ORIGINAL ARTICLE

Saraca asoca Bark-derived Procyanidins Mitigate Doxorubicin-induced Cardiotoxicity

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Abstract

Background: Doxorubicin (DOX) is a commonly utilized chemotherapeutic agent for treating hematological malignancies and solid tumors. However, its widespread use is hindered by the significant side effect of oxidative injury-related cardiotoxicity. **Objective:** This study explores the potential of procyanidins (PCNs) derived from *Saraca asoca* bark, known for its robust free radical scavenging properties, in preventing DOX-induced cardiotoxicity in rats. **Materials and Methods:** Rats were treated intraperitoneally with a cumulative dose of 15 mg/kg DOX, both with and without prior administration of PCNs. **Results:** The findings revealed that DOX caused cardiac dysfunction, myocardial injury, and increased oxidative stress in cardiac tissues. The cardiac impairment included increased QT-interval and ST-interval in electrocardiograph (ECG) and reduced left ventricular developed pressure. DOX-induced myocardial injury manifested as elevated levels of creatine kinase, alanine aminotransferase, and aspartate aminotransferase in serum, along with observable myocardial lesions. Pretreatment with PCNs at a dosage of 150 mg/kg daily effectively mitigated DOX's adverse effects, such as myocardial injury and impaired heart function. PCN pretreatment ameliorated cytoplasmic vacuolization, increased left ventricular developed pressure, and improved ECG parameters. The cardioprotective impact of PCNs correlated with reduced lipid peroxidation and enhanced cardiac antioxidant capacity in DOX-treated rats also administered PCN. In addition, an *in vitro* cytotoxic study demonstrated that PCNs did not compromise DOX's antineoplastic activity against A549 adenocarcinoma cells. **Conclusion:** Collectively, these findings indicate that PCNs shield cardiomyocytes from DOX-induced cardiotoxicity by suppressing oxidative stress.

Key words: Cardiotoxicity, doxorubicin, oxidative stress, procyanidin, *Saraca asoca* bark

INTRODUCTION

Doxorubicin (DOX), an expansive antitumor antibiotic, is commonly employed in the treatment of hematological malignancies and solid tumors. However, its clinical utility is impeded by dose-dependent severe cardiotoxicity.[1] Since the discovery of DOX-induced cardiomyopathy and congestive heart failure in the 1970s, extensive research has delved into understanding the mechanisms underlying anthracycline cardiotoxicity.[2] Studies have proposed various mechanisms, including the formation of free oxygen radicals, nitric oxide expression, myocardial mitochondrion damage, and

