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Effect of Different Fractions of Methanolic Extract of *Cassia alata* Leaves against Doxorubicin Induced Cardiotoxicity in Rats

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ABSTRACT

The Cardioprotective effects of Petroleum ether (Pet-ether), *n*-butanol (*n*-BuOH), chloroform ($CHCl_3$) and ethyl acetate fractions (EtOAc) from methanolic extract of *Cassia alata* leaves were investigated in normal and doxorubicin-induced cardiotoxicity in rats. 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. *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 (25 mg/kg i.p.) induced cardiomyopathy by significant elevation in *serum creatine kinase* (CK), *lactate dehydrogenase* (LDH), triglycerides, total cholesterol& lipid peroxidation (LPO) activities with a corresponding decrease in SOD, CAT and GSH level in tissue homogenate. Oral administration of different fraction of *Cassia alata* leaves methanolic extract (100&200mg/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 *Cassia alata* leaves. Present study showed that n-butanol (*n*-BuOH) fraction of *Cassia alata* leaves methanolic extract significantly restores most of the biochemical and histopathological parameters. These results indicate that *Cassia alata* leaves methanolic extract has significant cardioprotective activity.

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-hydrotetratriacontane, n-dotriacontanol, n-triacontanol, palmitic

acid ceryl ester, 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 different fractions from methanolic extract of *Cassia alata* leaves against doxorubicin-induced cardiotoxicity 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 *Cassia alata* L. leaves 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& fractionation of methanolic extract of *Cassia alata*

The dried powder of leaves was defatted with petroleum ether (60-80^{0}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. The methanolic extract of *Cassia alata* leaves (MECA) was taken in a round bottom flask of simple condenser and further fractionated using solvents of increasing polarity *viz.* petroleum ether (400 ml×3wash), *n*-butanol (400 ml×3wash), chloroform (400 ml×3wash) & ethyl acetate (400 ml×3wash) as depicted in fig no. 01.

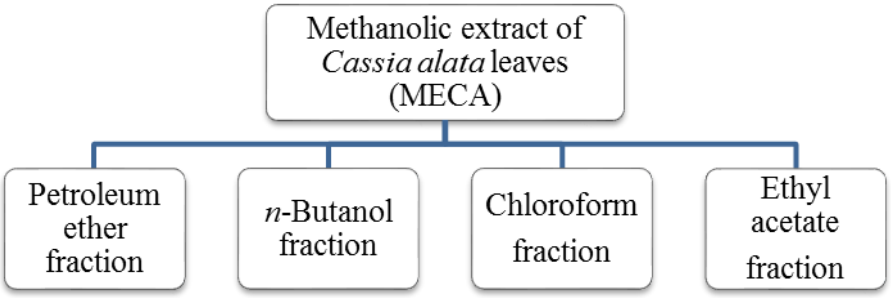

Fig No. 01- Fractionation of methanolic extract of *Cassia alata* leaves.

From all the four fractions the solvent was removed using a rotary vacuum evaporator under reduced pressure. The methanol extract was thus fractionated into petroleum ether soluble fraction, *n*-butanol soluble fraction, chloroform soluble fraction & ethyl acetate soluble fraction.

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 *ab 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]. From acute toxicity study 100 & 200mg/kg doses were selected for actual study. In cardioprotective studies sub-fractions of *Cassia alata* leaves methanolic extract were pre-treated before inducing cardiac damage with DOX [20].The entire fractions of MECA were prepared in the form of emulsion with gum acacia for oral administration to animals at doses 200 mg/kg body weight. The rats were divided into following groups with 6 animals in 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 Pet. ether fraction of MECA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Group-IV Received Pet. ether fraction of MECA (200mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Group-V Received η-butanol fraction of MECA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Group-VI Received η-butanol fraction of MECA (200mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Group-VII Received chloroform fraction of MECA (100mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Group-VIII Received chloroform fraction of MECA (200mg/kg p.o.) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Group-IX Received ethyl acetate fraction of MECA (100mg/kg p.o) for 28 days & single dose of DOX (25mg/kg i.p.) on 27th day.

