Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(4): 354-360

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Cardioprotective Activity of Ethanolic Extract of *Citrus grandis* (L.) Osbeck Peel on Doxorubicin and Cyclophosphamide Induced Cardiotoxicity in Albino Rats

Samir Baniya[1*], Dhananjaya D.R.[1], Ankit Acharya[2], Bishal Dangi[1], Arjun Sapkota[1]

[1]*Department of Pharmacology, Mallige College of Pharmacy, Bangalore-560090, Karnataka, India*
[2]*Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, B.G. Nagara-571448, Karnataka, India*

ABSTRACT

The present study was designed to evaluate the cardioprotective potential of *Citrus grandis* (L.) Osbeck (CGO) peel extract in rats. *Citrus grandis* (L.) Osbeck peel extract was evaluated for protection against cyclophosphamide (200 mg/kg body wt., *i.p.*) and doxorubicin (15 mg/kg body wt., *i.p.*) induced cardiotoxicity in male albino rats. Biomarkers like lactate dehydrogenase (LDH), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), total cholesterol (TC), triglyceride (TG) and creatinine kinase (CK-MB) along with heart weight index and antioxidant enzymes was considered to determine the cardioprotective property. Histopathological study was also carried out on heart of experimental animals. The CGO peel extract was found to contain alkaloids, flavonoids, steroids and triterpenoids, saponins, phenolic compounds and tannin as chemical constituents. Cyclophosphamide (CYP) and doxorubicin (DOX) treated groups exhibited significant increase in LDH, ALT, AST, ALP, TC, TG and CK-MB level and decrease in catalase (CAT), superoxide dimutase (SOD) when compared to control group. Pretreatment with different doses of CGO significantly reduced the serum biomarkers and increased the tissue antioxidant level when compared to DOX and CYP alone treated groups. Moreover, treatment with CGO also improved CYP induced changes in histopathology of heart which may be due to its antioxidant property. The *Citrus grandis* (L.) Osbeck exerted protective effect against CYP and DOX induced cardiotoxicity in rats, which may be due its lipid lowering and antioxidant properties. These findings might be helpful to understand the beneficial effects of CGO extract against myocardial injury although further study is needed to confirm its mechanism.

Keywords: *Citrus grandis* (L.) Osbeck, cyclophosphamide, cardiotoxicity, doxorubicin.

INTRODUCTION

Cardiovascular diseases (CVDs) are the major health problem of advanced as well as developing countries of the world. [1] Cardiotoxicity is a well-known side effect of several cytotoxic drugs, especially of the anthracyclines that have been reported to cause cardiomyopathy, congestive heart failure and ECG alteration. [2] Myocardial infarction (MI) is a key factor for the burden of CVDs. The assessment of the incidence and case fatality of myocardial infarction is important determinants of the decline in coronary disease mortality. The global burden of disease due to CVDs is escalating, principally due to sharp rise in the

***Corresponding author: Mr. Samir Baniya,**
Department of Pharmacology, Mallige College of Pharmacy, Bangalore-560090, Karnataka, India; **Tel.:** +91-9742599813; **E-mail:** samir_baniya1000@yahoo.com
Received: 24 May, 2015; **Accepted:** 08 June, 2015

developing countries which are experiencing rapid health transition. [3]

Cyclophosphamide (CYP) is a potent alkylating agent that is widely used in anticancer treatments due to its DNA alkylation and preparative regimens for blood stem cell transplantation. However, CYP can cause a variety of adverse effects, including fatal cardiotoxicity, in high-dose regimens. [4] It is inactive until metabolised in the liver by the P-450 mixed function oxidases to 4-hydroxycyclophosphamide, which forms aldophosphamide reversibly. [5] Two different types of acute cardiac effects from high dose of CYP are described: a myocarditits that can be asymptomatic, and congestive heart failure, which may be fatal. [6-7]

