Research article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Cardioprotective effect of 'Qolest' a polyherbal formulation against doxorubicin induced cardiotoxicity in wistar rats

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ABSTRACT

Albino Wistar rats were used in this study. The activities of serum marker enzymes (CK, CK-MB, LDH, AST, ALT and ALP), lipid profiles such as cholesterol, triglycerides and HDL level were increased significantly in DOX group. In addition, it also exhibited electrocardiographic changes such as reduced R wave and ST segment elevation. Pretreatment with Qolest decreases serum enzyme levels and lipid profiles brought to the near normal values. Pretreatment with Qolest significantly protected the myocardium from the toxic effect of DOX, by increasing the levels of antioxidant enzymes such as GSH, SOD and CAT towards normal and decreased the increased level of malondialdehyde. It also reduced the severity of cellular damage of the myocardium confirmed by histopathology. The restoration of the endogenous antioxidant system clearly depicts that Qolest have produced its protective effect by scavenging the reactive oxygen species (ROS). The results of this study indicated that the cardioprotective effect of Qolest might be attributed to its antioxidant property.

KEY WORDS: Antioxidant, Cardiotoxicity, Doxorubicin, Cardioprotective, Free radicals, Qolest.

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INTRODUCTION

Doxorubicin, an anthracycline derivative is one of the effective and useful antineoplastic agent commonly used for the treatment of variety of tumors including solid and malignant lymphoma.[1] However, its clinical use is restricted due to its cardiotoxic effect.[2]

The mechanism of doxorubicin induced cardiotoxicity is not completely understood, but recent studies have postulated the involvement of oxygen free radicals in the development of cardiotoxicity. Doxorubicin, by the virtue of its semiquinone group the drug is reported to increase the generation of superoxide radicals and hydrogen peroxide there by damage the heart by exceeding the oxygen radical detoxifying capacity of cardiac mitochondria and scarcoplasmic reticulum.[3]

The mechanisms of cytotoxicity of anthracyclines in cancer cells are diverse including[4]:-

(1) inhibition of both DNA replication and RNA transcription;

(2) free radical generation, leading to DNA damage or lipid peroxidation;

(3) DNA alkylation;

(4) DNA cross-linking;

(5) Interference with DNA unwinding or DNA strand separation and helicase activity;

(6) Direct membrane damage due to lipidoxidation; and

(7) Inhibition of topoisomerase II.

Like all other anticancer agents, however, anthracyclines are a double-edged sword because their use can lead to development of tumor cell resistance, and they can be toxic to healthy tissues. In particular, anthracyclines are known to cause a cardiomyopathy that leads to a form of congestive heart failure that is usually refractory to common medications.[5,6,7]

Therefore, antioxidant therapy may be useful in the management of doxorubicin induced cardiotoxicity. Earlier studies have reported that Cardipro, a polyherbal formulation has shown protective effect in doxorubicin induced cardiotoxicity.[8] Abana has been reported to possess the cardioprotective property[9] and preventive role of lipistat against doxorubicin induced myocardial toxicity in rats.[10]

Qolest is a polyherbal formulation containing extracts of well known plants and possess antioxidant property. The herbal composition of Qolest contains (each 450 mg capsules) Guggulu (*Commiphora Mukul),* Maricha *(Piper Nigrum),* Chitraka *(Plumbago Zeylanica),* Arjuna *(Terminalia Arjuna),* Guduchi *(Tinospora Cordifolia) and* Medhika *(Trigonella Foenum–Gracecum).* Earlier studies have shown that some of the constituents of Qolest such as *Terminalia Arjuna* prevents Doxorubicin induced cardiotoxicity[11], *Piper longum* by the virtue of its antioxidant property prevents adrimycin

induced cardiotoxicity.[12] *Plumbago Zeylanica* has been reported to possess antioxidant and free radical scavenging property[13] and other constituents like *curcuma longa*[14]and *Tinosporia cordifolia*[15] significantly protect the ischemia-reperfusion induced myocardial injuries due to their antioxidant property.

In view of this, doxorubicin induced cardiotoxicity is linked to oxidative stress, therefore the present study has designed to investigate the antioxidant and cardioprotective effect of Qolest against doxorubicin induced cardiotoxicity in wistar rats.

MATERIALS AND METHODS

Animal selection - Albino Wistar rats of either sex weighing 150-200 g were selected for cardioprotective activity. They were procured from animal house of KLES College of pharmacy, Hubli. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27 ^{o}C +- 2 ^{o}C under 12 hours dark / light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water ad libitum was provided. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) of KLE University's College of Pharmacy, Hubli (Reference Number KLEU/COPHBL/17/2010-11) and CPCSEA Registration no. 1712/AC/08/CPCSEA Dated O7.O4.2007.

Materials - Qolest was a generous gift from pink health, India. Doxorubicin & other chemicals used analytical grade & procured locally. Analytical kits are obtained from ERBA Diagnostics, Daman, India.

Dosage fixation- Dosage of the Doxorubicin administered intraperitonially was prepared in the saline (0.9% sodium chloride saline solution). Each dose of Doxorubicin containing 2.5mg/kg body weight for a cumulative dose of 15mg/kg body weight for the present study, were chosen based on previous reports[10].Qolest suspension was prepared in 0.5% CMC by using distilled water. Dose of Qolest 40 mg/kg & 80 mg/kg body weight were selected according to therapeutically equivalent dose.

