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RESEARCH ARTICLE

Protective effects of *Spirulina* microalgal extracts on Doxorubicin-induced cardiotoxicity in rats

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ABSTRACT

The current study was conducted to assess the cardio protective potential of *Spirulina* microalgal extract (SME) in male Wistar rat model of doxorubicin-induced cardiotoxicity. The study consisted of sixgroups, comprising of six rats in Group I and eight rats in Groups II to VI. Group I rats received PBS orally daily for five weeks, Group II to VI rats received doxorubicin (DOX) @ 3.5 mg/kg bw intraperitoneally twice a week from 3rd to 5th week of the experiment period. In addition to DOX, Group III received vitamin E @ 200 mg/kg and Selenium @ 400 µg/kg bw orally; Group IV, V and VI rats received SME @ 250 mg/kg; 500 mg/kg and 1000 mg/kg bw orally daily for five weeks, respectively. The study revealed a significant negative variation in feed and water intake, body weight, heart weight, hematological, biochemical and pathomorphological parameters of heart in Group II, when compared to Group I. The changes caused by DOX were alleviated in SME treatment groups in a dose dependent manner. Similarly, amelioration of DOX induced cardiotoxicity was also observed in vitamin E and selenium group. Histopathologically, the SME treatment groups showed substantial improvement in toxicity lesions ofthe heart compared to Group II rats. The cardio protective efficacy of SME @ 250 mg/kg was lower than that of vitamin E and selenium, while the 500 mg/kg SME group showed slightly better protection and 1000 mg/kg SME showed highest protection, surpassing the effects of vitamin E and selenium.

Keywords: Cardiotoxicity, Doxorubicin, *Spirulina* microalgal extract

INTRODUCTION

Cardiotoxicity is a pathological condition in which damage to the electric or muscular systems of the heart results in its malfunction. As it ages, the heart weakens and becomes less effective at pumping blood. Chemotherapy and/or radiotherapy may result in cardiotoxicity[1]. Doxorubicin (DOX) is an antibiotic anthracycline that was discovered in the early 1960s from the pigment-producing bacterium *Streptomyces peucetius* and used for more than 30 years to fight cancer; however, it is currently produced chemically[2].

Dogs receiving doxorubicin treatment have shown clinically relevant cardiotoxic side effects. The most researched idea for these effects is that Doxorubicin interacts with and releases iron from storage proteins, resulting in the production of reactive superoxide molecules or oxidative free radicals[3]. The occurrence of cardiotoxicity and nephrotoxicity linked to many antineoplastic medicines currently used to treat patients is causing oncologists increasing concern, as it has been noted that such persistent adverse effects may affect the long-term results of survivors. New targeted therapies as well as unique processes linked to conventional cytotoxicities have been described[4].

Spirulina platensis is a filamentous cyanobacterium (blue-green alga) that belongs to the Oscillatoraceae family. It is rich in proteins, lipids, carbohydrates and some vital elements like zinc, magnesium, manganese, selenium. Phycocyanin, β-carotene, tocopherol, γ-linolenic acid and phenolic compounds in *Spirulina* are responsible for the antioxidant activity[5]. *Spirulina* also contains an important enzyme superoxide dismutase that acts indirectly by slowing down the rate of oxygen radical generating reactions. The antioxidant properties of *Spirulina* and its capacity to scavenge hydroxyl radicals and to inhibit lipid peroxidation have attracted the attention of many researchers[6].

A lot of antioxidant combinations have been recommended as chemo-deterrents for DOX persuaded toxicity. *Spirulina*, besides inhibiting protein oxidation and lipid peroxidation, also inhibits reactive oxygen species induced apoptosis. The present experiment is intended to delineate the protective perspective of the *Spirulina* microalgal extract against DOX-prompted cardiac injuries in Wistar rats.