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molecular signaling alterations.[3] Among these hypotheses, free oxygen radicals derived from adriamycin semiquinones are widely acknowledged as major contributors to DOX-induced heart failure.[4] The heart's vulnerability to oxidative stress, attributed to its relatively low expression of antioxidant enzymes such as catalase and superoxide dismutase, intensifies the impact of DOX-induced cardiotoxicity.[5] To comprehend the involvement of free radicals in DOX cardiotoxicity, numerous antioxidant compounds have been investigated for their potential to alleviate histological changes in cardiac myocytes. Antioxidants such as ascorbic acid and Vitamin E have demonstrated protective effects against cardiac cell damage.[6,7] Dexrazoxane, an iron chelator with no interference in pharmacokinetics and pharmacodynamics, has gained approval as prophylaxis for DOX cardiotoxicity in cancer patients.[8] Dexrazoxane's potential hematological and hepatological toxicities have spurred further research to identify agents that can ameliorate DOX cardiotoxicity without compromising its antineoplastic activity. *Saraca asoca*, commonly known as "Ashoka," is a significant tropical medicinal plant predominantly found in the Western Ghats of India. Belonging to the *Fabaceae* family, it is a small evergreen tree. Traditional uses of *S. asoca* encompass the treatment of a variety of ailments including gynecological disorders, dyspepsia, blood disorders, biliousness, tumors, abdominal enlargement, colic, piles, ulcers, and bone fractures. More than 30 compounds have been identified in different parts of *S. asoca*, primarily comprising flavonoids, tannins, steroids, fatty acids, and glycosides. Both the flowers and bark of *S. asoca* are notable sources of gallic acid and ellagic acid. Extracts, fractions, and isolated compounds derived from various parts of *S. asoca* have exhibited a wide array of pharmacological activities.[9] Procyanidins (PCNs) derived from grape seeds, composed of catechin unit oligomers, exhibit antibacterial, anti-allergic, and antigenotoxic activities.[10] Recognized as efficient phytochemical antioxidants, PCNs have been employed to mitigate the incidence of cardiovascular disease and cancer.[11] Recent studies have highlighted the ability of PCNs to effectively scavenge 1,1-diphenyl-2picrylhydrazyl and superoxide in relevant radical-producing systems, both *in vitro* and in cell-free systems.[12] Moreover, an 8-week dietary treatment with a grape seed extract rich in PCNs significantly enhanced the ferric-reducing antioxidant power in plasma compared to the control group.[13] Proanthocyanidins in the bark of *S. asoca* Rob. de Wilde were reported earlier by Middelkoop and Labadie.[14] Given PCNs' capacity to neutralize reactive oxygen species, we hypothesized that they may offer potential protection against DOX-induced injury in cardiac tissue. This study aims to investigate the potential cardioprotective effects of PCNs using animal models. The assessment of the cardioprotective efficacy of PCNs involved the evaluation of biochemical, electrocardiographic, and histomorphological parameters that serve as indicators of oxidative stress and cardiotoxicity in rats subjected to pretreatment with DOX.[15] In addition, an *in vitro* examination employing A549 adenocarcinoma cells was conducted to investigate the potential impact of PCNs on the antitumor activity of DOX.

MATERIALS AND METHODS

Reference compounds of the highest grade (purity >99.0%), namely, catechin, gallic acid, PCN B2, (-)-epicatechin, (-) epigallocatechin gallate, (-)epicatechin gallate, and DOX were purchased from Sigma-Aldrich (Mumbai, India). Methanol, acetonitrile, and phosphoric acid (Merck, Mumbai, India) were of high-performance liquid chromatography (HPLC) grade. Milli Q grade water used throughout the experiment was prepared using a Millipore purification system (Millipore, Milli Q gradient A10). Stock solutions of different extracts were prepared by dissolving extract in water-methanol (1:1, 1.0 mg/mL) and filtered through a 0.45-µm membrane filter. Stock solution of catechin, gallic acid, PCN B2, (-)-epicatechin, (-)epigallocatechingallate, and (-)epicatechingallate were prepared in HPLC grade methanol (1.0 mg/mL, each). Working solutions of lower concentration were prepared by appropriate dilution of the stock solutions. Solutions of extract and standards were stored at 4 ± 1°C and were brought to room temperature just before use. 100 g of powdered bark of *S. asoca* were extracted in a Soxhlet apparatus successively with petroleum ether 40–60°C for 10 h, yielding 0.65 g extract, followed by ether for 20 h, yielding 0.34 g extract, and ethyl acetate for 24 h, yielding 3.4 g extract, methanol for 20 h, yielding 10.4 g extract. Ethyl acetate extract was chromatographed on a polyamide column using methanol as eluent. All fractions containing phenolics were pooled and are hereafter referred to PCN mixture. The chemical composition of the PCN mixture was investigated by HPLC.

