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CACTUS GRANDIFLORUS, A HOMEOPATHIC PREPARATION HAS PROTECTIVE EFFECT AGAINST DOXORUBICIN INDUCED CARDIOMYOPATHY IN RATS

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ABSTRACT

To evaluate the cardioprotective action of *Cactus grandiflorus* in doxorubicin-induced cardiomyopathy in rats on the basis of biochemical and histopathological analysis and compare the cardioprotective potential of *Cactus grandiflorus* with the standard drug Vitamin E with drug toxicity on liver and kidney by means of blood parameters. Clinical use of doxorubicin is limited due to its dose dependent cardiotoxicity inspite of its broad spectrum activity against variety of malignancies and solid tumors. Doxorubicin induced acute cardiotoxicity may be caused with the initiation of chemotherapy whereas chronic toxicity develop generally after completion of therapy and occurs in patients after cumulative dose of ≥500 mg doxorubicin/m. This study was aimed to investigate the alcoholic extract (mother tincture) of *Cactus grandiflorus* on doxorubicin induced cardiomyopathy. Male wistar albino rats were used in vivo model for the study of seven days. Two different doses (0.5 mL and 1.1 mL) of *Cactus grandiflorus* mother tincture were given orally for seven days in this post treatment study. doxorubicin 15 mg/kg was given ip on the first day. There was significant decrease in level of antioxidant enzymes like glutathione superoxide dismutase (SOD) and catalase (CAT) in animals treated with DOX 15 mg/kg and elevated level of lactate dehydrogenase (LDH), serum glutamate oxaloacetatae transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) alkaline phosphates and serum creatinine. Post treatment with *Cactus grandiflorus* resulted in increase in level of antioxidant enzymes and decrease in level of LDH, SGOT, SGPT alkaline phosphates and serum creatinine. Histological investigation had shown that doxorubicin-treated group had scattered myocardial cells with multiple vacuolar myopathic changes. The samples from the groups treated with 0.5 mL and 1.1 mL *Cactus grandiflorus* with doxorubicin showed a significant reduction in the number of vacuolated cells, s of sample from the saline *Cactus grandiflorus* only and vitamin E only groups showed normal myocardium. These results suggested mother tincture of Cactus grandiflorus had protective effect against doxorubicin-induced cardiotoxicity and had potential as a cardioprotective agent.

Key words: Cactus grandiflorus, Doxorubicin, Homeopathic preparation, Cardiotoxicity.

INTRODUCTION

Doxorubicin the most extensively used anthracycline has been the mainstay for a wide spectrum of malignancies. Unfortunately, its clinical utility is seriously limited by its acute (manifest within 48 hours of administration) and chronic (months to years after therapy) cardiotoxicities (Singal PK *et al*., 2000).

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If the cumulative dose of doxorubicin exceeds 550 mg/m^{2}body surface, the risk of developing cardiomyopathy, cardiac dilation, and finally decompensated heart failure sharply increases (Minotti G *et al*., 2004). Doxorubicin-induced cardiotoxicity has been proposed to be mediated by diverse mechanisms such as inhibition of nucleic acid and protein synthesis (Buja LM *et al*., 1973; Robbie M *et al*., 1984) release of vasoactive amines (Singal PK *et al*., 2000), alteration of calcium transport (Solem LE *et al*., 1996), lysosomal alteration (Singal PK *et al*., 1985), reduced mitochondrial calcium and ATP content (Zhou S *et al*., 2001), disruption

of muscle gene expression (Gamblels HA *et al*., 2002) and functional alteration of membrane bound enzymes. However, the most prevalent presumption on doxorubicin-induced cardiotoxicity is based on free radical formation through enzymatic and nonenzymatic pathways (Olson R.D *et al*., 1990; Dorshow JH, 1995) as well as lipid peroxidation (Bagchi D *et al*., 1995). According to this, the B ring of the adriamycinone part of the molecule is reduced to semiquinone, and under aerobic condition doxorubicin is regenerated, forming a superoxide radical ($O_2^{\bullet}$). Even a small dose of doxorubicin is sufficient to produce large amount of free radicals (Dorsow JH, 1983). Hence, doxorubicin was used to induce cardiotoxicity in animals and this animal model is widely used to investigate the cardioprotective effect of the drugs. The use of antioxidants to prevent doxorubicin induced toxicity had accepted widely. Various strategies and therapies had devised to combat the toxic effect of doxorubicin. This study attempted to investigate the cardioprotective effect of *Cactus grandiflorus* mother tincture and vitamin E. Vitamin E is a known antioxidant and had shown to be cardioprotective in doxorubicin-induced cardiotoxicity (Sonnveld P, 1978; Puri Maulik SK *et al*., 2005). Hence we used this as a reference drug.

Cactus grandiflorus is good source of polyphenols. So far, no sufficient work has been done revealing a variety of pharmacological effects of this drug especially in cardiovascular diseases and cancer. Most of pharmacological effects are not scientifically evaluated. Only reported by homeopathic practioners. Cactus is thought to elevate arteriolar tension by increasing the muscular energy of the heart and causing arteriolar contraction. This theory has not been confirmed by human data. Early research with commercial preparations of the active compound proved it to be physiologically inert. More recently, in studies with rats and dogs, hordenine showed a positive inotropic effect on the heart, with increased systolic and diastolic blood pressures and peripheral blood flow volume. Flavonoids and their derivatives (rutin, rutinoside, and kaempferitin) are thought to improve capillary function by decreasing abnormal leakage (Orlano Jones, 1980). Studies on isolated frog heart and papillary muscle from guinea pig had shown positive inotropic effect. Antihypertensive and antiarrhythmic activity is due to flavonolglycosides which are present mainly in stem and flowers extract. Flavones affect the myocardium's calcium metabolism, thereby increasing its contractile power and promoting its normal rhythm (Anonymous 1).

