eISSN: 2231-0541 CAS CODEN: PHARN8 An ELSEVIER Covered Journal

PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 4 | Issue 6 | November-December 2013 | Pages 1131-1141

Original Research Article

EFFECT OF TRIPHALA- AN AYURVEDIC HERBAL FORMULATION ON DOXORUBICIN INDUCED CARDIO TOXICITY IN RATS

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Received: 05-08-2013 Revised: 03-09-2013

Accepted: 02-09-2013 Available online: 01-11-2013

ABSTRACT

The objective was to evaluate the cardioprotective activity of triphala in doxorubicin induced cardiotoxicity in albino wistar rats. Twenty- four rats were divided into four groups (n=6). Group I (normal) received normal saline (p.o.) for 10 days. The group II rats received doxorubicin HCl (15mg/kg, i.p.) on 7th day. The rats in group III and IV were pretreated with triphala (500mg/kg, p.o. and 1000 mg/kg, p.o.) followed by doxorubicin on 7thday. On 11th day, ECG studies, serum creatinine phosphokinase (CPK), CK-MB and lactate dehydrogenase (LDH) levels as well as anti-oxidant parameters like superoxide dismutase, catalase and lipid peroxidation along with histopathological changes were studied. The doxorubicin treated rats have shown abnormal ECG values along with elevated (*** P <0.001) serum creatinine phosphokinase (CPK), CK-MB, LDH as well as lipid peroxidation levels while significantly reduced values (*** P <0.001) of SOD and catalase were observed as compared to group I rats. The triphala and doxorubicin treated rats have shown significant alterations when compared to the doxorubicin alone treated rats suggesting the cardio protection. Histological observations of rat hearts further correlated the cardioprotective effect of triphala. The result of this study provides the experimental evidence for triphala's cardioprotective effects.

Key words: Triphala, cardiotoxicity, catalase, lactate dehydragenase, electrocardiogram.

INTRODUCTION

Cardiotoxicity is a condition arising due to extensive damage to the myocardium. Hence, the heart may be unable to pump blood throughout the body. This can be due to the use of chemotherapy drugs and other medications.[1] Doxorubicin is mainly used in the treatment of solid tumors. It causes breaks in DNA strands by activating Topoisomerase II and generating quinine type free radicals. The oxidative damage is complex effecting mitochondria, lipid peroxidation levels, sarcoplasmic reticulum, and other structures. Mitochondrial oxidative damage and calcium overload, both potent inducers of the mitochondrial permeability transition are common events during DOX treatment.[2,3]

Triphala is the most commonly used ayurvedic herbal formulation and comprises of the fruits of three trees including Indian gooseberry, Bellericmyrobalan and Chebulicmyrobalan. It is believed to promote health, boost immunity and longevity. It is rich in anti-oxidants which play an essential role in the treatment of a wide variety of conditions like infections, obesity, anemia, fatigue, constipation, and in infectious diseases like tuberculosis, pneumonia, and AIDS. It is also considered to be a blood cleanser and gentle laxative. The individual herbs, used in the formulation are reported to have several other health benefits. Triphala has been reported to be a rich source of vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, beta-sitosterol and flavonoids. As per the literature survey, no work has been done on cardio protective activities of triphala. Hence, it is selected to study its cardioprotective effects.[4,5]

MATERIAL AND METHODS

Animals- Healthy albino wistar rats, weighing 200-250g were maintained in the animal house of PES college of Pharmacy, Bangalore for experimental purpose. All these animals were housed in temperature controlled room 25±1°C, relative humidity 45-55% and a 12 h light/ 12h dark cycle. They were acclimatized for 7 days and fed with standard rat chow Prior approval was taken from the Institutional Animal Ethical Committee (IAEC approval No-***PESCP/IAEC/* 10 /11**).