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MATERIALS AND METHODS

Animals

The study was conducted on 46 healthy adult male Wistar rats, weighing around 150 ± 20g, which were procured from CCSEA approved animal breeding facility, KCC BIO-LABS, Sy. No. 8/1, Tumakuru, Karnataka (Reg- 2066/PO/RcBiBt-S/Bt-L/19/ CCSEA dated 18th June 2019). All the rats were acclimatized to standard laboratory conditions at Small Animal House facility, Hassan for fifteen days prior to the initiation of the experiment and maintained at 25 ± 2^{O}C housing temperature and relative humidity of 50 to 70 per cent and to laboratory conditions of a 12-hour light/dark cycle throughout the study period and provided with regular standard pellet diet along with free access to deionized drinking water *ad libitum* throughout the course of the experiment. The animal care and handling were carried out according to the guidelines set by CCSEA. The study was approved by the Institutional Animal Ethical Committee (HVC/ IAEC/08/2023).

Preparation of drug and Mode of administration

Doxorubicin hydrochloride was procured from Khandelwal Laboratories Private Limited, Mumbai, India. It was injected intraperitoneally at 3.5 mg/kg body weight. Export grade twenty percent *Spirulina* platensis microalgal extract in liquid form was received from Department of Studies in Food Technology, Davangere University, Karnataka. It was used at the dose rates of 250, 500 and 1000 mg/kg body weightorally. Vitamin E and Selenium (Selvit-E®) were purchased from a registered chemist and administered orally at dose rate of 200 mg/kg and 400 µg/ kg, respectively, to the rats of Group III.

Experimental protocol

The study included six treatment groups comprising six rats in Group I and eight rats in each Group II to VI. Group I served as normal control and received Phospate buffered saline at 5 ml/kg/day orally throughout the experiment; Group II disease control rats received DOX at 3.5 mg/kg body weight, intraperitoneally, twice a week for three weeks (from 3rd week to end of 5th week); Group III rats along with DOX treatment received vitamin E (200 mg/kg) and Selenium (400 µg/kg) orally daily for five weeks; Group IV rats along with DOX treatment received SME at 250 mg/kg bw daily by oral gavage for five weeks; Group V rats along with DOX treatment received SME at 500 mg/kg bw daily by oral gavage for five weeks; while Group VI rats along with DOX treatment received SME at 1000 mg/kg bw daily by oral gavage for five weeks.

Parameters assessed

Body weight (g), feed (g) and water (ml) consumption were assessed every week. At the end of the study (36th day), all rats were sacrificed humanely by using overdose of Ketamine and Xylazine (as I/M injection) and subjected to detailed post-mortem examination. Blood was collected from retro-orbital plexus in EDTA and serum vitals for hematological and biochemical analysis, respectively.

Histopathological analysis

Table 1. The mean (±SE) body weight (g) and heart weight (g) values of rats of different experimental groups in the study.

Groups	Day 0	1st week	2nd week	3rd week	% change	4th week	% change	5th week	% change	Absolute weight of Heart (35th day)	% change
Group I (n = 6)	223.00±14.01^{A}	233.16±9.82^{AB}	240.33±8.93^{ABC}	253.16±9.28^{BC}		256.00±11.28^{BCb}		263.16±10.24^{Cc}		0.82±0.03^{b}	
Group II (DC, n = 8)	230.00±9.53^{A}	238.50±9.58^{A}	246.50±9.61^{A}	231.62±7.67^{AB}	-8.50	214.37±6.40^{BCa}	-16.26	197.25±5.74^{Ca}	-25.04	0.66±0.02^{a}	19.51
Group III (RC, n = 8)	229.75±8.00^{ABC}	240.62±7.67^{AB}	246.75±7.42^{A}	240.62±5.71^{A}	3.88	223.25±6.10^{BCa}	4.14	210.87±6.71^{Cab}	6.90	0.75±0.05^{ab}	13.63
Group IV (SME 250, n = 8)	232.12±7.97^{ABC}	245.00±7.62^{A}	252.00±7.04^{A}	238.62±4.99^{AB}	3.02	219.75±6.80^{BCa}	2.50	215.50±6.47^{Cab}	9.25	0.68±0.05^{ab}	3.03
Group V (SME 500, n = 8)	231.25±7.90^{ABC}	244.75±7.03^{AB}	251.50±6.90^{A}	239.62±5.18^{AC}	3.45	227.62±7.60^{BCa}	6.18	218.62±10.06^{Cab}	10.83	0.72±0.02^{ab}	9.09
Group VI (SME 1000, n = 8)	236.12±7.54^{AC}	253.87±8.35^{A}	262.62±8.05^{B}	249.62±7.57^{AC}	7.77	238.50±9.01^{ACb}	11.25	231.12±9.91^{Cb}	17.17	0.76±0.05^{ab}	15.15