Doxorubicin (DOX) is a potent anthracycline antibiotic used for the treatment of variety of tumours including solid and hemopoietic malignancies. However, the clinical uses of DOX have been restricted owing to its serious cardiotoxic effects. A number of mechanisms have been proposed for cardiotoxic effect of DOX, including mitochondrial dysfunction, calcium overload, inhibition of several membrane-bound molecules, interaction with nucleic acid and nuclear components, alteration of fatty acid oxidation that leads to the depression of energy metabolism in the cardiac tissue and induction of apoptosis. [8]

Since, from the ancient period nature has been a source of medicinal treatments and plant-derived products continue to play an essential 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 been increased because of their numerous possible cardioprotective mechanisms besides antioxidant activity. [9] Herbal drugs have received greater attention as an alternative to clinical therapy and the demand for these herbal remedies has greatly increased in recent time. Their utilization is often based on long-term clinical experience. Despite the usage of plants in folk medicine over ages, only lately has pharmacology and toxicity of these plants begun to receive attention from scientists. [10]

Citrus grandis (L.) Osbeck is a citrus fruit belonging to the family Rutaceae, which is originated in tropical and subtropical Southeast Asia, best suited to hot humid tropics. Most of the studies on pomelo fruits have been focused on the antioxidant activity of compounds found in the juice, tissues, peel and seeds. Molecules responsible for the antioxidant activity are flavonoids, coumarins, and carotenoids. Chemical constituents like flavanones, flavones and flavonols present in citrus fruits have been shown to be powerful antioxidants. Flavonoids also have the potency to stimulate the immune system, induce protective enzymes in the liver or block damage to genetic materials. [11]

Carotenoids, apart from being responsible for the color of a wide variety of foodstuffs, have many beneficial properties for human health. These compounds have been implicated in the prevention of, or protection against, serious human health disorders such as cancer, heart and cardiovascular diseases, macular degeneration, cataracts, osteoporosis and hypertension. Researchers found that level of flavonoids were higher in the flavedos, the peels, than in the juices and therefore more study should be conducted on these materials commonly consider as wastes. [12]

Literature survey reveals that *Citrus grandis* Osbeck is extensively used in Chinese system of medicine. [13] However its cardioprotective activity has not been investigated scientifically so far. The present study was planned to evaluate the cardioprotective activity of peel extract of *Citrus grandis* (L.) Osbeck on doxorubicin and cyclophosphamide induced cardiotoxicity with a view to provide scientific evidence.

MATERIALS AND METHODS

Drugs and chemical used

Doxorubicin was procured from the Sapthagiri Pharma, Bangalore, India. Cyclophosphamide was procured from HCG pharma, Bangalore, India. All other reagents used were of analytical grade.

Animals

Adult male Wistar rats (160-200 g) were used for this purpose. The animals were randomized into experimental, normal and control groups, housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. The animals were maintained under standard condition in an animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethics Committee, Mallige College of Pharmacy, Bangalore, Reg. no.1432/PO/a /11/CPCSEA.

Collection and authentication of plant material

The fruit of *Citrus grandis* (L.) Osbeck was collected from surrounding farm of Hesargattha, Bangalore. The plant was identified and authenticated by senior botanist at FRLHT (Foundation for Revitalization of Local Health Traditions), Jarakabande Kaval, post Attur, Yelahanka, Bangalore -560106.

Extraction procedure

Fruits peels were cleaned, blended into powder by a blender after being dried at 60°C for the period of 8 h in an oven. The coarse powder of *Citrus grandis* (L.) Osbeck peel (500 gm) was subjected to extraction with 80% ethanol (1000 ml) by Soxhelet extraction for the duration of 8 h. During the process of extraction, the alternate filling and emptying of the body of the extractor goes on continuously till it gets exhausted and it was confirmed by discoloration of solvent in the side tube of extractor (Siphon). Then after, the residue was removed by filtration and concentrated under steam bath. The concentrated extract was transferred to china dishes and finally placed in a vacuum oven at 45°C. The extract was semisolid, brownish black in color and it was stored in an airtight amber colored bottle until use.

