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EVALUATION OF METHANOLIC EXTRACT OF *CASSIA FISTULA* BARK FOR CARDIOPROTECTIVE ACTIVITY

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ABSTRACT
Cassia fistula of the family Leguminaceae is traditionally used in cardiopathy and heart disease. The cardioprotective effect of the methanolic extract of *C. fistula* (MECB) bark against doxorubicin (DXR) induced cardiotoxicity in wistar rat was studied. DXR 10 mg/kg i.p. single dose to male Wistar rats causes cardiac damage and increases the levels of cardiac biomarker enzymes Viz. AST, ALT, LDH and CKMB. The histopathological changes in DXR treated animals showed sever degeneration of the myofibrils with focal necrosis and vacuolated cytoplasm. Pretreatment with MECB at a dose of 400 mg/kg significantly decreased the elevated levels of serum enzymes histological disturbances and electro cardiogram changes to normal myocardium functioning. The result suggests that MECB has cardio protective effect in DXR induced myocardial damage rats.

KEYWORDS: Cardioprotective, *Cassia fistula*, vitamin E, doxorubicin

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INTRODUCTION

Cardiomyopathy is the leading cause of death and is poised to become the most significant health problem worldwide. In the United States, dilated cardiomyopathy occurs in approximately five to eight people per 100,000; it causes approximately 10,000 deaths and 46,000 hospitalizations each year. It is the most common reason for heart transplantation[1].

Nature has been a source of medicinal treatments for years and plants derived products continue to play an important role in the primary health care of about 80-85% of the world's population. Recently, the keen interest in medicinal plants for cardioprotection has increased because of their numerous possible cardioprotective mechanisms besides antioxidant activity[2]

Herbal medicine is increasingly gaining acceptance from the public and medical professionals due to advances in the understanding of the mechanisms by which herbs positively influence health and quality of life[3]. Various plants like *Trichopus zeylanicus*[4] *Curcuma longa, Withania somnifera, Ocimum sanctum*[5] showed the potent cardioprotective effect have containing potent antioxidants.

Cassia fistula Linn (Family: Leguminaceae) is widely distributed throughout India, different parts of this plant have been demonstrated to possess several pharmacological activities such as antioxidative[6], hepatoprotective[7], antibacterial[8] and antidiabetic[9]. Bark has the highest antioxidant potential followed by the old leaves, the young leaves and the twigs. The antioxidant activity directly related to the total phenolic contents of the extract where the presence of established antioxidant such as xanthenes, flavans, flavonols and di-anthraquinones are potentially responsible for the activities[10]. Doxorubicin (DXR) is an anthracyclin antibiotic and has broad spectrum of antitumor activity. Several studies have shown a cumulative dose dependent and irreversible cardiotoxicity that limits its use as a broad spectrum anti cancer drug[11]. But, its clinical usefulness is still restricted due to its specific toxicities to cardiac tissue[12]. The presence of less developed antioxidant defence mechanisms, heart is particularly vunnerable to injury by anthracycline induced reactive oxygen species. Libration of free radicals is central to the mechanism of DXR induced damage to the myocardium[13]. Polyherbal formulations/antioxidant compounds have shown protective effects in DXR induced carditoxicity without reducing their therapeutic efficacy. Moreover, there is a growing interest in the usage of natural antioxidants as a protective strategy against the cardiovascular related problems in experiments such as ischemia reperfusion[14] and DXR induced cardiotoxicity[15]. The plant CF is used traditionally and ancient practices in cardiopathy and heart disease[16]. The present study is to evaluate scientifically the cardioprotective effect of the methanolic extract of *Cassia fistula* bark (MECB) against DXR induced cardio toxicity in Wistar rats.

MATERIALS AND METHODS

Collection of plant material

The bark of *Cassia fistula* were collected from the Jamboti of Belgaum, Karnataka, India and identified and authenticated at Regional Medical Research Centre (ICMR), Belgaum, where herbarium of the specimen is deposited. (vouchar No.RMRC-515)

Preparation of extract

The fresh bark of *Cassia fistula* was chopped, shade dried and coarsely grounded to powdered. The powdered material was soaked and defatted by using petroleum ether. The defatted material was extracted with methanol using soxhlet extractor for 8 hours. The solvent was evaporated under reduced pressure using rotary vacuum evaporator to get methanolic extract of *Cassia fistula* bark (MECB). The extract was stored at -4^{o}C for further use.