Mean ± SE bearing different superscripts (abc; Within column and ABC; Between columns) are statistically significant at $p < 0.05$

Table 2. The mean (±SE) values of various hematological parameters of rats in different groups on final day (35th day) of the study.

Groups	Hb (g/dl)	% change	TEC (10^6/µl)	% change	TLC (10^3/µl)	% change	PCV (%)	% change	Platelet (10^3/µl)	% change
Group I (NC, n = 6)	13.92±0.33^c		6.21±0.60^c		8.91±0.32^b		43.94±0.29^c		541.33±53.90^b	
Group II (DC, n = 8)	8.41±0.32^a	-39.58	3.92±0.20^a	-36.87	4.08±0.47^a	-54.20	22.19±0.66^a	-49.49	292.37±22.06^a	-45.99
Group III (RC, n = 8)	10.77±0.49^b	28.06	5.11±0.31^{bc}	30.35	5.46±0.87^a	33.82	33.84±1.12^b	52.50	373.37±40.63^a	29.70
Group IV (SME 250, n = 8)	10.70±0.31^b	27.22	5.15 ±0.36^{bc}	31.37	5.75±0.42^a	40.93	33.86±1.92^b	52.59	391.62±32.01^a	33.94
Group V (SME 500, n = 8)	10.49±0.43^b	24.73	4.96±0.33^{ab}	26.53	5.22±0.70^a	27.94	30.93±1.75^b	39.38	357.5±48.15^a	22.27
Group VI (SME 1000, n = 8)	10.98±0.47^b	30.55	5.27±0.40^{bc}	34.43	5.64±0.54^a	38.23	34.11±1.54^b	53.71	351.12±41.52^a	20.09

One way ANOVA with Duncan's post hoc test (SPSS); Mean values with different superscript differ significantly at $p < 0.05$

Representative heart tissue from rats of all the groups were subjected to histopathological studies. The tissue was fixed using 10 per cent Neutral Buffered Formalin solution and sections were prepared using paraffin blocks and stained with hematoxylin and eosin, Masson'strichrome and phosphotungstic acid haematoxylin after dewaxing[7].

Histopathological scoring for heart

The heart samples were examined in random microscopic areas semi-quantitatively under high power fields and the number of changes was assessed by counting twenty non overlapped fields for the same slide of each animal. The extent of damage and the severity of lesions in the heart were assessed semi-quantitatively[8] with slight modification as follows; Score 0 - No abnormalities detected; Score 1-1 to 15% of the examined fields revealed histological alterations; Score 2 - 16 to 30% of the examined fields revealed histological alterations; Score 3 - 31 to 60% of the examined fields revealed histological alterations and Score 4 -> 60% of the examined fields revealed histological alterations.

Statistical analysis

Statistical analysis of the data collected for various parameters was done using one-way ANOVA with Duncan's multiple-range test and two-way ANOVA with Bonferroni post hoc test (p<0.05)[9].

RESULTS

General observation

Group I rats remained healthy and active throughout the period of experiment. Group II rats exhibited clinical signs such as weakness, dullness, depression, anorexia, reduced body weight and water intake, diarrhea, ruffled hairs, dehydration, arched back after 2nd dose of doxorubicin. The rats of Group III to VI manifested similar clinical signs as those of disease control rats, but with reduced intensity and frequency.