Materials and Methods

Experiment

The animal care and handling were done according to the guidelines issued by Committee for the Purpose of Control and Supervision on Experiments on Animals, 10-13 weeks old male wistar albino rats weighing 150-200 g were selected from central animal house facility Jamia Hamdard University. They were housed under controlled conditions of temperature (23±2ºC), humidity (50% ± 5%) and light (10 and 14 h of light and dark, respectively).The animals had free access to food and water. Eight animals were housed in sterile polypropylene cage containing sterile paddy husk as bedding. The study was approved by Ethics Committee (proposal no. 478, dated- Nov 2008) Jamia Hamdard, New Delhi-110062.

Drug and its mode of administration

Cactus grandiflorus mother tincture (10x) (Dr Reckeweg & Co, Germany) and Vitamin E (Bio-3Sg®) were purchased from market. Marketed preparation of doxorubicin (Adrim®) was used and was given by ip route. *Cactus grandiflorus* was given by oral route with the help of oral feeding needle.

Animal model

Male wistar rats, body weight 150-200 g were maintained on a normal rat chow diet. The rats were divided in seven groups: Saline, doxorubicin only, *Cactus grandiflorus* only, doxorubicin+*Cactus grandiflorus* 0.5 mL, doxorubicin+*Cactus grandiflorus* 1.1 mL, Vitamin E only and doxorubicin+vitamin E.

Doxorubicin was given 15 mg/kg ip single dose on first day while *cactus grandiflorus* mother tincture three times a day by oral feeding needle. Saline group rats were injected with the same volume of normal saline shown in Table 1. After the treatment blood was withdrawn from the tail vein of rat for the estimation of the biochemical parameters such as LDH, SGOT, SGPT, alkaline phosphates, creatinine in serum (shown in Table 2) and TBARS, GSH, SOD and CAT in heart tissue (Table 3). After that rats were sacrificed for histopathology.

Thiobarbituric Acid Reactive Substances (Okhaa, H *et al*., 1979)

Lipid peroxidation is a free radical mediated event. The primary products of such damage are a complex mixture of peroxides which then breakdown to produce carbonyl compounds. The malondialdehyde (MDA) is one such carbonyl compound, which forms a characteristic chromogenic adduct with two molecules of thiobarbituric acid (TBA). The colorimetric reaction of TBA with MDA, a secondary product of lipid peroxidation has been widely accepted for measuring lipid peroxidation. One millileter of suspension medium was taken from the 10% tissue homogenate and 0.5 mL of 30% tricholoroacetic acid (TCA) was added to it, followed by 0.5 mL of 0.8% TBA reagent. The tubes

were then be covered with aluminum foil and kept in shaking water bath for 30 minutes at 80 °C. After 30 minutes tubes were taken out and kept in ice-cold water for 30 minutes. These were then be centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 540 nm at room temperature against appropriate blank. Blank consist of 1 mL distilled water, 0.5 mL of 30% TCA and 0.5 mL of 0.8% TBA.

Tissue Glutathione (Sedlak J and Lindsay RH, 1968)

This spectrophotometric procedure is based on the method of Ellman i.e. 5, 5'-dithiobis-(2-nitrobenzoic acid), DTNB, is reduced by–SH groups to form one mole of 2-nitro-5-mercaptobenzoic acid per mole of –SH. The nitro mercaptobenzoic acid anion has an intense yellow color that is determined spectrophotometrically at 412 nm. A known weight of heart tissue ranging from (300-600 mg) was homogenized in 5-8 mL of 0.02 M ethylenedieamine tetra acetic acid (EDTA) and then 4.0 mL of cold distilled water was added to it. After mixing it well, 1 mL of 50% TCA was added and shaken intermittently for 10 minutes using a vortex mixer. After 10 minutes the contents were transferred to centrifuge tubes (rinsed in EDTA) and centrifuged at 6000 rpm for 15 minutes. Following centrifugation, 2 mL of the supernatant was mixed with 4.0 mL of 0.4 M Tris buffer (pH 8.9). The whole solution was mixed well and 0.1 mL of 0.01M DTNB was added to it. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank. Blank: The method will be same as for the test except that 0.02 M EDTA was added in place of tissue homogenate.

Superoxide Dismutase (Markund S and Marklund G, 1974)

Pyrogallol auto-oxidizes rapidly in aqueous solution; higher the pH faster is autoxidation and several intermediate products are formed. Thus the solution first becomes yellow-brown with a spectrum showing should between 400-425 nm. After a number of minutes the color begins to turn green and finally after a few hours, a yellow color appears. So the autoxidation is studied essentially during the first step and the rate is taken from the linear increase in absorbance at 420 nm, which is seen for a number of minutes after an induction period of some 10 seconds. Super oxide anion radical ($O_2^{\cdot-}$) catalyses the autoxidation of pyrogallol. A simple and rapid method for assay of SOD is described, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. The supernatant was assayed for SOD activity by following the inhibition of pyrogallol autoxidation. 100 µl of cytosolic supernatant was added to Tris HCl buffer (pH 8.5). The final volume of 3 mL was adjusted with the same buffer. At least 25 µl of pyrogallol was added and changes in absorbance at 420 nm were recorded at 1 minute interval for 3 minutes. The increase in absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD. One unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation per 3 mL of assay mixture.

