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Protective Effect of Beta-Caryophyllene on Doxorubicin Induced Multiple Organ Toxicity in Rats

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ABSTRACT

The major limiting factor in doxorubicin's long-term administration is the development of cumulative dose-dependent cardiomyopathy and congestive heart failure. Also, doxorubicin causes deterioration in hepato-renal function. It significantly increases the levels of blood urea nitrogen, creatinine, alanine transaminase, and aspartate transaminase distortion in normal renal and hepatic histology. The present study was undertaken to find out the protective role of beta-caryophyllene (BCP), an anti-oxidant against doxorubicin-induced multiple organ toxicities in experimental animals. In this study, male Wistar rats were divided into four groups. The first group, control group, was administered with vehicle (2.5% tween 20); the second group received doxorubicin (15 mg/kg intraperitoneally at a single dose), third and fourth groups (treatment groups) received BCP plus doxorubicin (15 mg/kg) at doses of 100 mg/kg and 200 mg/kg respectively. BCP was given orally for 15 days and doxorubicin was given on 13th day of treatment. Cardiac function was assessed by measuring electrocardiogram changes and cardiac biomarkers—doxorubicin-induced significant lengthening of QT-interval and ST-elevation, which was completely prevented by BCP treatment. Doxorubicin caused oxidative stress as indicated by a significant decrease in reduced superoxide dismutase, glutathione level, and catalase activity with an increase in malondialdehyde compared to control. Doxorubicin and BCP significantly reversed these values compared to doxorubicin in heart, kidney, and liver. The histopathological examination has also shown signs of toxicity in doxorubicin treated groups, and healing effect was noticed in treatment groups.

Keywords: Beta-caryophyllene; Doxorubicin; Cardiac biomarkers.

1. INTRODUCTION

Cancer is a disease that begins in the cells of the body. In a normal situation, the cells grow and divide as the body needs them. This orderly process is disturbed when new cells form when the body doesn't need them, and old cells don't die when they should. These extra cells lump together to form a growth or tumor. The most prevalent cancers in men are lung, esophagus, stomach, oral, and pharyngeal, while in females, it includes breast, cervix, followed by those of the stomach and esophagus.[1]

Doxorubicin (DOX) is an anthracycline antibiotic that has been successfully used as one of the first-line anti-cancer drugs against solid and hematological malignancies.[2] However, its clinical application may be hampered by dose-dependent cardiotoxicity. Recent studies have suggested that DOX-induced cardiotoxicity involves the formation of O_2 and H_2O_2 free radicals and amplification of mitochondrial dysfunction.[3,4]

Also, DOX use has been constrained due to its multi-organ toxic effects, including its effects on the liver and kidney, the main drug detoxifying excretory organs in the body. The most acceptable theory attributes the initiation of such toxicity to oxidative stress. Other factors contributing to organ toxicity includes DOX generation of the inflammatory cascade and eventually, programmed cellular death, apoptosis.[5-9]

Anti-oxidants are widely used in dietary supplements and have been investigated to prevent diseases such as cancer and coronary heart diseases. The sesquiterpene

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BCP an anti-oxidant, is a major plant volatile compound found in large amounts in the essential oils of *Cannabis sativa* and also common in many different spice and food plants, such as oregano, species of Cinnamomum, and *Piper nigrum*.[10-13] Beta-caryophyllene is an effective inhibitor of lipid peroxidation, probably due to its free radical-scavenging activity against hydroxyl radicals, superoxide anions, and lipid peroxides. We investigated the BCP for its protective effect on DOX-induced multiple organ toxicities in male Wistar rats because of the above activities.

2. MATERIALS AND METHODS

2.1. Toxicity Studies

An acute oral toxicity test was performed as per OECD 423 guidelines (Annex-2d). All the animals were randomly distributed into four groups. Four fixed doses of 5, 50, 300 and 2000 mg/kg body weight BCP was administered p.o. to Wistar rats. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Rats were weighed daily for the observation of any change in morphological behaviors.

2.2. Drugs and Chemicals

DOX (50 mg/25 mL) was procured from Fresenius Kabi Oncology Ltd., Himachal Pradesh (batch no: 882AE014), manufacturing license no: MB/94/6 and BCP was procured from Sree Bankey Beheri Lal Board Mills, Ghaziabad, Uttar Pradesh. Lactate dehydrogenase, alkaline phosphatases, aspartate transaminases, alanine transaminase, creatinine, and blood urea nitrogen kits were procured from ERBA Diagnostics, USA. Reagents and chemicals for the estimations of malondialdehyde, superoxide dismutase, catalase, reduced glutathione and total protein were procured from SD. Fine Chemicals.