Experimental animals

Experimental animals were maintained according to the Institutional Animal Ethical Committee (IACE), regulated by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Healthy male Wistar rats weighing 200-230 g were purchased. The animals were housed in polypropylene cages, maintained under standard conditions 12 hr light-dark cycle. The animals were fed standard died procured from Hindustan Lever Ltd., Banglore and water *ad libitum.*

Experimental protocols

The experimental protocol applied for cardioprotective effect of MECB and in DXR induced cardiotoxicity. Five groups each containing six animals were selected for the study.

Group 1- 5% Tween 80 in normal saline
Group 2- 5% Tween 80 in normal saline + Doxorubicin (DXR) (10 mg/kg b.w. in saline)
Group 3- 400 mg/kg b.w. of MECB dissolved in saline with 5% Tween 80
Group 4-400 mg/kg b.w. of MECB dissolved in saline with 5% Tween 80 +
DXR (10 mg/kg b.w. in saline)
Group 5- 5% Tween 80 in normal saline + vitamin E (50IU/kg b.w.)

Animals were pretreated with saline or extract for 14 days. DXR was administered subcutaneously to the groups 2^{nd} and 4^{th} on 12^{th} and 13^{th} day at an interval of 24 hours. On completion of the experimental period, animals were anesthetized with thiopentone sodium (50 mg/kg). The blood was collected from retro-orbital sinus under light ether anesthesia serum separated for biochemical estimation. Plasma and serum were separated by centrifugation. Rats were sacrificed and the heart was excised immediately, immersed in physiological saline and dissected out for histopathological examination.

Biochemical analysis

Serum creatine kinase isoenzymes (CKMB) and lactate dehydrogenase (LDH) were measured kinetically at 340 nm according to standard methods using diagnostic kits from Sigma Chemical Co. (St.Louis.mo). Serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were determined according to the method of Bergemeyer et al (17) using diagnostic kits from ERBA diagnostics Mannheim GmbH Germany.

Examination of electrocardiogram (ECG)

Cardiac parameters were estimated by using electrocardiogram. Seven days after the treatment with DXR administration, the Lead II ECGs of all animals were recorded using Biopac Student Lab PRO 3.7 software (Model No. MP35) make BIOPAC Systems, Inc. 42 Aero Camino, Goleta, CA 93117.

Histopathological study

Multiple sections studied from heart specimen of saline and MECB extract treated groups showed normal features, while DXR treated group showed focal necrosis with oedema and swelling of muscle fibers. Muscle fibers showed eosinophilia of cytoplasm. There is focal hemorrhage. Multiple sections studied from heart specimen of group 4 showed normal features and slight cytoplasmic vacuolation in groups of muscle fibers, while group 5 treated with vitamin E and DXR showed normal features and mild cytoplasmic vacuolation in isolated tissue.

Statistical analysis

Results were expressed as mean ± S.E.M. The statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA, using Tukey multiple comparisons test as *post hoc* test.

RESULTS

Acute administration of DXR 10 mg/kg single dose i.p. induces cardiotoxicity and the cardioprotective effect of MECB was established by significant decrease in cardio biomarker enzymes, ECG recording and histopathological study.

Biochemical estimation of DXR treated group showed increased in the levels of serum enzymes viz. AST, ALT, LDH and CKMB as reported previously. The increased concentration of serum enzymes is a well accepted quantitative index of myocardial damage caused by DXR treatment. Pre and concurrent treatment with MECB at dose (400 mg/kg single p.o.) showed significant ($P<0.001$) decreased in the levels of serum enzymes viz. AST, ALT, LDH and CKMB in DXR induced myocardial damage group (**Table 1**).

Histopathological study showed severs degeneration of the myofibrils with focal necrosis and vacuolated cytoplasm was seen clearly in DXR treated animals. DXR intoxication also induced eosinohillic cytoplasmic and focal hemorrhage with inflammatory cell infiltration. Treatment with MECB at dose of 400 mg/kg in DXR induced cardiac damage animals showed mark improvement and preserved the myocardial structure (**Fig 1**).

ECG findings of treatment with the MECB at dose of 400 mg/kg showed no significant changes in control group. Whereas DXR treatment group showed significant changes in ECG pattern: a significant prolongation in the QT and ST intervals. There is no significant prolongation in QRS complex where observed. Thus it indicates severe myocardial damage, especially affecting the ventricular conduction, Treatment with MECB in DXR induced animals (group 4) showed significant ($p<0.001$) improvement in the ECG (**Table 2**).