Chromatographic separation was conducted utilizing a Shimadzu HPLC system comprising of quaternary pump, an in-line vacuum degasser, an auto sampler, a column heater, and a photodiode array detector (PDA). The injection volume was maintained at 1 µL. To optimize the mobile phase viscosity, the column oven temperature was set at 37°C. The separation of compounds was executed on an RP-18 column (50 × 2.1 mm internal diameter, 1.8 µm pore diameter, 100 Å pore size) employing gradient elution. The mobile phase consisted of a blend of aqueous phosphoric acid (0.1%, v/v) (Solvent A) and acetonitrile (Solvent B) at a flow rate of 0.75 mL/min. Gradient programming was established as follows: initial conditions of 5% B were maintained for 0 min, followed by a gradient to 20% B over 6 min, reaching 80% B at 7 min, and maintaining this composition for 2 min before returning to initial conditions at 9.10 min. The total run time was extended to 11 min to ensure complete elution of all components. Chromatographic peaks were monitored in the range of 200–350 nm using a PDA. For quantitative analysis of gallic acid, catechin, PCN B2, (-)-epicatechin, (-)-epigallocatechingallate, and (-)-epicatechingallate in *S. asoca* bark methanolic extract, a wavelength of 210 nm was selected. This wavelength provided the optimal baseline separation with maximum

absorbance compared to chromatograms recorded at 230 nm or 280 nm. Barks when extracted with methanol showed trace levels of gallic acid (0.45 min), 0.30 mg of catechin(2.45 min), 0.90 mg of PCN B2 (3.25 min), 1.25 mg of (–)-epicatechin (3.50 min), and 3.56 mg of (–)-epicatechin gallate (5.20 min) per g of dry bark, as shown in Figure 1.[16]

Animal model

Male Sprague–Dawley rats weighing 250–300 g was procured from the National Center for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad. The IAEC approved the animal experimental protocols. The rats were maintained under standard conditions, including a temperature range of 20–25°C, relative humidity between 50% and 60%, and a 12-h light: dark cycle. Throughout the experimental period, the rats were provided with a standard diet and water.

Experimental design

Conscious rats were randomly allocated into four groups. The control group (n = 6) received daily intragastrical irrigation of distilled water (4 mL/kg) for 24 days and intraperitoneal injections of 0.9% saline (10 mL/kg) on days 7, 14, and 21. The PCN group (n = 8) and the DOX group (n = 8) followed a similar protocol to the control group, with the substitution of distilled water for PCN (150 mg/kg daily) in the PCN group and saline for DOX (5 mg/kg) in the DOX group. The PCN + DOX group (n = 8) underwent the same regimen as the PCN group, alternating with DOX injections (5 mg/kg).[17]

Observations and measurements

Throughout the experimental period, the general appearances of the rats were monitored. Seventy-two hours after DOX injection, the rats were weighed and anesthetized with pentobarbital (60 mg/kg, i.p.) for electrocardiography (ECG) and hemodynamic assessment. Blood samples were collected at the time of euthanasia for creatine kinase, alanine aminotransferase, and aspartate aminotransferase analyses. The hearts were excised, weighed, and divided for biochemical and histopathological examinations.

ECG and hemodynamic assessments

For ECG and hemodynamic evaluations, all animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). A polyethylene cannula was inserted into the left ventricle through the left atrium and mitral valve for arterial pressure, while electrodes were inserted in the limbs for ECG.[18] Data were recorded for 3–5 min per animal using an Electro-Physiology System. The ST-interval and QT-interval were measured in five consecutive waves to assess rhythm