Experimental design- After one week of acclimatization, the animals were divided into 5 groups of 6 animals each Group I: CONTROL (Control group): Animals were treated with normal saline 5 ml/kg body weight alone; on the same regimen as doxorubicin.

Group II: DOX (Doxorubicin treated): Doxorubicin was administered intraperitonially in 6 equal injections (each dose containing 2.5 mg/kg body weight) alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg body weight.

Group III: QOLEST TREATED: Qolest suspended freshly in 0.5% CMC will be pretreated orally (40 mg/kg body weight, According to Therapeutically equivalent dose) for 2 weeks, and then alternatively with vehicle for next 2 weeks.

Group IV: QOLEST + DOX (Qolest plus doxorubicin treated): Qolest was pretreated daily (40 mg/kg/oral) for 2 weeks, and then alternatively with doxorubicin injection (for a cumulative dose of 15 mg/kg body weight) for next 2 weeks.

Group V: QOLEST + DOX (Qolest plus doxorubicin treated): Qolest was pretreated daily (80 mg/kg/oral) for 2 weeks, and then alternatively with Doxorubicin injection (for a cumulative dose of 15 mg/kg body weight) for next 2 weeks.

Enzyme assay- Thirty six hour after the last treatment, orbital blood samples were obtained under light ether anesthesia using heparinized micro capillaries for the estimation of biomarkers (CK, CK-MB and LDH). Control as well as treated animals was observed for a period of 3 weeks after the last injection for the general appearance, behavior and mortality. At the end of 3 weeks post treatment period, animals were anaesthetized with light anesthetic ether and ECG patterns were recorded using computerized data acquisition system (Biopac MP 35).

The animal were killed under ether anesthesia and a midline abdominal incision was performed and heart tissue was quickly dissected out, washed in ice cold saline, dried on filter paper and weighed. A 30% w/v heart tissue homogenate was prepared in 0.9% buffered potassium chloride (pH 7.4) for the estimation of glutathione,[16] malondialdehyde,[17] SOD[18] and CAT. Orbital blood samples were collected before sacrificing the animals and used for estimation of cardiac enzymes marker (ALT, AST and ALP) and lipid Profiles (cholesterol, triglyceride, and HDL) by Kit methods. The remaining portion of the heart tissue was used for histopathological studies.

Statistical analysis- The results are expressed as the mean±S.E.M. The results obtained from the present study were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Data was computed for statistical analysis by using Graph Pad PRISM 5 Software.

RESULTS AND DISCUSSION

Chronic administration of doxorubicin induced cardiac toxicity and effect of effect of Qolest was established by significant increase in cardiac biomarker enzymes and endogenous antioxidants and heart tissue histopathology.

General observation- The general appearance of all groups of animals was recorded throughout the study. In the later days, DOX treated animals developed a pink tinge, alopecia and the animal's fur

became scruffy. These rats also had red exudates around the eyes and nose, soft watery feces and enlargement of the abdomen. These conditions were more severe at the end of study period.

There were no deaths in the normal and Qolest control groups but a mortality rate of 75% was observed in DOX group while in Qolest 40 and 80 mg/kg treated animals mortality was found to be 50% and 37.5% respectively. DOX treated group showed a decrease in their feed and water consumption during the drug treatment period as compared with the normal group. Qolest control rats showed no significant changes in feed and water consumption as compared with normal. This consumption was improved in the treatment duration. In Qolest treated group feed and water consumption was significantly increased as compared to DOX.

Effect on body weight and body weight gain: It is depicted from Table No.1 that the body weight and body weight gain is gradually decreased in DOX treated group compared to normal group while Qolest treated group significantly increases the body weight gain as compared to DOX control group. Qolest control rats showed no significant changes in the body weight gain as compared with normal. In treatment group i.e. Qolest (40 mg/kg) + DOX significantly increased ($P < 0.01$) in the body weight gain as compared to DOX while in Qolest (80 mg/kg) + DOX significantly increased ($P < 0.001$) in the body weight gain.

Table 1. Initial and final body weight and body weight gain

Treatment	Initial Body weight (g)	Final Body weight (g)	Body weight gain (g)
Normal	169.0 ± 3.209	179.4 ± 1.364	10.40 ± 2.638
DOX	169.4 ± 3.010	151.4 ± 3.234	-18.00 ± 2.168^{ns}
Qolest	168.8 ± 2.478	177.6 ± 1.435	8.6 ± 0.8967^{ns}
Qolest (40 mg/kg)+ DOX	171.2 ± 3.666	164.8 ± 5.024	-6.400 ± 3.043**
Qolest (80 mg/kg)+ DOX	171.0 ± 3.479	168.6 ± 1.600	-3.600 ± 1.600***

Values are mean ± SEM; n=6 in each group, ns= not significant, when compared to normal,

** P <0.01 and ***P<0.001 when compared to DOX.

Effect of Qolest on heart weight, liver weight, kidney weight in doxorubicin induced Cardiotoxicity: It is depicted from Table No.2 that the ratio of heart weight to body weight in DOX treated rats was significantly increased as compared with normal rats. The heart weight to body weight ratio in Qolest control rats was not significant as compared with normal rats. The ratio of heart weight to body weight in pretreatment group i.e. Qolest (80 mg/kg) + DOX is significantly ($P <0.05$) decreased as compare with DOX rats.

