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Evaluation Of Cardioprotective Potential of Aqueous Extract of Hibiscus Rosa Sinensis Against Doxorubicin Induced Cardiotoxicity in Albino Rats

Harish[1], Sharma M[2], Vishwakarma P[3], Saini M, Goel R[4]
[1]Junior Resident, Department of Pharmacology, Lala Lajpat Rai Memorial Medical College, Meerut, Uttar Pradesh, India
[2,3,4,5]Department of Pharmacology, Lala Lajpat Rai Memorial Medical College, Meerut, Uttar Pradesh, India

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*Corresponding Author:

Harish
Junior Resident, Department of Pharmacology, Lala Lajpat Rai Memorial Medical College, Meerut, Uttar Pradesh, India

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A B S T R A C T

Background: Cardiotoxicity is one of the most terrifying side effects of anticancer agents like Doxorubicin. *Hibiscus rosa sinensis* (*Malvaceae* family) has been found to have antiulcer, antidiabetic, anticonvulsant, anti-inflammatory, antipyretic and antibacterial activity. Since not much literature was available on cardioprotective potential of *Hibiscus rosa sinensis*, the present study was undertaken to investigate the cardioprotective potential of aqueous extract of *Hibiscus rosa sinensis*.

Material and Methods: Following approval from institutional animal ethics committee of L.L.R.M. Medical College registered under CPCSEA, India, this study was undertaken in Department of Pharmacology. During the study period of 21 days, 30 rats was randomized into five groups of 6 rats each. **Group 1** was given Normal saline 1ml/kg orally (control) for 21 days. **Group 2** in addition to normal saline was administered Doxorubicin (20mg/kg intraperitoneally) on 21st day of study. **Group 3** was treated with Carvedilol 30 mg/kg orally for 21 days followed by Doxorubicin (20mg/kg intraperitoneally) on 21st day. **Group 4 and 5** received aqueous extract of *Coriander sativum* orally in 2 graded doses for 21 days and Doxorubicin (20mg/kg intraperitoneally) on the 21st day. The rats were observed for 48 hours and then sacrificed under ketamine (75mg/kg) and xylazine (10mg/kg) anesthesia given intraperitoneally. Blood samples (volume ≈ 5ml) were collected from abdominal aorta for performing biochemical tests. The animals were sacrificed and heart was dissected out for histopathological study. The data obtained was organized and analyzed by suitable statistical methods i.e. ANOVA followed by Post Hoc test.

Results: In the present study, serum Creatinine phosphokinase-MB (CK-MB), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were used as biochemical markers for assessing cardiac injury.

The present study revealed increased levels of serum markers of cardiotoxicity such as CK-MB, LDH, AST and ALT on Doxorubicin administration (20mg/kg i.p). Biomarker levels were significantly lower in rats pretreated with *Hibiscus rosa sinensis.*

Degree of protection with *Hibiscus rosa sinensis* against Doxorubicin induced cardiotoxicity was also evident clearly on histopathological examination of cardiac tissue.

Conclusion: Excessive formation of free radicals and oxidative stress resulted following doxorubicin administration. *Hibiscus rosa sinensis* significantly reduced doxorubicin induced damage to rat myocardium. No significant adverse effects were seen among the rats after the administration of aqueous extract of *Hibiscus rosa sinensis* for 21 days. This cardioprotective potential of *Hibiscus rosa sinensis* might be attributed to the antioxidant property of chemical compounds present in them and can serve as a good source for the production of a cardioprotective herbal medicine.

Keywords: *Hibiscus rosa sinensis* (HRS), Doxorubicin (DOX), Cardiotoxicity, Carvedilol

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death globally, taking an estimated 17.9 million lives each year. CVDs are a group of disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, heart failure, cardiomyopathy etc.[1] The death rates due to cardiomyopathies are 6 per 1,00,000 population and disability

adjusted life years are 166 per 1,00,000 population.[2] Incidence of death due to drug related cardiomyopathy is 2 % with 10 % decline in left ventricular ejection fraction.[3]

The use of herbal plants is increasing as protective agents against a number of cardiovascular diseases. By eliminating the generation of free radicals, phytochemicals from natural sources have decreased the risks of heart disease and gained fundamental importance in modern drug systems. Cardio-protection includes "all mechanisms and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage".[4]

Herbal plants like Tulsi (*Ocimum sanctum*) and Arjuna (*Terminalia arjuna*) have shown cardioprotective potential.[5] *Hibiscus rosa sinensis* (*Malvaceae* family) has been found to have antiulcer, antidiabetic, anticonvulsant, anti-inflammatory, antipyretic, antibacterial activity as well as cardioprotective activity.[6]

Since not much literature is available on cardioprotective potential of *Hibiscus rosa sinensis*, hence, the present study was undertaken to investigate the cardioprotective potential of aqueous extracts of *Hibiscus rosa sinensis*.

