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Cardioprotective Potential of *Cassia alata*(L.) Leaves Methanolic Extract against Doxorubicin Induced Cardiotoxicity in Rats



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ABSTRACT

Myocardial infarction is the common presentation of ischemic heart disease. Herbal medicines are getting more importance in the treatment of heart diseases because the modern synthetic medicines have limitation in their use due to side effects. Present study was designed to investigate the protective effect of *Cassia alata* against cardiotoxicity induced by doxorubicin. *Cassia alata* L. (family: Leguminosae) is an exotic plant introduced in India from the West Indies for its medicinal importance. Almost all parts of the plant are recorded to be therapeutically valuable in the Ayurvedic, Unani and allopathic systems of medicine. Administration of doxorubicin (15 mg/kg i.p.) induced cardiomyopathy by significant elevation in serum creatine kinase MB (Ck-MB), lactate dehydrogenase (LDH), triglycerides & cholesterol activities with a corresponding decrease in SOD, CAT, GSH level in tissue homogenate. Oral administration of *Cassia alata* leaves methanolic extract (100, 200 & 400mg/kg) prior to doxorubicin produced a significant reduction in mortality & restoration of altered cardiac marker enzymes. The histopathological studies also supported the protective properties of *C. alata* leaves, animals pre-treated with *C. alata* leaves extract showed a marked protective effect with decreased necrotic zones and revealed normal cardiac muscle bundles. Present study showed that *C. alata* leaves extract significantly restores most of the the biochemical and histopathological parameters. These results indicate that *C. alata* leaves methanolic extract has significant cardioprotective activity.



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INTRODUCTION

Myocardial infarction is the common presentation of the ischemic heart disease. It occurs when myocardial ischemia surpasses the critical threshold level for an extended time resulting in irreversible myocardial cell damage. Although clinical care is improved, public awareness is raised and health innovations are widely used, myocardial infarction still remains the leading cause of death worldwide[1]. According to the World Health Organization, it will be the major cause of death in the world by the year 2020 [2]. In India, the number of patients being hospitalized for myocardial infarction, commonly known as heart attack, is increasing in the past 35 years and male patients have shown a more striking increase [3].

Herbal medicines are represented as the most important field of alternative medicines all over the world. *Cassia alata* L. (family: Leguminosae) is an exotic plant introduced into India from the West Indies for its medicinal importance. Almost all parts of the plant are recorded to be therapeutically valuable in the Ayurvedic, Unani and Allopathic systems of medicine. Extracts from the leaves of this species has shown several pharmacological properties such as antimicrobial and antifungal activities [4-7], antiseptic [8], anti-inflammatory and analgesic[9], anti-hyperglycemic[10]. The plant is a source of chrysoeriol, kaempferol, quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O- β -D-glucopyranoside, kaempferol-3-O- β -D-lucopyranosyl-(1->6)- β -D-glucopyranoside, 17-hydroxytriacontane, n-dotriacontanol, n-triacontanol, palmitic acid cerylester, stearic acid, palmitic acid [11]. Several other flavonoids [12-14] and anthraquinones [15-17] have been isolated from the plant. There is only a report on the constituents of its volatile oil [18].

Doxorubicin-induced cardiac toxicity is characterized by ventricular wall thinning and dilation of the left ventricular chamber. The variety of pathogenic mechanisms such as mitochondrial dysfunction, apoptosis of cardiac myocytes and alteration in calcium handling have been shown to be involved in doxorubicin-induced cardiomyopathy. The present study was conducted to evaluate the Cardioprotective potential of the *Cassia alata* leaves against doxorubicin-induced cardiac toxicity in rats.

The existing drugs can cure most of the diseases. Still there is a never ending search for finding new drugs in the hope that it would yield drugs with lesser side effects and better therapeutic

benefits than the existing drugs. Heart plays a vital role in regulation of physiological processes. There are numerous plants and polyherbal formulations claimed to have cardioprotective activities. Growing concerns in the recent past over the toxic effects of various synthetic drugs have forced pharmaceutical researchers and physicians to use herbal drugs. Present study deals with pharmacological evaluation of leaves of *Cassia alata* L. with special reference to Cardioprotective potential in animal models.