Catalase (Calibrne AL, 1985)

In the ultraviolet (UV) range H_2O_2 shows a continuous increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance (ΔA) per unit time is a measure of the catalase activity. Heart tissue was homogenized in 50 mM/L potassium phosphate buffer with a ratio of 1:10 w/v. The homogenate was centrifuged at 10,000 revolution per minute (rpm) at 4º C in a cooling centrifuge for 20 minutes. Catalase activity was measured in supernatant obtained after centrifugation. Supernatant (50 µL) was added to cuvette containing 2.95 mL of 19 mM/L solution of H_2O_2 prepared in potassium phosphate buffer. The change in absorbance was monitored at 240 nm wavelength at 1 minute interval for 3 minutes. Presence of catalase decomposed H_2O_2 leading to a decrease in absorbance.

Histopathological studies (Beure B and Kandaswamy N, 1990)

The heart of two rats from each group were excised and fixed in 10% formalin and stained with hematoxylin and eosin. Sections were examined using an Olympus BX51 fluorescence microscope.

Statistical Analysis: All values were expressed as the mean ± SD. Significant differences between three groups were stastically analysed using a one way ANOVA analysis of variance, followed by Dunnett's test. Value of $p<0.05$ was considered statistically significant.

RESULTS

Cactus grandiflorus (0.5 mL and 1.1 mL p.o.) post treatment for 7 days showed cardioprotective activity as substantiated by significant decline in the elevated levels of serum marker enzymes LDH and SGOT; abating lipid peroxide levels; and also by maintaining the level of other endogenous antioxidants with restoration in myocardial endogenous antioxidants levels and also by minimizing vacuolation and maintaining the integrity of the myofibrils as observed under light microscope. *Cactus grandiflorus* probably have decreased the doxorubicin-induced oxidative stress by virtue of its antioxidative properties. Cardioprotection at the two dose levels (0.5 mL and 1.1 mL of *Cactus grandiflorus*) was comparable i.e. showed dose dependent response. The protection produced by the highest dose of test drug

Table 1. Treatment Plan

Groups N=8	Treatment	Dosage, Route of administration and Duration
1	Saline	1 ml / kg normal saline i.p. single dose, 1st day.
2	DOX only	15 mg/ kg DOX i.p. single dose, 1st day.
3	CG only	0.5 ml CG oral three times a day for 7 days and DOX 15 mg/kg i.p on 1st day.
4	DOX + CG 0.5 mL	1.1 ml CG oral three times a day for 7 days and DOX 15 mg/kg i.p. on 1st day.
5	DOX + CG 1.1 mL	0.5 ml CG oral three times a day for 7 days.
6	Vitamin E only	100 mg/kg oral three times a day for 7 days.
7	DOX + Vitamin E	100 mg/kg oral three times a day for 7 days and DOX 15 mg/kg i.p. on 1st day.
CG=*Cactus grandiflorus* mother tincture, DOX=Doxorubicin, N=no of rats in each group		

Table 2. Biochemical observations of the blood parameters of different groups

Treatment groups	LDH (IU/L)	SGOT (IU/L)	SGPT (IU/L)	Alkaline phosphates (KA units)	Creatinine (mg/ml)
Saline	122.431 ± 4.185	32.91 ± 2.387	8.93 ± 1.197	8.491 ± 1.5321	0.879 ± 0.0604
DOX only	393.476 ± 8.99 P<0.01	82.66 ± 5.842 P<0.01	26.16 ± 3.080 P<0.01	14.55 ± 0.584 P<0.01	1.84 ± 1.08 P<0.01
CG only	120.216 ± 2.661 P>0.05	32.95 ± 1.792 P>0.05	9.05 ± 0.696 P>0.05	8.25 ± 0.4975 P>0.05	0.885 ± 0.033 P>0.05
DOX + CG 0.5 mL	122.93 ± 3.273 P>0.05	31.45 ± 1.653 P>0.05	9.14 ± 0.9376 P>0.05	8.26 ± 0.3144 P>0.05	0.878 ± 0.0918 P>0.05
DOX + CG 1.1 mL	272.72 ± 5.419 P<0.01	71.66 ± 3.698 P<0.01	21.75 ± 2.386 P<0.01	11.38 ± 0.5326 P<0.01	1.28 ± 0.184 P<0.01
Vitamin E only	230.306 ± 4.213 P<0.01	55.416 ± 1.365 P<0.01	15.10 ± 1.402 P<0.01	10.58 ± 1.711 P<0.01	0.952 ± 0.054 P<0.01
DOX + Vitamin E	232.715 ± 4.949 P<0.01	58.12 ± 1.757 P<0.01	15.44 ± 1.387 P<0.01	10.62 ± 0.538 P<0.01	0.958 ± 0.125 P<0.01
CG=*Cactus grandiflorus* mother tincture Data represent mean ± SEM of eight rats per group. Data were analyzed using ANOVA followed by Dunnett's test p value (All groups are compared with Saline group).					

Table 3. Biochemical observations of the heart tissue of different groups

Treatment groups	TBARS (nM of MDA/mg protein)	Tissue GSH (µg/mg protein)	SOD (µg/mg protein)	CAT (nM of H2O2 consumed/minute/mg protein)
Saline	1.143 + 0.0878	24.16 + 1.270	13.78 + 0.5168	9.643 + 0.9927
DOX only	8.436 + 1.363 P<0.01	17.64 + 0.663 P<0.01	9.68 + 0.2950 P<0.01	5.387 + 0.6053 P<0.01
CG only	1.405 + 0.0954 P>0.05	22.97 + 0.885 P>0.05	13.39 + 0.8315 P>0.05	9.635 + 0.644 P>0.05
OX + CG 0.5 mL	1.453 + 0.0922 P>0.05	23.64 + 0.847 P>0.05	13.28 + 0.6697 P>0.05	9.855 + 0.453 P>0.05
DOX + CG 1.1 mL	6.883 + 0.732 P< 0.01	19.06 + 1.087 P<0.01	10.01 + 0.3558 P<0.01	6.246 + 0.5879 P<0.01
Vitamin E only	3.688 + 0.397 P< 0.01	20.97 + 0.7424 P<0.01	11.85 + 0.1204 P<0.01	8.282 + 0.7120 P<0.01
DOX + Vitamin E	4.010 + 0.012 P< 0.01	21.95 + 1.789 P<0.01	12.02 + 0.0579 P<0.01	8.596 + 0.3445 P<0.01
CG=*Cactus grandiflorus* mother tincture Data represent mean + SEM of eight rats per group. Data were analyzed using ANOVA followed by Dunnett's test p value (All groups are compared with Saline group)				