The ratio of liver weight to body weight in DOX treated rats was significantly increased as compared

with normal rats. The liver weight to body weight ratio in Qolest control rats was not significant as compared with normal rats. The ratio of liver weight to body weight in pretreatment group i.e. Qolest (40 and 80 mg/kg) + DOX is significantly ($P < 0.05$ and $P < 0.01$) decreased as compare with DOX rats.

The ratio of kidney weight to body weight in DOX treated rats was significantly increased as compared with normal rats. The kidney weight to body weight ratio in Qolest control rats was not significant as compared with normal rats. The ratio of kidney weight to body weight in pretreatment group i.e. Qolest (80 mg/kg) + DOX is significantly ($P < 0.05$) decreased as compare with DOX rats.

Table 2: Effect of Qolest on heart weight, body weight, liver, kidney weight and ratio of heart weight to body weight in doxorubicin induced cardiotoxicity in rats

Treatment	Body Weight (g)	Heart Weight (g)	Liver Weight (g)	Kidney Weight (g)	Heart/ Body ratio ($x10^{-3}$)	Liver/Body ratio ($x10^{-3}$)	Kidney/Body ratio ($x10^{-3}$)
Normal	179.4 ± 1.364	0.6500 ±0.0285	7.106 ± 0.1745	1.373 ± 0.0325	3.623	39.60	7.653
DOX	151.4 ± $3.234^{\#\#\#}$	0.8400 ± $0.0564^{\#\#}$	8.900 ± $0.1381^{\#\#\#}$	1.555 ± $0.0210^{\#\#}$	5.548 $^{\#\#\#}$	58.78 $^{\#\#\#}$	10.270 $^{\#\#\#}$
Qolest	177.6 ± 1.435^{ns}	0.6550 ± 0.0225^{ns}	7.134 ± 0.2689^{ns}	1.383 ± 0.0124^{ns}	3.692^{ns}	40.21^{ns}	7.795^{ns}
Qolest(40mg/ kg) + DOX	164.8 ± 5.024^{*}	0.7775 ± 0.0330^{ns}	8.126 ± 0.2731^{*}	1.490 ± 0.0178^{ns}	4.717^{ns}	49.30^{*}	9.041^{ns}
Qolest(80mg/ kg) + DOX	168.6 ± 1.600^{**}	0.7050 ± 0.0184^{*}	7.862 ± 0.1275^{**}	1.433 ± 0.0384^{*}	4.181^{*}	46.63^{**}	8.499^{*}

[Values are mean ± SE from 6 rats]

Values are mean ± SEM, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ when compared to normal, ns= not significant, * $P<0.05$ and ** $P<0.01$, when compared to DOX.

Estimations of biomarkers- It is depicted from Table No.3, the rats administered with DOX shows a significant increased in the levels of CPK, CK-MB and LDH as compared to normal. Qolest control rats showed not significant changes in the enzyme levels as compared with normal. In treatment group i.e. Qolest (40 mg/kg) + DOX significantly decreased ($P<0.001$) in the level of CPK, CK-MB and LDH as compared to DOX treated group while Qolest (80 mg/kg) + DOX significantly decreased ($P < 0.001$) in the level of CPK,CK-MB and LDH as compared to DOX. Qolest (80 mg/kg) + DOX group shows more protective effect as compared with Qolest (40 mg/kg) + DOX treated group.

Table 3—Effect of Qolest on CPK, CK-MB and LDH in doxorubicin induced cardiotoxicity in rats

Treatment	CPK (IU/L)	CK-MB (IU/L)	LDH (IU/L)
Normal	125.2 ± 6.978	10.24 ± 2.249	190.8 ± 6.080
DOX	253.8 ± 6.655$^{###}$	230.0 ± 20.12$^{###}$	364.0 ± 4.435$^{###}$
Qolest	111.3 ± 2.472^{ns}	11.11 ± 2.762 ^{ns}	183.5± 4.724 ^{ns}
Qolest (40 mg/kg) + DOX	164.7 ± 5.954***	96.14 ± 14.76 ***	318.8.0 ± 8.487***
Qolest (80 mg/kg) + DOX	138.0 ± 1.826***	58.08 ± 8.541 ***	277.5± 7.424***

[Values expressed in IU/L are mean ± SE from 6 rats]

Values are mean ± SEM, $^{###}$P<0.001 when compared to normal, ns= not significant, ***P<0.001 when compared to DOX.

Serum enzyme levels- It is depicted from Table No.4, rats administered with DOX shows a significantly increased in the levels of AST, ALT and ALP as compared to normal. Qolest control rats showed no significant changes in the enzyme levels as compared with normal. In treatment group i.e. Qolest (40 mg/kg) + DOX significantly decreased the levels of AST (P<0.05), ALT (P<0.01), ALP (P<0.001) while Qolest (80 mg/kg) + DOX significantly decreased (P < 0.001) in the levels of AST, ALT and ALP as compared to DOX. Qolest (80 mg/kg) + DOX group shows more protective effect as compared with Qolest (40 mg/kg) + DOX treated group.