MATERIALS AND METHODS

STUDY DESIGN

The present study was conducted in the Department of Pharmacology, L.L.R.M. Medical College, Meerut for evaluation of cardioprotective potential of aqueous extract of *Hibiscus rosa sinensis* against Doxorubicin induced cardiotoxicity in albino rats.

After obtaining approval from the Institutional Animal Ethics Committee (registration no.819/GO/ReRc BiBt/S/04/CCSEA), albino rats weighing 150-200gm was procured and maintained under standard laboratory conditions (alternating periods of light and darkness of 12h each and at 25 ^{O}C), with free access to standard rat pellet diet and tap water *ad libitum*. Pregnant female rats were not included in the study. All animal experiments were carried out in accordance of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. As per OECD guidelines, doses of aqueous extract of *Hibiscus rosa sinensis* to be used in the study were calculated on the basis of previously documented LD50 on rats (OECD-423).

Method of preparation of extract:

Aqueous extract of *Hibiscus rosa sinensis*:

Dried *Hibiscus rosa sinensis* flowers was procured from market in pulvurised form and incubated with 10 volumes (w/v) of boiling water for 20 min and cooled to room temperature. This 10% solution was then clarified by centrifugation, sterilized by filtration through 0.2 micron filters (millipore), frozen, and lyophilized to dryness. This dried extract was then suspended in water to a final concentration of **50 mg/ml**.[7]

STUDY OUTLINE:

Evaluation of cardioprotective activity:

The study was conducted on albino rats weighing 150-250 gm. During the study period of 21 days, 30 rats were randomized into five groups of 6 rats each.

- **Group 1** – This group was given Normal saline 1ml/kg orally (control) for 21 days.
- **Group 2** – This group in addition to normal saline was administered Doxorubicin (20mg/kg intraperitoneally) on 21st day of study.[8]
- **Group 3** – This group was treated with Carvedilol 30 mg/kg orally for 21 days followed by Doxorubicin (20mg/kg intraperitoneally) on 21st day.
- **Group 4 and 5** These groups received aqueous extract of *Hibiscus rosa sinensis* orally in 2 graded doses (250 & 500mg/kg) for 21 days and Doxorubicin (20mg/kg intraperitoneally) on the 21st day.

After giving Doxorubicin (20mg/kg intraperitoneally) on 21st day, rats were observed for 48 hours and then sacrificed. The albino rats were sacrificed using Ketamine (75mg/kg) anesthesia, given intraperitoneally. Blood sample was collected from abdominal aorta (5ml) for performing blood test i.e. Creatine kinase-MB(CK-MB), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT). Also the heart was dissected out for the purpose of taking histological samples. The cardiac and histological markers were measured and compared with control group and the group treated with a standard cardioprotective drug (Carvedilol).

HISTOPATHOLOGICAL EXAMINATION

The heart was excised from the animal and washed with normal saline. Whole of the heart was placed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene, respectively, followed by embedding in paraffin wax from which blocks were prepared. Sections of 5µm thickness were prepared from the blocks using microtome. These were processed in alcohol-xylene series and were stained with Harris Haematoxylin and Eosin stain and then subjected to histopathological examination

STATISTICAL ANALYSIS

Mean ± SE was calculated for each group to observe the general trend of the group. The statistical analysis was carried out using one way analysis variation (ANOVA) followed by Post-Hoc Test. P- values were estimated by referring to appropriate tables[9].