Table 4. Observations of the heart weight and heart weight/ body weight of different groups

Treatment groups	Heart Weight (g)	Heart weight/body weight ratio	Mortality %
Saline	1.13 ± 0.04676	0.005 ± 0.000189	0%
DOX only	0.340625±0.0113 P<0.01	0.00212 ± 0.000125 P<0.01	37.5%
CG only	1.09737 ± 0.06928 P>0.05	0.004 ± 0.0001 P<0.01	0%
DOX + CG 0.5 mL	1.034375 ± 0.06863 P<0.01	0.004 ± 0.0001 P<0.01	12.5%
DOX + CG 1.1 mL	0.4575 ± 0.02455 P<0.01	0.003 ± 0.000189 P<0.01	0%
Vitamin E only	0.5755 ± 0.02521 P<0.01	0.0037 ± 0.000163 P<0.01	0%
DOX + Vitamin E	0.587875 ± 0.01125 P<0.01	0.0035 ± 0.0001830 P<0.01	0%
CG=*Cactus grandiflorus* mother tincture Data represent mean ±SEM of eight rats per group. Data were analyzed using ANOVA followed by Dunnett's test p value (All groups are compared with Saline group)			

(1.1 mL *Cactus grandiflorus*) was fairly comparable to that of the standard drug i.e. vitamin E (100 mg/kg). The only treatment of 0.5 mL *Cactus grandiflorus* and vitamin E (100 mg/kg) did not yield any significant difference in terms of biochemical and histopathological observations with that of the normal control animals. Post treatment with vitamin E (100 mg/kg) for 7 days blunted the rise in biochemical parameters (LDH, SGOT and TBARS) showing cardioprotection, restored the myocardial endogenous antioxidants levels (blood and tissue GSH, SOD and CAT) and also minimized vacuolation and maintained the integrity of the myofibrils as observed in the light microscopy.