MATERIALS AND METHODS

Plant collection

The *Cassia alata* leaves were collected in the month of Nov-Dec 2011 from Salem district of Tamil-Nadu. The *Cassia alata* plant material was authenticated by Botanist Dr. Kumresh, HOD, Dept. of Botany from Govt. College of Art, Commerce & Science; Salem. The leaves were dried under shade and then powdered with a mechanical grinder. The powder of plant was passed through sieve No. 30 and stored in airtight containers for further use.

Extraction procedure:

The dried powder of leaves was defatted with petroleum ether (60-80⁰ C) in a Soxhlet apparatus & further defatted material extracted with methanol. The solvent was recovered by distillation under low pressure and the resulting semisolid mass was dried using rotary flash evaporator & stored in airtight container for further use.

Chemical used

Doxorubicin (Oncodria, Sun Pharma Lab. Ltd.)

Experimental setup

Albino rats of Wistar strain weighing 150-200gm were procured from listed suppliers National Institute of Bioscience, Pune. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use. The experimental protocol was approved by Institutional Animal Ethics Committee (Reg. No. 1092/ac/07/CPCSEA/02/2012). Acute oral toxicity test was carried out according to OECD guidelines 423[19]. Acute toxicity study 100, 200 & 400mg/kg doses were selected for actual

study. In cardioprotective studies, the test extracts were pre-treated before inducing Cardiac damage with DOX [20]. The methanolic extract was prepared in the form of emulsion with gum acacia. The rats were divided into following groups with 6 animals each.

Group-I Received vehicle control gum acacia (2%, w/v acacia 5 ml/kg p.o.).

Group-II Received vehicle control gum acacia (2%, w/v acacia 5 ml/kg p.o) for 28 days once daily & single dose of DOX (25mg/kg i.p.) on the 27th day.

Group-III Received methanolic extract of *Cassia alata* (100mg/kg p.o) for 28 days once daily & single dose of DOX (25mg/kg i.p.) on the 27th day.

Group-IV Received methanolic extract of *Cassia alata* (200mg/kg p.o) for 28 days once daily & single dose of DOX (25mg/kg i.p.) on the 27th day.

Group-V Received methanolic extract of *Cassia alata* (400mg/kg p.o) for 28 days once daily & single dose of DOX (25mg/kg i.p.) on the 27th day.

At the end of the experiment period (29 days), 48 h after DOX injection, all the rats were anesthetized and then sacrificed by cervical decapitation & blood was collected. Serum was separated from blood and heart specimens were fixed in 10% formalin for histopathological examination.

Statistical analysis

All the values are presented as mean \pm SEM (standard error of mean) for six rats. Statistical significance of differences between control and treatment groups were assessed by One-way ANOVA followed by Dunnett's multiple comparison test using the "Stat" statistics computer program. A difference in the mean values of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Medicinal plants have recently become a focus of interest because they may play key roles in treating a majority of heart disease with minimal or no side effects. Therefore, present study was designed to examine the cardioprotective actions of *Cassia alata* L.leaves methanolic extracts against DOX (Doxorubicin) induced cardiotoxicity. The anthracycline antibiotic DOX is one of the most effective chemotherapeutic agents against a wide variety of cancers. Present study has shown that intraperitoneal administration of DOX produced signs of cardiomyopathy as it was

manifested by excessive fluid accumulation that found in pleural, pericardial and peritoneal cavities together with ventral edema and enlargement of liver and kidneys.^[21] Cardiac dysfunction associated with DOX is due to cardiac cell apoptosis resulted from reactive oxygen species (ROS) produced by DOX [22].

It was found that creatine kinase isoenzyme and lactate dehydrogenase are most specific highly sensitive markers for myocardial cell injury [23]. Animal treated with DOX shows extremely elevated level of these enzymes. The mechanism for the release of these markers seems to be from oxidative damage of DOX to cardiac tissue and the subsequent release of its contents into circulation. Normalization of CK-MB and LDH elevated levels and increasing percentage of survivors by *Cassia alata* leaves methanolic extracts confirms the cardioprotective effects. The results observed in pre-treatment of *Cassia alata* leaves methanolic extract with respect to induction of cardiotoxicity using doxorubicin were given in Table No. 1. Rats treated with DOX developed significant ($P < 0.001$) heart damage and it was well indicated by change in levels of cardiac marker enzymes in serum & tissue homogenate. A marked elevation in triglycerides & total cholesterol level were observed in the group treated with DOX and they were significantly high when compared with the normal values. The CAT, SOD, GSH levels in the heart homogenate were also significantly altered in the group received DOX alone.