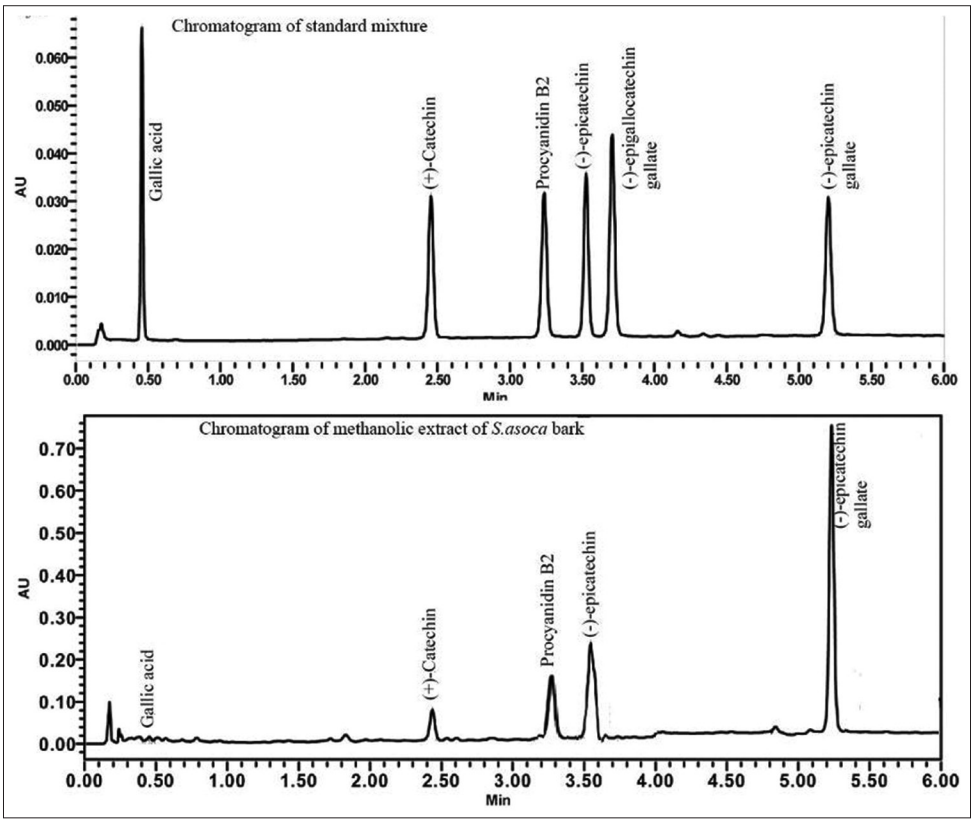

Figure 1: Representative chromatograms of a standard mixture of gallic acid, (+)-catechin, procyanidin B2, (-)-epicatechin, (-)-epigallocatechin gallate, and (-)-epicatechin gallate (upper panel); methanolic extract of *Saraca asoca* bark (lower panel)

disorders, and left ventricular developed pressure was measured for cardiac function evaluation.

Cardiac antioxidant activity determination

On washing cardiac tissue samples with cold saline, 1 g of the heart sample underwent homogenization in 10 mL of 20 mM Tris-HCl buffer at 4°C. Subsequently, the homogenates were centrifuged at 3000× g for 10 min. The concentrations of malondialdehyde (a lipid peroxidation marker) and the activities of superoxide dismutase (antioxidant enzymes) were determined using the malondialdehyde assay kit and superoxide dismutase assay kit, respectively, following the manufacturer's protocol. Specifically, superoxide dismutase activity was assessed through the xanthine oxidase-xanthine system, while malondialdehyde content was measured using the thiobarbituric acid method.[19,20]

Serum enzymatic assays

Blood samples collected at the time of sacrifice were utilized for evaluating serum levels of alanine aminotransferase, aspartate aminotransferase, and creatine kinase. An autoanalyzer (Hitachi Medico, Tokyo, Japan) was employed for these assessments.

Histopathological examinations

Cardiac tissues, fixed for 48 h in 10% formalin and embedded in paraffin after dehydration with ethanol, underwent 5-mm sectioning stained with hematoxylin and eosin. A pathologist, blinded to the treatments, examined the slides through light microscopy. The sections were graded on a scale of 0 (no change) to 3 (severe).[21]

Effect of PCN on antitumor activity of DOX *in vitro*

Human A 549 lung adenocarcinoma cells were plated in a 96-well plate with approximately 5000 cells per well, followed by incubation in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum in a humidified atmosphere with 5% CO2 at 37°C. After a 24-h incubation, cells were exposed to PCN solution (150 µg/mL) and/or DOX injection (1 µg/mL) for an additional 48-h incubation. Cytotoxicity was assessed using the crystal violet method, where cells were washed with saline, fixed with 11% glutaral, and stained with 0.1% crystal violet solution for 30 min. Absorbance quantification at 595 nm was performed after removing the crystal violet-containing medium and re-solubilizing the intracellular dye in 10% acetic acid.[22]

Statistical analysis

Results were expressed as mean ± standard error of the mean and subjected to analysis of variance followed by Duncan's Multiple Range test. One-tailed Wilcoxon rank-sum test was employed for the analysis of weight ratios and histological scores. $P < 0.05$ was considered statistically significant.