Preliminary Phytochemical Analysis

Ethanolic extract of the peel of *Citrus grandis* was subjected to chemical tests for the identification of their active constituents. Test for the presence of carbohydrates, glycosides, resins, tannin, alkaloid, fixed oil, flavonoids, terpenoids, protein, saponins, anthraquinone and amino acid were conducted as per the standard procedure. [14]

Determination of Acute Toxicity

Acute toxicity study was carried out using male Wistar rats (160-200 g) by up and down/staircase method as per OECD guidelines. The CGO extract was orally administered to different groups of rats (n=3) at the dose of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight respectively. Animals were observed for 48 h to study the general behaviour of animals, signs of discomfort and nervous manifestation. The CGO extract was found devoid of mortality of animals at the dose of 5000 mg/kg body weight. Hence the 1/10th (500 mg/kg, *p.o*) and 1/20th (250 mg/kg, *p.o*) of the dose were selected for the screening of cardioprotective activity. [15]

Pharmacological Screening

Model I: Cyclophosphamide induced cardiotoxicity in rats

Experimental protocol [16]

Animals were divided into four groups of six animals each. The groups were as follows:

Group 1: Normal control (rats administered with 0.9 % normal saline daily for 10 days).

Group 2: Cyclophosphamide control (animals were administered with single dose of CYP (200 mg/kg, *i.p.*) on first day of the experimental period).

Group 3: animals received single dose of CYP (200 mg/kg, *p.o.*) on first day followed by ethanolic extract of CGO at (250 mg/kg, *p.o.*) alone for 10 days.

Group 4: animals received single dose of CYP (200 mg/kg, *p.o.*) on first day followed by the administration of ethanolic extract (500 mg/kg, *p.o.*) of CGO continuously for 10 days.

Biochemical parameters

Animals were sacrificed 24 h after the last dose and blood samples were collected from retro-orbital plexus of rats under anesthesia with chloroform. Blood samples were centrifuged at 3000 rpm for 10-15 min and serum was obtained. The endogenous biological markers such as lactate dehydrogenase (LDH) [17], creatine kinase isoenzyme MB (CK-MB) [18], alanine transaminase (ALT) [19], aspartate transaminase (AST) [20], alkaline phosphatase (ALP) [21], total cholesterol (TC) [22], triglyceride (TG) [23] in serum and antioxidant enzymes (CAT [24] and SOD [25]) will be determined in heart tissue homogenate.

Histopathological Studies

The animals were sacrificed and heart was isolated, washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin-wax and sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope. [16]

Model II: Doxorubicin (DOX) induced cardiotoxicity in rats

Experimental protocol [26]

The rats were divided into four groups of six animals each. The groups were as follows:

Group 1: Normal (rats treated with 1% Na CMC-2 ml/kg/day, *p.o.*)

Group 2: Control (rats treated with DOX with total cumulative dose of 15 mg/kg, *i.p.* for 2 weeks in six divided dosage).

Group 3: Rats pretreated with ethanolic extract of *Citrus grandis* (L.) Osbeck (CGO) 250 mg/kg, *p.o.* along with DOX treatment.

Group 4: Rats pretreated with CGO (500 mg/kg, *p.o.*) along with DOX treatment. Group 2, 3 and 4 will receive DOX at alternate days for a period of 2 weeks. The days selected for DOX injection will be on the 8th, 10th, 14th, 16th, 18th, 21st day after the 7 days pretreatment with the extract. On the 22nd day parameters will be studied for general appearance, heart weight and heart/body weight ratio.

Biochemical parameters

Animals were sacrificed 24 h after the last dose and blood samples were collected from retro-orbital plexus of rats under anesthesia with chloroform. Blood samples were centrifuged at 3000 rpm for 10-15 min and serum was obtained. The endogenous biological markers such as lactate dehydrogenase (LDH) [17], creatine kinase isoenzyme MB (CK-MB) [18], alanine transaminase (ALT) [19], aspartate transaminase (AST) [20], alkaline phosphatase (ALP) [21], total cholesterol (TC) [22], triglyceride (TG) [23] in serum and antioxidant enzymes (CAT [24] and SOD [25]) will be determined in heart tissue homogenate.