Feed and water consumption

Mean weekly feed and water consumption in Group I rats was normal to progressive. Group II rats showed statistically significant decrease (decrease of 42.1%, 71.45%, 79.17% in feed consumption and 10.63%, 43.15%, 45.96% in water consumption during 3rd, 4th and 5th week respectively) after 2nd dose of doxorubicin treatment as compared to Group I. Mean weekly feed consumption in Group V and VI rats significantly increased by 28.39%, and 31.26% than Group II rats during third week. Animals in the remaining treatment groups did not show any significant increase in mean weekly feed consumption as compared to Group II rats. Mean weekly feed consumption in Group IV, V and VI rats was significantly increased by 28.57%, 29.87% and 25.33% than Group II rats during 5th week. Though there was no significant difference in mean weekly water consumption in Group IV to VI rats during third and fourth week of experimental period, they showed slight improvement compared to Group II rats.

Body weight and organ weight

The mean body weights and heart weight in grams with standard error of mean at different time intervals of 0 day and 1st, 2nd, 3rd, 4th and 5th week of the experiment have been presented in Table 1. Group

I rats remained healthy and active throughout the period of experiment and demonstrated steady and progressive enhancement in their body weight over the course of experiment. Body weight of Group II rats decreased significantly from 4th to 5th week in comparison to normal control rats. The animals of Group VI in the present study showed reduction in the body weight from 4th to 5th week but in a slower manner as compared to Group II. Group II showed a significant decrease in absolute weight of heart in comparison to Group I rats, where as in Group IV, V, III and VI, heart weight was slightly higher from Group II.

Hematology parameters

The mean values of hematological parameters with

Table 3. The mean (±SE) values of LDH AND CK-MB in various experimental groups on final day (35th day) of the study.

Groups	LDH (U/L)	CK-MB (U/L)
Group I (NC, n=6)	278.58±28.60^{a}	631.36±53.13^{a}
Group II (DC, n=8)	881.25±51.32^{c}	915.80±41.05^{b}
Group III (RC, n=8)	562.76±46.57^{b}	706.32±34.82^{a}
Group IV (SME 250, n=8)	511.24±38.96^{b}	713.72±17.59^{a}
Group V (SME 500, n=8)	527.54±23.09^{b}	721.24±22.34^{a}
Group VI (SME 1000, n=8)	504.66±25.85^{b}	708.12±27.16^{a}

One way ANOVA with Duncan's post hoc test (SPSS); Mean values with different superscript differ significantly at $p < 0.05$