Grouping of animals: The animals were randomly divided into 4 groups with 6 animals each. (n=6), Group I- Vehicle (distilled water + 1% gum acacia) was given orally for 10 consecutive days. Group II- Doxorubicin (15mg/kg i.p.) was administered once on 7th day. Group III- Ttriphala500mg/kg, p.o. was administered for 10 days and once on 7th day doxorubicin was administered. Group IV- Triphala1000 mg/kg, p.o. was administered for 10 days and doxorubicin once on 7th day.

Recording of ECG- On 11th day, 24 h after the last administration of the drugs, the rats were subjected to ECG estimation. For this purpose the rats were anesthetized using thiopentone sodium (35 mg/kg, i.p.), and ECG was recorded using a Lab chart 7 reader.

Sample collection and biochemical assays- Blood was drawn by retro orbital puncture method and transferred to non-heparinized eppendorf tubes and centrifuged at 4000 rpm for 15 min at 4°c and supernatant serum was pipetted by micropipette and transferred to sterile labeled eppendorf tubes and these were stored in the freezer at -20°c and these were used for the estimation of CPK, CK-MB and LDH by using kits manufactured by ERBA diagnostics.

Cardiac biochemical estimation- After the blood withdrawal from retro orbital sinus the rats were sacrificed by cervical dislocation under anesthesia. The thorax was opened immediately and the heart was exposed and isolated immediately and washed in ice cold normal saline followed by 0.15M Tris-Hcl (pH-7.4) and blotted dry and weighed. A 10% w/v of homogenate was prepared in 0.15M tris-Hcl buffer. Further, the homogenate was centrifuged at 1500rpm for 15 min at 4°C. The supernatant thus obtained was used for estimation of superoxide dismutase, lipid peroxidation and Catalase estimation.

Estimation of superoxide dismutase

The SOD activity was assayed by the method of Kakkar*et al.,* (1984). The principle of the assay was based on the inhibition of nitrobluetetrazolium (NBT) reduction. The reduction of nitro blue tetrazolium was inhibited by SOD, which was measured colorimetrically at 560 nm. "One unit of SOD activity is defined as that amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions".[9]

Estimation of Lipid peroxidation

Lipid peroxidation in cardiac tissues was estimated colorimetrically by measuring thiobarbituric acid reactive species (TBARS). Here, the sample under test is heated with thiobarbituric acid (TBA) at low pH to form a pink chromogen $(TBA)_2$-malondialdehyde adduct, the fluorescence of which is measured at 532nm against blank. This test is often said to measure malondialdehyde (MDA) formed in peroxidizing lipid systems and hence expressed as nmole of MDA/g wet weight.[10]

Catalase estimation

The estimation of catalase was based on the method devised by Sinha et.al.(1972). It is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 570-610 nm.[11]

Histological examination- Heart from each sacrificed rat was taken and fixed in 10% v/v neutral formalin and processed to paraffin wax. Sections (5 microns) were stained with haematoxyllin and eosin and were examined under light microscope.

Statistical analysis- Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Dunnet's test for post comparison using software Graph pad prism version 5.0. Results were expressed as mean ± SEM. P values <0.05 were considered to be statistically significant

RESULTS

There is a significant increase in the P duration, QT interval, RR interval, PR interval and heart rate (BPM) while not significant for QRS interval in group II rats as compared to group I (control). The rats in group III have showed significant decrease in P duration (s), QRS interval, RR interval and heart rate (BPM) when compared to group II rats while the QT interval was non- significant. While, rats in group IV have showed significant decrease in P duration, RR interval and heart rate (BPM) when compared to group II rats, while QRS interval and QT interval were found non- significant given in table I

A significant increase has been seen in heart weight (g), serum creatinine kinase –MB (mg/gl),serum creatinine kinase (IU/L), serum LDH(mg/dl) and total protein content (g/dl of protein) in group II rats while compared to group I. The rats treated with triphala and doxububicin have showed significant cardioprotection by decreased heart weight, serum CK-MB, serum LDH and total protein content levels as compared to group II, shown in table II. A significant decrease in SOD and catalase levels was seen in group II rats as compared to group I (normal) while a significant increase was found in lipid peroxidation levels (LPO) in group II as compared to group I rats. The rats in group III and IV did not show significant increase in SOD levels as compared to group II rats while a significant increase in catalase (CAT) levels and a significant reduction in lipid peroxidation (LPO) levels were found in these groups as compared to group II rats, shown in table III.