RESULTS

General appearance

The administration of PCN did not induce any significant alterations in the overall appearance of the rats. Conversely, following DOX treatment, the rats exhibited signs of illness and weakness, accompanied by a disheveled fur coat. Notably, ascites was observed in rats treated with DOX. In comparison to animals in the DOX group, the volume of ascites in rats receiving PCN + DOX was significantly lower ($P < 0.05$).

Physiological parameters

DOX injection led to a noteworthy reduction in heart weight, body weight, and the heart–body weight ratio ($P < 0.05$) in comparison to the control group [Table 1]. In the PCN + DOX group, both body weight and the heart–body weight ratio were significantly higher than in the DOX-only group ($P < 0.05$) and were comparable to those in the control group. Although pretreatment with PCN mitigated the DOX-induced decrease in heart weight, the heart weight in the PCN + DOX group remained lower than that in the control group, as shown in Figure 1.

Cardiac function

To assess DOX-induced cardiotoxicity, ST-interval and QT-interval durations were measured using ECG, as these parameters have been suggested to correlate with cardiac dysfunction in the previous studies (Dragojevic-Simic *et al*., 2004; Fisher *et al*., 2005). DOX administration significantly prolonged ST-interval and QT-interval ($P < 0.05$) compared to the control group [Table 2]. PCN treatment had no significant impact on ECG parameters in the PCN group. However, it effectively protected against DOX-induced prolongation of ST-interval and QT-interval in the DOX + PCN group rats ($P < 0.05$). Left ventricular developed pressures were similar between the control group and the PCN group. DOX injection alone induced a significant decline in the left ventricular developed pressure ($P < 0.05$), indicating severe cardiac dysfunction. In the PCN + DOX group, left ventricular developed pressure showed no significant difference compared to the control group.

Serum biochemistry

Serum levels of creatine kinase, aspartate aminotransferase, and alanine aminotransferase are established markers of myocardial damage in clinical practice.[23] In Table 3, levels of these enzymes were significantly higher ($P < 0.05$) in the

Table 1: Procyanidins from *Saraca asoca* bark significantly lower the doxorubicin-induced changes in ascites and improve the heart weight of rats (means±standard error of the mean; *n*=8 in each group)

Group	Ascites (mL)	Heart weight (g)	Body weight (g)	Heart weight/Body weight ratio (mg/g)
Control	0.0±0.0	1.25±0.05	375.0±8.2	3.33±0.05
PCN	0.0±0.0	1.24±0.04	377.2±10.2	3.29±0.06
DOX	22.4*±2.0	0.65*±0.04	245.5*±6.8	2.65*±0.06
PCN+DOX	11.5*†±2.3	0.96*†±0.05	311.6*±5.5	3.08*†±0.05

DOX: Doxorubicin, PCN: Procyanidins from *Saraca asoca* bark. *P<0.05 compared with the control group, †P<0.05 compared with the DOX group, and significantly different than control and PCN alone

Table 2: Procyanidins from *Saraca asoca* bark mitigate the doxorubicin-induced changes in electrocardiographic and hemodynamic parameters in rats (means±standard error of the mean; *n*=8 in each group)

Group	ECG		Hemodynamic	
	ST-interval (ms)	QT-interval (ms)	Heart rate (beats/min)	LVDP (mmHg)
Control	15.0±0.4	142.2±4.50	340.5±12.5	98.0±4.0
PCN	14.8±0.4	144.0±5.20	348.6±16.0	95.6±4.2
DOX	30.4*±0.6	188.5*±4.34	254.2*±10.8	63.6*±5.6
PCN+DOX	20.5*†±0.6	155.4*†±4.30	325.8†±12.4	82.3†±4.5

DOX: Doxorubicin, PCN: Procyanidins from *Saraca asoca* bark, LVDP: Left ventricular developed pressure. *P<0.05 compared with the control group, †P<0.05 compared with the DOX group, and significantly different than control and PCN alone