OBSERVATIONS AND RESULTS

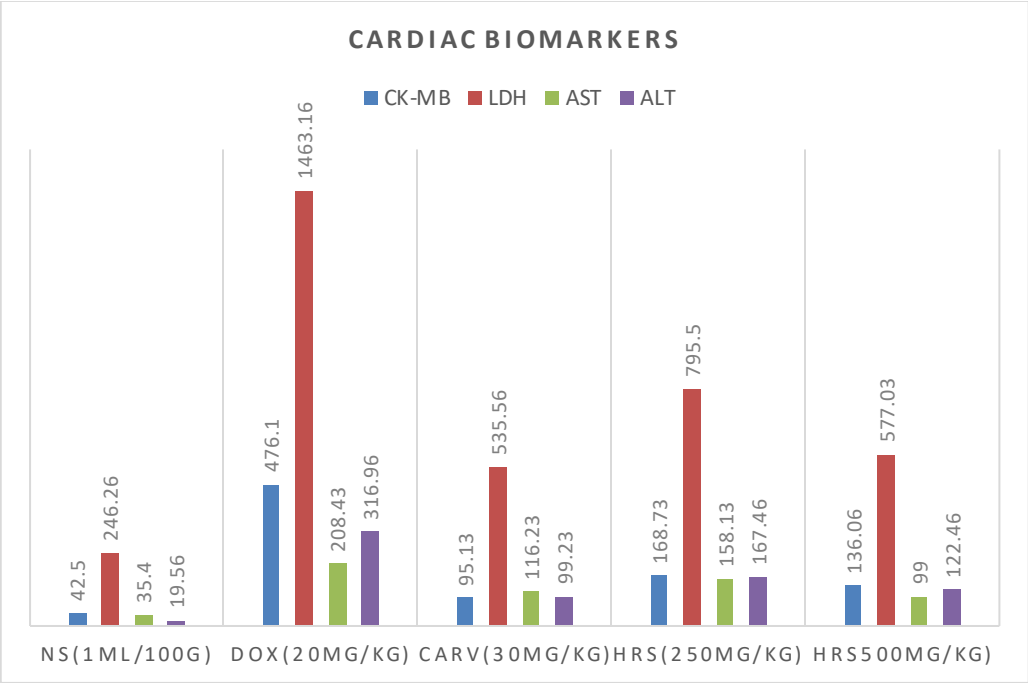
The present study revealed increased levels of serum markers of cardiotoxicity such as CK-MB, LDH, AST and ALT following Doxorubicin administration (20mg/kg i.p). These findings were consistent with study done by El-Agamy DS et al., (2016)[10]. The myocardial tissue of saline-treated rats showed clear integrity of the myocardial cell membrane and an absence of inflammatory cell infiltration. In contrast, Doxorubicin-injected rats exhibited separation of cardiac muscle fibers and infiltration of inflammatory cells. Rats treated with Hibiscus rosa sinensis (250mg/kg/day p.o + DOX 20mg/kg i.p single dose) for 21 days demonstrated gentle widening of the muscle fibres with focal loss of myofibres, inflammation and absence of myonecrosis. Rats treated with Hibiscus rosa sinensis (500mg/kg/day p.o + DOX 20mg/kg i.p single dose) for 21 days showed no inflammation, no edema and almost normal cardiac architecture which was similar to that seen in Carvedilol treated groups.

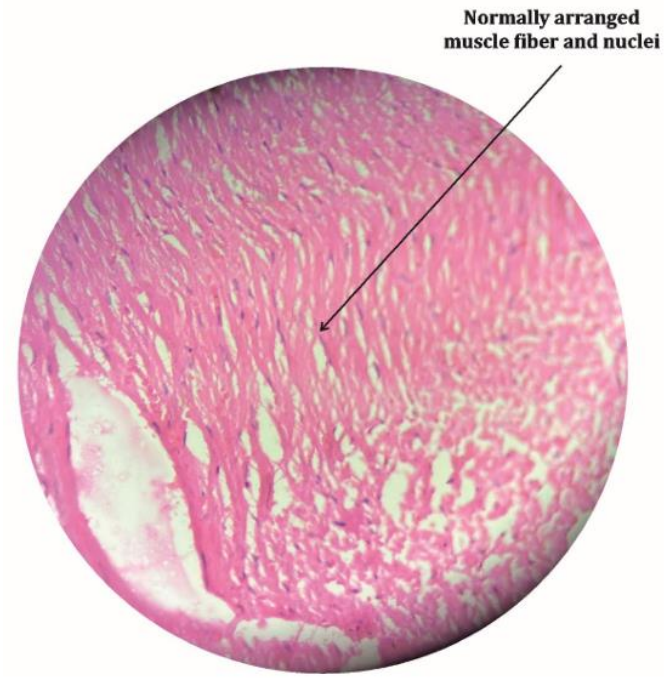
Table 1: Effect of Carvedilol, Aqueous extract of *Hibiscus rosa sinensis* in their respective doses on Doxorubicin (DOX) induced changes in CK-MB, LDH, ALT and AST levels (n = 6).