Heart Weight Index (HWI)

After blood withdrawal, all the rats were sacrificed by cervical dislocation; the hearts were dissected out, washed in ice cold saline, weighed after blotting with filter paper and heart weight index (HWI) was calculated.

$$\text{Heart weight index (HWI)} = \frac{\text{Heart weight (mg)}}{\text{Body weight (mg)}}$$

Table 1: Chemical constituents of *C. grandis* (L.) Osbeck peel extract

S. No.	TEST	Ethanolic extract of *Citrus grandis* (L.) Osbeck
1.	CARBOHYDRATE	-
2.	GLYCOSIDE	-
3.	ALKALOIDS	+
4.	STEROIDS AND TRITERPENOIDS	+
5.	FIXED OILS	-
6.	SAPONINS	+
7.	PROTEIN AND AMINO ACIDS	-
8.	TANNINS	+
9.	FLAVONOIDS	+

(+) indicate presence while (-) stand for absence

RESULTS AND DISCUSSION

Extraction and Phytochemical Investigation

Successive Soxhlet extractions of CGO peel was performed. The extract powder was yellow orange in colour and hygroscopic in nature. The CGO extract was subjected to different preliminary chemical tests to determine the chemical constituents present in the extract. The results has indicated the presence of alkaloids, flavonoids, steroids and triterpenoids, saponins, phenolic compounds and tannin as shown in Table 1.

Model I: Cyclophosphamide induced Cardiotoxicity

The administration of cyclophosphamide did not cause mortality in any of the groups. The general appearance of all groups of animals was recorded throughout the study. In CYP treated group, the animals became weak and without much movement in the cage and developed a pink tinge. The rat also had red exudates around the eyes and nose along with soft watery faces. These observations were markedly reduced in CGO (250 and 500 mg/kg) treated groups.

Estimation of serum enzyme markers

Rats administered with CYP (200 mg/kg) shows a significant (P<0.001) increase in the levels of LDH, AST, ALT, ALP, TC, TG and CK-MB as compared to normal. Pretreatment with CGO (250 mg/kg) in CYP treated rats showed significant (P<0.001 for LDH, P<0.01 for ALT and P<0.05 for AST and TC) decrease compared to that of CYP alone treated animals. Likewise, CGO (500 mg/kg) showed significant (P<0.001 for LDH, ALT, TC, P<0.01 for AST, TG and P<0.05 for ALP) decrease compared to that of CYP alone treated animals as shown in Table 2.

Cyclophosphamide exposure can disrupt the redox balance tissues causing biochemical and physiological disturbances resulting from oxidative stress. As a result, biomarkers are released into the blood stream and serve as the diagnostic markers of myocardial tissue damage. [27] The elevated levels of these enzymes are associated with certain types of heart damage such as myocardial infarction, myocarditits and heart failure. The administration of CGO showed dose dependent reduction in CYP induced elevated biomarkers of cardiac injury. This effect confirms that CGO is responsible for restricting the leakage of biochemical markers due to its membrane stabilizing property. This action of CGO could be attributable to its phytoconstituents such as naringin and neohesperidin which are known to reduce the risk of heart failure. [28]

Endogenous enzymatic and non-enzymatic antioxidant levels

Catalase (CAT)

Normal basal level of catalase activity in normal control rats was found to be 35.33±0.88 U/mg of protein. Cardiotoxicity rats showed significantly decreased (P<0.001) levels of catalase (17.50±0.76 U/mg of protein). The catalase level in CGO (250 mg/kg) treatment was significantly (P<0.05) increased as compared to CYP alone treated group, whereas CGO (500 mg/kg) treatment significantly (P<0.001) increased the levels of catalase to the near normal values (24.17±1.07) as shown in Table 3.

Cardiac antioxidant enzyme Catalase was significantly reduced in CYP treated group. This enzyme was utilized for scavenging super oxides and hydrogen peroxides which are produced by excessive dose of CYP. This decrease of catalase activity may be due to exhaustion in combating the previously observed oxidative stress. This study revealed that CGO mitigated the decrease in the activity of catalase enzyme. This explains and confirms the protein carbonyls get accumulated in cardiac tissues. This can be explained on the basis of its high content of several antioxidants including flavonoids and phenolic compounds.