Table 3: Procyanidins from *Saraca asoca* bark protect against doxorubicin-induced changes in serum creatine kinase, aspartate aminotransferase, and alanine aminotransferase activities in rats (means±standard error of the mean; *n*=6 in each group)

Group	Creatine kinase nmol/(s.L)	Aspartate aminotransferase nmol/(s.L)	Alanine aminotransferase nmol/(s.L)
Control	2150±185	452±90	1540±231
PCN	1988±242	420±82	1428±173
DOX	5580*±293	1250*±108	3626*±248
PCN+DOX	3025†±283	480†±94	1886†±210

DOX: Doxorubicin, PCN: Procyanidins from *Saraca asoca* bark. *P<0.05 compared with the control group, †P<0.05 compared with the DOX group, and significantly different than control and PCN alone

DOX group compared to the control group. PCN pretreatment significantly attenuated the DOX-induced elevation of these enzymes. There was no significant difference in enzyme levels between the control and PCN groups.

Heart antioxidant activity

The concentrations of malondialdehyde (a marker of lipid peroxidation) and superoxide dismutase (an antioxidant enzyme) are examined [Table 4]. PCN administration alone did not significantly alter malondialdehyde levels or superoxide dismutase activity. In the DOX group, there was a significant increase in malondialdehyde concentration ($P < 0.05$) compared to the control group. PCN pretreatment significantly reduced malondialdehyde levels by approximately 60% in the PCN + DOX group compared to the DOX group ($P < 0.05$). Similarly, superoxide dismutase activity decreased significantly in the DOX group compared to the control group ($P < 0.05$), and this decrease was attenuated by PCN pretreatment.

Table 4: Effect of procyanidins from *Saraca asoca* bark alleviate doxorubicin-induced myocardial oxidative stress (means±standard error of the mean; *n*=6 in each group)

Group	Malondialdehyde (nmol/mg protein tissue)	Superoxide dismutase (U/mg protein)
Control	12.0±1.4	40.2±2.0
PCN	9.8±1.2	41.4±1.9
DOX	42.0*±2.4	25.0*± 2.1
PCN+DOX	14.2†±1.4	42.5 †±1.9

DOX: Doxorubicin, PCN: Procyanidins from *Saraca asoca* bark, *P<0.05 compared with the control group, †P<0.05 compared with the DOX group, and significantly different than control and PCN alone

Histopathological changes

The histological evaluation of rat hearts treated with DOX alone or in combination with PCN is summarized in Table 5. No pathological damage was observed in the cardiac slides from the control [Figure 2] and PCN groups. In contrast, DOX administration induced significant histopathological changes, including cytoplasmic vacuolization and myofibrillar disorganization in cardiac tissues [Figure 2 and Table 5]. Coadministration of DOX with PCN markedly attenuated the myocardial damage [Figure 2 and Table 5], with no remarkable loss of myofibrillar cells observed. However, occasional disorganization of cardiac myofibrils was noted in the PCN + DOX group.

Antitumor activity

Figure 3 illustrates the cytotoxicity of DOX, demonstrating that nearly 50% of A549 cells were killed at a concentration of 1 μg/mL. PCN (150 μg/mL) exhibited some level (≈20%) of cytotoxicity to adenocarcinoma cells. Importantly, PCN did not suppress the antitumor activity of DOX. The cells' survival rate in wells treated with PCN + DOX was similar to that with DOX alone, indicating that PCN did not compromise the efficacy of DOX in the tested adenocarcinoma cells.