DISCUSSION

Doxorubicin induced myocardial damage have been well established in patients and in experimental animal models [19]. The mechanism behind the cardiotoxicity induced by DXR is via generation of free radicals, with lipid peroxidation in myocardium. It is well known that antioxidants could attenuate DOX-induced cardiotoxicity by eliminating free radicals [20]. It was reported *Cassia fistula* bark having antioxidant property [6]. Therefore this study examined the potential protective effect of MECB on DXR induced cardiotoxicity.

In the present study administration of DXR 10 mg/kg i.p single dose induced cardiac toxicity as reported in the literatures, loss of cardiac myocytes and the attenuation of left ventricular function. The serum AST, ALT, CKMB and LDH enzymes concentration are the important measures of the identification of cardiac damage. DXR caused elevation in these serums enzyme when compared with the control group. These enzymes are not specific indicators of myocardial damage but increase concentration of these enzymes together may be mark of myocardial injury [21].

Pre and concurrent treatment of MECB at a dose of 400 mg/kg p.o. single dose significantly decreased the levels of these serum enzymes. These results showed that MECB may preserve the myocardial tissues against DXR induced toxicity.

The ECG tracing were recorded for evaluating the effect of treatment with MECB and/or DXR on the heart rate and rhythm. The ECG of the control animals was normal. Dosing MECB daily had no significant effect on the ECG of the experiment rats. The ECG in the DXR treated group showed arrhythmias and conduction abnormalities in the rats. There was significant increase in the heart rate, as well as significant ST and QT segment prolongation in DXR-treated rats in comparison to control animals. The increased heart rate of DOX treated rats can lead to increase in oxygen consumption and can influence myocardial injury. There is no significant increase in prolongation of QRS complex too, when compared with control group. These results were same, as reported from previous studies [22]. Treatment with MECB in DXR induced cardiac damage animals showed significant improvement in ECG recording indicates restoration of normal cardiac functions.

Histopathological studies showed sever degeneration of the myofibrils with focal necrosis and vacuolated cytoplasm was seen clearly in DXR treated animals. DXR intoxication also induced eosinohillic cytoplasmic and focal hemorrhage with inflammatory cell infiltration. Similar observations have also been made in previous studies on acute DXR induced cardiotoxicity [23-25]. Pretreatment with MECB in DXR induced cardiac damage animals showed mark improvement and preserved the normal myocardial structure.

CONCLUSION

In conclusion the results of the present study showed MECB having cardioprotective effect by virtue of its antioxidant, anti-inflammatory and antitumor property. It is difficult to establish the mechanism of action from the present study against DXR induced cardiotoxicity. Further study is required to evaluate that MECB as a useful adjunct in combination with DXR chemotherapy.

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Table 1: Effect of methanolic extract of *Cassia fistula* bark (MECB) on doxorubicin induced changes in the activities of CKMB, LDH and transaminase in serum

GROUPS	AST(U/ML)	ALT(U/ML)	LDH(U/ML)	CKMB(U/ML
NORMAL	0.2962 ± 0.003	0.4565 ± 0.003	1.858 ± 0.078	2.223 ± 0.114
DXR	0.4933 ± 0.004##	0.6397 ± 0.003##	4.824 ± 0.078##	4.940 ± 0.005##
MEC B	0.2970 ± 0.004**	0.4517 ± 0.002**	2.034 ± 0.078**	2.374 ± 0.022**
MECB+DXR	0.3352 ± 0.014**	0.5192 ± 0.012**	3.202 ± 0.078**	3.298 ± 0.139**
DXR+VIT-E	0.3845 ± 0.003**	0.4878 ± 0.008**	3.396 ± 0.078**	3.868 ± 0.0178**

Values are expressed as the mean ± SEM from 6 animals in each group. ##P<0.001; when compared with Normal, **P< 0.001; when compared with DXR administered rats. Values are expressed as follows: Serum AST, ALT, CKMB and LDH (u/ml)

Table 2: Effect of methanolic extract of *Cassia fistula* bark (MECB) extract on doxorubicin induced changes in ECG parameters

Groups	QRS (Time in sec.)	QT Interval (Time in sec.)
Normal control	0.045 ± 0.0007	0.0650 ± 0.0010
MECB	0.043 ± 0.0007	0.0658 ± 0.0008
DXR	0.057 ± 0.0012##	0.0755 ± 0.0007##
MECB + DXR	0.048 ± 0.0008**	0.0672 ± 0.0007**
Vitamin E + DXR	0.049 ± 0.0008**	0.0675 ± 0.0009**

Values are expressed as the mean ± SEM from 6 animals in each group. ##P<0.001; when compared with Normal **P< 0.001; when compared with DXR treated group.