2.3. Animals

Male wistar rats weighing 150-200g were used. They were procured from National Center for Lab Animal Sciences, National Institution of Nutrition, Hyderabad, India and housed in a group of six under an environmentally controlled room with 12-h light/dark cycle with free access to food and water. After seven days of acclimatization period, they were randomly selected for different experimental groups. All the experimental procedures were carried out in accordance with the committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental procedures were approved by the institutional animal ethical committee (IAEC Reg.no: I/IAEC/LCP/019/2014/WR-30♂).

2.4. Induction of Experimental Multiple Organ Toxicities

Doxorubicin (DOX) was injected to rats (15 mg/kg, i.p.) at a single dose to induce experimental multiple organ toxicities. Animals were sacrificed 48 h after the dose of DOX.

2.5. Experimental Design

After acclimatization, the animals were randomly divided into the following groups consisting of 6 rats each.

Group I: Injected with 2.5% Tween 20 orally and serve as normal control

Group II: Injected single dose of DOX 15 mg/kg intraperitonially

Group III (DOX + BCP 100mg/kg): Oral administration of BCP 100 mg/kg for 15 days along with DOX, which is intraperitoneally injected on 13th day of treatment.

Group IV (DOX + BCP 200mg/kg): Oral administration of BCP 200 mg/kg for 15 days along with DOX, which is intraperitoneally injected on 13th day of treatment.

2.6. Electrocardiography

At the end of the experiment, the rats were anesthetized with urethane (1 g/kg, i.p.) and needle electrodes were inserted under the skin for the limb lead at position II. Electrocardiograph was recorded continually; ST-segment elevation or depression (expressed in mv) and lengthening of QT-interval in normal and experimental animals were considered by using Power lab 2.7.

2.7. Biochemical Analysis

After recording the ECG, the animals were sacrificed, and blood samples were collected. Serum was separated from each sample and used for the biochemical analysis. Immediately after sacrifice, heart, liver and kidney tissues were excised in ice-cold condition. They were blotted free of blood and tissue fluids. Then they were weighed and stored at −80 °C till further use for the analysis (Cryo Scientific, India).

2.7.1. Biochemical parameters in serum

The collected serum was used for the estimation of cardiac marker enzymes lactate dehydrogenase (LDH) and alkaline phosphatase (ALP), hepatic biomarkers i.e., aspartate transaminase (AST) and alanine transaminase (ALT), renal biomarkers i.e., blood urea nitrogen (BUN) and creatinine using commercially available standard enzymatic kits (ERBA Diagnostics, USA). Endogenous antiperoxidative enzymes such as superoxide dismutase (SOD), catalase, GSH, and Total Protein were estimated.[14,15]

2.7.2. *Biochemical parameters in heart, liver and kidney tissues: Lipid peroxidation (LPO) and anti-oxidant enzymes*

The excised heart and kidney tissues were homogenized in chilled Tris– HCl buffer (0.1 M) pH 7.4 and excised liver tissues in Phosphate buffer solution pH 7.4. The homogenate was then centrifuged at 7000 rpm for 15 minutes. The clear supernatant obtained was used for the assay of lipid peroxidation (MDA), endogenous anti-peroxidative enzymes such as superoxide dismutase (SOD), catalase, GSH, and total protein.

2.8. Statistical Analysis

All the values are expressed as mean ± SEM Statistical significance between more than two groups was tested using one-way ANOVA followed by the Tukey's multiple comparision test as appropriate using computer based fitting program (Prism, Graphpad). Differences were considered to be statistically significant when p <.05.

3. RESULTS

3.1. Effect of BCP on Heart, Liver and Kidney Weight

The heart weight decreased significantly (p<.01) in DOX administered rats when compared with normal control rats (Table 1). In rats pretreated with BCP and then treated with DOX, showed a significant recovery (p<.01) for BCP 200 mg/kg in the heart weight when compared to DOX treated rats.

The liver and kidney weights decreased significantly (p<.01) in DOX administered rats when compared with normal control rats (Table 1). In rats pretreated with BCP and then treated with DOX, showed a significant recovery (p<.01) for BCP 200 mg/kg in the liver and kidney weights when compared to DOX treated rats.

