Cardioprotective Effects of Peonidin against Doxorubicin-induced Cardiotoxicity in Wistar Rats: Electrophysiological, Biochemical and Histopathological Evaluation

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Abstract

Doxorubicin (DOX) is a widely used chemotherapeutic agent known for its potent anticancer effects; however, its clinical utility is limited due to severe cardiotoxicity. The present study investigates the cardioprotective potential of peonidin against DOX-induced myocardial injury in Wistar rats. Wistar rats (155-180 g) were divided into four groups such as normal control with no treatment, DOX treatment, DOX with 200 mg/kg of peonidin and DOX with 400 mg/kg of peonidin administered subcutaneously (15 mg/kg) on the 8th and 9th days to induce cardiotoxicity.

Peonidin was orally administered for seven days before DOX treatment. Blood samples were collected for biochemical analysis and histopathological and electrophysiological assessments were performed to evaluate myocardial damage and inflammation. DOX administration results in significant cardiac dysfunction, as evidenced by prolonged ST interval, decreased R wave amplitude, increased left ventricular end-diastolic pressure (LVEDP) and decreased left ventricular systolic pressure (LVSP).

Elevated levels of cardiac biomarkers (LDH, CK and troponin) and inflammatory mediators (IL-6 and TNF-α) further confirm myocardial injury. Peonidin pre-treatment demonstrates a dose-dependent cardioprotective effect, with the 400 mg/kg dose showing significant improvements in ST interval, R wave amplitude, LVSP and ± dp/dtmax. Biochemical analysis reveals a substantial reduction in LDH, CK and troponin levels in the 400 mg/kg group.

Histopathological examination further confirms reduced necrosis and preserved myocardial integrity in peonidin-treated rats. It was concluded that peonidin exhibits potent cardioprotective effects against DOX-induced myocardial injury through antioxidative, anti-inflammatory and membrane-stabilizing mechanisms.

Keywords: Peonidin, Doxorubicin, cardioprotective activity, *in vivo* study, histopathological study.

Introduction

Doxorubicin is a commonly administered anthracycline chemotherapeutic drug used to treat multiple cancers, among them breast cancer, leukemia and sarcomas. Clinical application of this drug is profoundly restricted by the fact that it can cause substantial cardiac damage that may result in long-term cardiovascular complications like cardiomyopathy and heart failure[10]. The degree of cardiac injury correlates with the dose and with an increased cumulative dose, the risk of dysfunction of the heart is enhanced. Clinically, doxorubicin-induced cardiac damage can be acute or chronic cardiotoxicity. Acute toxicity happens within hours to weeks following its administration and manifests as arrhythmias, electrocardiogram (ECG) abnormalities and transient left ventricular dysfunction[6].

Chronic cardiotoxicity, which is more prevalent, arises months to years following treatment and results in insidious heart failure, which is determined by decreased ejection fraction and compromised systolic function. Because of its irreversible character, avoiding doxorubicin-induced cardiac damage is one of the greatest challenges in oncology. Various approaches like dose restriction, cardioprotective drugs (e.g. dexrazoxane) and antioxidant treatment are being studied to limit its effect. Natural products possessing potent antioxidant and anti-inflammatory activities including flavonoids and anthocyanidins, are also under investigation for their capacity to prevent doxorubicin-induced cardiotoxicity[8].

Peonidin is a natural anthocyanidin compound and is a type of flavonoid that contributes to red, purple and blue colors of different plant food and flower items. It is a water-soluble compound and is naturally found mostly in glycosylated form as peonidin-3-glucoside. Its color expression and stability, like those of other anthocyanidins, are pH, metal ion complexation and co-pigmentation effect, sensitive to other polyphenols. Peonidin has garnered major scientific attention as it exhibits antioxidant, anti-inflammatory and putative therapeutic effects[5]. Peonidin has a number of biological activities responsible for its possible health benefits. It is a strong antioxidant that traps free radicals, lowering oxidative stress and protecting cells against damage to lipids, proteins and DNA.

It has strong anti-inflammatory activity through down-regulation of inflammatory cytokines like tumour necrosis

factor-alpha (TNF-α), interleukin-6 (IL-6) and NF-κB and is thus useful in the case of arthritis, cardiovascular diseases and metabolic syndromes[4]. Peonidin also exhibits anti-cancer activity in that it inhibits the proliferation of cancer cells and induces apoptosis in models of colon, breast and skin cancer.

It possesses neuroprotective activity, protecting neurons from oxidative damage and possibly lowering the risk of neurodegenerative diseases such as Alzheimer's and Parkinson's. Peonidin exhibits significant cardioprotective activity by reducing oxidative stress, improving endothelial function and lowering the risk of atherosclerosis. Its potent antioxidant properties help to neutralize ROS and prevent lipid peroxidation and oxidative damage to cardiovascular tissues.

The anthocyanidin compounds are widely recognized as cardioprotective compounds based on their potent antioxidant and anti-inflammatory properties. Anthocyanidins prevent oxidative stress through scavenging of ROS and promotion of endogenous antioxidant defences, thus safeguarding cardiomyocytes from damage[9]. Anthocyanidins also enhance endothelial function, modulate nitric oxide (NO) generation and inhibit lipid peroxidation, which together help to ensure cardiovascular health. They also regulate major signaling pathways participating in apoptosis and mitochondrial function, minimizing the possibility of doxorubicin-induced cardiotoxicity[7].