Superoxide Dimutase (SOD)

As shown in Table 3, the SOD level in the Normal, CYP alone, CYP+CGO (250 mg/kg), CYP+CGO (500 mg/kg)(n=6) treated rats were 24.33±0.84, 10.33±0.66, 12.33±0.61, 12.83±0.60(U/mg protein), respectively. The SOD level in the CYP treated rats were significantly (P<0.001) decreased compared with the normal rats. The SOD level in CGO 250 mg/kg and 500 mg/kg treatment was not significantly increased when compared with CYP treated group.

Histopathological changes

Figure 1 illustrates the histopathological photographs of heart tissues of normal and experimental animals. Normal group showed regular cell distribution and normal myocardial architecture. CYP induced myocardial lesions observed were very prominent in CYP alone treated rats compared to the normal control.

CYP produced massive change in the myocardium showing degeneration of myocardial tissue, vacuolization of the cardiomyocytes, infiltration of inflammatory cells and myofibrillar loss. CGO (250 mg/kg) appeared to have significant protective effects on rat cardiac myocytes treated with CYP, i.e. myocardial damage was focalized and damaged cells with granular cytoplasm were surrounded by those that appeared normal. Furthermore, only limited numbers of isolated cells exhibited cytoplasmic vacuolization or myofibrillar loss. Necrotic cardiomyocytes were very rare, and the presence of mononuclear cells and fibroblasts was decreased in CGO (500 mg/kg) when compared with the CYP only-treated group. This reduced inflammatory cell infiltration and normal cardiac muscle fiber architecture further confirmed the cardioprotective activity of *C. grandis* (L.) Osbeck.

Model II: Doxorubicin induced Cardiotoxicity

The general appearance of all groups of animals was recorded throughout the study. In DOX-treated group, the animal fur became scruffy and developed a light yellow tinge. These animals also showed red exudates around the eyes, soft watery faces and appeared sicker, weaker and lethargic when compared to normal. These

observations were markedly reduced in the CGO (250 and 500 mg/kg) treated groups.

Body weight and heart/body weight ratio

In DOX-treated rats, body weight, heart weight and heart/body weight ratio was decreased at the end of the study when compared with the normal. CGO 250 and 500 mg/kg demonstrate significant increase in body weight gain and heart weight (P<0.01) and increase in the heart/body weight ratio (P<0.001) when compared to the DOX treated group. This decrease in the body weight of doxorubicin treated rats may be due to reduced food intake and inhibition of protein synthesis (shown in Table 4).

Estimation of serum enzyme markers

As shown in Table 5, rats administered with DOX (15 mg/kg) showed a significant (P<0.001) increase in the levels of LDH, AST, ALT, ALP, TC, TG and CK-MB as compared to normal rats. This might be due to the generation of reactive oxygen species (ROS) in the myocardium which triggers intrinsic mitochondria-dependent apoptotic pathway in cardiomyocytes. [29] Pretreatment with CGO (250 mg/kg) in DOX treated rats showed significant (P<0.001 for ALT, ALP, P<0.01 for LDH, AST and CKMB and P<0.05 for TG) decrease compared to that of DOX alone treated animals.

Table 2: Effect of ethanolic extract of *C. grandis* (L.) Osbeck peel on LDH, AST, ALT, ALP, TC, TG and CK-MB in CYP induced cardiotoxicity in rats.

Enzymes	Normal	CYP treated	CYP treated + CGO (250 mg/kg)	CYP treated + CGO (500 mg/kg)
LDH (IU/L)	169.2±0.83	219.3±2.67	197.2±0.79***	184.7±1.78***
AST (IU/L)	26.67±0.88	92.67±1.74	87.50±0.76*	56.17±0.65**
ALT (IU/L)	46.33±1.60	116.5±2.27	107.8±1.16**	95.83±1.57***
ALP (IU/L)	86.67±0.88	119.7±2.90	114.5±1.43	110.5±1.47*
TC (mg/dl)	30.33±1.17	66.17±1.07	60.67±1.25*	54.67±1.30***
TG (mg/dl)	55.50±0.76	129.5±1.47	126.8±1.72	121.5±1.08**
CK-MB (U/L)	23.33±0.88	66.67±2.24	62.83±0.87	61.17±1.22

Each value are expressed as mean ± SEM for 6 animals in each group,*P<0.05; **P<0.01; ***P<0.001 as compared to CYP treated group. One-way ANOVA followed by Tukey's test.