3.2. Effect of CAR on Electrocardiograph:

Normal control and BCP pretreated rats showed normal pattern of ECG whereas rats treated with DOX showed a significant (p < .001) increase in ST segment as compared to control rats indicating the infarcted myocardium and also there is a prolongation of QT-interval. BCP pretreatment in DOX treated rats showed significant decrease for BCP 100 mg/kg (p < .001) and BCP 200 mg/kg (p < .001) in ST segment and QT-interval as compared to DOX alone treated rats (Table 2).

3.3. Effect of BCP on Cardiac markers

In the present study, the cardiac markers estimated are LDH and ALP. The activities of these enzymes were increased significantly (p<.001) in DOX treated rats as compared to normal control group rats (Table 3). Pre-treatment with BCP in DOX treated animals significantly decreased (P<.001) the LDH and ALP activities.

3.4. Effect of BCP on Hepatic Markers

In the present study, the hepatic markers estimated are SGOT and SGPT. The activities of these enzymes were increased significantly (p <.001) in DOX treated rats as compared to normal control group rats (Table 4). Pre-treatment with BCP in DOX treated animals significantly decreased (p <.001) the SGOT and SGPT activities.

Table 1: Effect of BCP on Organ weight

Group	*Heart Weights (g)*	*Liver Weights (g)*	*Kidney Weights (g)*
Group I	0.74 ± 0.024	8.65 ± 0.914	1.63 ± 0.067
Group II	0.53 ± 0.055**	5.95 ± 0.434	1.15 ± 0.025***
Group III	0.67 ± 0.030	8.55 ± 0.687	1.51 ± 0.061$^{###}$
Group IV	0.76 ± 0.023$^{\$\$}$	9.64 ± 0.831$^{\$}$	1.73 ± 0.0439$^{\$\$\$}$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests. ***p<.001, **p<.01 indicates comparison of negative control group with control group.
###P<.001 indicates comparison of low dose group with negative control.
$\$\$\$P$<.001, $\$\$P$<.01, $\$ P$<.05 indicates comparison of high dose group with negative control.

Table 2: Effect of BCP on ST segment elevation, QT interval

Group	*Treatment*	*QT Interval*	*ST Segment Elevation (mV)*
I	Control	0.04 ± 0.002	0.14 ± 0.016
II	Negative Control (DOX)	0.06 ± 0.003***	0.37 ± 0.008***
III	BCP (100mg/kg)	0.05 ± 0.001$^{\#}$	0.25 ± 0.009$^{###}$
IV	BCP (200mg/kg)	0.04 ± 0.001$^{\$\$\$}$	0.19 ± 0.006$^{\$\$\$}$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p<.001, **p<.01 indicates comparison of negative control group with control group.
###p<.001, ##p<.01, #P<.05 indicates comparison of low dose group with negative control.
$\$\$\$p$<.001, $\$\$p$<.01 indicates comparison of high dose group with negative control.

Table 3: Effect of BCP on cardiac biomarkers (LDH and ALP)

Group	Treatment	LDH	ALP
I	Control	26.80 ± 7.067	162.0 ± 38.78
II	Negative control (DOX)	85.60 ± 6.022***	411.6 ± 39.51***
III	BCP (100 mg/kg)	37.20 ± 7.235###	228.8 ± 27.21#
IV	BCP (200 mg/kg)	27.00 ± 5.050$$$	183.80 ± 35.82$$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p <.001 indicates a comparison of the negative control group with the control group.
###p <.001, #p <.05 indicates a comparison of low dose group with a negative control.
$$$$P$<.001, $$$P$<.01 indicates a comparison of high dose group with negative control.

Table 4: Effect of BCP on hepatic biomarkers (AST and ALT)

Group	Treatment	AST	ALT
I	Control	101.40 ± 10.10	60 ± 11.19
II	Negative Control (DOX)	199.8 ± 18.46***	137.60 ± 16.09***
III	BCP (100mg/kg)+DOX	81.80 ± 15.52###	64 ± 6.641###
IV	BCP (200mg/kg)+DOX	92.50 ± 9.133$$$	108.8 ± 15.07$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p <.001 indicates a comparison of the negative control group with the control group.
###p <.001 indicates a comparison of low dose group with negative control.
$$$$p$ <.001, $$p$ <.05 indicates a comparison of high dose group with a negative control.