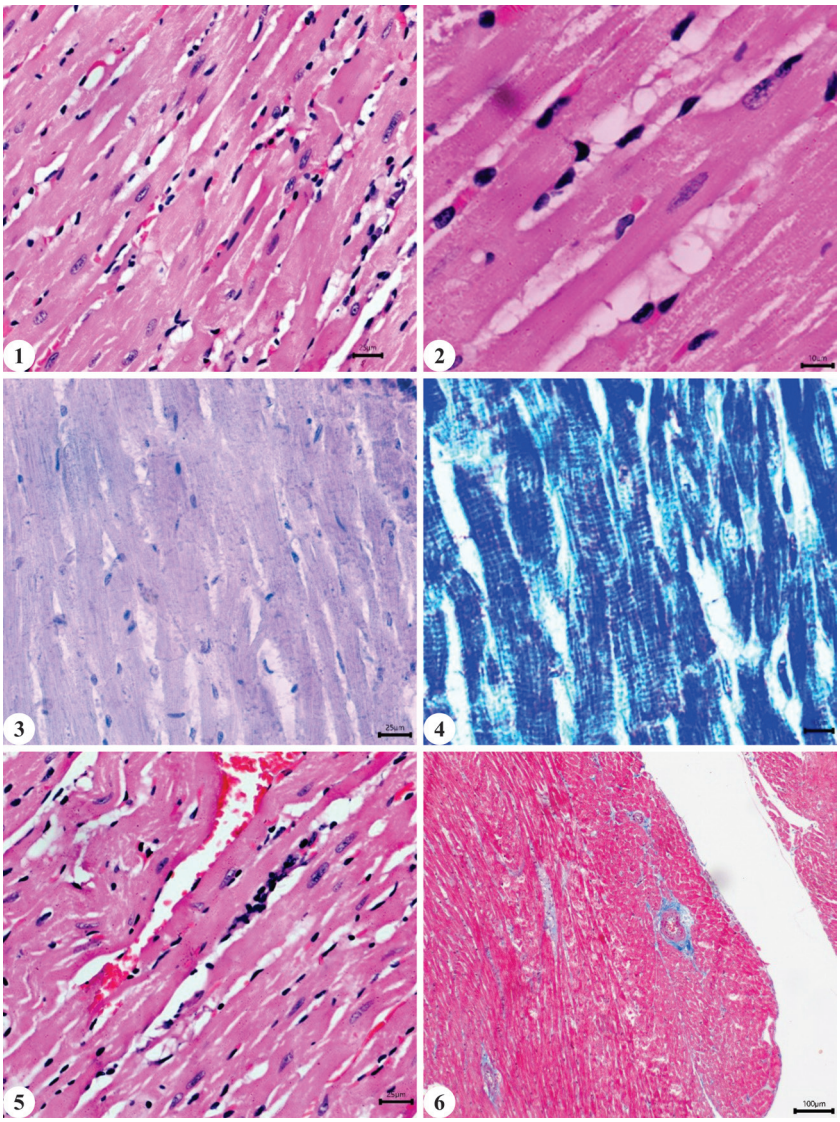

Fig. 1. Heart from disease control (Group II) rat showed increased sarcoplasmic eosinophilia with loss of cross striations along with macro-vacuolisation (H&E X400); **Fig. 2.** Higher magnification of heart from disease control (Group II) rat showing macro vacuoles separating the myocytes fiber bundle. Also absence of cross striations and nucleus in few fibers (H&E X1000); **Fig. 3.** Heart from disease control (Group II) rat showing loss of cross striations as compared to Group I rat (PTAH X400); **Fig. 4.** Heart from normal control (Group I) rat showing normal cross striations (PTAH X1000); **Fig. 5.** Heart from disease control (Group II) rat showing moderate congestion and infiltration of inflammatory cells (H&E X400); **Fig. 6.** Heart from disease control (Group II) rat showing moderate degree of interstitial and peri-vascular fibrosis (MT X100).