GROUP	Treatment (mg/kg)	CK-MB(IU/L) (Mean±SE)	LDH(IU/L) (Mean±SE)	AST(IU/L) (Mean±SE)	ALT(IU/L) (Mean±SE)
1.	Normal saline (1ml/100g p.o)	42.50±5.31	246.26±17.02	35.40±6.87	19.56±2.73
2.	Doxorubicin (20mg/kg i.p)	476.10±54.59$^{\gamma}$	1463.16±1.32$^{\gamma}$	208.43±12.19$^{\gamma}$	316.96±59.55$^{\gamma}$
3.	Carvedilol (30mg/kg p.o)	95.13±4.68$^{\beta}$	535.56±45.55$^{\beta}$	116.23±21.47$^{\beta}$	99.23±14.91$^{\beta}$
4.	*Hibiscus rosa sinensis* (250 mg/kg p.o)	168.73±2.97$^{\alpha}$	795.50±4.89$^{\alpha}$	158.13±2.22$^{\alpha}$	167.46±3.04$^{\alpha}$
5.	*Hibiscus rosa sinensis* (500 mg/kg p.o)	136.06±4.71$^{\beta}$	577.03±33.23$^{\beta}$	99.00±11.10$^{\beta}$	122.46±6.35$^{\beta}$

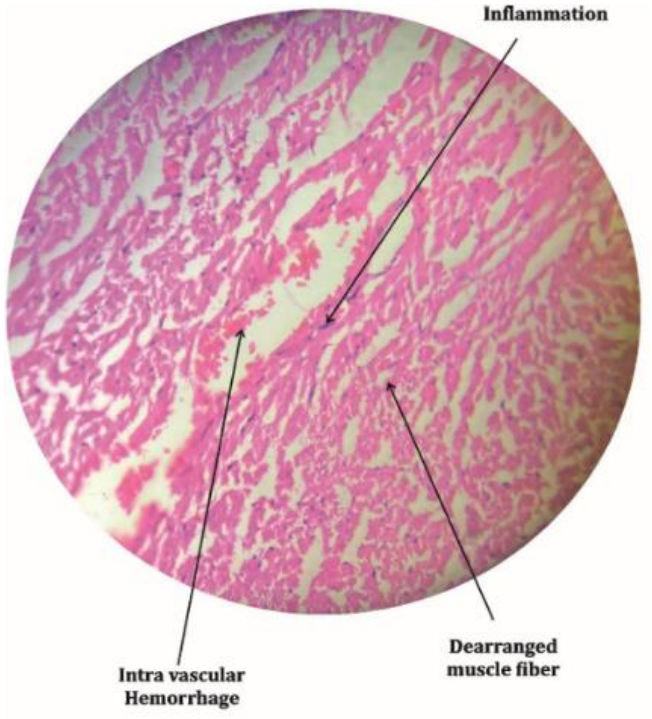
$^{\alpha}p< 0.01$ as compared to DOX treated group.
$^{\beta}p < 0.001$ as compared to DOX treated group.
$^{\gamma}p < 0.001$ as compared to normal saline treated group.