Table 5: Effect of BCP on kidney biomarkers

Group	Treatment	Creatinine	BUN
I	Control	27.01 ± 6.772	24.80 ± 2.574
II	Negative Control (DOX)	108.5 ± 9.142***	79.16 ± 5.733***
III	BCP (100mg/kg)+DOX	51.32 ± 5.453###	35.14 ± 3.273###
IV	BCP (200mg/kg)+DOX	73.75 ± 9.725$	59.48 ± 6.098$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p <.001 indicates a comparison of the negative control group with the control group.
###p <.001 indicates a comparison of the low dose group with the negative control.
$$$p<.001, $p<.05 indicates a comparison of high dose group with the negative control.

Table 6: Effect of BCP on Anti-oxidant parameters of Heart homogenates

Group	Treatment	SOD	Catalase	GSH
I	Control	13.43 ± 0.402	4.31 ± 0.246	6.46 ± 0.304
II	Negative Control	4.23 ± 0.423***	0.73 ± 0.071***	1.84 ± 0.215***
III	BCP (100mg/kg)	10.53 ± 0.295###	3.02 ± 0.094###	3.61 ± 0.349##
IV	BCP (200mg/kg)	8.01 ± 0.432$$$	1.72 ± 0.153$$	3.00 ± 0.166$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p <.001 indicates a comparison of negative control group with control group.
###p <.001, ##P<0.01 indicates a comparison of low dose group with negative control.
$$$p<.001 indicates the comparison of high dose group with negative control.

3.5. Effect of BCP on Kidney Parameters

In the present study, the kidney bio-markers estimated are creatinine, BUN. The activities of these enzymes were increased significantly (p <.001) in DOX treated rats as compared to normal control group rats (Table 5). Pre-treatment with BCP in DOX treated animals significantly decreased (P <.001) the creatinine and BUN activities.

3.6. Effect of BCP on Anti-oxidant Parameters and Lipid Peroxidation

The levels of MDA (end product of lipid peroxidation) along with the activities of the anti-oxidant enzymes SOD, GSH and catalase in normal and experimental rats are listed in Table 6-9. DOX treated rats showed significantly (p <.001) elevated levels of MDA, a marker for oxidative stress, and significantly reduced the levels of GSH (p <.001) in heart, liver, and kidney tissues as compared to control rats.

Anti-oxidant enzymes (catalase and SOD) were significantly (p <.001) lowered in the heart, liver, and kidney tissues of DOX injected rats as compared to normal control rats. Pre-treatment with BCP in DOX intoxicated rats significantly (p <.001) reduced LPO levels compared to DOX alone treated rats. The prior administration of

Table 7: Effect of BCP on Anti-oxidant parameters of Liver homogenates

Group	Treatment	SOD	Catalase	GSH
I	Control	55.71 ± 3.900	11.24 ± 0.5945	20.48 ± 1.679
II	Negative Control	21.53 ± 1.445***	4.038 ± 0.5206***	9.594 ± 1.128***
III	BCP (100mg/kg)	38.92 ± 2.849##	8.588 ± 0.6043###	16.79 ± 0.7014##
IV	BCP (200mg/kg)	35.01 ± 3.819$	6.657 ± 0.6696$	13.05 ± 1.597

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p <.001 indicates comparison of negative control group with control group.
###p <0.001, ##p <0.01 indicates a comparison of low dose group with a negative control.
$$$$p$ <0.001 indicates comparison of high dose group with negative control.

Table 8: Effect of BCP on Anti-oxidant parameters of Kidney homogenates

Group	Treatment	SOD	Catalase	GSH
I	Control	48.51 ± 1.842	11.33 ± 0.5463	12.13 ± 0.6819
II	Negative control	19.67 ± 2.315***	5.637 ± 0.6410***	5.056 ± 0.5881***
III	BCP (100 mg/kg)	38.78 ± 2.273###	8.994 ± 0.4785##	10.00 ± 0.5935###
IV	BCP (200 mg/kg)	32.67 ± 2.874$$	6.469 ± 0.3249	7.805 ± 0.5520

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***P<.001 indicates comparison of negative control group with control group.
###P<.001, ##P<.01 indicates comparison of low dose group with negative control.
$$$$P$<.001 indicates comparison of high dose group with negative control.