Fig 1. Level of GSH in different Groups

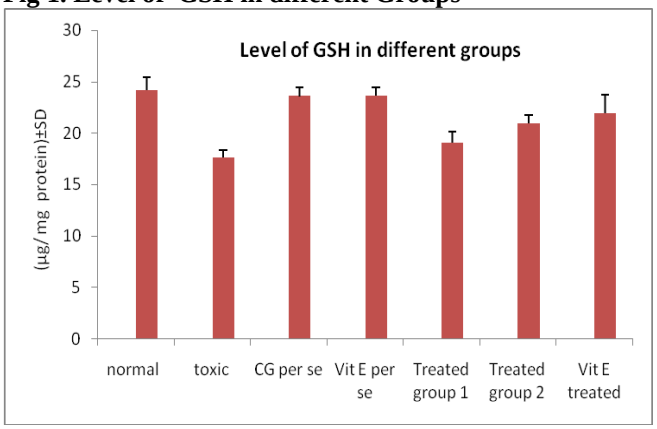


Fig 2. Level of TBARS in different Groups

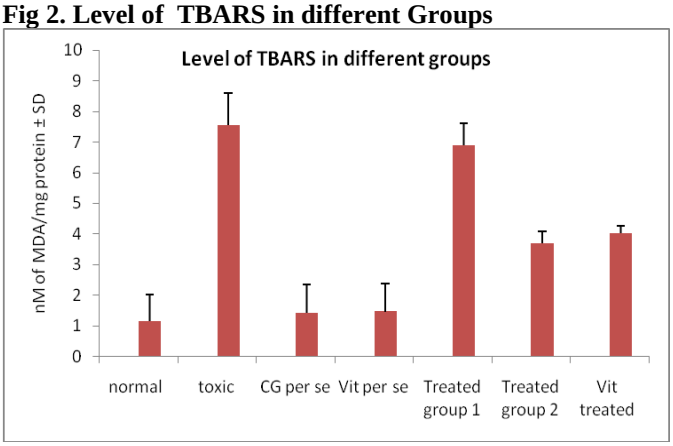


Fig 3. Level of SOD in different Groups

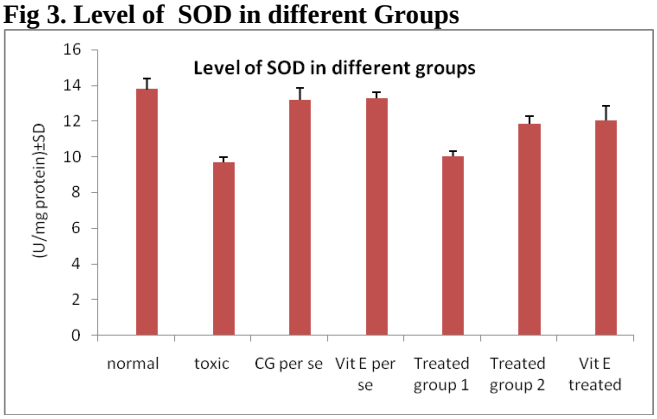


Fig 4. Level of Catalase in different Groups

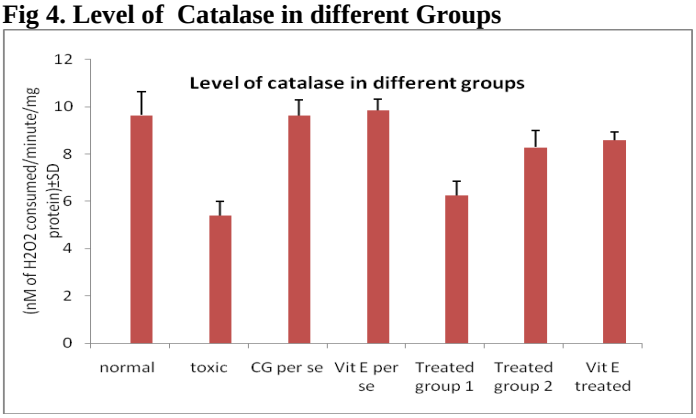


Fig 5. LDH level in different Groups

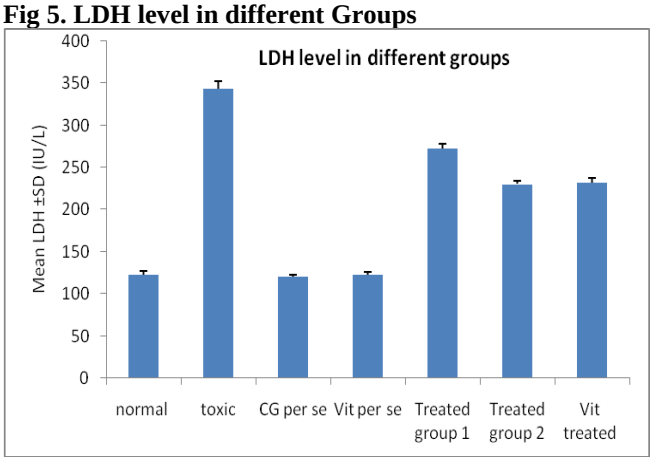


Fig 6. Level of SGOT in different Groups

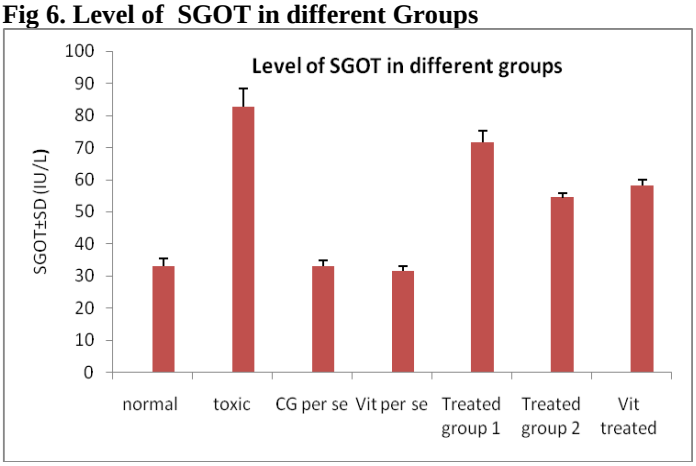


Fig 7. Level of SGPT in different Groups

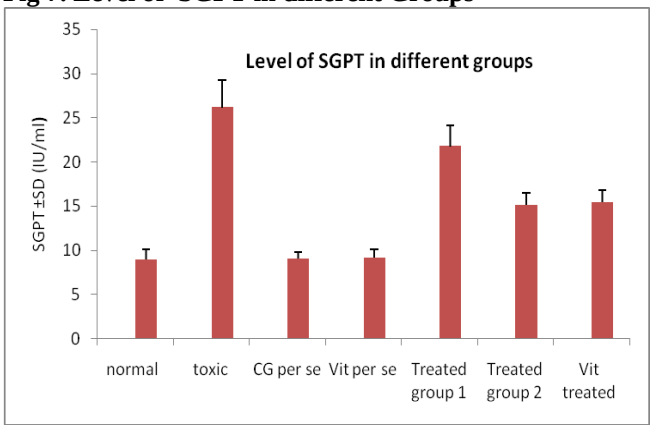


