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Protective effect of *Syzygium cumini* seeds against doxorubicin induced cardiotoxicity in rats

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Abstract

Syzygium cumini Linn. seeds possessing potent antioxidant and cardioprotective properties were evaluated against Doxorubicin induced cardiotoxicity in rats. Intra peritoneal injection of DOX(1.5 mg / kg/bw) administered once a day for 15 days, was revealed by elevated serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT), HDL-cholesterol and changed LDL-cholesterol, and triglycerides in comparison to control and associated with increase in the levels of myocardial malondialdehyde (MDA) with simultaneously decrease in the levels of glutathione (GSH), glutathione-s-transferase(GST),glutathione reductase(GR), glutathione peroxidase (GPx),catalase(CAT) and super oxide dismutase (SOD). Daily oral administration of aqueous suspension of *Syzygium cumini* seeds extract (in the dose of 100 mg/kg/b.w. for 15 days) produced normalization in the serum levels of heart marker enzymes. *Syzygium cumini* seeds were found to be more effective in restoring lipid profile changes in rats and antioxidant enzyme activities in heart tissue. So the study shows that *Syzygium cumini* seeds possess antioxidant and cardioprotective effects.

Keywords: *Syzygium cumini*, Doxorubicin, Antioxidant, Cardio protective, Marker enzyme, Lipid peroxidation, Lipoprotein.

Introduction

Doxorubicin (DOX, Adriamycin) is a member of the anthracyclin drug family, and one of the most frequently used drug to treat many forms of cancer such as leukemia, lymphoma and solid tumors[1].The mechanism of cytotoxic action of DOX is based on intercalation of anthracyclin portion with DNA, there by changing the conformational shape and interferes with topoisomerase-II interaction, which is critical to DNA function[2-3]. Several documents reported that the superoxide and hydrogen peroxide free radical plays a predominant role in oxidative stress induced by DOX, resulting in peroxidation of unsaturated fatty acid of myocardial membrane and hamper the antioxidant defense mechanisms against the formation of excessive free radicals[4-6].

Medicinal plants are rich source of effective cardio-protective agent and its derivatives play an important role in cardio-toxicity treatment. Several medicinal plants have been screened based on the integrative approaches on drug development from Ayurveda and Unani traditional system of medicines[7]. *Syzygium cumini* Linn. (Synonyms: *Syzygium jambolana* (Lam.), *Eugenia jambolana* Willd.) belongs to a family of *myrtaceae*, widely distributed throughout Southeast Asia and Eastern Africa ,it is Commonly known as *Naval* in Tamil, black plam in English, Jamun in Hindi, Neredu in Telgu, Jamboda in Gujrati, Kala Jam in Bengali, Jambu in Marathi and Jambuh in Sanskrit . Seeds have been reported to posses, alkaloid, tannins, glycoside, flavonoid and sterols. The phytochemical constituents have been structurally elucidated as jambosine, gallic acid, ellagic acid, corilagin and related tannin, 3,6-hexahydroxydiphenoylglucose and its isomer 4,6-hexahydroxy- diphenoylglucose, 1-alloylglucose, 3-galloylglucose, quercetin, kaempferol, myricetine and β-sitosterol respectively[8-11].

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Syzygium cumini seeds have extensively used for various ailments such as anti-inflammatory[12], hypolipidaemic[13], anti-diabetic and antioxidant[14], neuro-protective[15] and recently it has been reported for the DNA protection against radiation[16]. Although the *Syzygium cumini* seeds were used for several biological activities; the possible cardio-protective activities of *S. cumini* (Jamun) seeds have not been reported so far. Therefore, in the present investigation, we aimed to evaluate the cardio protective effect of the *Syzygium cumini* (Jamun) seeds against the DOX induced acute myocardial stress in rats.

Material and methods

Drugs and chemicals

Doxorubicin (adrim®) was purchased from Dabur Pharma Limited, New Delhi, India. All other chemicals and solvents used were of the highest purity and analytical grade.

Collection of plant material

The seeds of *Syzygium cumini* were collected locally during month of July and Aug of the year 2007 and were authentified by Prof. P. Jayaraman, (Plant Anatomy Research Centre Pharmacognosy Institute, Tambaram, Chennai), Specimen voucher No.-PARC/ 2007/97.

Extraction of plant material

The plant seed were freed of pericarp, shade dried and powdered in a mixer and the extract was prepared described earlier Briefly, 100 gm of the seed powder was extracted with 70% ethanol at 50 to 60 °C in a soxhlet apparatus for 72 h. The liquid extract was cooled and concentrated by its liquid content in vacuum and freeze dried. An approximate yield of 15% was obtained. The extract of *Syzygium cumini* seeds will be called as SC.