Table 9: Effect of BCP on Lipid peroxidation of Heart, Liver and Kidney homogenates

Group	Treatment	Heart	Liver	Kidney
I	Control	69.19 ± 4.360	26.66 ± 2.654	50.80 ± 4.673
II	Negative control	115.8 ± 6.810***	52.11 ± 3.991***	99.60 ± 9.288***
III	BCP (100 mg/kg)	73.08 ± 3.641###	28.39 ± 1.802###	53.80 ± 3.967###
IV	BCP (200 mg/kg)	91.80 ± 3.704$	39.36 ± 3.455$	69.80 ± 5.687$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p <.001 indicates a comparison of the negative control group with the control group.
###p <.001, ##p <.01 indicates a comparison of low dose group with negative control.
$$$$p$ <.001 indicates the comparison of high dose group with negative control

Table 10: Effect of BCP on Total protein

Group	Treatment	Heart	Liver	Kidney
I	Control	18.22 ± 0.06306	17.09 ± 0.9957	19.98 ± 1.846
II	Negative Control	12.08 ± 0.7482***	6.000 ± 1.119***	9.532 ± 0.7008***
III	BCP (100 mg/kg)	17.30 ± 0.7691###	14.47 ± 1.193###	17.27 ± 1.726##
IV	BCP (200 mg/kg)	15.10 ± 0.7031$	12.65 ± 1.093$$	16.12 ± 1.140$

Values are expressed as mean ± SEM of 6 animals. Superscript symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests.
***p <.001 indicates a comparison of the negative control group with control group.
###p <.001 indicates a comparison of low dose group with negative control.
$$$$p$ <.001 indicates the comparison of high dose group with negative control.

BCP for 15 days along with DOX administration on 13th day resulted in a significant (p <.001 for SOD, P<.01 for catalase, GSH) increase in levels of GSH, SOD, and catalase compared to DOX group.

3.7. Effect of BCP on Doxorubicin Induced Changes in the Activity of Total Protein

The levels of total protein were decreased significantly (p <.001) in DOX-induced rats when compared with control rats, and this reduction was reversed significantly (p <.001) in treated groups (Group III and IV) (Table 10).

3.8. Effect of BCP on Doxorubicin Induced Changes in Histology of Heart, Liver and Kidney

3.8.1. Heart

Histopathology of hear have revealed normal pericardium and myocardium layers without any inflammation or necrosis in control animals whereas very severe vacuolar degeneration noticed from myocardium to endocardium region of heart in negative control group. The animals treated with beta caryophyllene have showed

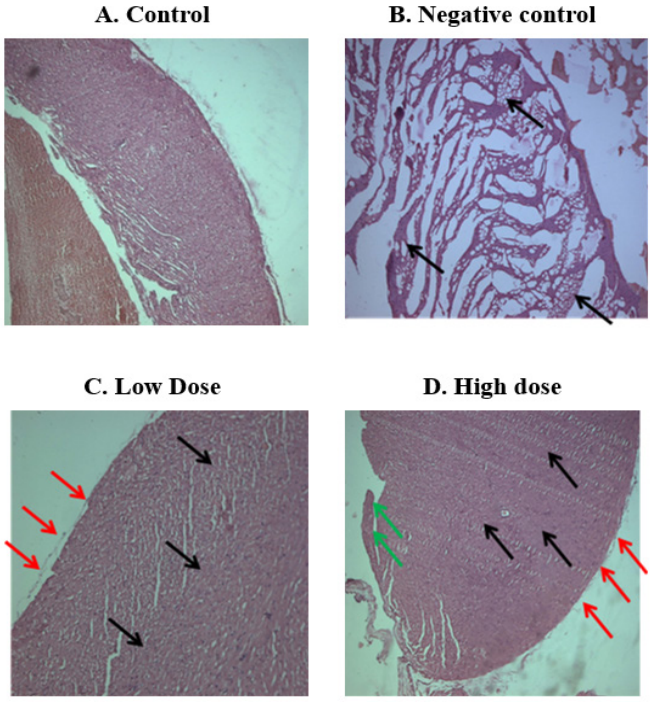

Fig. 1: Histopathology of Heart. (a) Control animals showing normal pericardium and myocardium layers (b) Negative group animals showing necrosis (c) Animals treated with low dose of beta caryophyllene showing normal architecture (d) Animals treated with high dose of beta caryophyllene showing normal pericardium, myocardium and endocardium regions.