DISCUSSION

The clinical utility of DOX is hindered by its associated adverse effects, notably cardiomyopathy and congestive heart failure. A substantial body of research underscores the significant correlation between DOX-induced cardiotoxicity and oxidative stress resulting from DOX disposition. In our current investigation, the administration of DOX at a cumulative dose of 15 mg/kg elicited cardiac damage exemplified by cytoplasmic vacuolization and myofibrillar disorganization. Profound cardiac dysfunction was evident through reduced left ventricular developed pressure and an increase in ST-interval and QT-interval. These findings align with prior studies reporting similar alterations in ECG and hemodynamics following DOX exposure.[23] Consistent with established patterns of serum biochemistry changes induced by DOX, our results affirmed that DOX treatment elevated creatine kinase, aspartate aminotransferase, and alanine aminotransferase levels, indicating myocardial damage. Furthermore, our study demonstrated a significant increase in malondialdehyde levels (a marker of lipid peroxidation and oxidative injury) and a decrease in superoxide dismutase content in cardiac tissues following DOX treatment. These observations align with findings from other animal models exposed to DOX, supporting the notion that free oxygen radicals play a pivotal role in DOX-induced cardiotoxicity.[24] Given the documented efficacy of antioxidants such as Vitamin E and N-acetylcysteine in mitigating DOX-induced cardiotoxicity through scavenging

Table 5: Improved myocardial histopathology in rats pretreated with procyanidins from *Saraca asoca* bark and challenged with doxorubicin (means±standard error of the mean; *n*=6 in each group)

Group	Scores	Evaluation
Control	0.0±0.0	No change
PCN	0.0±0.0	No change
DOX	2.1*±0.2	Severe
PCN+DOX	1.3*†±0.1	Mild

DOX: Doxorubicin, PCN: Procyanidins from *Saraca asoca* bark. *P<0.05 compared with the control group, †P<0.05 compared with the DOX group, and significantly different than control and PCN alone

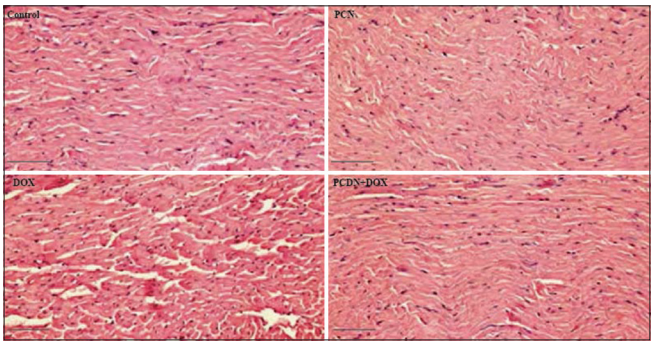

Figure 2: Typical photomicrographs of myocardial tissue from Control, procyanidins from *Saraca asoca* bark (PCN), doxorubicin (DOX), and procyanidins from *Saraca asoca* bark + doxorubicin (PCN+DOX) groups. Cytoplasmic vacuolation and myofibrillar disorganization in the doxorubicin group were effectively attenuated in the procyanidins + doxorubicin group (hematoxylin and eosin ×200, scale bar 50 μm)

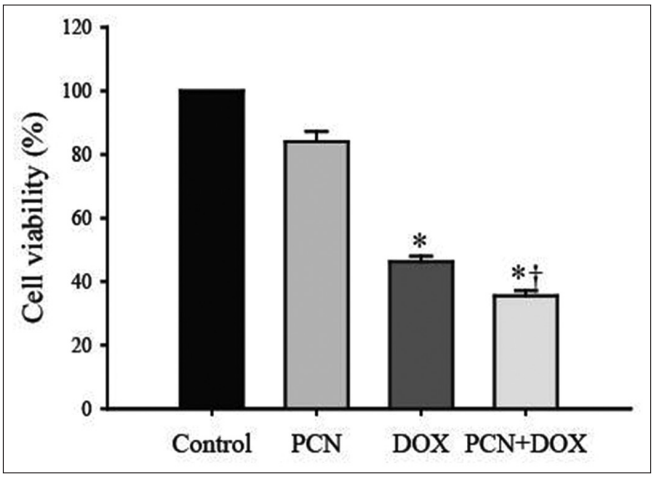

Figure 3: The effect of procyanidins (PCN) on the efficacy of doxorubicin (DOX) against human lung carcinoma cells (A549). The cells were treated with PCN at a concentration of 150 μg/mL and/or DOX at 1 μg/mL for a duration of 48 h. Mean values along with standard errors of the mean are presented, with a sample size of 6 in each group.*P<0.05 compared with the control group, †P<0.05 compared with the DOX group, and significantly different than control and PCN alone

free oxygen radicals, our study explored the chemopreventive potential of PCNs in DOX-treated rats.[25]