Animals

Animals healthy Wistar adult male rats between 2 and 3 months of age and weighing about 160–180 g were used for the study. They were housed individually in polypropylene cages, maintained under standard conditions (12-h light:12-h dark cycle; 25-30 °C; 35–60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee clearance (CPCSEA/12/12/00-PH-07-03).

Experimental protocol

The experimental animals were randomized into four groups of six rats each as follows:

Group I: Control rats received distilled water (1 ml/kg body weight), orally for 15 days.

Group II: Rats were injected intraperitoneally with a single dose of DOX (1.5 mg/kg body weight for 15 days) and receive distilled water.

Group II: Rats received SC extract by oral gavage (100 mg/kg body weight for 15 days).

Group IV: Rats were administered DOX as in Group II, and pretreatment with SC extract (100 mg/kg body weight) before one hour DOX treatment by oral gavage for 15 consecutive days.

After the 15 days experimental period (i.e., on the 16th day), all the animals were anesthetized and were scarified and isolated heart. The heart tissue was excised immediately and thoroughly washed with physiological saline. The heart tissue homogenates prepared in 0.1M Tris HCl buffer, pH 7.4, were used for the determination of lipid peroxides (LPO), reduced glutathione (GSH), glutathione peroxidase(GPx), glutathione-S-tranferase (GST), catalase (CAT), and superoxide dismutase (SOD). The serum separated was used for the determination of alanine amino transferase(ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), LDL-C, HDL-C and triglycerides.

Biochemical assays

Enzymatic indices of cellular damage

The activity of creatine phosphokinase (CPK) was assayed by the method of Okinaka *et al*.,(1961) and was expressed by IU/L. Lactate dehydrogenase (LDH) was assayed by the method of Praful B. Godkar (1994) and was expressed by IU/L. Aspartate transaminase (AST) and alanine transaminase (ALT) were estimated[17].Their activities were expressed in terms IU/L. Protein content was estimated[18].The Serum HDL- cholesterol and LDH- cholesterol was estimated[19-20]. The triglycerides were estimated[21].

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Lipid peroxidation

Tissue lipid peroxide level was determined as TBARS[21]. The absorbance was measured photo-metrically at 532 nm and the concentrations were expressed as nmol malonaldehyde (MDA) min^{-1} mg^{-1} protein.

Antioxidants

SOD was assayed[23]. The degree of inhibition of auto oxidation of epinephrine at an alkaline pH by SOD was used as a measure of enzyme activity. Catalase (CAT) level was estimated as per method described[24]. Glutathione peroxidase (GPx) was assayed as per the method[25], based on the reaction between glutathione remaining after the action of GPx and 5,5'-dithio-bis(2-nitro benzoic acid) to form a complex that absorbs maximally at 420 nm., Glutathione-*S*-transferase (GST) was assayed[26]. Glutathione reductase (GR) that utilizes NADPH to convert oxidized glutathione (GSSG) to the reduced form was assayed and total reduced glutathione (GSH) was determined by the method[27-28].

Statistical Analysis

The results were expressed as mean ± standard deviation (S.D.) for six animals in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the SPSS 13.0 software package for Windows. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test. *P*-values < 0.05 have been considered as statistically significant.

Results and Conclusion

Biochemical Studies

Effect of *Syzygium cumini* on antioxidant enzyme

Table I show the activities of glutathione dependent antioxidant enzyme (GPx and GST) and antiperoxidative enzymes (CAT and SOD) in the heart tissue of normal and experimental groups of rats. SOD, CAT, GPx GST and GR level were significantly reduced ($p<0.05$) in DOX induced animals (Group-II), when compared with control animal (Group-I). The ethanolic extract of SC (Group IV) at a dose of 100 mg/kg/b.wt. treated showed significant increase ($p<0.05$) in SOD, CAT, GPx, GST and GR activity in heart muscle tissue, when compared to induced animal (Group-II). SC (Group-III) at dose of 100 mg/kg/b.wt. treated animals also showed non significantly ($p<0.05$) increased in level of SOD, GPx, GST , GR and non significantly ($p<0.05$) decreased activity of CAT when compared to control animal (Group-I).

Effect of *Syzygium cumini* on marker Enzyme

Table 2 show the level of diagnostic marker enzyme (CPK, LDH, AST, ALT) in serum significantly increased ($p<0.05$) in Group-II animal (DOX induced), when compared to control (Group-I.). The ethanolic extract of the SC (Group IV) at the dose of 100 mg/kg/b.wt. Significantly ($p<0.05$) decreased in the enzyme level in serum, when compared to DOX treated animals (Group-II). SC (Group-III) at a dose of 100mg/kg/b.wt. Produced a non significantly ($p<0.05$) decreased in the enzyme activity of CPK, AST, ALT and non significantly increased activity of LDH, when compared to control animal (Group-I).