Table 4—Effect of Qolest on ALT,AST and ALP levels in doxorubicin induced cardiotoxicity in rats

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal	18.67 ± 0.9888	30.33 ± 2.996	92.83 ± 5.375
DOX	65.17 ± 2.822$^{###}$	196.8 ± 6.585$^{###}$	229.5 ± 6.946$^{###}$
Qolest	18.17 ± 0.7923^{ns}	29.67± 2.246^{ns}	87.33 ± 4.924^{ns}
Qolest (40 mg/kg) + DOX	52.17 ± 3.439*	164.0± 5.285**	185.3 ± 4.514***
Qolest (80 mg/kg) + DOX	37.67 ± 5.090***	116.3 ± 12.56***	155.8 ± 3.628***

[Values expressed in IU/L are mean ± SE from 6 rats]

Estimation of lipid profiles- It is depicted from Table No.5, rats administered with DOX shows a significantly increased in the levels of cholesterol and triglyceride as compared to normal and there is slightly difference in HDL levels as compared to normal group. Qolest control rats showed no significant changes in the lipid levels as compared to normal but in the HDL level there is slightly increased in Qolest group compare to normal and DOX treated groups. In treatment group i.e. Qolest (40 mg/kg) + DOX significantly decreased the level of cholesterol (P < 0.05) and triglyceride (P < 0.001) while Qolest (80 mg/kg) + DOX significant decreased (P < 0.001) in the levels of cholesterol and triglyceride as compared to DOX and significant increased (P < 0.05) in the level of HDL as compared to DOX. Qolest (80 mg/kg) + DOX group shows more protective effect as compared with Qolest (40 mg/kg) + DOX treated group.

Table 5—Effect of Qolest on serum cholesterol, triglyceride and HDL levels in doxorubicin induced cardiotoxicity in rats

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)
Normal	55.83 ± 2.937	133.7 ± 3.748	27.00 ± 1.826
DOX	109.3 ± 3.528$^{###}$	317.3 ± 9.885$^{###}$	33.17 ± 0.7923$^{\#}$
Qolest	53.33 ± 2.499^{ns}	128.5 ± 3.519^{ns}	41.50 ± 1.088$^{###}$
Qolest (40 mg/kg) + DOX	90.00 ± 3.830*	247.2 ± 5.461***	35.50 ± 0.8466^{ns}
Qolest (80 mg/kg) + DOX	67.33 ± 6.601***	179.3 ± 5.903***	38.67 ± 2.216*

[Values expressed in IU/L are mean ± SE from 6 rats]

Values are mean ± SEM $^{\#}$ P<0.05and $^{###}$P<0.001 when compared to normal, ns= not significant, *P <0.05 and ***P<0.001 when compared to DOX.

Effect of Qolest on different ECG patterns- Pretreatment of Qolest 40 mg/kg and 80 mg/kg + DOX administration showed a protective effect against DOX induced altered ECG patterns and eliminated the acute fatal complication by protecting the cell membrane damage. Qolest (80 mg/kg) + DOX group shows more protective effect as compared with Qolest (40 mg/kg) + DOX treated group.

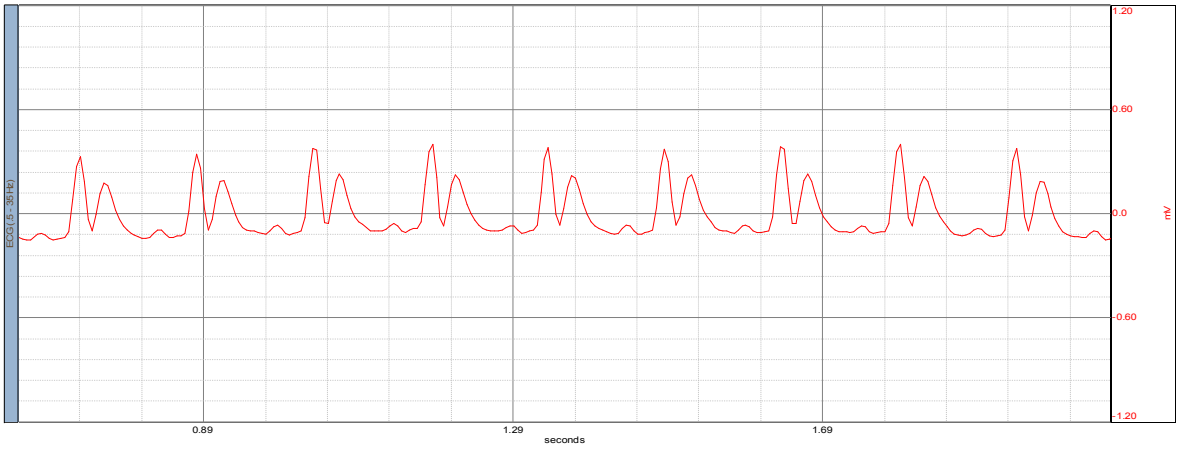
The data of the experimental animals such as heart rate, P wave, QRS complex, QT interval and ST interval are shown in Table No.6 and Fig. no.1

Table 6—Effect of Qolest on ECG patterns in doxorubicin induced cardiotoxicity in rats