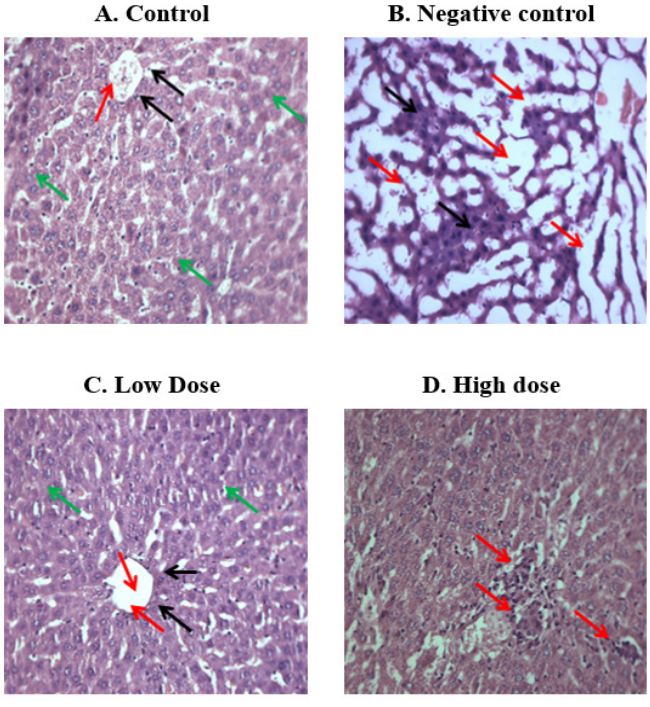

Fig. 2: Histopathology of Liver. (a) Control animals showing normal portal and periportal layers (b) Negative group animals showing degeneration and necrosis (c) Animals treated with low dose of beta caryophyllene showing normal hepatic architecture (d) Animals treated with high dose of beta caryophyllene showing normal hepatic lobules and centrilobular region.

pericardium and myocardium layers without inflammation or necrosis with normal endocardium containing valvular region (Figure 1).

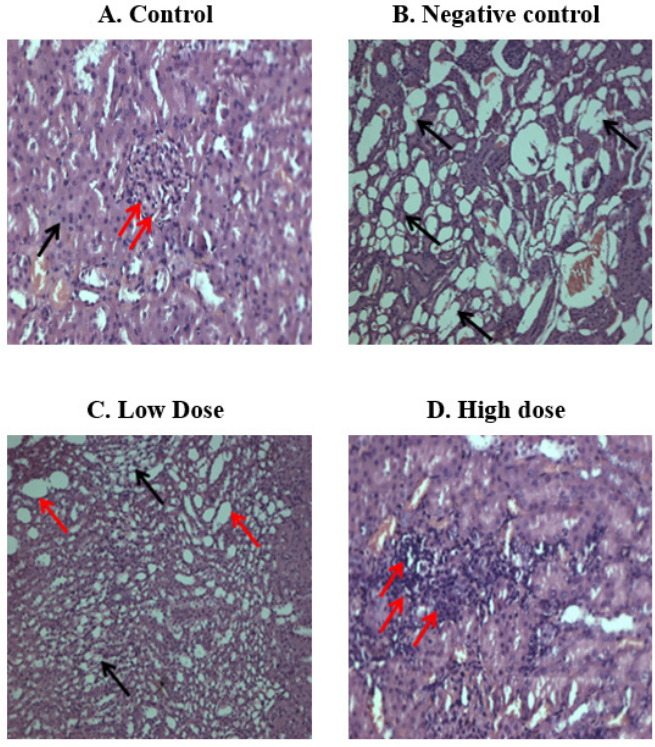

Fig. 3: Histopathology of the Kidney. (a) Control animals showing normal appearance of glomerulus, tubular region and interstitium between the tubules (b) Negative group animals showing severe vacuolar degeneration along with dilatation of tubules and hemorrhages (c) Animals treated with low dose of BCP showing mild tubular dilatation and degeneration without any inflammation or necrosis (d) Animals treated with high dose of BCP showing moderate tubular dilatation and tubulonephritis.

3.8.2. Liver

Liver histopathology has revealed normal portal and periportal regions without any inflammation or necrosis in control animals, whereas very severe degeneration of hepatocytes along with extensive dilation of the sinusoidal region observed in the entire portion of liver in the negative control group. The animals treated with beta-caryophyllene have showed normal hepatic architectures and centrilobular regions without any inflammation or necrosis (Figure 2).

3.8.3. Kidney

Histopathology of the kidney has revealed normal appearance of a glomerulus, tubular region and interstitium between the tubules in control animals, whereas very severe vacuolar degeneration along with dilatation of tubules and hemorrhages noticed in most of the tubular region of kidney in negative control group. The animals treated with BCP have showed mild to moderate tubular dilatation and degeneration without any inflammation or necrosis. Moderate Tubulonephritis was observed in high dose kidney (Figure 3).