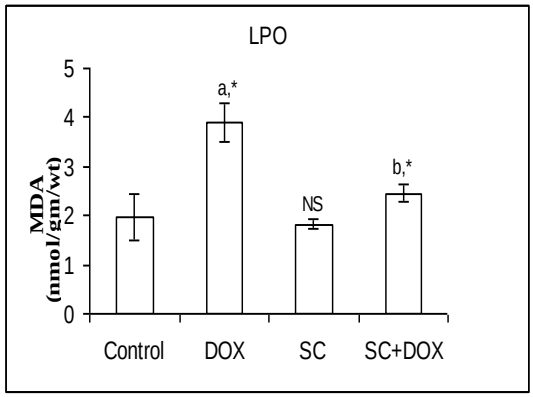

Fig 1

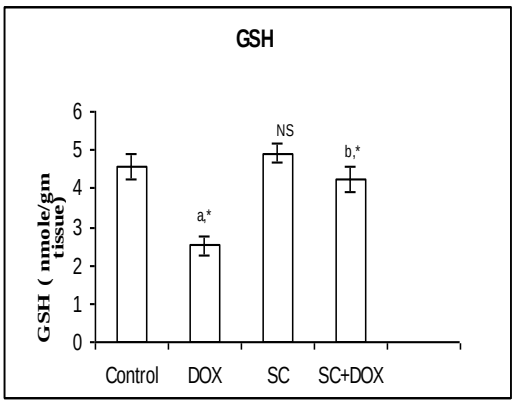

Fig 2

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Fig. 1: Levels of MDA in heart of the experimental animals. Results are given as mean ± S.D. for six rats. Comparisons are made between: a-Group I and Group II; b-Group II and Group IV.* statistically significant ($P < 0.05$); NS – non-significant

Fig. 2: Levels of GSH in the heart of the experimental animals. Results are given as mean ± S.D. for six rats. Comparisons are made between: a-Group I and Group II; b-Group II and Group IV. * Statistically significant ($P < 0.05$); NS – non-significant.

Table 1: Effect of doxorubicin and *Syzygium cumini* Linn. on the activities of cardiac enzymic antioxidants

Group	Group I control	Group II DOX-administered[B]	Group III SC treated[A]	Group IV [A+B]
Glutathione –dependent antioxidant enzymes				
GPx	2.43±0.15	1.24±0.13 ^{a,*}	2.44±0.28 ^{NS}	1.97±0.10 ^{b,*}
GST	0.77±0.07	0.40±0.036^{a,*}	0.81±0.047 ^{NS}	0.75±0.048 ^{b,*}
GR	1.23±0.10	0.70±0.044^{a,*}	1.29 ±0.02 ^{NS}	1.19±0.06 ^{b,*}
Antiperoxidant				
SOD	06.81±0.32	2.96±0.38 ^{a,*}	7.0±0.17 ^{NS}	5.96±0.29^{b,*}
CAT	11.01±0.71	5.08±0.61 ^{a,*}	10.43±0.67 ^{NS}	9.05±0.54 ^{b,*}

Results are expressed as mean ± S.D. for six rats. Units - SOD: Units/mg protein, one unit is equal to the amount of enzyme that inhibits auto-oxidation of epinephrine by 50%; CAT: µmoles H_2O_2 consumed min^{-1} mg^{-1} protein; GPx: µmoles GSH oxidized min^{-1} mg^{-1} protein; GST: nmoles CDNB (1-chloro-2,4-dinitrobenzene) conjugated min^{-1} mg^{-1} protein; GR: nmoles NADPH oxidized min^{-1} mg^{-1} protein. Comparisons are made between: a-Group I and Group II; b-Group II and Group IV. * Statistically significant ($P < 0.05$); NS – non-significant.

Table 2: Effect of doxorubicin and *Syzygium cumini* Linn. on the activities of cardiac marker enzymes in serum

Group	Group I Control	Group II DOX-Administered [B]	Group IIISC- treated[A]	Group IV [A+B]
CPK (IU L^{-1})	88.31±4.05	183.58±9.84 ^{a,*}	83.68±3.76^{NS}	102.28±5.29 ^{b,*}
LDH (IUL^{-1})	143.04±3.85	326.27±17.71 ^{a,*}	147.10±7.95 ^{NS}	161.92±12.04^{b,*}
AST (IUL^{-1})	71.11±3.91	129.52±10.45 ^{a,*}	67.83±3.34 ^{NS}	79.75±5.89 ^{b,*}
ALT (IUL^{-1})	28.04±2.06	76.39±7.03^{a,*}	25.70±1.42^{NS}	36.93±3.09 ^{b,*}

Results are expressed as mean ± S.D. for 6 rats. Comparisons are made between: a-Group I and Group II; b-Group II and Group IV. * Statistically significant ($P < 0.05$); NS – non-significant.