Treatment	Heart Rate (Beats/Min)	P wave (sec)	QRS complex (sec)	Q-T interval (sec)	S-T interval (sec)
Normal	408.8 ± 2.638	0.04333 ± 0.001054	0.0425 ± 0.001118	0.0600 ± 0.001291	0.01833 ± 0.001054
DOX	351.9 ± 0.2007^{c}	0.03667 ± 0.001054^{b}	0.0375 ± 0.001118^{a}	0.0825 ± 0.001118^{c}	0.03667 ± 0.001054^{c}
Qolest	406.7 ± 2.836^{ns}	0.0425 ± 0.001118^{ns}	0.04333 ± 0.001054^{ns}	0.06083 ± 0.0009804^{ns}	0.0185 ± 0.0009220^{ns}
Qolest (40 mg/kg) + DOX	379.0 ± 2.530***	0.0375 ± 0.001118^{ns}	0.03883 ± 0.0008333^{ns}	0.06833 ± 0.001054***	0.02417 ± 0.0008333***
Qolest (80 mg/kg) + DOX	393.5 ± 2.907***	0.0410 ± 0.001317*	0.04167 ± 0.001406*	0.0625 ± 0.001118***	0.0190 ± 0.0008944***

[Values expressed in seconds(sec) are mean ± SE from 3 rats]

Values are mean ± SEM, a P<0.05, b P<0.01 c P<0.001 when compared to normal. ns= not significant, * P <0.05, **P<0.01 and ***P<0.001 when compared to DOX.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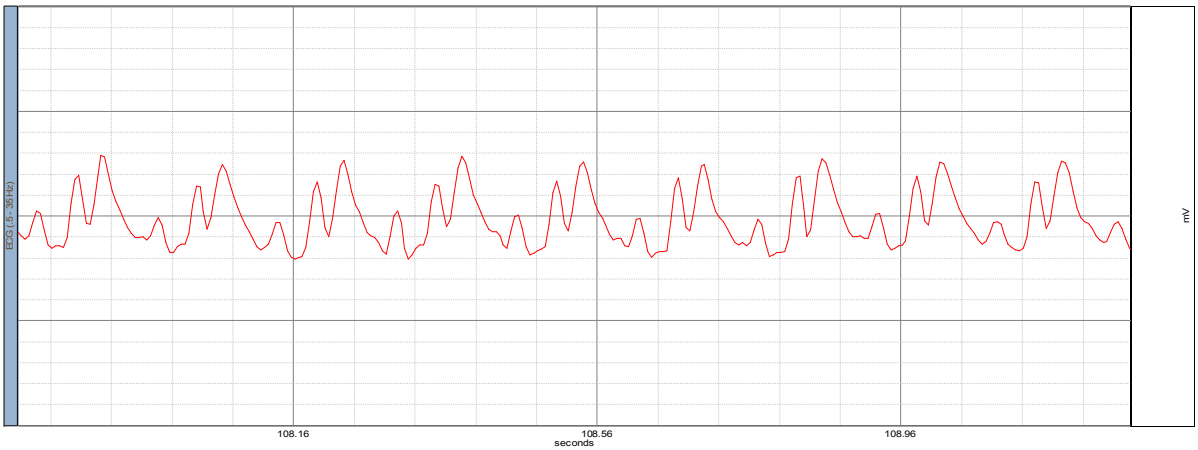
0.0
-0.60
-1.20
mV
0.89
1.29
1.69
seconds

(A)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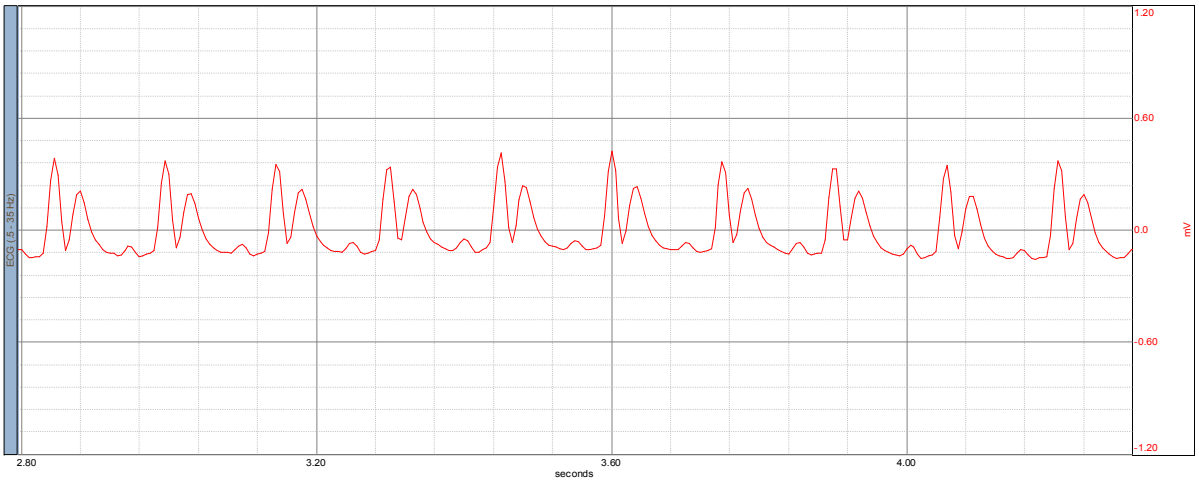
108.96
seconds