GRAPH



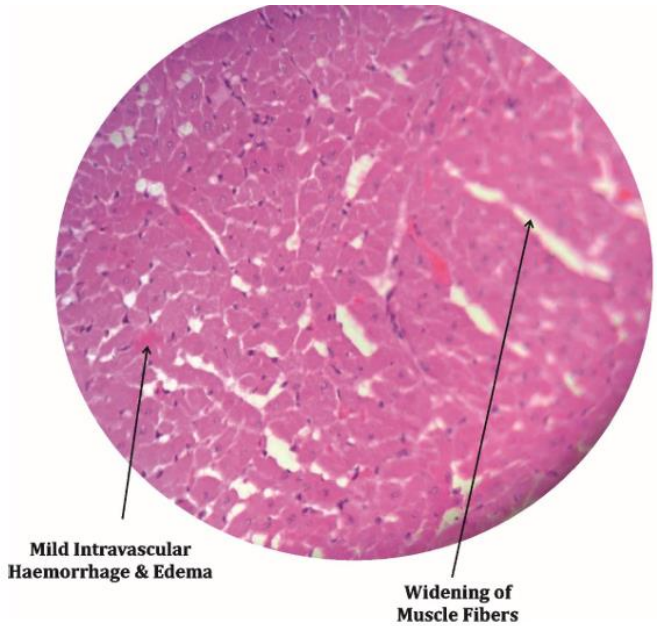


SLIDE 1: NORMAL SALINE GROUP

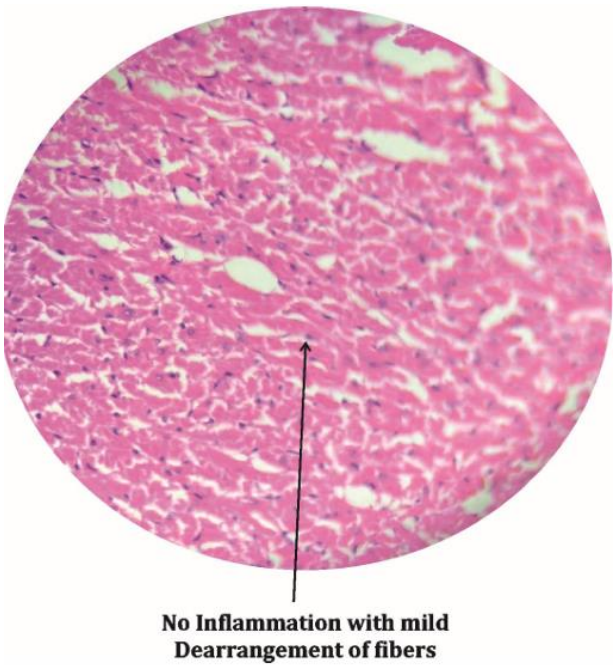


SLIDE 2: DOX TREATED

SLIDE 3: CARVEDILOL TREATED



SLIDE 4: HRS 250mg/kg treated



SLIDE5: HRS 500mg/kg treated

DISCUSSION

Cardiovascular diseases (CVD) are non-communicable diseases encompassing a range of conditions that affect the heart and blood vessels. One important factor associated with CVD is cardiotoxicity, which refers to damage to the heart caused by toxic substances. It typically manifests as electrophysiological disturbances and myocardial (heart muscle) damage, which ultimately leads to heart failure[11]. Among cardiotoxic agents, chemotherapeutic drugs are particularly notable. A major group within this category is the anthracyclines, which includes the widely used anticancer drug doxorubicin (DOX). Doxorubicin-induced cardiotoxicity is a progressive condition that begins with injury to myocardial cells, followed by a reduction in left ventricular ejection fraction (LVEF). If not detected and managed early, it can lead to symptomatic heart failure.

The underlying mechanisms of doxorubicin cardiotoxicity are complex. A key contributor is the generation of reactive oxygen species (ROS), along with disturbances in iron metabolism and calcium (Ca^{2+}) signalling. Free radicals are highly reactive for instance, hydroxyl radicals can cause damage to membrane phospholipids, DNA, Molecules and proteins, in which the former would result in the formation of peroxyl radicals. Thus, when there are insufficient natural antioxidant components in the body, such as superoxide dismutase (SOD) enzyme and glutathione peroxidase (GSH-px), an oxidative chain reaction may occur, resulting in tissue damage. A critical molecular target is topoisomerase 2β (Top2β), an enzyme involved in uncoiling DNA during replication, transcription, and recombination. Doxorubicin inhibits Top2β, leading to mitochondrial dysfunction, activation of cell death pathways, and accumulation of ROS, all of which contribute to cardiac damage[12].

Carvedilol is taken as standard drug in present study. Carvedilol is a third generation β receptor antagonist, used to treat symptomatic congestive heart failure. Carvedilol has antioxidant, anti inflammatory and membrane stabilizing properties, thereby reduces arterial blood pressure by decreasing vascular resistance and tone. Carvedilol also inhibits ROS mediated loss of myocardial contractility, stress induced hypertrophy, apoptosis and activation of neutrophils[13]. Because of these properties carvedilol is considered to be a standard drug for comparison of cardioprotective activity as evidenced by study done by Varshney P et al.,(2016)[8].