Group-X Received ethyl acetate fraction of MECA (200mg/kg p.o) for 28 days & single dose of DOX (25mg/kg i.p.) on 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 sample were collected from all groups. 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 ± SEM (standard error of mean) for six rats. Statistical significance of differences between the control and treatments groups were assessed by One-way ANOVA followed by Dunnett's multiple comparison tests 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 different fraction from methanolic extract of *Cassia alata* L. leaves 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 and LDH elevated levels and increasing percentage of survivors by different fraction from methanolic extract of *Cassia alata* leaves confirms the cardioprotective effects. The results observed in pre-treatment of different fraction from methanolic extract of *Cassia alata* leaves with respect to induction of cardiotoxicity using doxorubicin were given in Table No.01 Rats treated with DOX developed significant 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 different fraction from methanolic extract of *Cassia alata* leaves at dose levels of100 &200mg/kg body weight significantly controlled the altered level of biochemical cardiac markers. From the result, it was observed that *n*-butanol fraction of *Cassia alata* leaves methanolic extract at dose 100mg.kg shows mark activity as compared to other fractions, *n*-butanol fraction shows significant increase ($p<0.05$) in serum SOD & CAT level as compared to DOX-treated. *Creatine kinase* plays important role in myocardial infarction. From the result, it was observed rat treated with DOX shows significant increase in CK level as compared to control. However pre-treatment with different fractions of *Cassia alata* leaves methanolic extracts at doses 100, 200 mg/kg shows decreased in the activity of serum CK. It was observed that as compare to all the fractions *n*-butanol fraction shows significant activity by increase ($p<0.01$) in CK level as compared to control. High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage. Pretreatment with different fraction *cassia alata* methanolic extract leaves resulted in significant decrease in the cholesterol level when compared to rats administrated with DOX group.

Table no.01 shows the level of glutathione (GSH) & lipid peroxides (LPO) in heart of control and experimental rats. It has been observed that there was a significant decrease in the level of

the tissue GSH in doxorubicin-treated group as compared to normal control animals. Pretreatment with different fraction of *Cassia alata* methanolic extracts raised the myocardial GSH level as compared toxic group. From the result lipid, peroxidation was found to be significantly increased in animals subjected to DOX exposure. The level of lipid peroxides was decreased in groups of rat pretreated with different fraction of *Cassia alata* methanolic extract when compared to toxic group. It was observed that n-butanol fraction at dose 100mg/kg shows significant ($P<0.05$) decreased in lipid peroxidation activity when compare with DOX-treated group indicating the cardioprotective potential of *Cassia alata* leaves.

The histopathological studies also supported the protective properties of different fraction of *Cassia alata* leaves methanolic extract. The areas of necrosis, inflammatory cell infiltration and degeneration of myocytes were observed in the toxic group (Fig no.03). The group of animals pre-treated with *n*-butanol fraction *cassia alata* leaves methanolic extract showed a marked protective effect with decreased necrotic zones and revealed normal cardiac muscle bundles (Fig No.04). There was mild edema but no infarction and inflammatory cells and the cardiac fibers were within the normal limits. Cardiac fibers were within the normal limits.

Table No. 01 Effect of different fraction of *Cassia alata* leaves methanolic extract on cardiac marker enzymes against DOX-induced cardiotoxicity

Treatment/Dose	CK (IU/L)	SOD (IU/L)	CAT (IU/L)	LDH (IU/L)	TG (mg/dl)	TC (mg/dl)	GSH (ug/mg protein)	LPO (nM of MDA/min/mg protein)
Control	97.67±8.7	111.2 ± 9.5	25.50 ± 1.4	96.3±8.61	58.9±9.3	82.1 ± 12.0	8.62 ± 0.32	0.49 ± 0.03
DOX	176.45±1.7	45.23±3.12	8.0 ± 0.77	185.5±4.54	104.4±16.6	220.3±19.62	5.09 ± 0.47	4.14 ± 1.22
Pet.Ether fraction of MECA (100mg/kg)	160.45±7.8	39.22±3.15	10.31±0.23	152.5±7.32	89.5±23.9	186.3±22.33	4.88 ± 0.32	4.23 ± 0.05
Pet.Ether fraction of MECA(200mg/kg)	172.45±6.7	50.23±2.15	7.8±0.68	143.2±4.52	91.5±25.2	211.1±25.94	4.4±0.24	4.15± 0.03
n-BuOH fraction of MECA(100mg/kg)	118.43±8.9[b]	78.54±1.2[a]	17.12±1.50[b]	122.3±5.21[a]	64.7±12.5[b]	114.2±5.55[b]	7.45±0.12[b]	1.02 ± 0.88[b]
n-BuOH fraction of MECA(200mg/kg)	130.43±9.7[a]	82.13±1.6[a]	16.11±0.15[a]	126.6±6.76[a]	70.2±12.9[a]	117.1±5.00[b]	8.12±0.25[b]	2.02 ± 1.44[a]
$CHCl_3$ fraction of MECA(100mg/kg)	162.66±9.2	55.18±9.6	10.34 ± 0.7	165.6±5.66	100.5±6.3	220.4±25.56	6.67 ± 0.01	3.00 ± 0.04
$CHCl_3$ fraction of MECA(200mg/kg)	144.87±5.3[a]	42.65±5.3	15.31±0.24[a]	128.6±1.30[a]	78.6±2.3[a]	170.4±32.46	5.37 ± 0.13	3.5± 0.02
EtOAc fraction of MECA(100mg/kg)	140.47±2.5[a]	69.12±3.5[a]	8.93 ± 0.60	156.42±6.87	101.2±1.2	131.1±1.11[a]	6.29 ± 0.04	3.30 ± 0.04
EtOAc fraction of MECA(200mg/kg)	166.58±3.2	60.32±1.5	12.35±0.24	165.26±5.36	91.8±3.4	163.2±23.09	6.36±0.36[a]	2.05 ± 0.02[a]