Fig 8. Level of Creatinine in different Groups

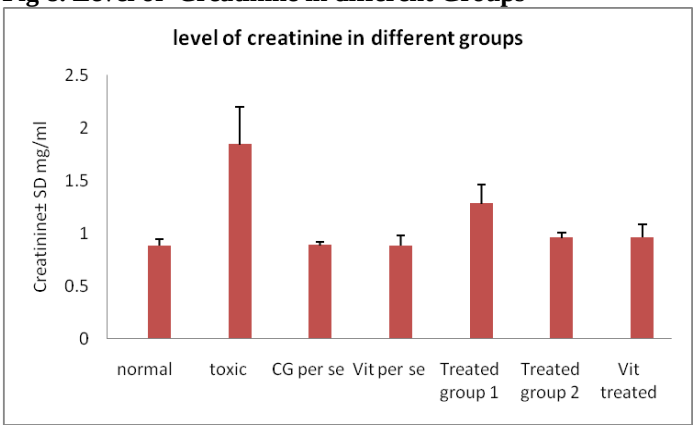


Fig 9. Level of alkaline phosphates in different Groups

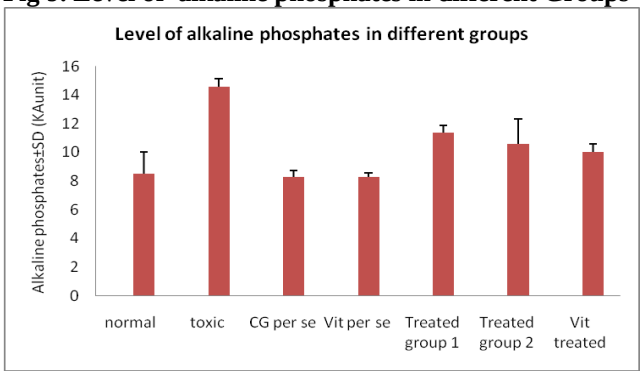
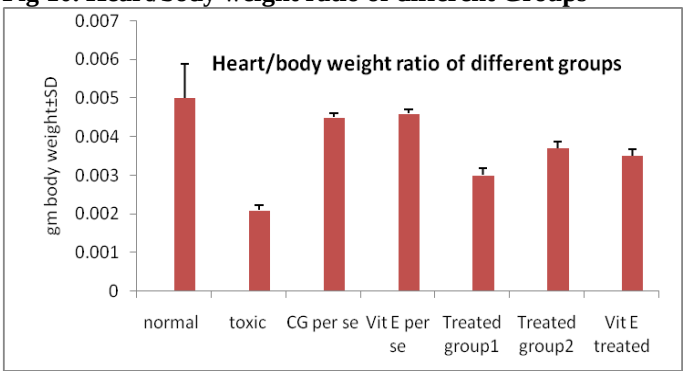
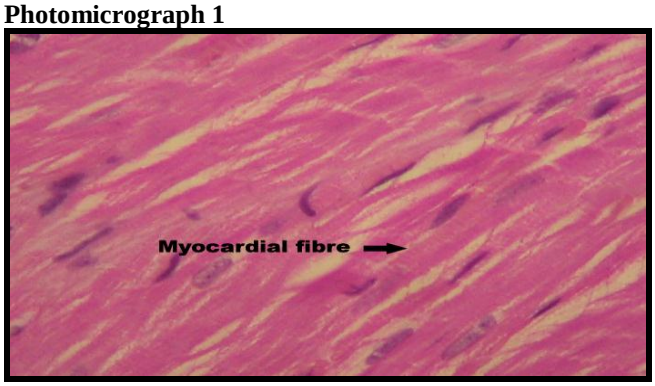


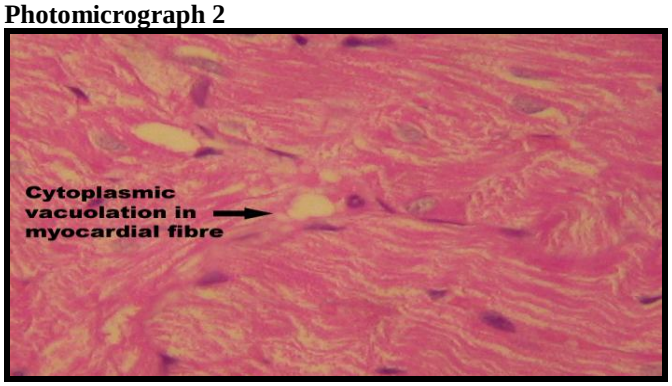
Fig 10. Heart/body weight ratio of different Groups



Photomicrograph 1



Photomicrograph 2



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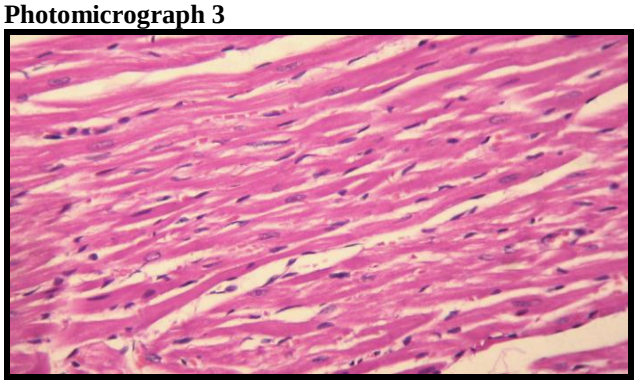
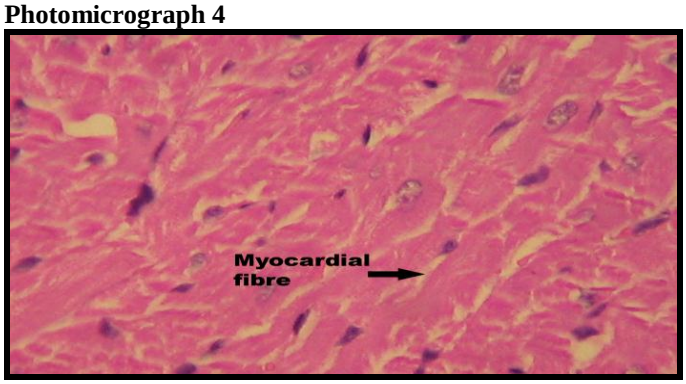
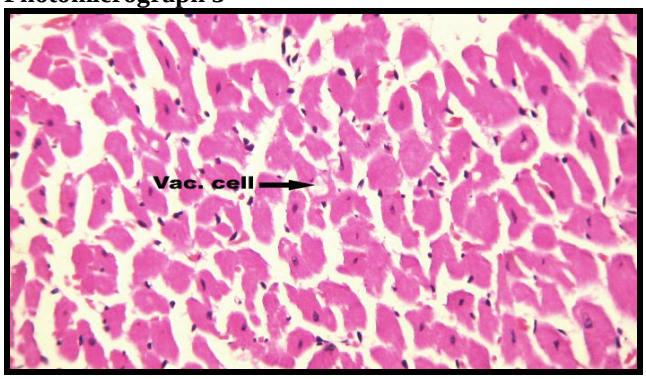