Doxorubicin is a clinically important drug used in the treatment of several solid tumor and malignancies. Several lines of evidence indicated that DOX induce myocardial lesion, both in human and experimental animals However, clinical uses of DOX is limited by various unwanted side effects, such as cardiotoxicity[29-30]. It has been reported that ROS plays a predominate role in DOX induced cardiotoxicity[31].cPrevious literature demonstrated that semiquinone radical is formed during DOX metabolism and in the presence of oxygen , DOX semi-quinone free radical is rapidly oxidized, forming superoxide free radical through an enzymatic mechanism, by utilizing cellular oxidoreductase[32]. Further, Superoxide free radical undergoes dismutation to form hydrogen peroxide (H_2O_2) by super oxide dismutase (SOD), which later, in the presence of metal ion (iron) leads to the formation of hydroxyl radical by Fenton reaction[33].

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Several studies revealed that generation of free radical decreased the antioxidant defence mechanism thereby damage cellular constituent, Glutathione (GSH), extensively found in cells, protects cells against electrophilic attacks provided by drugs such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, liver and heart, the elevation of MDA levels, which is one of the end products of lipid per oxidation in the heart muscles tissue, and the reduction of cardiac GSH levels are important indicators in DOX-intoxicated rats. DOX is capable of generating superoxide free radicals; there by suppressed the GSH synthesis and increasing MDA levels due to peroxidation of polyunsaturated fatty acid of myocardial membranes[34]. In the present study, elevation of MDA along with the decrease of GSH level in the heart tissue of rats was observed and which is in agreement with previous reports[35]. DOX produces acute injury to the myocardial membrane which causes significant elevation of marker enzyme (CPK, LDH, AST, and ALT) activities in serum could be regarded as a sign of damage to the heart muscles membrane**,** which suggests the event of enhanced lipid peroxidation. Several literature revealed that the elevation of lipid peroxidation lead to depletion of GSH in DOX induced rats, leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals[36-37]. In present study *Syzygium cumini* extract prevent the leakage of marker enzymes by scavenging lipid peroxides and improve the GSH levels thereby protecting integrity of membrane. The antioxidant enzymes, constituting the first line of defense mechanism to prevent and neutralize the reactive oxygen species (ROS) induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as (GR and GST) and anti-peroxidative enzymes (CAT, SOD, GPx), whose activities are dependent on the level of reduced glutathione (GSH), it has been well documented that depletion of GSH and elevation of lipid peroxides leads to decrease in antioxidant enzyme levels in DOX induced rats[38]. Glutathione peroxidase (GPx) is an endogenous antioxidant present in most of tissues. DOX treatment decline the level of glutathione peroxidase (GPx) and it has an important role in protecting the heart from peroxidative attack. Glutathione disulfide reductase (GR) is a flavoprotein that involve in conversion of oxidised Glutathione GSSH into reduced GSH by the oxidation of NADH to NAD+ and CAT is peroxisomal enzyme which converts the hydrogen peroxide (H_2O_2) in to water (H_2O) and O_2[39]. CAT and SOD are cytoplasmic enzyme and showed significant decrease in treated rats with DOX, therefore, a reduction in the activities of antioxidant enzyme observed by us in DOX, induced cardiac damage can lead to an excess availability of superoxide and hydrogen peroxide (H2O2) and which in turn generate hydroxyl radicals resulting in propagation of lipid peroxidation process , which is in agreement with the previous report. The present study clearly shows that the *Syzygium cumini* seeds posses free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by DOX, *Syzygium cumini* seed significantly reversed these all biochemical changes and hence, it is possible that the mechanism of the cardio-protection may be due to in the presence of potential phytochemical constituent in *Syzygium cumini* seeds[40]. Lipid consists of cholesterol (HDL and LDL cholesterol), triglyceride (neutral fat), In the present studies shows that DOX reduced the rate of lipolysis whereas markedly elevation of LDL-C, HDL-C, triglycerides levels in DOX induced cardiomyopathy, which is agreement with previous studies[41]**.** Supplement of *Syzygium cumini* extract brought back near to normal lipid profile. It has been well documented that these constituent posses potent antioxidant activity and has been reported for its ability to scavenge the reactive oxygen species such as OH and superoxides and also inhibits the lipid peroxidation[42-43]. Previous studies also suggest that the seeds of *Syzygium cumini* have been found to have high total phenolic contents (72.0–167.2 mg/g) [44]**.** So, we can conclude that oral administration of *Syzygium cumini* seeds have been shown to modulate the biochemical changes observed in DOX induced cardiotoxicity in animals. The cardio protective effect of the *Syzygium cumini* was further concluded by the histopathological examinations. The results show that *Syzygium cumini* seeds have potent antioxidant as well as cardio-protective activity. A further research on *Syzygium cumini* seeds against DOX induced rats is in progress.

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