The groups received the pre-treatment of *Cassia alata* leaves methanolic extract at dose levels of 100, 200 & 400mg/kg body weight significantly controlled the altered level of biochemical cardiac markers. The extract at a dose level of 200mg/kg exhibited a sharp decrease in the serum enzyme levels. As well as pretreatment with *Cassia alata* leaves methanolic extract shows significant increase in SOD, CAT & GSH level in tissue homogenate as shown in Table no. 01. A single dose of DOX-induced significant increase in MDA in heart homogenates while pretreatment with *Cassia alata* leaves methanolic extract shows significant decrease in MDA ($P < 0.001$) as shown in Table no. 01.

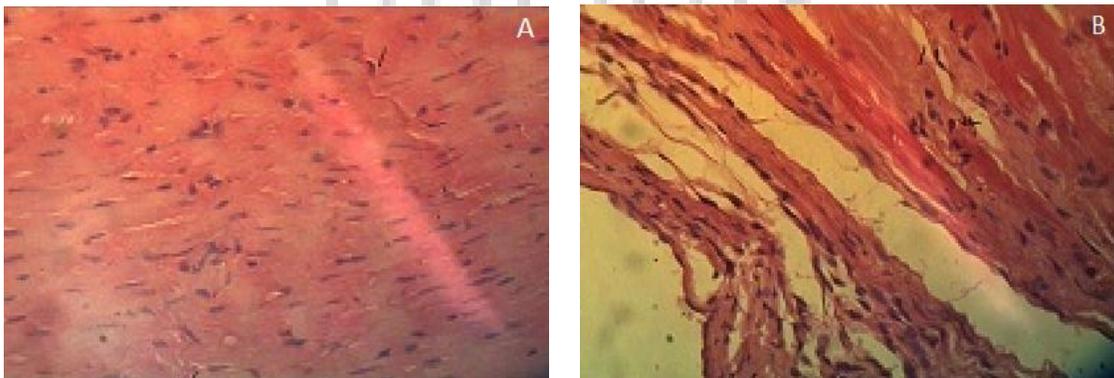
The histopathological studies also supported the protective properties of *Cassia alata* leaves. 33 (Fig. No. 01). The areas of necrosis, inflammatory cell infiltration and degeneration of myocytes were observed in the toxic group. The group of animals pre-treated with *cassia alata* extract showed a marked protective effect with decreased necrotic zones and revealed normal cardiac

muscle bundles. There was mild edema but no infarction and inflammatory cells and the cardiac fibers were within the normal limits. A cardiac fibers were within the normal limits.

Table no. 1. Effect of methanolic extract of *Cassia alata* leaves on cardiac markers of control and doxorubicin induced cardiotoxicity in rat.

Groups	CK-MB (IU/L)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (mmol/g tissue)	LDH(U/l)	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	MDA (mmol/mg tissue)
Group-I	258.4±1.96	252±2.47	14.4±0.50	11.2±0.37	104.6±2.27	65.2±1.74	77.6±1.20	1.62±0.14
Group-II	360±13.0	190.4±2.11	4.6±0.40	4.4±0.24	197.6±2.04	182.6±3.12	205±4.53	5.82±0.10
Group-III	318.6±6.16*	205.4±6.16*	6.4±0.24*	6.2±0.37*	175.6±5.66	159.8±5.58*	148.4±8.37*	4.8±0.22
Group-IV	277±2.00**	227±8.00**	10.8±0.37**	9.0±0.31*	131.8±3.77*	105.6±4.15**	102.6±5.67**	2.94±0.12**
Group-V	293.8±5.14*	220.2±8.81**	7.2±0.37*	8.0±0.31**	146.6±4.25*	110.6±5.32*	109.4±4.62**	4.1±0.26

Values are expressed as Mean ± SEM of 6 rats in each group. P values: * <0.05, ** <0.01, *** <0.001 compared with control & toxic. The data was analyzed by one way ANOVA followed by Dunnett Multiple Comparison Test and values P<0.05 were considered significant.