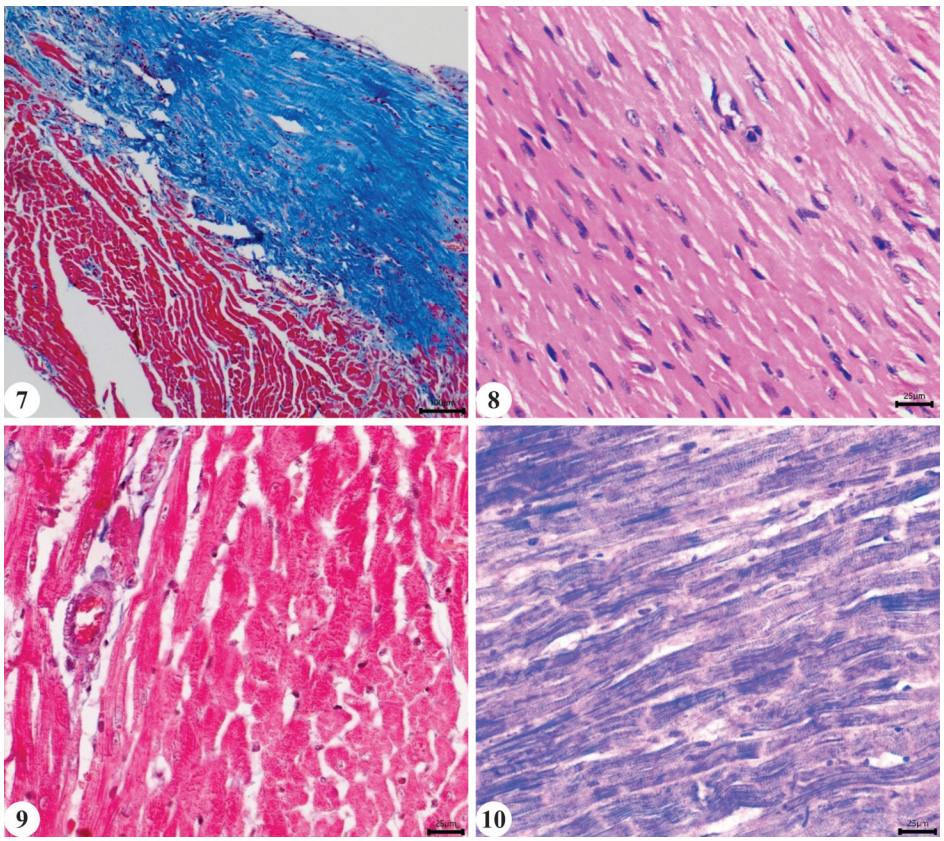

Fig. 7. Heart from disease control (Group II) rat showing thickened pericardial layer with blue staining collagen fibers (MT X100); **Fig. 8.** Heart from Group VI (SME 1000) rat showing reduced sarcoplasmic eosinophilia with normal feature of nucleus and reduced granularity as compared to Group II rats (H&E X400); **Fig. 9.** Heart from Group VI (SME 1000) rat showing reduced peri-vascular fibrosis in comparison to Group II rats (MT X400); **Fig. 10.** Heart from Group VI (SME 1000) rat showing improved appearance of cross striations in comparison to Group II rats (PTAH X400).

standard error of mean on 35th day of the experiment is presented in Table 2. The mean values of all the hematological parameters such as Total erythrocyte count (TEC), Haemoglobin (Hb), Packed Cell Volume (PCV), Total leucocyte count (TLC) and Platelets of Group II rats were significantly decreased when compared to Group I rats. The mean values of TEC in Group III, IV and VI; the mean values of Hb and PCV in Group IV to VI rats were increased significantly as compared to Group II disease control rats. The mean TEC values of Group V rats, TLC and Platelets values of Group III to VI rats though not significantly different was slightly increased as compared to Group II rat.

Serum Biochemistry

The mean values with standard error of mean on 35th day of the experiment is presented in Table 3.

Lactate dehydrogenase (LDH)

There was a significant (p<0.05) increase in serum LDH levels of Group II rats by 216.33% than Group I rats. Among the treatment groups, there was no significant difference in mean serum LDH levels. The mean values of serum LDH levels of Group III, IV, V and VI rats were significantly decreased (p<0.05) than Group II rats.

Creatine Kinase - Myocardial Band (CK-MB)

There was a significant (p<0.05) increase in serum CK-MB levels of Group II rats by 45.05% than Group I rats. The mean values of serum CK-MB levels of Group III, IV, V and VI rats were significantly decreased (p<0.05) in comparison to Group II rats and were comparable to that of Group I rat.

Gross pathology

The rats from the disease control group (Group II) showed mild degree of ascites and pleural effusion; blood mixed contents were observed in the stomach and intestine of three out of eight rats. The heart was pale and rounded and kidney appeared pale with cortico-medullary haemorrhages in comparison to Group I rat. The heart of Group III to Group VI rats did not reveal any visible gross abnormalities.

Histopathology

The heart of the disease control group (Group II) showed histopathological changes in the myocytes histology where in most of the cardiac myocytes exhibited moderate to severe disorganization and discontinuity with increased sarcoplasmic eosinophilia, wavy appearance of myofibres, loss of cross striations

Table 4. The mean (±SE) HP score of heart of rats in different groups on final day (35th day) of the study.

