classified into amorphous head, banana shaped head, head without hook and bent at cephalocaudal junction. The sperm head abnormalities were increased from 53 to 150 within 35 days in the group of rats that received Doxorubicin (3mg/kg). A significant decrease in the percentage of abnormal sperms was observed in the group pre-treated with the Spirulina (250mg/kg) from 15% to 7.4% in comparison with the Doxorubicin treated group ($P < 0.001$) (Table 2).

Table 2

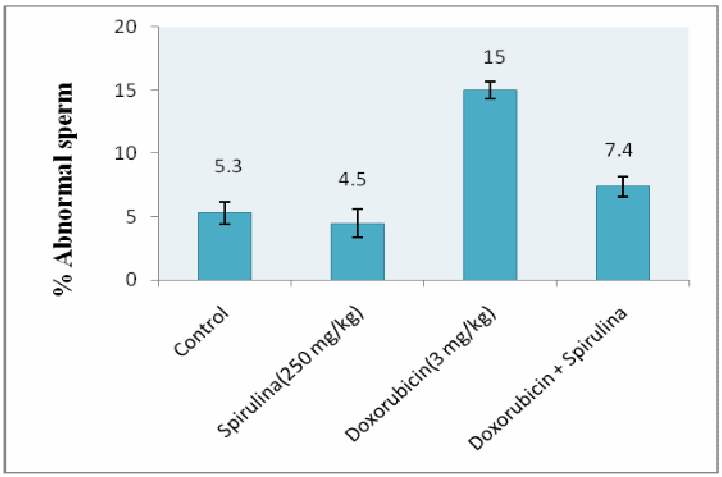
Effect of doxorubicin alone and along with Spirulina on Sperm Head Abnormalities.

Groups	Number of sperms examined/ number of animals	Normal sperms	Sperm Head Abnormalities				Total sperm head abnormality	%Abnormal sperm (mean ± SEM)
			Amorphous head	Banana shaped head	Head Without hook	Bent at cephalocaudal junction		
Control	1000/5	947	17	3	18	15	53	5.3±0.9
Spirulina (250 mg/kg)	1000/5	955	17	4	13	11	45	4.5±1.1
Doxorubicin (3mg/kg)	1000/5	850	41	23	59	27	150	15±0.7*
Doxorubicin (3mg/kg)+ Spirulina (250 mg/kg)	1000/5	926	24	5	27	18	74	7.4±0.8*

Values are expressed as mean ± SEM. Doxorubicin was compared with Control. Doxorubicin + Spirulina was compared with Doxorubicin. P <0.001.*

Fig 2

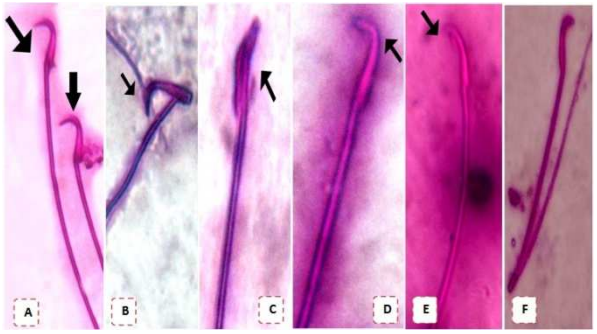
Effect of Spirulina administration on sperm head abnormalities expressed as of abnormal sperm.



Values are expressed as mean ± SEM. Doxorubicin was compared with Control. Doxorubicin + Spirulina was compared with Doxorubicin.* P <0.001.

Fig 3

Sperm head abnormalities -(A) Normal sperm; (B) sperm head bent at cephalocaudal junction; (C) amorphous head sperm; (D) sperm with banana shaped head; (E) sperm without hook; (F) sperm with bent tail.