4. DISCUSSION

The pathogenesis of multiple organ toxicities has not

yet been fully understood, but studies on DOX-induced cardiotoxicity provide a good insight into this pathology and clearly indicate the involvement of oxidative stress. In the present study, we found that BCP exerted a strong protective effect against DOX-induced multiple organ toxicities in rats. Augmentation of endogenous anti-oxidants, maintenance of the myocardial anti-oxidant status and significant restoration of most of the altered hemodynamic parameters may contribute to its protective effect. Electrocardiograph abnormalities such as ST-segment elevation could be due to the consecutive loss of cell membrane in injured myocardium.[16] In the present study, we noted that there was significant elevation in ST-segment in DOX-treated rats, but pre-treatment with BCP markedly restrained DOX-induced ST-segment elevation suggestive of its cell membrane protecting effects.

Myocardium contains an abundant amount of diagnostic marker enzymes for cardiotoxicity, also liver and kidney shows diagnostic marker enzymes for their toxicities. Once metabolically damaged, they release their intracellular contents into the extracellular fluid.[17] Hence, the serum levels of these marker enzymes reflect the alterations in membrane integrity and membrane permeability. The results in the present study showed a significant elevation of serum levels of alanine transaminase, aspartate transaminase, blood urea nitrogen, creatinine, lactate dehydrogenase and alkaline phosphatase in DOX-treated rats. Pre-treatment with BCP (100 mg/kg and 200 mg/kg) significantly lowered the DOX-induced elevation of serum levels of these diagnostic marker enzymes in the heart. But, pre-treatment with a higher dose (200 mg/kg) of BCP did not show a therapeutic effect in liver and kidney. It demonstrated that BCP could maintain membrane integrity, thereby restricting the leakage of these enzymes at a low dose.

Administration of supramaximal doses of DOX had been reported to induce severe oxidative stress.[18] Overproduction of ROS can cause severe impairment of cellular functions and necrotic lesions in the myocardium, liver and kidney tissues of rats. In the other hand, superoxide dismutase, catalase, and reduced glutathione constitute a mutually supportive enzyme team of defense against oxidative injury.[19] Previous studies found that BCP showed a strong anti-oxidant activity. In the present study, we found that BCP significantly elevated the decreased activities of superoxide dismutase, catalase and reduced glutathione in DOX-injected rats. These findings suggested that BCP could considerably improve cellular antioxidative defense against oxidative stress; it might work as a preventive anti-oxidant by scavenging superoxide anions, or an anti-oxidant by scavenging lipid free radicals.

In the present study, increase in malondialdehyde levels was observed in the heart, liver and kidney tissues after the administration of DOX. Malondialdehyde is a major lipid peroxidation end product; the increased level of malondialdehyde indicates activation of the lipid peroxidative process, resulting in irreversible damage to hearts of animals subjected to DOX-induced stress.[20] The results in the present study indicated that pre-treatment with BCP (100mg/kg) could inhibit DOX-induced elevation of malondialdehyde content.

The ECG has been considered the single most important initial clinical test for the diagnosis of cardiotoxicity. DOX administration in rats showed a pathological Q wave. DOX administration in rats also showed a decrease in P wave intensity, QRS complex, R–R interval, and an increase in the heart rate. DOX administration also resulted in increased 'ST-segment'. This is inconsistent with the observations of the earlier reports. ST-segment elevation reflects the potential difference in the boundary between ischemic and non-ischemic zones and consequent loss of cell membrane function following DOX administration.[21] BCP (100 mg/kg and 200 mg/kg) pre-treatment in DOX treated rats prevented the ECG's pathological alterations suggestive of its cell membrane protective effect. Histopathological results shown that severe toxicity is noticed in tissues treated with DOX, and treatment with a low dose of CAR has shown healing effect.

In summary, the present study provides experimental evidence that BCP has strong anti-oxidant activity, and it can maintain cell membrane integrity and improve cardiac systolic/diastolic dysfunction induced by high-dose DOX administration. Beta-caryophyllne treated rats showed an increased level of anti-oxidant enzymes, suggesting that BCP has cardioprotective, hepatoprotective and nephroprotective activity.

5. CONCLUSION

In conclusion, the present study demonstrated that intraperitoneal injections of DOX produced cardio, hepato and renal toxicities in rats as evidenced by the release of various injury bio-markers in serum. This finding might be scientific support to understand the beneficial effects of BCP on the protection against injury caused in the heart, liver and kidney, in which oxidative stress has long been known to contribute to the pathogenesis. Finally, BCP is an effective anti-oxidant in heart tissue at both doses but not effective at a higher dose in hepatic and renal tissues.