(B)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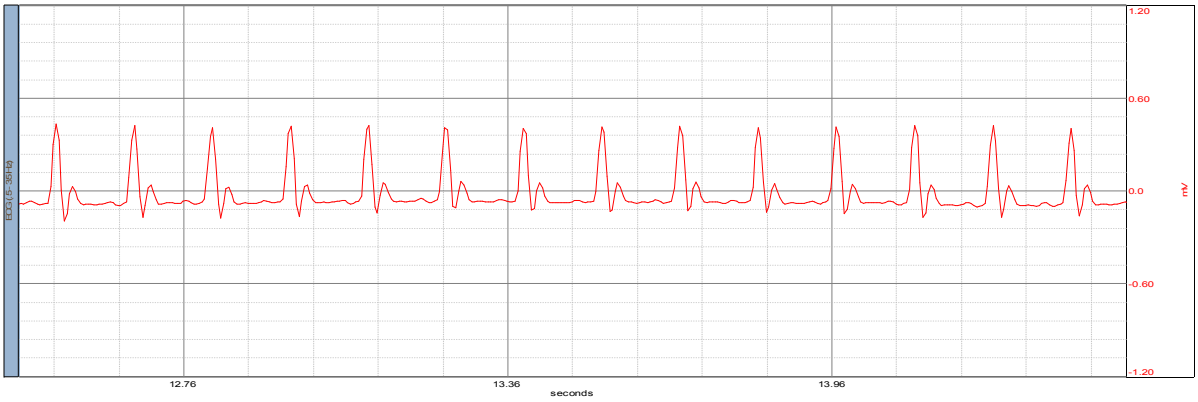
-1.20
mV
2.80
3.20
3.60
4.00
seconds

(C)



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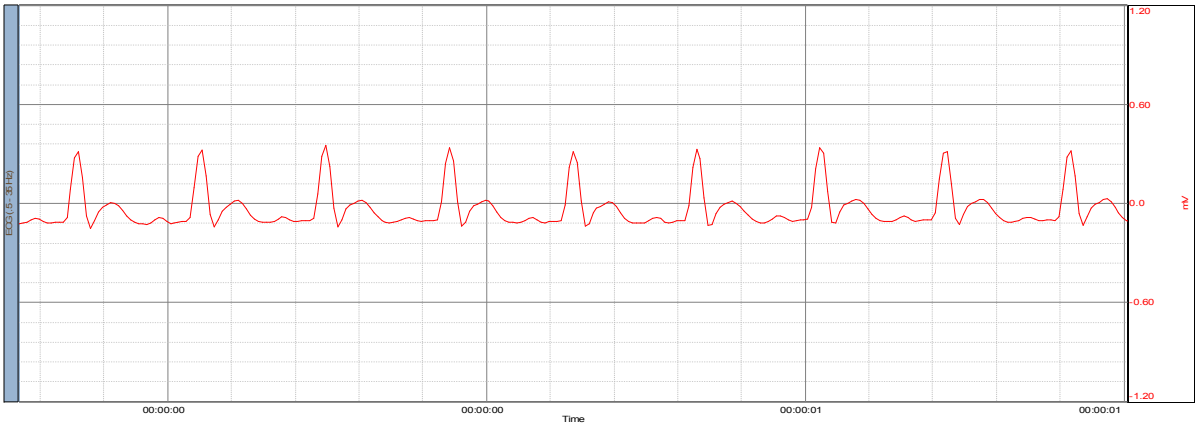


(E)

Fig No.1: Effect of Qolest on electrocardiographic pattern of (a) Normal; (b) DOX; (c) Qolest; (d) Qolest(40 mg/kg) + DOX and (e) Qolest (80 mg/kg) + DOX in rats.

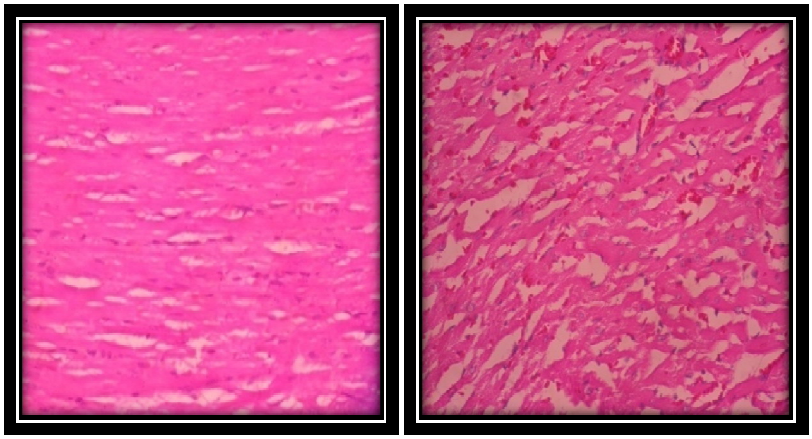
Evaluation of antioxidant activity- It is depicted from Table No.7 that the antioxidant heart enzyme levels in DOX treatment group showed a significant increase in lipid peroxidation (nmol MDA/min/mg of wet tissue) while a significant decrease in reduced glutathione (nmol/min/mg of wet tissue), superoxide dismutase (Unit^x/mg protein) and catalase (Unit^y/mg protein) when compared to normal group.