Table.1.Showing effect of vehicle, Doxorubicin and Triphala on rat ECG and Heart rate

Group	Treatment	P duration (s)	QRS interval (s)	QT interval (s)	RR interval (s)	PR interval (s)	Heart rate (BPM)
I	Vehicle p.o.	0.0239 ±.0017	0.0237 ±.001	0.05167 ± 004	0.1607 ±.007	0.0460 ±.0001	358.9 ±11.55
II	DOX	0.03245 ±.0015[b]	0.02539 ± .002	0.07210 ±.005[b]	0.2596 ±.002[c]	0.0561 ±.003[a]	233.1 ±0.866[c]
III	TRI 500 + DOX	0.02717 ±.0016[d]	0.02092 ±.005[d]	0.06695 ±.001	0.1796 ± 004[f]	0.0490 ±.002	339.1 ±9.151[f]
IV	TRI1000 +DOX	0.02648 ±.0017[d]	0.02165 ± .001	0.07249 ± .001	0.1513 ±.003[f]	0.0483 ±.001[d]	392.4 ±4.041[f]

Group II was compared with group I, a= P<0.05, b=P<0.01 and c=P<0.001. Group III and IV were compared with group II, d= P < 0.05, e= P < 0.01 and f= P < 0.001, Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Dunnet's test.

Table.2.Showing effect of vehicle, doxorubicin and triphala on heart weight, serum CK-MB, serum LDH and protein content in rats

Group (n=6)	Treatment		Heart weight (g)	Serum CK-MB (mg/dl)	Serum creatinine kinase (IU/L)	Serum LDH (mg/dl)
	Dose and Route	Duration				
I	Vehicle,	11 days	1.091 ±0.049	366.95 ±16.22	155.44 ± 2.73	842.56 ±11.99
II	DOX	On 7th day	1.371 ±.0855[a]	876.72 ±29.54[c]	556.091 ± 17.43[c]	2633.1 ± 130.0[c]
III	Triphala + DOX	11days On 7thday	1.178 ±0527	502.26 ±29.33[f]	217.63 ± 19.66[d]	1530.63 ±157.4[f]
IV	Triphala + DOX	11days On 7th day	0.988 ±.0554[e]	403.73 ±10.22[f]	266.49 ±22.69[f]	1461.91 ±90.4[f]

Group II was compared with group I, a= P<0.05, b=P<0.01 and c=P<0.001. Group III and IV were compared with group II, d= P < 0.05, e= P < 0.01 and f= P < 0.001, Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Dunnet's test.

Table.3.Showing effect of vehicle, doxorubicin and triphala on SOD, lipid peroxidation (LPO), catalase and total protein content in rats

Group (n=6)	Treatment		SOD (units/mg of protein	LPO (MDA/mg of protein)	Catalase (units/mg of protein)	Total protein content (g/dl of protein)
	Dose and Route	Duration				
I	Vehicle,p.o.	11 days	48.271 ± 2.33	21.777 ± 0.495	62.01 ± 1.867	56.89 ± 1.454
II	DOX	On 7th day	25.598 ± 1.314^{c}	84.557 ± 4.784^{c}	35.65 ± 2.026^{c}	32.38 ± 1.211^{c}
III	Triphala DOX	11days On 7th day	31.672 ± 2.428	51.571 ± 4.903^{e}	41.91 ± 0.934	37.192 ± 2.284
IV	Triphala DOX	11days On 7th day	27.985 ± 2.776	36.198 ± 5.150^{f}	46.72 ± 3.071^{e}	41.69 ± 1.304^{e}

Group II was compared with group I, a= P<0.05, b=P<0.01 and c=P<0.001. Group III and IV were compared with group II, d= P < 0.05, e= P < 0.01 and f= P < 0.001, Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Dunnet's test.