In the present study, serum Creatinine phosphokinase-MB (CK-MB), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were used as biochemical markers for assessing cardiac injury. The myocardium is rich in diagnostic marker enzymes for cardiotoxicity, and when damaged, it releases its intracellular contents into the extracellular fluid. Therefore, serum levels of these marker enzymes reflect alterations in membrane integrity and permeability. Cytosolic enzymes CK-MB, LDH, AST, and ALT, which serve as diagnostic markers, leak from damaged tissue into the bloodstream when the cell membrane becomes permeable or ruptures. AST and ALT are important for the regulation of metabolism. Necrosis and inflammation increase AST levels in the heart and liver. In Doxorubicin-treated rats, ALT and AST levels were significantly elevated compared to normal rats. These enzymes are valuable in diagnosing Doxorubicin-induced myocardial cardiotoxicity.

Hibiscus rosa-sinensis contains numerous compounds including quercetin, glycoside, riboflavin, niacin, carotene, anthocyanin, anthocyanidin, malvalic acid, gentisic acid, margaric acid and lauric acid. *Hibiscus rosa sinensis* possess strong antioxidant properties[14]. Potential antioxidant mechanism is preventing oxidative damage to the myocardial tissues.

Hibiscus rosa sinensis has anti-inflammatory, anti-ulcer, anti-diabetic, nootropic, hepatoprotective, anti-hypertensive and hypolipidemic and many more activities. This species have various flavonoids, glycosides, alkaloids and various phytochemicals that makes it more important candidate for the researchers. Guddeti V et al demonstrated anti inflammatory activity of *Hibiscus rosa sinensis* by reducing the paw edema in rats against standard drug diclofenac due to antioxidant property of its constituents[15]. Notably, CK-MB levels were significantly lower in rats pretreated with Hibiscus rosa sinensis. Hibiscus rosa sinensis provided cardioprotection as indicated by the limited rise in serum markers of cardiac damage. Furthermore, Hibiscus rosa sinensis at 500 mg/kg conferred more cardioprotection than at 250 mg/kg..

A study by Mohammed HS et al., (2020) demonstrated a significant increase in LDH levels in rats treated with Doxorubicin 48 hours post treatment[16]. Similarly increased level of LDH was also noticed in the present study. Pretreatment with *Hibiscus rosa sinensis* significantly reduced the elevated LDH levels, indicating a reduction in the severity of cardiotoxicity.

The serum marker results correlated with histopathological observations in the myocardial tissue of animals treated with Doxorubicin, test drugs and normal saline. Another study by Gauthaman KK et al., (2006) on the Cardioprotective effect of the Hibiscus rosa sinensis flowers in an oxidative stress model of myocardial ischemic reperfusion injury in rat also showed cardioprotective activity, similar to the findings of the present study[17]. The exact cardioprotective mechanism of aqueous extract of Hibiscus rosa sinensis has not been evaluated in the present study but it can be postulated to be due to presence of phytochemicals constituents with antioxidant potential. However, comprehensive and extensive research, conducted over a larger sample size is needed to explore the pharmacokinetic and pharmacodynamic profiles of these extracts, which will provide the foundation for future clinical trials.

CONCLUSION

Doxorubicin, a drug belonging to anthracycline group, is widely used as chemotherapeutic agent for treatment of cancers like breast carcinoma, hodgkin lymphomas and solid tumors. Excessive formation of free radicals and oxidative stress resulting in serious cardiotoxicity limits its clinical use. A continuous search to overcome this problem is going on. In the present study it was found that pretreatment with aqueous extract of *Hibiscus rosa sinensis* significantly reduced the doxorubicin induced damage to rat myocardium. No significant adverse effects were seen among rats after the administration of aqueous extract of *Hibiscus rosa sinensis* for 21 days. This study also provides scope for further evaluation of *Hibiscus rosa-sinensis* using their hydroalcoholic extracts at different dose levels, assessing additional biochemical parameters, and extending the duration of the tests. This cardioprotective potential of *Hibiscus rosa sinensis* might be attributed to the antioxidant property of chemical compounds present in them and can serve as a good source for the production of a cardioprotective herbal medicines. However, an extended study using larger number of animals with removal of confounding factors like pretreatment cardiac enzymes levels, sequential administration of doxorubicin and subsequent analysis is required so that substantial data can be generated for facilitating further evaluation of these agents through clinical trials. Further molecular level of investigation could be done using different animal models and other biochemical parameters to elucidate the possible mechanism of action of *Hibiscus rosa sinensis.*

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