Histopathological investigation:- The histology of the heart tissue from control and Qolest treated rats showed normal morphological appearances. Where in group 2 disruption of loss of myofibrils and vacuolization of the cytoplasm were observed. The histology of heart tissues from group 5 showed less loss of myofibrils and vacuolization of the cytoplasm. Fig. no. 2

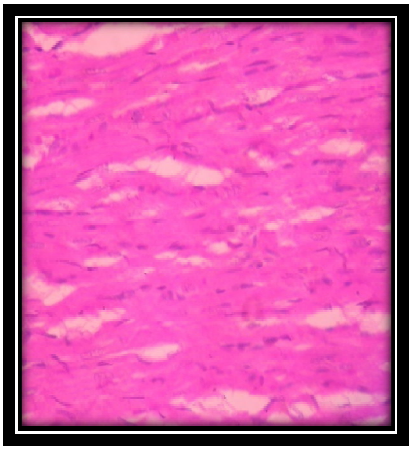
Table 7—Effect of Qolest on malondialdehyde, glutathione, catalase and superoxide dismutase in doxorubicin induced cardiotoxicity in rats [Values are mean ± SE from 6 rats]

Treatment	GSH (n mole/min/mg of wet tissue)	Lipid peroxidation (n mol of MDA/min/ mg of wet tissue)	CAT (Unit^{x}/ mg protein)	SOD (Unit ^{y} /mg protein)
Normal	5.733 ± 0.4408	1.580 ± 0.01528	59.29 ± 1.085	33.95 ± 1.377
DOX	1.648 ± 0.06700$^{###}$	6.332 ± 0.06997$^{###}$	32.67 ± 0.9360$^{###}$	19.05 ± 0.1234$^{###}$
Qolest	5.953 ± 0.5414^{ns}	1.497 ± 0.02704^{ns}	60.45 ± 1.061^{ns}	34.95 ± 0.9611^{ns}
Qolest (40 mg/kg)+ DOX	3.380 ± 0.4811*	3.647 ± 0.3624***	37.17 ± 0.8640*	26.83 ± 1.0960***
Qolest (80 mg/kg) + DOX	4.815 ± 0.5140***	2.723 ± 0.1689***	48.79 ± 0.8330***	30.78 ± 0.5046***

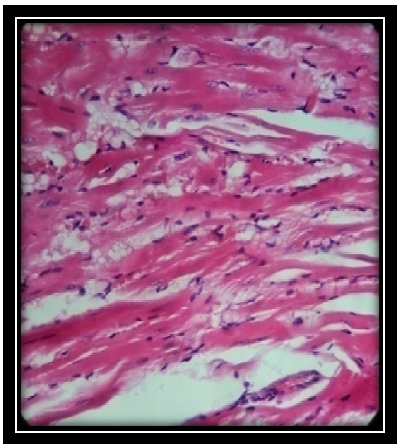
Values are mean ± SEM, $^{###}$P<0.001 when compared to normal.
ns= not significant, *P <0.05 and ***P<0.001 when compared to DOX.



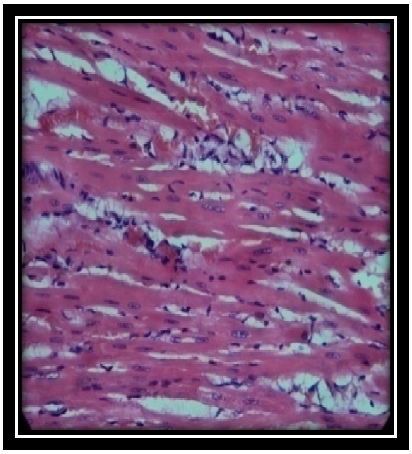
Normal **DOX**



Qolest



Qolest (40 mg/kg) + DOX



Qolest (80 mg/kg) + DOX

Fig. No. 2: Histopathological studies of various treated groups

The present study was done to investigate the influence of Qolest on DOX induced myocardial toxicity. Our results suggest that Qolest prevents DOX induced cardiomyopathy in rats.

The DOX treated rats showed increase in heart weight and decrease in body weight. Decreased in the body weight may be due to the reduced intake of food. Increase in the heart weight may be attributed to the loss of myofibrils, dilation of sarcoplasmic reticulum, swelling of mitochondria and increased number of lysosomes.[19]

The experimental study reveals severe biochemical changes as well as oxidative damage in the cardiac tissue after the chronic treatment with DOX

DOX induced cardiotoxicity is due to destruct myocardial cells. As a result of this, Creatine kinase (CK), CK-MB, Lactate Dehydrogenase (LDH) and Transaminase enzymes (AST, ALT) were released into blood stream and serve as the diagnostic markers of myocardial tissue damage.[9,12] The amount of these cellular enzymes present in the blood reflects the alteration in plasma membrane integrity and/or permeability. The prior administration of Qolest 40 mg/kg and 80 mg/kg + DOX showed significant reduction in DOX induced elevated serum marker enzymes. This reduction in the enzyme level confirms that Qolest is responsible for maintenance of normal structural and architectural integrity of cardiac myocytes, thereby restricting the leakage of these enzymes, which can be accounted for membrane stabilizing property of Qolest.