DISCUSSION

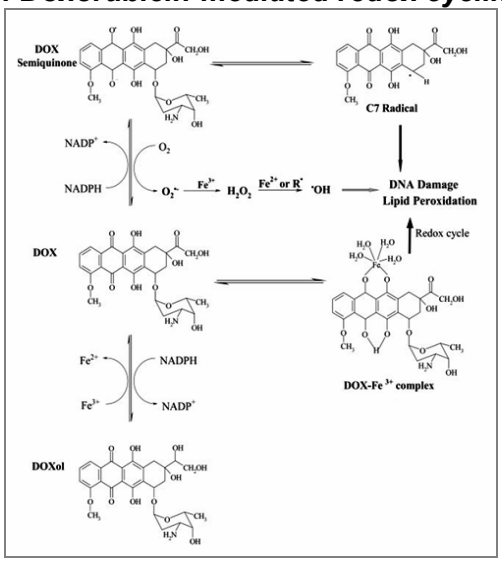
Doxorubicin is one of the widely used cytotoxic agents known to disturb spermatogenesis and testicular functions. It has been shown that Doxorubicin-induced cytotoxicity is mainly concentrated in early spermatogenic cells, which undergo rapid proliferation and differentiation [15]. It has been reported that Doxorubicin-induced testicular toxicity may be the consequence of oxidative stress [16,17,18].

Two different pathways of free radical formation by Doxorubicin have been described. The first implicates the formation of a semiquinone free radical [4,19,2]. The semiquinone radical can be transformed to a C7 radical that can also mediate cellular damage. The reduction of doxorubicin by two electrons generates a secondary alcohol metabolite, Doxorubicinol [21].

In the second pathway, Doxorubicin free radicals come from a non-enzymatic mechanism that involves reactions with iron. For example, Fe^{3+} reacts with Doxorubicin in a redox reaction, after which the iron atom accepts an electron and a Fe^{2+}- Doxorubicin free radical complex is produced [4,19,20]. This iron- Doxorubicin complex can reduce oxygen to hydrogen peroxide and other active oxygen species [4,22].

Fig 4

Illutration of Doxorubicin-mediated redox cycling [21].



Increased oxidative damage to sperm membranes, proteins and DNA is associated with lterations in signal transduction mechanisms that can be detrimental to male fertility [16,17,18]. Thus, the combination of the drug delivery together with a potent antioxidant may be the appropriate approach to reduce the toxic side effect of Doxorubicin [23].

Spirulina, blue green microalgae, has been used since ancient times as a source of food because of its high protein and nutritional value. The chemical composition of Spirulina indicates that it has phenolic acid, tocopherol and β-carotene, which are known to exhibit antioxidant properties [24]. Previous studies have shown that C-phycocyanin has the ability to chelate metals including free iron [25,26]. The cardioprotective effect of Spirulina could also be due to the scavenging of hydroxyl, peroxynitrite and chelating of free iron [12].

Spirulina treatment reduced the Cyclophosphamide induced testicular spermatogenic cell damage due to genotoxic effect, thereby showing its protectivbe effec on germ cells [11]. Spirulina extracts have been shown to inhibit buccal cancers in animal models and buccal squamous cell carcinoma [27]. Oral supplement of Spirulina prevents oral cancer in human. Spirulina was also reported to act as an antimutagen against Cyclophosphamide, Cisplatin and Mitomycin-C [12].

Furthermore, its chemopreventive efficacy against skin tumor has been proved. Toxicological studies of several spirulina species have not revealed any toxic effect during and after different acute, chronic and reproductive tests. The antigenotoxic effect of Spirulina on Cisplatin and urethane were proved [24].

Using micronucleus testing, it was shown that Spirulina significantly reduced both chromosomal damage and lipid peroxidation induced by Cyclophosphamide, mitomycin-C, Cisplatin and urethane in mice [10,24]. When applied to bone marrow cells, the test revealed that Spirulina is radioprotective in mice [28]. Finally, SP behaves as an anticlastogenic agent as shown by the Tradescantia bioassay [29].

In the present investigation, DOX was found to decrease the sperm count and increase the frequency of sperms with abnormal head. The sperm head abnormalities possibly result due to the interference with the DNA integrity and/or the expression of the genetic material. Administration of Spirulina prior to DOX was found to restore the sperm counts and decrease the incidence of sperms with head abnormalities.

From the results, it clearly demonstrated that intervention of Spirulina prior to DOX reduces the germ cell toxicity induced in rat by decreasing sperm head abnormalities, and increasing sperm count.

CONCLUSION

From these study it was concluded that Spirulina was potential candidate as a protective agent to Doxorubicin induced genotoxicity effect in germ cells. The combined treatment of Doxorubicin and Spirulina holds promise as a safe and effective chemotherapeutic strategy.

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