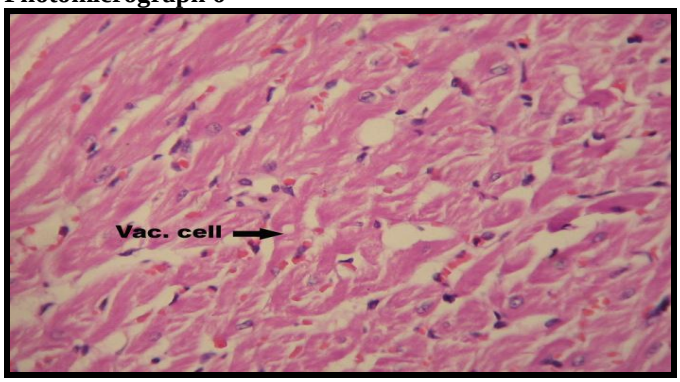
Photomicrograph 4



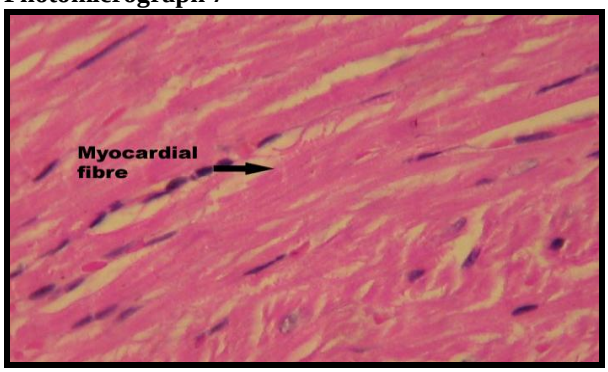
Photomicrograph 5



Photomicrograph 6



Photomicrograph 7



Histopathological Observations

Samples stained in Haematoxyline and Eosin (HE) from heart muscle of all the groups were examined under high power (x 400) of light microscope with special reference to integrity of myocardial fibres and presence of histological evidence of acute DOX-induced cardiac damage. Following findings had been observed:

Photomicrograph 1: High power photomicrograph of normal control animal (Group I) had shown regular and well oriented myocardial fibres (HE x 400).

Photomicrograph 2: High power photomicrograph of DOX-treated 15 mg/kg single dose i.p. on first day (Group II) had shown severe disorganization of myocardium and multiple focal cytoplasmic vacuolation (HE x 400).

Photomicrograph 3: High power photomicrograph from animal treated with 0.5 ml oral *Cactus grandiflorus* mother tincture three times a day (Group III) had shown regular and well oriented myocardial fibres. No necrotic, inflammation or fibrosis was seen (HE x 400).

Photomicrograph 4: High power photomicrograph from animal treated with vitamin E 100 mg/kg once a day oral (Group VIII) had shown normal morphology of myocardial fibres (HE x 400).

Photomicrograph 5: High power photomicrograph from animal post treated with 0.5 ml oral *Cactus grandiflorus* mother tincture three times a day after DOX 15 mg/kg single dose i.p. on first day (Group V) had shown fair presence of disorganized and vacuolated myocardial fibres (HE x 400).

Photomicrograph 6: High power photomicrograph from animal post treated with 1.1ml oral *Cactus grandiflorus* mother tincture three times a day after DOX 15 mg/kg single dose i.p. on first day (Group VI) has shown a single vacuolated fiber. (HE x 400).

Photomicrograph 7: High power photomicrograph from animal pretreated with vitamin E 100 mg/kg oral once a

day followed by DOX (Group VI) shown cardiac muscle fibres of normal shape, size and configuration with minimal disorganization of myocardial fiber (HE x 400).

COMMENTS ON HISTOLOGICAL CHANGES

Samples from heart muscle from animals belonging to normal saline, toxic control, drug per se, drug treated groups and Vitamin E per se and treated were examined with special reference to myocardial fiber integrity and histological evidence of acute doxorubicin induced cardiac damage.

The Toxic Control group showed individual myocardial cells with small and large vacuolar myopathy. There was however no evidence of necrosis of the myocardium seen. The samples from the group post treated with drug showed only rare isolated myocardial fibers with small vacuoles. The group post treated with Vitamin E after Doxorubicin showed a normal myocardium and no evidence of vacuolar myopathy. A normal histological appearance of the myocardium was also seen in normal control and drug per se groups. The results indicate a partial protective effect of the test drug against Doxorubicin induced cardiac myopathy.

DISCUSSION

Free oxygen free radicals are implicated in the pathophysiology of diverse cardiovascular disease (Bagchi D *et al*., 2003). Reactive oxygen species and reactive nitrogen species associated lipid peroxidation, oxidation of low density lipoproteins (LDL), activation of matrix metalloproteinases are suggested to be the major contributor to the pathogenesis and progression of cardiovascular diseases (Dröge W, 2002; Giorano FJ, 2005). Free radical and oxidative stress also appear to be a common mediator of apoptosis and necrosis, directly or via lipid peroxidation (Kumr D and Jugdutt BI, 2003). Although the results are somewhat inconsistent in human, animal studies have established that ROS scavenging antioxidants may play an important role in the prevention and combat against ROS-mediated cardiac abnormalities (Kumr D and Jugdutt BI, 2003; Kaul *et al*., 1993).

Cactus grandiflorus is good source of polyphenols. So far, no sufficient work has been done revealing a variety of pharmacological effects of this drug especially in cardiovascular diseases and cancer. Most of pharmacological effects are not scientifically evaluated. Only reported by homeopathic practioners. Cactus is thought to elevate arteriolar tension by increasing the muscular energy of the heart and causing arteriolar contraction. This theory has not been confirmed by human data. Early research with commercial preparations of the active compound proved it to be physiologically inert. More recently, in studies with rats and dogs, hordenine showed a positive inotropic effect on the heart, with increased systolic and diastolic blood pressures and peripheral blood flow volume. Flavonoids and their derivatives (rutin, rutinoside, and kaempferitin) are thought to improve capillary function by decreasing abnormal leakage (Orlano Jones, 1890). Studies on isolated frog heart and papillary muscle from guinea pig had shown positive inotropic effect. Antihypertensive and antiarrhythmic activity is due to flavonolglycosides which are present mainly in stem and flowers extract. Flavones affect the myocardium's calcium metabolism, thereby increasing its contractile power and promoting its normal rhythm (Anonymous 1).