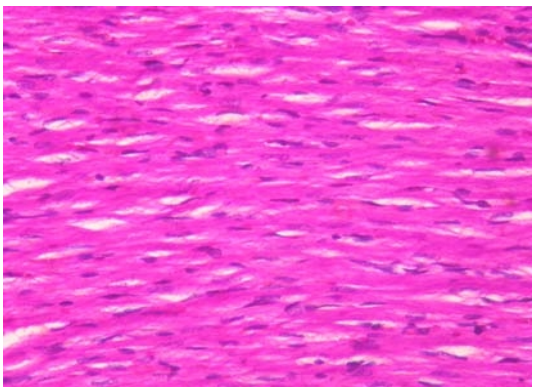

Fig A

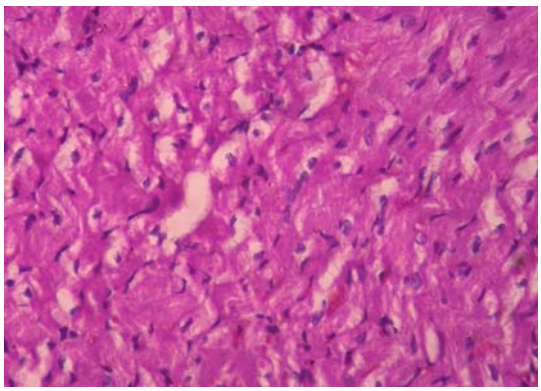

Fig B

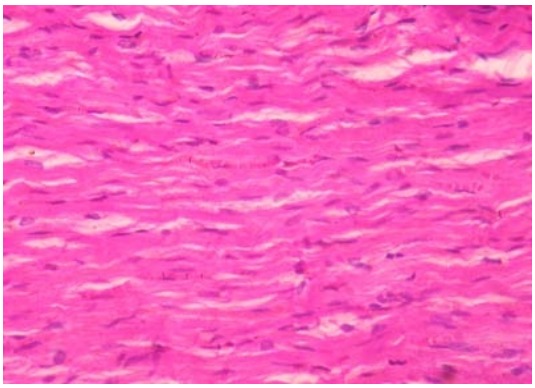

Fig C

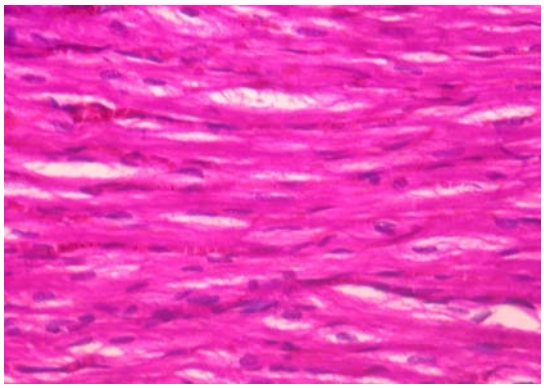

Fig D

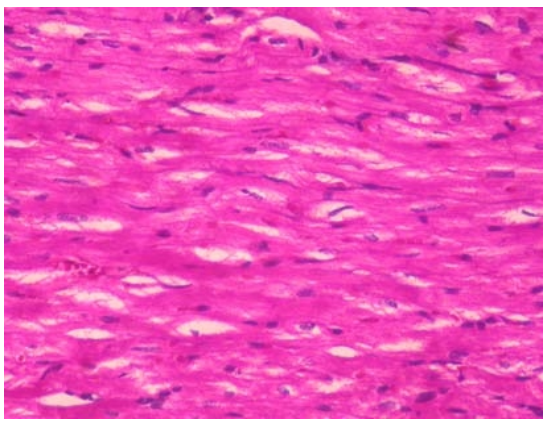

Fig E

Fig 1: Histopathology of saline treated group (A); DXR treated group (B); saline + MECB treated group (C); MECB + DXR treated group (D); saline + vitamin E treated group (E).

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