Original Research

Cardiotoxicity Induced by Doxorubicin in Wistar Rats and its Amelioration with Ascorbic Acid and *Spirulina*

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Abstract

The study was designed to evaluate the cardiotoxicity of Doxorubicin in Wistar rats and amelioration by ascorbic acid and spirulina. Female rats were divided into four groups. Animals were slaughtered at the end of the experiments (28th day) and collected blood for analysis. The biochemical assays showed significant (P<0.05) increase in creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), cardiac troponins (cTn) in Doxorubicin treated rats. There was considerable amelioration in ascorbic acid and spirulina groups. Grossly, heart revealed severe congestion, endocardial haemorrhages. Histopathological changes in heart of doxorubicin group revealed severe disruption, separation, degeneration, necrosis, fragmentation of muscle fibers with endocardial haemorrhages, edema, and focal infiltration of lymphocytes. The ameliorative groups 3 and 4 showed mild to moderate improvement. The tissue enzymes assays revealed a significant (P<0.05) increase in TBARS and significant (P<0.05) decrease of GSH and SOD activities in group 2. The ameliorative groups 3 and 4 showed mild to moderate improvement.

Key words: Amelioration, Ascorbic Acid, Cardiotoxicity, Doxorubicin, *Spirulina*, Wistar Rats

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Introduction

There are many classes of drugs and among them Doxorubicin comes under antibiotic class of anticancerous drugs which is derived from algae (Arcamone *et al.*,1969). It is very active against a wide spectrum of cancers and is mainly used in the treatment of lymphomas and leukemias (Blum *et al.*,1974 and Billingham *et al.*, 1978). Doxorubicin induces acute and chronic toxicity and produces a broad range of physiological, haematological and biochemical dysfunctions resulting in reduced performance and death. Cardiotoxicity

is a worrisome side effect of Doxorubicin and appears clinically as a dose-related cardiomyopathy with heart failure (Bristow *et al.,* 1978). It causes myelosuppression and dose-dependent, reversible leukopenia and/or granulocytopenia (neutropenia) and it affects heart, kidney and liver but mainly it causes cardiotoxicity with congestive heart failure, depressed cardiac function and finally cardiac failure (Bristow *et al.,* 1978). Many others reported that the Doxorubicin caused dose related toxicity through free radical formation (Maini, 2000 & Maria Volkova and Raymond Russell, 2011) a major mechanism of adriamycin cardiotoxicity resulting cardiac DNA or membrane damage (Roy *et al.,* 1999 and Sailaja Rao *et al.,* 2011). The damage caused due to oxidative stress can be prevented to a larger extent by the use of antioxidants (Naidu *et al.,* 2002). Vitamin C and E, carotene, bilirubin, glucose, glutathione peroxidase, catalase, superoxide dismutase, transferrin and ceruloplasmin are known to act as protectors against free radical damage (Khan *et al.,* 2005; Khan *et al.,* 2006 and Rock *et al.,* 1996). Herbal drugs have gained importance in recent years and numerous plants and algae are claiming to be having cardioprotective action of virtue of their antioxidant properties (Belay, 2002 and Chularojmontri *et al.,* 2005). The pathophysilogical changes following in Doxorubicin administration in rats are comparable to those taking place in human (Deman *et al.,* 2001). In this context the present investigation was undertaken to study the cardioprotective effects of vitamin C and *Spirulina* in Doxorubicin induced cardiotoxicity.

Materials and Methods

Experimental Animals

Female Wistar rats (weighing 150-200g) and feed were procured from National Centre for Laboratory Animal Sciences, National Institute of Nutrition (NIN), Hyderabad and the experiment was carried out according to the guidelines and prior approval of Animal Ethics Committee. Animals were placed in solid bottom polypropylene cages in the laboratory animal house and they were allowed to acclimatize for about 20 days. The animals were fed on *ad libitum* feed and water throughout the experiment.

Experimental Design

Animals were divided into four groups consisting of 6 in each group. The experimental study was designed as follows for the period of 28 days: Group 1-Control, Group 2-Doxorubicin – Toxic control @ 2mg/kg body wt. by intravenous injection for 5 days followed by weekly once for 2 weeks., Group 3-Pretreatment with ascorbic acid @ 500mg/g feed by enteral route for 7 days followed by intravenous injection of Doxorubicin as mentioned in group 2. Ascorbic acid supplementation was continued during these 19 days, Group 4-Pretreatment with *Spirulina* @ 1000mg/kg feed by enteral route for 7 days followed by intravenous injection of Doxorubicin as mentioned in group 2. *Spirulina* supplementation was continued during these 19 days.