Table 3: Effect of ethanolic extract of *C. grandis* (L.) Osbeck peel on antioxidant enzyme of heart tissue homogenate in CYP induced cardiotoxicity in rats.

Group no.	Groups	CAT (U/mg protein)	SOD (U/mg protein)
1	Normal control	35.33±0.88	24.33±0.84
2	CYP treated	17.50±0.76	10.33±0.66
3	CYP treated + CGO (250 mg/kg)	22.00±1.06*	12.33±0.61
4	CYP treated + CGO (500 mg/kg)	24.17±1.07***	12.83±0.60

Each value are expressed as mean ± SEM for 6 animals in each group,*P<0.05; **P<0.01; ***P<0.001 as compared to CYP treated group. One-way ANOVA followed by Tukey's test.

Table 4: Effect of ethanolic extract of *C. grandis* (L.) Osbeck peel on body weight, heart weight and heart/body weight ratio in doxorubicin induced cardiotoxicity in rats.

Group no.	Groups	Body weight (g)	Heart weight (g)	Heart/body weight ratio (*10^{-3})
1	Normal control	180.0±1.366	0.66±0.0102	3.69±0.050
2	DOX treated	162.8±1.138	0.43±0.0110	2.692±0.082
3	DOX treated + CGO (250 mg/kg)	168.2±1.046	0.54±0.0088	3.242±0.066**
4	DOX treated + CGO (500 mg/kg)	172.7±0.802	0.58±0.0217	3.375±0.132***

Each value are expressed as mean ± SEM for 6 animals in each group, *P<0.05; **P<0.01; ***P<0.001 as compared to DOX treated group. One-way ANOVA followed by Tukey's test.

Table 5: Effect of ethanolic extract of *C. grandis* (L.) Osbeck peel on LDH, ALT, AST, ALP, TG, TC and CK-MB in doxorubicin induced cardiotoxicity in rats.

Enzymes	Normal	DOX Treated	DOX treated + CGO (250 mg/kg)	DOX treated + CGO (500 mg/kg)
LDH (IU/L)	95.33±0.88	130.0±1.71	121.0±1.80**	105.5±1.76***
ALT (IU/L)	75.17±1.44	128.7±1.99	119.0±2.30**	95.50±1.64***
AST (IU/L)	81.50±2.23	126.0±1.65	103.7±2.57***	93.67±1.28***
ALP (IU/L)	107.2±1.99	144.8±1.51	127.7±1.22***	108.2±1.99***
TG (mg/dl)	122.2±1.88	152.3±2.04	144.8±1.53	141.0±1.03***
TC (mg/dl)	112.3±1.83	146.8±6.14	135.3±1.54*	118.2±1.27***
CK-MB (U/L)	25.67±0.66	30.67±1.11	26.0±0 .96**	22.50±0.76***

Each value are expressed as mean ± SEM for 6 animals in each group, *P<0.05; **P<0.01; ***P<0.001 as compared to DOX treated group. One-way ANOVA followed by Tukey's test.

Table 6: Effect of ethanolic extract of *C. grandis* (L.) Osbeck peel on antioxidant enzyme of heart tissue homogenate in doxorubicin induced cardiotoxicity in rats.

Group no.	Groups	CAT (U/mg protein)	SOD (U/mg protein)
1	Normal control	64.50±1.232	37.17±0.945
2	DOX treated	44.0±1.29	24.0±1.065
3	DOX treated + CGO (250 mg/kg)	49.17±1.682	26.50±0.562
4	DOX treated + CGO (500 mg/kg)	52.17±0.945**	27.33±0.714

Each value are expressed as mean ± SEM for 6 animals in each group, *P<0.05; **P<0.01; ***P<0.001 as compared to DOX treated group. One-way ANOVA followed by Tukey's test.