6. REFERENCES

1. Gennari A, Salvadori B, Donati S, Bengala C, Orlandini C, Danesi R, Del Tacca M, Bruzzi P, Conte PF. Cardiotoxicity of

epirubicin/paclitaxel-Containing regimens: role of cardiac risk factors. J Clin Oncol. 1999;17:3596-3602.

2. Patil RR, Guhagarkar SA, Devarajan PV. Engineered nanocarriers of DOX: a current update. Crit Rev Ther Drug Carrier Syst. 2008;25:1–61.
3. Menna, P, Salvatorelli E, Minotti G. Anthracycline degradation in cardiomyocytes: a journey to oxidative survival. Chem Res Toxicol. 2010;23:6–10.
4. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev. 2004;56:185–229.
5. Tulubas F, Gurel A, Oran M, Topcu B, Caglar V, Uygur E. The protective effects of omega-3 fatty acids on doxorubicin-induced hepatotoxicity and nephrotoxicity in rats. Toxicol Ind Health. 2013;31:638-44.
6. El-Sheikh AA, Morsy MA, Mahmoud MM, Rifaai RA, Abdelrahman AM. Effect of coenzyme-q10 on Doxorubicin-induced nephrotoxicity in rats. Adv Pharmacol Science. 2012;2012:981461.
7. Korga A, Dudka J, Burdan F, Sliwinska J, Mandziuk S, Dawidek-Pietryka K. The redox imbalance and the reduction of contractile protein content in rat hearts administered with L-thyroxine and Doxorubicin. Oxid Med Cell Longev. 2012;681367.
8. Park J, Kanayama A, Yamamoto K, Miyamoto Y. ARD1 binding to RIP1 mediates doxorubicin-induced NF-kappaB activation. Biochem Biophys Res Commun. 2012;422:291–7.
9. Zhang YW, Shi J, Li YJ, Wei L. Cardiomyocyte death in doxorubicin-induced cardiotoxicity. Arch Immunol Ther Exp (Warsz). 2009;57:435–45.
10. Hendriks H, Malingre T, Battermann S, Boss R. Mono- and sesquiterpene hydrocarbons of the essential oil of Cannabis sativa. Phytochemistry. 1975;14:814–815
11. Mockute D, Bernotiene G, Judzentiene A. The essential oil of Origanumvulgare L. ssp. vulgare growing wild in Vilnius district (Lithuania). Phytochemistry. 2001;57:65–69.
12. Jayaprakasha GK, Rao JM, Sakariah KK. Volatile constituents from Cinnamomumzeylanicum fruit stalks and their antioxidant activities. J Agric Food Chem. 2003;51:4344–4348.
13. Orav A, Stulova I, Kailas T, Muurisepp M. Effect of storage on the essential oil composition of piper nigrum l. Fruits of different ripening states. J Agric Food Chem. 2004;52:2582–2586.
14. Ramesh CV, Malarvannan P, Jayakumar R, Jayasundar S, Puvanakrishnan R. Effect of a novel tetrapeptide derivative in a model of isoproterenol induced myocardial necrosis. Mol cell Biochem. 1998;187:173–182.
15. Lowry OH, Rosenbrough NJ, Farr AI, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;93:265–275.
16. Rajadurai M, StanelyMainzen Prince P. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistar rats. Toxicology. 2007;230:178–188
17. Suchalatha S and Shyamala Devi CS. Protective effect of *Terminalia chebula* against experimental myocardial injury induced by isoproterenol. Indian J Exp Biol. 2004;42:174.
18. Doroshow JH, Locker GY, Myers CE. Enzymatic defenses of the mouse heart against reactive oxygen metabolites. J Clin Invest. 1980;65:128-135.
19. Ji LL, Stratman FW, Lardy HA. Anti-oxidant enzyme systems in rat liver and skeletal muscle. Influences of selenium deficiency, chronic training, and acute exercise. Arch biochem biophys. 1988;263:150-160.
20. Angelo AI, Giulia DC, Francesca B, Edzard E. Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. Int J Cardiol. 2005;98:1-14.
21. Kirsten Schimmel AV, Gietema JA, Veldhuisen DJ van, Graaf WT van der, Vries EGE, Sleijer DT. Long-term chemotherapy-related cardiovascular morbidity. Cancer Treat Rev. 2004;26:429-447.

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