Data were expressed as mean ± S.D. (n=6) and analyzed by one way ANOVA followed by Dunnett's comparison test.P values: [a]<0.05, [b]<0.01, [c]<0.001, when compared to DOX treatment.

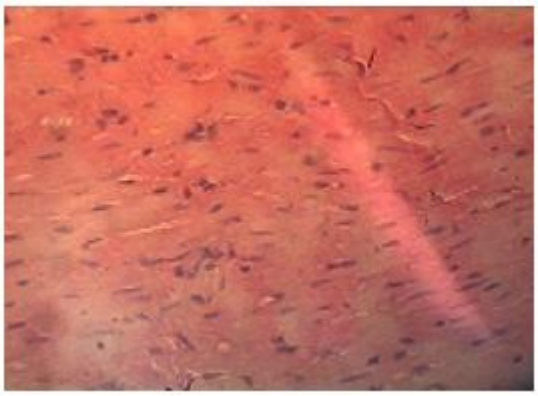

Fig.02 Normal architecture of the cardiac cells was observed with no evidence of microscopic changes in the control group.

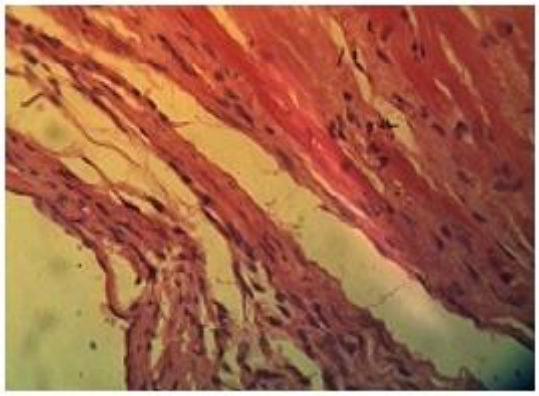

Fig.03 Doxorubicin-treated group (25mg/kg i.p.) showing focal confluent necrosis of muscle fiber with inflammatory cell infiltration, edema with fibroblastic proliferation and phagocytosis was seen.

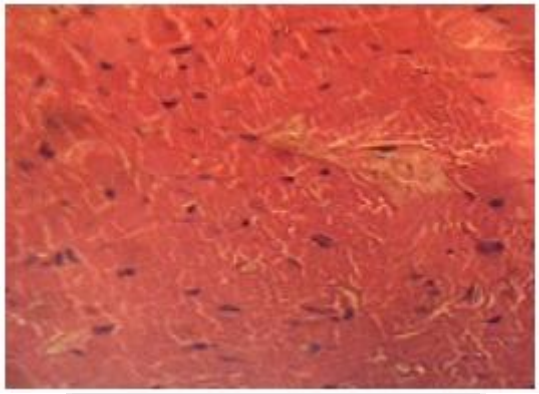

Fig.04 Photomicrograph of heart section taken from rat treated with *n*-Butanol fraction of *Cassia alata* leaves methanolic extract (100mg/kg) + DOX (Haematoxylin and Eosin x100). Section shows maximum recovery of cardiac myocytes, showed a protective effect with minimal of no pale yellow coloration and maximum fiber integrity without inflammatory cells.

CONCLUSION

Among the various extract of *Cassia alata* leaves methanolic extract *n*-butanol fraction exhibit strong cardioprotective activity. Preliminary phytochemical investigation of C*assia 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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