It is well known that the rat model of doxorubicin-induced cardiotoxicity has been widely used to evaluate cardioprotective drugs and to study myocardial consequences of ischemic disorders (Puri Maulik SK *et al*., 2005; Haed S *et al*., 2006; Yilmz S *et al*., 2006) doxorubicin at 20 mg/kg i.p. (a dose which causes heart injury during the first five days but shows low mortality rate) has been used earlier to develop acute model of MI (Bagchi D *et al*., 2003; Haed S *et al*., 2006). All these findings have prompted us to investigate possible cardioprotective effect of *Cactus grandiflorus* in doxorubicin-induced model of acute cardiotoxicity in rats. In this study, rats were administered 0.5 mL and 1.1 mL *Cactus grandiflorus* mother tincture orally after doxorubicin administration. The dose of *Cactus grandiflorus* is calculated on the basis of human dose and by the use of conversion factor.

The elevation of LDH and SGOT is a well known quantitative index of compromised cell integrity and is considered as an indicator of myocardial damage induced by doxorubicin (Saad SY *et al*., 2001). Results of the present study also indicate that, doxorubicin (15 mg/kg i.p.) elevated serum levels of LDH and SGOT, which is believed to be due to damage caused to sarcolemma by doxorubicin and rendering the cells containing these enzymes leaky. It also confirmed that MI was developed in our model. This is well agreed by vast majority of investigators where doxorubicin has increased the serum levels of LDH and SGOT (Saad SY *et al*., 2001; Adel-Wahab MH *et al*., 2003) *Cactus grandiflorus* (0.5 mL and 1.1 mL) post treatment significantly brought down the elevated levels of these enzymes back to near normal values indicating the possible cardioprotective effect. Glutathione is one of the important nonenzymatic defenses essential for maintaining cell integrity because of its reducing properties (Maxwell SR *et al*., 1997; Łuczaj W, Skrzydlewska E, 2004). In our study following doxorubicin treatment, there was significant decrease in both blood and cardiac tissue GSH levels leaving the heart more susceptible to oxidative injury. Similar findings were observed by previous workers where doxorubicin-treated rats showed severe depletion in both blood and tissue GSH levels. In the present study, *Cactus grandiflorus* post-treatment significantly restored the

decreased levels of blood and tissue GSH in all the concentrations.

It was reported earlier that reactive oxygen species generated after doxorubicin-mediated injury induces lipid peroxidation (Saad SY *et al*., 2001; Adel-Wahab MH *et al*., 2003). Which either triggers apoptosis or necrosis depending on its extent (Kumr D and Jugdutt BI, 2003). In accordance with these reports, we have found a considerable increase in MDA level, the index of lipid peroxidation, in doxorubicin treated animals and *Cactus grandiflorus* post treatment was potentially effective in lowering this level. The possible mechanism involved in reducing lipid peroxidation by *Cactus grandiflorus* in this model could be its strong free radical scavenging properties.

In the present study, doxorubicin alone treatment to a great extent reduced the levels of antioxidative enzymes i.e. SOD and CAT. However, *Cactus grandiflorus* supplementation in two doses in doxorubicin challenged groups considerably restored the levels of both SOD and CAT to in a dose dependent manner. *Cactus grandiflorus* post treatment efficiently maintained the activities of SOD and CAT of rats exposed to various oxidative challenges. Doxorubicin--nduce cardiotoxicity is also manifested by altered cardiac histopathological features including marked interstitial oedema, focal cytoplasmic vacuolation, focal myocardial fibrosis, disorganization of myocardium, myofibrillar loss and myocardial necrosis (Yilmz S *et al*., 2006; Saad SY *et al*., 2001). Our observations corresponding to doxorubicin-induced alterations in myocardial morphology are in accordance with these findings. The doxorubicin-treated group showed scattered myocardial cells with multiple vacuolar myopathic changes. There was however no evidence of necrosis of the myocardium seen. The samples from the groups treated with 0.5 mL and 1.1 mL *Cactus grandiflorus* with doxorubicin showed a significant reduction in the number of vacuolated cells, Samples from the saline *Cactus grandiflorus* only and vitamin E only groups showed normal myocardium.

It is quite obvious that *Cactus grandiflorus* polyphenols must have alleviated doxorubicin-mediated cardiac injury by virtue of its strong radical scavenging and iron chelating properties. In addition to this, it would be quite exciting to look for some other probable mechanisms underlying for the cardioprotective activity of *Cactus grandiflorus* and its polyphenols and to explore how these protective effects are mediated at the cellular level. Thus, our findings indicate that *Cactus grandiflorus* has cardioprotective effect against doxorubicin-induced cardiotoxicity model in rats. Further studies to unravel the basic mechanism would be worth investigating. Likewise, the active components of *Cactus grandiflorus* responsible for the cardioprotective effects need to be identified in further studies.

CONCLUSION

After above results and discussion it can be said that doxorubicin induced cardiotoxicity is an antioxidant deficient affair. Treatment with *Cactus grandiflorus* mother tincture can improve the deficient antioxidant status of heart. This preparation also has no deleterious effect on liver and kidney in both doses. Its beneficial effect in doxorubicin cancer therapy should be evaluated in further studies.

Conflict of Interest

We declare that the research involved in this manuscript had been carried out at an educational institute as a part of dissertation work. We did not receive any funds that could influence our work. Our institute had not paid us any honoraria, consultancy fees and the findings of this study have been submitted as a part or as a whole to the patenting authorities in any country.

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