ECG studies

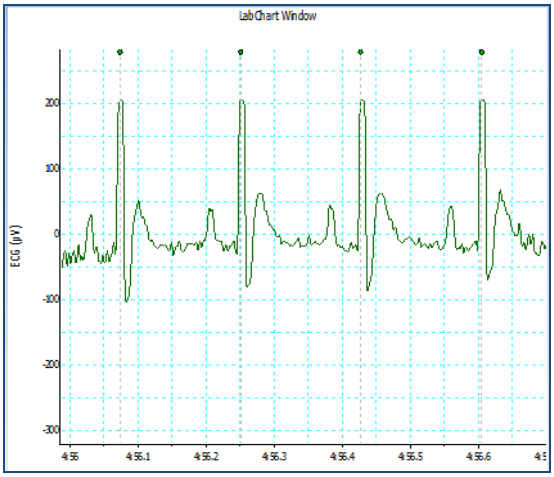

Fig.1.Effect of vehicle on rat ECG

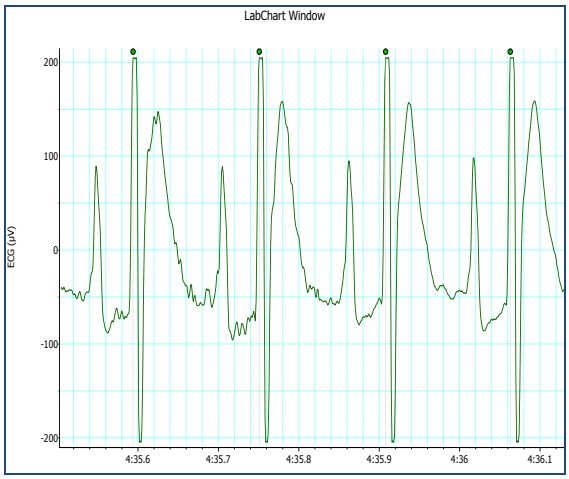

Fig.2.Effect of DOX on rat ECG

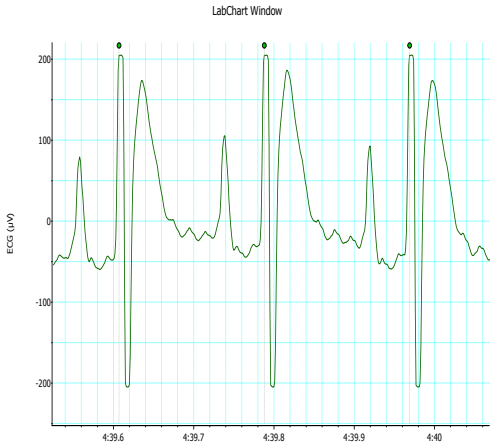

Fig.3.Effect of TRI 500mg/kg + DOX on rat ECG

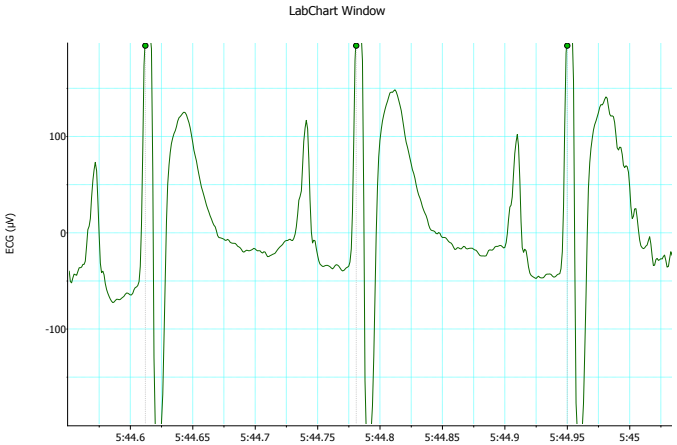

Fig .4.Effect of TRI 1000mg/kg + DOX on rat ECG

HISTOPATHOLOGICAL EXAMINATION