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Chemicals

Adriamycin (Doxorubicin hydrochloride) was obtained from Pfizer, Canada which was manufactured by Pharmacia, Italia, Italy. *Spirulina* was obtained from Parry nutraceutical Pvt. Ltd., Mumbai, India. Ascorbic acid as L-Ascorbic acid was obtained from S.D. Fine-Chem Limited, Mumbai, India. Other chemicals and reagents were obtained from Qualigens Pvt. Ltd., Mumbai, India.

Methods

Blood samples were collected and serum was separated and stored under refrigeration. Above samples were subjected to biochemical assays by using the standard kits procured from Qualigens and Span Diagnostics and analysed by UV/Vis spectrophotometer (Tech Comp UV 7500; Tech Comp Ltd. Kowloon Bay, Hongkong). Trophonin, CPK and LDH by using standard kits supplied by Chema diagnostics, Italy. Heart weights were recorded and compared with body weights as Heart weights & body weights ratio. One gram of tissue sample with 10 ml of 0.2M Tris HCl buffer (pH 7.2) was taken in a tissue homogenizer to get a 10% homogenate. In that homogenate tissue protein (Lowry *et al.*, 1951), thiobarbituric acid reacting substances (TBARS) (Balasubramanian *et al.*, 1988), reduced glutathione (GSH) (Moron *et al.*, 1979) and activity of superoxide dismutase (SOD) (Madesh and Balasubramanian, 1998) were estimated. The data was subjected to statistical analysis by applying one-way ANOVA. Differences between means tested using Duncan's multiple comparison test and significance was set at $P<0.05$. Hearts from different groups were collected, fixed in 10% neutral buffered formalin prior to processing. After overnight washing in running water and dehydration in ascending grades of alcohol, the tissue was embedded in paraffin and 5-micron thick sections were cut and stained with haemotoxylin and eosin (H & E) as per the method of Luna (1968) and examined under light microscope for the tissue changes.

Results and Discussion

Heart Weight/Body Weight Ratio (1x 0.001 g)

Heart weights (g) were taken immediately after sacrifice; heart weights (g) and heart weight / body weight ratio (1× 0.001g) were significantly ($P<0.05$) reduced in doxorubicin treated group (Group 1). The heart weight and heart weight / body weight ratio (1× 0.001g) was also reduced in ameliorative groups 3 and 4 but this reduction was moderate in comparison to group 2. In the doxorubicin-treated group, heart weights and Heart weight / Body weight ratio were significantly decreased compared to control. The adverse effect on body weight might be due to disruption of basal metabolism due to their toxic effect especially on heart tissue. These findings are in accordance with the reports of Kalender *et al*. (2001) and Kozluca *et al*. (1995) In group 3 and 4 there was moderate reduction in the heart weights and it was lower than group 1. This indicated that there was moderate ameliorative effects of ascorbic acid and *Spirulina* on heart weights.

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These findings are in accordance with the reports of McKee and Harrison (1995) and Wattanapitayakul *et al*. (2005).

Serum Biochemical Profile

The values indicated that CPK, LDH and troponins in group 2 were significantly (P<0.05) higher than group 1; groups 3 & 4 which were ameliorative groups (Table 1).

Table 1: Sero-biochemical parameters in different groups of rats

Groups	HW/BW Ratio (1x 0.001 g)	cTn (mg/dl)	CPK (U/L)	LDH (U/L)
Control	3.20 ± 0.16 ^{c}	14.95 ± 0.28 ^{a}	103.12 ± 5.83 ^{a}	214.19 ± 3.86 ^{a}
Doxorubicin	2.70 ± 0.07 ^{a}	50.75 ± 0.54 ^{d}	310.29 ± 26.61 ^{d}	669.33 ± 39.71 ^{d}
Dox + Ascorbic Acid	2.84 ± 0.09 ^{a}	42.99 ± 0.67 ^{c}	263.08 ± 18.13 ^{c}	455.15 ± 49.64 ^{c}
Dox + *Spirulina*	3.01 ± 0.06 ^{b}	37.57 ± 0.60 ^{b}	186.54 ± 7.21 ^{b}	375.94 ± 12.63 ^{b}

Means bearing common superscripts do not differ significantly (P<0.05)

Between groups 3 & 4, group 4 showed slightly lower CPK, LDH and troponins levels than group 3 (P<0.05). The values in control group (group 1) were significantly (P<0.05) lower in comparison to all other groups. The present study revealed that significant (P<0.05) increased in CPK, LDH and troponins activity doxorubicin toxic control. The increased activity of CPK, LDH and troponins might be due to the effect of toxic metabolites of Doxorubicin which bound to cellular macromolecules in the heart causing damage and necrosis which made to discharge of intracellular contents into the systemic circulation and also free radicals generated or due to the release of lysosomal enzymes that further aggravate the injury. These results were in accordance with the Senthilkumar *et al.* (2005). The ameliorative groups (3 and 4) showed moderate decrease in CPK, LDH and troponins activity as compared to toxic group but it was significantly greater than the control group. The decrease might be due to the improved heart function mediated by the ameliorative agents (Bhaskar *et al.*, 2002).