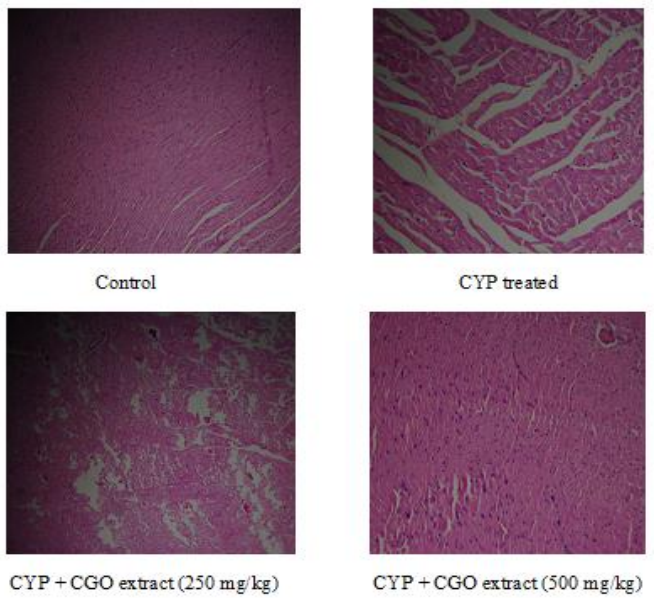

Fig. 1: Histopathology of myocardial tissue of various treatment groups

Likewise, CGO (500 mg/kg) showed significant (P<0.001 for LDH, AST, ALP, TC, TG and CK-MB) decrease compared to that of DOX alone treated animals. This protective action of CGO peel may be due to its strong antioxidant activity, as this plant contains flavonoids namely naringin, narirutin and neohesperidin, which are suggested to decrease the risk of cardiovascular diseases with their free radical scavenging properties. The mechanism involved may be due to the flavonoids that protect body against the damage done by free radicals by suppressing the free radicals formation; scavenging for loose free radicals around the body; and by reacting with the free radicals to produce an inactive compound. [13]

Endogenous enzymatic and non-enzymatic antioxidant levels

Catalase

Normal basal level of catalase activity in normal control rats was found to be 64.50±1.232 U/mg of protein. Cardiotoxicity rats showed significantly decreased (P<0.001) levels of catalase (44.0±1.29 U/mg of protein). Pretreatment of doses of CGO (250 mg/kg) was not significantly increased as compared to DOX alone treated group, whereas CGO (500 mg/kg) significantly (P<0.01) increased the levels of catalase to the near normal values (52.17±0.945) as shown in Table 6.

Superoxide Dimutase (SOD)

As shown in **table 6**, the SOD level in the normal, DOX alone, CGO (250 mg/kg) + DOX, and CGO (500 mg/kg)+ DOX (n=6) treated rats were 37.17 ± 0.945, 24.0±1.065, 26.50±0.562 and 27.33±0.714 (U/mg protein), respectively. The SOD level in the DOX treated rats were significantly (P<0.001) decreased compared with the normal rats. The SOD level in pretreated group of animals with CGO 250 mg/kg and 500 mg/kg was not significantly increased as compared to DOX alone treated group. The present study data indicates that ethanolic extract of *C. grandis* (L.) Osbeck peel protects against cyclophosphamide and doxorubicin induced cardiac toxicity in rats. Although the protection is seen with 250 mg/kg/day and 500 mg/kg/day doses, the best protective effect is indicated by 500 mg/kg/day dose. In CYP and DOX administration, we observe myocardial necrosis associated with decreased antioxidant defense status in the heart, histopathological changes and release on inflammatory markers. In addition, the present study provided experimental evidence that CGO maintained the antioxidant enzyme levels, improved cardiac performance and lessening histopathological changes following doses of CYP administrations. These findings might be a scientific support to understand the beneficial effects of CGO on cardioprotection against myocardial injury, in which oxidative stress has long been known to contribute to pathogenesis.

ACKNOWLEDGEMENTS

The authors are thankful to Principal, Mallige College of Pharmacy, Bangalore for providing excellent laboratories facilities and his valuable guidance during this work.

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Source of Support: Nil, Conflict of Interest: None declared.