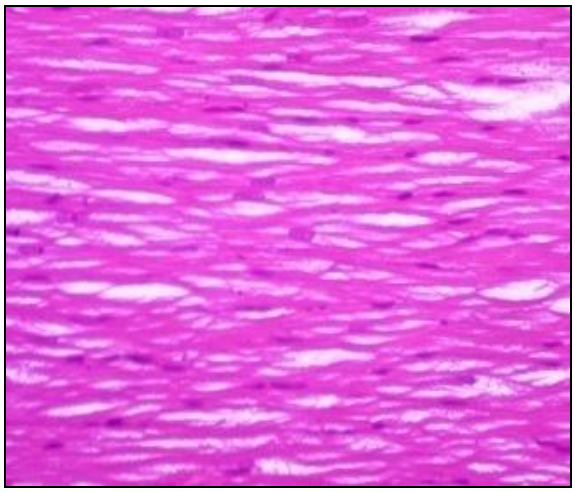

Fig .5.Effect of vehicle on rat heart (X400)

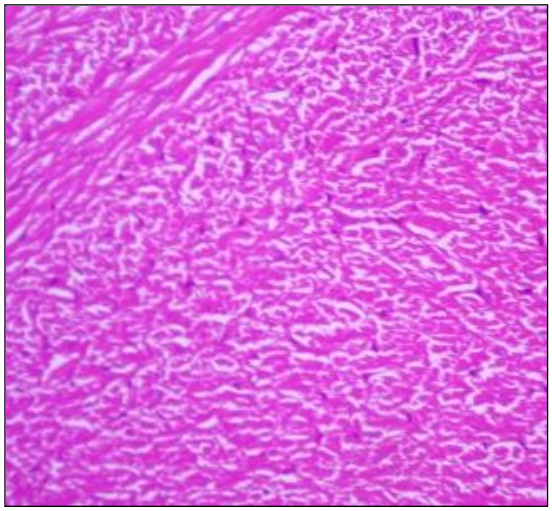

Fig.6.Effect of DOX on rat heart (X400)

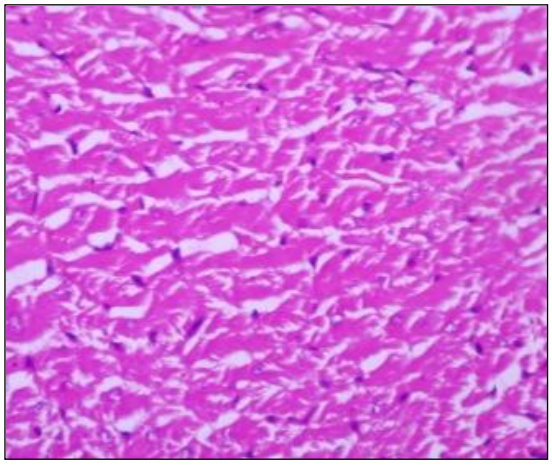

Fig.7. Effect of TRI 500mg/kg +DOX on rat heart(X400)

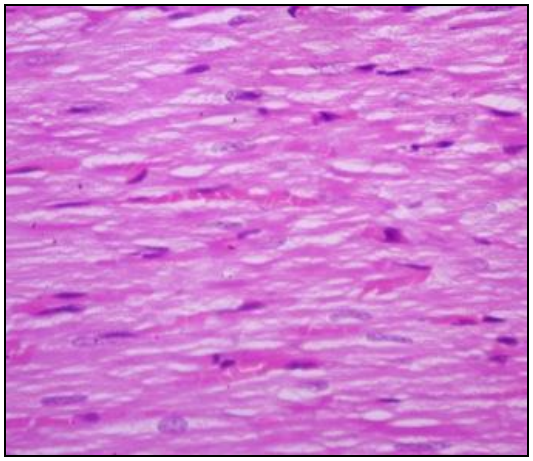

Fig .8.Effect of TRI 1000mg/kg +DOX on rat heart (X400)