Pretreatment of Qolest 40 mg/kg and 80 mg/kg + DOX shown decrease in the lipid profiles and increase in HDL cholesterol when compared with the DOX treated group. This lipid lowering effect is because of presence of *Terminalia arjuna* or *Commiphora mukul* or may be both. This lipid lowering effect of Qolest is due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid

secretion and stimulation of receptor mediated catabolism of LDL cholesterol. Lipid lowering activity and anti-cholesterolemic activity of Qolest can be accounted the stimulation of metabolism of LDL-cholesterol and increase in the uptake of LDL from blood by liver the present findings are similarly correlated to the earlier reports.[20]

The study show significant alteration of ECG patterns in DOX administered rats as compared to normal control rats. The characteristic findings were elevation of ST segment, reduction in P waves, QRS complex and decrease in heart rate. In addition there was a prolongation of QT interval. Moreover ECG changes are an indicator of the severity of DOX induced myocardial damage.[21] The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by ST elevation. Pretreatment of Qolest 40 mg/kg and 80 mg/kg + DOX administration showed a protective effect against DOX induced altered ECG patterns and eliminated the acute fatal complication by protecting the cell membrane damage.

The mechanism of cardiotoxicity induced by a DOX is not clearly known from the present study, although large body of evidence supports that DOX administration is associated with a decrease in endogenous antioxidants and increase in oxygen free radicals resulting in increased oxidative stress, which is followed by development of a variety of subcellular changes in the myocardium, typical of DOX-induced cardiac injury.[11,12]

Cardiac tissue damage may be due to increased oxidative stress and depletion of antioxidants as reported earlier.[13].

In our study, DOX treated rats showed increase in heart tissue MDA levels with decrease in levels of GSH, SOD and CAT which confirms the oxidative stress and cardiac damage. Qolest prevented the DOX induced changes in MDA and enzyme levels. Significant increase in the GSH, SOD and CAT activity and decrease in lipid peroxidation in heart tissue of Qolest 40 mg/kg and 80 mg/kg + DOX treated groups was found.

The antioxidant enzymes SOD, CAT and GSH play an important role in mitigating free radical-induced cell injury. In heart, GSH is extremely important because of its ability to use and remove organic and inorganic peroxide. Depletion of glutathione in heart tissue of rats is known to result in enhanced lipid peroxidation which cause increased glutathione consumption, as observed in present study. The prior administration of Qolest protects the myocytes against DOX induced myocardial toxicity by decreasing their susceptibility to free radicals.

DOX induced decline in both SOD and CAT activity along with GSH contents promotes the formation

of OH^- radicals, initiation and propagation of lipid peroxidation. The activities of antioxidant enzymes are in close relationship with the induction of lipid peroxidation, found in the present study.

Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation. It is commonly accepted that SOD protects against the free radical injury by converting O_2-radical to H_2O_2 and prevent the formation of OH^- radicals through O_2-driven Fenton reaction and the H_2O_2 can be removed by Catalase. Administration of Qolest, improved the antioxidant status and thereby preventing the damage to the heart, mainly because of the antioxidant sparing action of Qolest.

Histopathological report suggests that vehicle treated rats, did not show any morphological changes and heart showed normal appearance. The cardiac muscle fibers were found to be of uniform size, shape and configurations with no inflammatory cell infiltrates were present Cardiomyopathy occurred in all rats injected with DOX, as illustrated by the appearance of enlarged, swollen mitochondria and vacuoles within the cytoplasm which is in line with an earlier report. DOX produced massive change in the myocardium showing a varying degree of vacuolar changes in the cardiac muscle fibers mainly in the form of degeneration of myocardial tissue, vacuolization of the cardiomyocytes, infiltration of inflammatory cells and myofibrillar loss. Pretreatment with Qolest 40 mg/kg and 80 mg/kg + DOX effectively inhibits DOX induced cardiac damage by reversal of infiltration of inflammatory cells and fragmentation of myofibrils. Qolest protects DOX induced myocardial damage in the cardiac tissue of rats either by restoring endogenous antioxidant activity or as an antioxidant both.

The findings of the study suggests that, the Qolest protects against DOX-induced cardiotoxicity in rats as evidenced by improved mortality and effusion scores, mitigation of ECG abnormalities, improved cardiac injury markers and restoration of the oxidant/ antioxidant status as well as lessening histopathological changes. This can be attributed, at least in part, to antioxidant activity.

Finally we conclude that the cardiotoxicity induced by DOX is in relationship with oxidative stress. Qolest has shown to be most effective in the functional recovery of the heart and restoration of biochemical and histopathological alteration which may be associated with its potent antioxidant property.

Our study suggests that Qolest may be considered as a potentially useful candidate in the combination with DOX to limit free radical mediated organ injury. Further molecular level of investigation is to be done using different animal model and using different biochemical parameters to assess the possible mode of action of Qolest as cardio protective agent. It is worthwhile to consider this aspect for clinical

application in patient of cardiac injury.

ACKNOWLEDGEMENT

The authors thank Dr. B. M. Patil, Principal, K.L.E. Society's, College of Pharmacy, Hubli, India for support.

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