Tissue Antioxidant Profile

There was significant (P<0.05) increase in myocardial TBARS (nmol/g protein) in the Doxorubicin treated group when compared to the control group. Significant (P<0.05) decrease in the level of myocardial TBARS was observed in group 4 and moderate in group 3 in comparison to the group 2. The calculated mean value in group 4 was slightly lower than the value of group 3 (Table 2). There was significant (P<0.05) decrease in SOD and GSH activities in the Doxorubicin treated group (group 2) when compared to the control group (group 1). Significant (P<0.05) increase was observed in the level of SOD and GSH in group 3 and group 4 in comparison to the group 2 (Table 2). The TBARS values of toxic group significantly (P<0.05) increased in comparison to other groups (Table 2). The increase in lipid peroxidation might be attributed to free radicals formed either by the reaction of drug toxic radicals with oxygen or by the interaction of superoxide

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radicals with hydrogen peroxide seemed to initiate lipid peroxidation suggesting that increased lipid peroxidation might be associated with cellular damage. The GSH and SOD levels of toxic group showed a significant (P<0.05) reduction in comparison to other groups (Table 2).

Table 2: Heart TBARS, SOD and GSH levels in different groups

Group	TBARS level (n moles/gm protein)	SOD activity (units/mg protein)	GSH activity (mg/g protein)
Control	156.84 ± 4.80^{a}	5.19 ± 0.98^{d}	10.51 ± 0.86^{c}
Doxorubicin	322.01 ± 7.88^{d}	2.28 ± 0.17^{a}	3.89 ± 0.31^{a}
Dox + Ascorbic Acid	276.56 ± 7.66 ^{c}	3.44 ± 0.47^{b}	5.62. ± 1.42^{b}
Dox + *Spirulina*	242.41 ± 7.66^{b}	4.09 ± 0.35^{c}	6.02 ± 1.17^{b}

Means bearing common superscripts do not differ significantly (P<0.05)

GSH and SOD efficiently scavenges toxic free radicals. Decreased glutathione and SOD levels might be due to its increased utilization in protecting 'SH' containing proteins from lipid peroxides (Subhashini *et al.*, 2007). Results were in accordance with earlier reports of various workers (Maini, 2000; Mohamed *et al.*, 2014 and Naidu *et al.*, 2002). The mean TBARS, GSH and SOD values of ameliorative groups significantly (P<0.05) lower than Doxorubicin treated group. Results were in accordance with earlier reports of Sharma *et al.* (2007); Viswanatha Swamy *et al.* (2011) and Yasir *et al.* (2009).

Pathology

The rats belonging to group 2 exhibited severe congestion of heart and endocardial haemorrhages. Histopathological sections revealed marked degenerative changes (Fig. 1), edema (Fig. 2), separation of cardiac muscle fibres along with interfibrillar haemorrhages was noticed in Doxorubicin treated group 2 (Fig. 3). In addition, and focal lymphocytic infiltration were noticed (Fig. 4). In group 3 mild disrupted muscle fibres and interfibrillar haemorrhages (Fig. 5) were noticed, while group 4 revealed very mild haemorrhages (Fig. 6). The lesions extended to myocardium which might be due to toxic metabolites of the Doxorubicin. In ameliorative groups moderate range of degeneration and disruption of muscle fibers (Fig. 2) was observed due to their antioxidant properties. The present findings were supported by earlier reports of Saad *et al.* (2001); Viswanatha Swamy *et al.* (2011); Mohamed *et al.* (2014) and Zhonghao *et al.* (2015).

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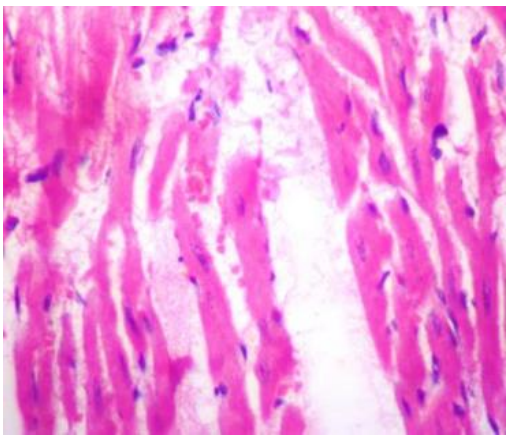

Fig.1: Heart showing marked degenerative changes in group 2. HE × 400.