PCNs have garnered significant attention as effective antioxidants in the therapy and prevention of oxidative stress-induced cardiovascular diseases. An early epidemiological study demonstrated an inverse relationship between the oral administration of flavonoids, including PCNs and catechins, and mortality from coronary heart disease. Recent research has also shown that grape seed proanthocyanidins can attenuate isoproterenol-induced myocardial damage in rats by resisting free radical attacks, preventing iron-catalyzed oxidative reactions, and stabilizing mitochondrial and lysosomal enzymes.[26]

In our study, oral administration of PCN (150 mg/kg) alone did not elicit significant effects on the parameters studied in control rats. However, this same PCN treatment demonstrated a distinct protective effect against DOX-induced cardiotoxicity. This protection was evidenced by lower cytoplasmic vacuolization in cardiomyocytes and improved cardiac function in the PCN + DOX group compared to the DOX group. In addition, pretreatment with PCN significantly attenuated the elevation of serum creatine kinase, aspartate aminotransferase, and alanine aminotransferase induced by DOX exposure. The protective effect was further supported by an increase in myocardial antioxidant potency in the PCN + DOX group.

A desirable cardioprotective agent for DOX chemotherapy must not compromise DOX's antitumor activity. Our *in vitro* cytotoxic study demonstrated that PCN did not diminish the antitumor activity of DOX in A549 cells. Interestingly, PCN treatment exhibited cytotoxicity to the carcinoma cells, suggesting potential antitumor activity. Some studies have implicated PCNs in cancer prevention and therapy by down-regulating growth factor receptor signaling in prostate cancer DU145 cells.[27] PCNs have also shown cytotoxicity to various tumor cells, including MCF-7 breast cancer, A-427 lung cancer, and K562 leukemia cells, without affecting normal cells.[28] The potential cytotoxicity of PCNs to tumor cells makes it a promising candidate for investigation as an adjunct in DOX chemotherapy.

The robust antioxidant capability of PCNs is attributed to their specific structure, particularly the catechol structure. This structure enables PCNs to bind with free oxygen radicals and chelate metals, such as copper and iron, involved in reactive oxygen species generation.[29] Apart from antioxidant properties, PCN has been shown to modulate the activity of antioxidant enzymes, such as cyclooxygenase and lipoxygenase, to limit free radical production.[30] The monomeric units of PCNs, catechins, have been reported to protect vascular endothelial cells by inhibiting endothelial NADPH oxidase activity.[31] In addition, PCNs stimulate various forms of cytochrome P450 and gene expression of CYP1A, involved in chelating transition metals associated with oxygen metabolism. Recent hypotheses propose that PCNs may enhance cellular redox status by modulating the glutathione synthesis pathway against oxidative stress.[32]

Despite the observed changes in antioxidant enzymes induced by DOX and/or PCNs, the precise molecular mechanism remains unclear, warranting further investigation. In conclusion, our study represents the inaugural demonstration of the distinct protective effects of PCNs derived from grape seeds against DOX-induced myocardial damage and cardiac dysfunction in rats. Notably, this protective action is attributed to the scavenging of free radicals. Importantly, our findings reveal that PCNs do not compromise the antitumor activity of DOX. This dual action, characterized by cardioprotection without hindering DOX's antitumor efficacy, underscores the potential of PCNs as a valuable adjunct in DOX chemotherapy.[33]

CONCLUSION

These results emphasize the promising role of PCNs as a cardioprotective agent, deserving further scrutiny and evaluation as a potential supplement in DOX-based chemotherapy regimens. This dual functionality, preserving cardiac function while not impeding DOX's antitumor activity, positions PCNs as a potential candidate for enhancing the safety profile of DOX treatment.

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