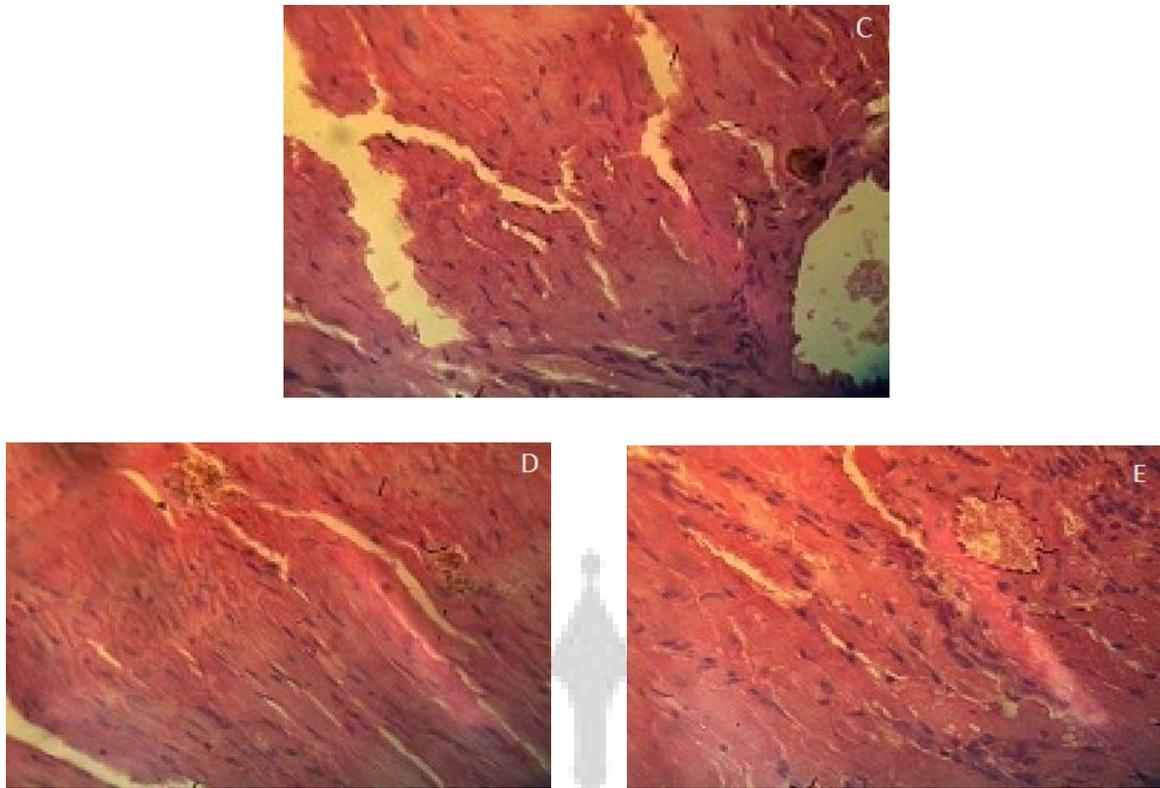


Fig. 1. A-Normal architecture of the cardiac cells was observed with no evidence of microscopic changes in the control group. B-Doxorubicin treated group (25mg/kg i.p.) showing focal confluent necrosis of muscle fiber with inflammatory cell infiltration, edema with fibroblastic proliferation and phagocytosis were seen. C- Group pre-treated with *C.alata* (100mg/kg) + DOX shows decrease in inflammatory cells. D-Group pre-treated with *C.alata* (200mg/kg) + DOX shows small foci of mononuclear collections without muscle damage, mild oedema and necrosis without inflammatory cells. E-Group pre-treated with *C.alata* (400mg/kg) + DOX showing mild degree of necrosis and less infiltration of inflammatory cells.

CONCLUSION

Preliminary phytochemical investigation of *cassia alata* leaves methanolic extracts shows presence of flavonoids, tannins, glycosides. Thus, the strong antioxidant and cardioprotective effect of the extract could be attributed to the presence of bioactive constituents present in the extract. Taking into consideration the reported activities and the various active chemical constituents, in the present study, it is proposed that *cassia alata* leaves are beneficial to protect myocardial infarction. *Cassia alata* leaves stand as a potential source for pharmaceutical

exploitation. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Cassia alata* leaves.

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