DISCUSSION

Doxorubicin is an anthracycline antibiotic and is used in treatment of solid tumors. In doxorubicin treated cardiotoxic rats the free radicals[12]generated as a result of doxorubicin triggers membrane peroxidation and disruption of cardiac myocytes, which may lead to increased release of creatinine kinase in the serum. The rats treated with triphala and doxorubicin has shown significantly decreased serum CK-MB and creatinine phosphokinase concentration when compared to doxorubicin induced cardiotoxic rats. This suggested that the cardioprotective effect of triphala. Further, elevated levels of serum lactate dehydrogenase (LDH) were seen in doxorubicin treated cardiotoxic rats. The increase of LDH level in serum and extracellular fluid suggests an increased leakage of this enzyme from mitochondria as a result of toxicity induced by treatment with doxorubicin. The rats treated with triphala and doxorubicin showed significantly decreased levels of LDH, which may be due to the cardioprotective activity of triphala on heart as compared to cardiotoxic rats.

Also, the heart tissues are particularly susceptible to free radical injury since it contains low levels of free radical detoxifying enzymes/molecules like SOD, catalase etc. In rats treated with doxorubicin a decreased level SOD was seen as compared to normal rats. This is because doxorubicin induced cardiotoxic effect on heart causes reduction in SOD levels, which act as free radical scavengers. This shows redox imbalance and is due to excessive consumption of SOD by the enormous amount of free radicals that are produced due to cardiotoxic action of doxorubicin, thus depleting the SOD concentration.

Rats treated with triphala and doxorubicin showed a significant increase in SOD levels as compared to cardiotoxic rats. This may be due to the anti-oxidant

activity of triphala. Catalase is an anti-oxidant enzyme which catalyzes the breakdown of hydrogen peroxide into hydrogen and water. In doxorubicin induced cardiotoxicity the catalase levels are depleted due to excessive consumption of catalase as well as due to mitochondrial damage. In rats treated with doxorubicin and triphala, the catalase levels increased significantly, showing cardioprotective action of triphala. Also, there is a significant increase in lipid peroxidation in doxorubicin treated group as compared to normal. This is due to cellular damage caused by reactive oxygen species. In rats treated with doxorubicin and triphala, the lipid peroxidation levels levels decreased significantly, showing cardio protective action of triphala.[13]

Further ECG studies showed significant alterations in group II (DOX treated) rats as compared to group I (normal) rats. There is a significant increase in the P duration (s) indicating atrial hypertrophy and atrial arrhythmias, QT interval (s) indicating myocardial infarction (MI) and elevated T wave indicating hyperkalemia, increased RR interval indicating ventricular tachycardia, increased PR interval (s) indicating hypokalemia.. There was no much significant change in QRS interval (s) seen. While in case of groups treated with triphala along with doxorubicin i.e. group III and IV respectively significant improvement in ECG was found like reduction in P interval, reduced QT interval, significantly normal RR interval, decreased PR interval as well as a decreased heart rate as compared to group II (DOX treated) animals.[14] This indicates normal heart conditions and alleviation of myocardial infarction (MI) and cardiomyopathic symptoms. This may support the cardioprotective nature of triphala.

The histopathological studies of rat heart further highlight the significant cardioprotective action of triphala. There are clear indications of myocardial damage in group II animals (DOX treated) with partial loss of arrangement of the cardiac muscle fibres, loss of integrity of myocardial cell membrane, loss of striations and loss of continuity of myofibrillar structures, increased interstitial spaces at focal areas and appearance of unremarkable vascular spaces. The rats in group III and IV pretreated with triphala and also administered with doxorubicin showed intact arrangement of cardiac muscle fibres, myocardial cell membrane and myofibrillar structure with striations along with continuity with adjacent myofibrils. The interstitial spaces also appeared intact and few congested vascular spaces were seen, as compared to group II rats. This indicates the cardioprotective action of triphala.

CONCLUSION

From the results of this study it is concluded that triphala possesses cardioprotective effects against

doxorubicin induced cardiotoxicity in experimental animals. This observed protective effect of triphala may be due preventing uptake and accumulation of doxorubicin in cardiomyocytes and also its antioxidant potential. The further research is warranted to know the exact mechanism of action.

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