Groups	Swollen myocardial fibers	Granular appearance of myofiber	Pyknotic nucleoli	Macro-vacuoles formation	Haemorrhage between myofibers	Wavy myofibers
Group I	0.13±0.08^{a}	0	0	0	0.13±0.08^{a}	0.06±0.06^{a}
Group II (DC, n = 8)	3.05±0.18^{d}	3.30±0.31^{d}	3.35±0.18^{e}	3.05±0.18^{d}	3.05±0.32^{d}	2.55±0.18^{c}
Group III (RC, n = 8)	2.70±0.31^{bc}	2.90±0.31^{bc}	2.95±0.18^{d}	2.75±0.29^{bc}	2.55±0.18^{c}	1.95±0.29^{b}
Group IV (SME 250, n = 8)	2.95±0.18^{cd}	2.95±0.18^{c}	2.85±0.22^{cd}	2.95±0.18^{cd}	2.45±0.29^{c}	1.95±0.22^{b}
Group V (SME 500, n = 8)	2.60±0.42^{b}	2.85±0.22^{bc}	2.65±0.18^{cb}	2.70±0.31^{bc}	2.35±0.22^{bc}	1.95±0.29^{b}
Group VI (SME 1000, n = 8)	2.65±0.18^{bc}	2.65±0.18^{b}	2.55±0.18^{b}	2.50±0.25^{b}	2.15±0.18^{b}	1.85±0.18^{b}

One way ANOVA with Duncan's post hoc test (SPSS); Mean values with different superscript differ significantly at $p < 0.05$

with granular appearance and pyknotic nuclei. Most of the myocytes were swollen, highly eosinophilic with condensed nuclei or with absence of nucleus. In multiple areas, the myocytes bundles were widely separated with macro-vacuoles. A mild to moderate degree of interstitial mononuclear cellular infiltration, oedema, haemorrhage and congestion were noted. A mild to moderate degree of fibrosis in between myocardial fibers and periarterial fibrosis was noticed in a few areas (Fig. 1 to 7).

In the present study, the Group III revealed microscopical changes similar to that of disease control rats but with mild improvement. The heart of the treatment control group (Group IV to VI) showed mild to moderate improvement in histopathological changes in the myocytes where in very few cardiac myocytes exhibited disorganizationas compared to Group II rat with mild degree of eosinophilia, loss of cross striations and a mild degree of granular appearance of myocytes. Very few of the myocytes were slightly swollen with pyknotic nuclei. The macro vacuoles formation was reduced as compared to Group II rats. A mild degree of interstitial mononuclear cellular infiltration with mild degree of haemorrhage and congestion were noted (Fig. 8 to 10). Among the treatment group, Group VI (SME 1000) showed comparatively less histopathological alterations in cardiac muscle when compared to Group IV (SME 250) and Group V (SME 500).

Histopathological score of heart

The mean HP severity score of all sectioned heart samples was assessed for lesions like swollen myocardial fibers, granular appearance of myofiber, pyknotic nucleoli, macro-vacuoles formation, haemorrhage between myofibers and wavy myofibers. The mean HP severity score values of heart with standard error of mean have been presented in Table 4. The mean HP score of Group II disease control rats for each of the recorded lesion was significantly (p<0.05) higher than mean HP score of Group I normal control rats. The mean HP score of Group III to VI were significantly (p<0.05) lower than mean HP score of Group II disease control rats.

DISCUSSION

Doxorubicin not only has a direct impact on the heart but also an indirect influence resulting from the augmentation of hemodynamic flow changes or thrombotic events[10]. DOX induced enhanced generation of ROS may directly damage mitochondria thus altering the synthesis of proteins associated with the mitochondrial electron transport chain and *Spirulina* has antioxidant properties and have capacity to scavenge hydroxyl radicals to inhibit lipid peroxidation[6].

Doxorubicin caused severe systemic illness in the rats including anorexia, reduced body weight and water intake, diarrhea, progressive physical exhaustion after the second dose of doxorubicin, persisting until the end of the study. Our findings of doxorubicin induced reduction in heart weight are in accordance with reports of previous workers[11-13]. DOX induced cardiotoxicity related decrease in hematological parameters like TEC, Hb, PCV, TLC and Platelets were similar to those previously reported[14-15]. This was linked to various factors such as disrupted RBC production leading to anemia and leukopenia[16] and bone marrow suppression caused by doxorubicin[17]. It has been documented that myelo suppression represents the primary dose-limiting toxicity of Doxorubicin[18]. Doxorubicin notably induces marrow depression resulting in peripheral blood leukopenia (granulocytopenia), thrombocytopenia and potential anaemia[19]. Additionally, bone marrow depletion, depression, or hypoplasia have been frequently observed in rats[20] which likely may be due to the rapid division of bone marrow cells owing to their high growth fraction[21].