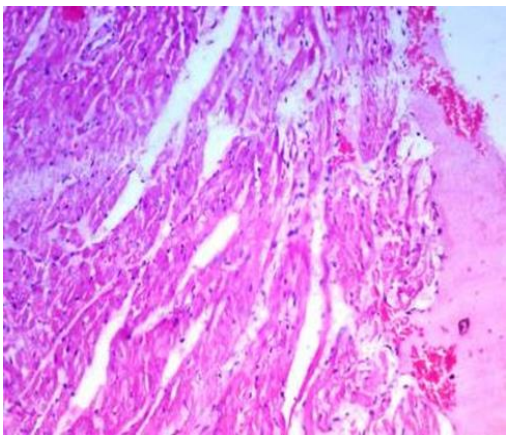

Fig. 2: Heart showing edema in group 2. HE × 200.

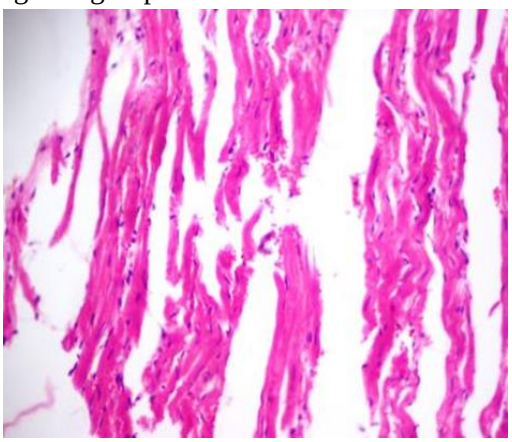

Fig. 3: Heart showing separation of cardiac muscle fibres along with interfibrillar haemorrhages in group 2. HE × 200.

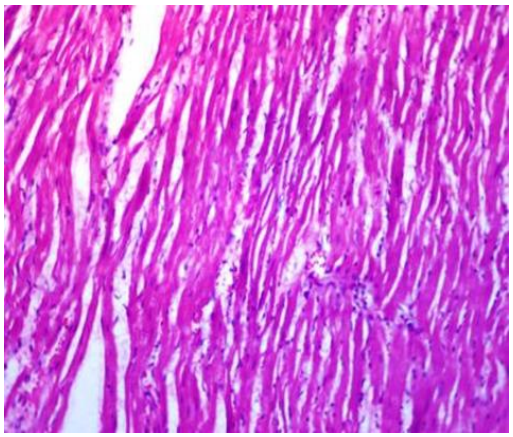

Fig. 4: Heart showing focal lymphocytic infiltration in group 2. HE × 200.

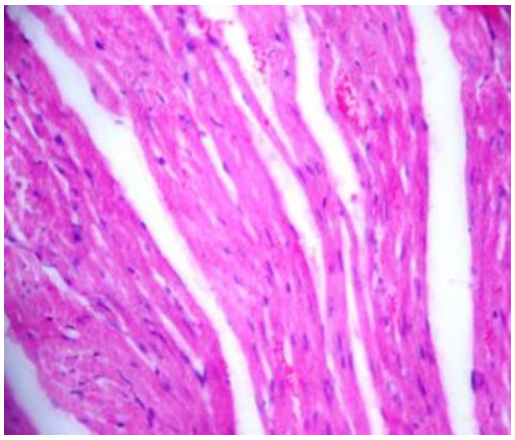

Fig. 5: Heart showing mild to moderate disrupted muscle fibres and interfibrillar haemorrhages in group 3. HE × 200.

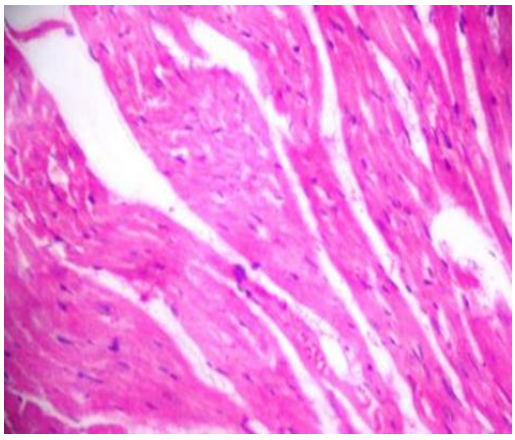

Fig. 6: Heart showing very mild haemorrhages in group 4. HE × 200.

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Conclusion

The present study is indicating that vitamin C @500mg/kg feed was found to be less protective affective and *Spirulina* @ 1000mg/kg feed offered moderate protection in counteracting the toxic effects of Doxorubicin. Keeping this in view, further studies can be advocated using different doses and different routes of administration so as to obtain best results combating the effects of anthracycline drugs which would prove beneficial for animals and humans.

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