A significant rise in the mean values of serum LDH and CK-MB was observed. This finding aligns with previous findings[15,22-23]. It has been previously highlighted the significance of these biochemical markers in detecting cardiac injury resulting from DOX-induced cardiotoxicity[23-24]. The cardiac damage likely resulted from increased permeability or rupture of cell membranes, leading to the leakage of cytosolic enzymes into the bloodstream and subsequent elevation of their serum concentrations. The release of these markers into the bloodstream is consistently linked to DOX-induced damage to the myocardial cell membrane[25]. These results are supported by earlier studies that demonstrated DOX administration caused cardiac injury and loss of functional integrity, as seen by markedly elevated serum levels of CK-MB, ALT, AST, and LDH[26].

The examination of heart tissue from rats in the disease control group (Group II) revealed extensive damage characterized by moderate toxicity lesions affecting cardiac myocytes. The myocardial fibers displayed diverse levels of damage, from loss of striations to complete necrosis and fragmentation, appearing granular, with some exhibiting focal necrosis and eosinophilia in the cytoplasm[23]. Additionally, myocyte wavy degeneration, interstitial haemorrhages, and interfibrillar congestion were observed, along with disruption and separation of cardiac muscle fibers and infiltration of mononuclear inflammatory cells, predominantly lymphocytes. These findings were documented in earlier studies and were opined to be due to toxic metabolites of Doxorubicin[22,25,27].

In the present study, a mild to moderate degree of fibrosis in between myocardial fibers and peri-arterial fibrosis was noticed. The DOX treated rat's heart stained with Masson'strichrome stain revealed fibrotic scarring in cardiomyocytes and in the surrounding interstitial tissues. This may be attributed to an increase in pro-fibrotic factor *TGF-β1*, the extracellular matrix content collagen I and the cardiac fibrosis-associated protein α-smooth muscle actin in DOX-treated rats[28].

It's also suggested that DOX induces cardiotoxicity and myocyte damage by triggering lipid peroxidation, which is influenced by increased levels of mitochondrial iron and cellular reactive oxygen species[29]. The sarcoplasmic vacuoles observed could be attributed to enlargement of sarcoplasmic reticula and terminal cisternae leading to altered intracellular water and electrolyte distribution[30]. Edema, both perivascular and interstitial, accompanied by vascular changes like dilated blood vessels and retracted endothelial cells, observed were consistent with prior research[29-30]. It was theorized that edema results from disruptions in cardiac electrolytes, as DOX increases sodium and calcium content in the cardiac ventricle during induced cardiotoxicity in rabbits[30-31].

Improvement in treatment groups (Group IV, V, VI) may be attributed to the antioxidant and anti-inflammatory properties of *Spirulina* microalgal extracts. This restoration could be attributed to the presence of antioxidants like β-carotene and C-phycocyanin in *Spirulina*. The findings suggest that *Spirulina* may shield cardiac myocytes from oxidative stress induced by DOX. Other previous research had also demonstrated the efficacy of *Spirulina* extract in countering free radical-induced lipid peroxidation caused by lead and in offering protective effects on major organs, including the heart[32]. It is widely acknowledged that C-phycocyanin, a key biliprotein in *Spirulina,* possesses notable antioxidant and radical scavenging properties[33].

The current research showed that among the treatment groups, notably, cardiac lesions in rats from Group VI were less severe compared to those in other treatment groups and the disease control group. The cardio protective effect of *Spirulina* in the present study suggests that *Spirulina* microalgal extract (SME) can potentially be used as a therapeutic agent or as a supplement in treatment of heart ailment.However, further clinical and mechanistic investigations are needed to further validate its efficacy.

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