Based on these protective effects, our research aims to assess the *in vivo* cardioprotective activity of peonidin, an anthocyanidin compound, to ascertain its ability to prevent doxorubicin-induced cardiac injury. This study is intended to give insights into the function of peonidin in lowering oxidative stress, enhancing cardiac function and inhibiting structural damage to the heart, thus validating its therapeutic value in cardiotoxicity treatment.

Material and Methods

Animals: In the present research, Wistar rats of 155-180 grams were employed for experimental purposes. All experimental and animal handling protocols were followed as per national guidelines for the ethical use of laboratory animals. Institutional Animal Ethics Committee (IAEC) approval was taken before conducting the study under approval number ANUCPS/IAEC/AH/P/11/2024, dated 23-07-2024. The rats were obtained from Mahaveer Enterprises, Hyderabad and were kept in a controlled laboratory setting with a natural day-night cycle to provide maximum living conditions during the study.

Experimental procedure: The *in vivo* cardioprotective effects of peonidin against doxorubicin induced cardiac damage was performed as the protocol reported in literature[1]. The rats kept under acclimatization for one week and then they were randomly allocated into four experimental groups with six rats in each group:

Normal Control (Group I): The rats in this group were fed a regular diet without any treatment for 7 days.
Doxorubicin Control (Group II): Rats of this group received doxorubicin at the dose of 15 mg/kg body weight subcutaneously in the form of physiological saline. The injection was administered on the 8th and 9th days with a 24-hour interval to cause myocardial infarction.
Doxorubicin + Peonidin 200 mg/kg (Group III): The rats in this group were pre-treated with peonidin at a dose of 200 mg/kg body weight daily for 7 days and then injected with doxorubicin at the same dose on the 8th and 9th days.
Doxorubicin + Peonidin 400 mg/kg (Group IV): Rats in group IV were pre-treated with peonidin at 400 mg/kg body weight orally once a day for 7 consecutive days prior to doxorubicin injections according to the same protocol as group III.

All the animals had blood collected at the end of the study via retro-orbital puncture under mild anaesthesia. The blood that was collected was centrifuged to isolate the serum and it was used for the estimation of lipid profile and liver function tests. The rats were subsequently sacrificed after collecting the blood and the hearts were isolated and washed in ice-cold saline and homogenized in Tris buffer. The homogenate was further centrifuged and the supernatant was harvested to assess the cardiac biomarkers for the evaluation of myocardial damage.

Electrophysiological assessments: The electrophysiological assessments were conducted to evaluate the impact of doxorubicin-induced cardiotoxicity and the protective effects of peonidin on cardiac function[11]. The procedure involves inserting a needle electrode subcutaneously between the paw pads of anesthetized rats to continuously record the electrocardiogram (ECG).

Biochemical Analysis: The supernatant fraction derived from homogenized cardiac tissue was utilized for the measurement of cardiac biomarkers and inflammatory mediators to assess myocardial injury and inflammation[2]. The cardiac biomarkers measured were lactate dehydrogenase (LDH), creatine kinase (CK) and troponin which are critical markers of cardiac muscle injury. Inflammatory mediators like IL-6 and TNF-α were also quantified to assess the level of inflammation within the cardiac tissue. The concentrations of LDH, CK and troponin were quantified by an automated biochemical analyzer for accurate and exact determination. On the other hand, IL-6 and TNF-α concentrations were analysed using an Elisa kit as per the manufacturer's instructions for best results.

Histological Assessment: Myocardial tissue was obtained from the left ventricle of all animals for histopathological examination and was stored in 10% formal saline[3]. The samples were prepared, cut into 3 µm thick sections and stained with haematoxylin and eosin. These slides were viewed under the light microscope to evaluate necrotic changes, which were scored by a predetermined scoring

system. The score was as follows: 0 for no necrotic cells, 1 for 1 to 10 necrotic cells, 2 for 10 to 50 necrotic cells, 3 for 50 to 100 necrotic cells and 4 for greater than 100 necrotic cells. Necrotic myocytes were defined by nuclear alterations such as pyknosis, karyorrhexis, or karyolysis and a hypereosinophilic cytoplasm with loss of striations. The density of necrotic myocytes was assessed independently in peripheral and subendocardial areas of the myocardium.

Statistical analysis: Statistical analysis was carried out by expressing the data as mean ± standard deviation (SD). To analyze differences between treatment groups, One-way Analysis of Variance (ANOVA) was carried out followed by Dunnett's multiple comparisons test and results were taken into consideration as statistically significant when $p < 0.05$.

Results and Discussion

Electrophysiology study: The protective effect of peonidin against DOX induced cardiotoxicity was evaluated by performing electrophysiology study of animal's groups in the study. DOX is known to cause cardiotoxicity, which leads to arrhythmias and conduction abnormalities, while peonidin is being studied for its potential protective effects. In electrophysiology study, the parameters such as heart rate, QT interval and conduction patterns were analysed and results help to determine whether peonidin can mitigate DOX-induced cardiac dysfunction.

In the DOX control group, administration of doxorubicin (15 mg/kg, subcutaneously on the 8th and 9th days) induces significant cardiac dysfunction and was confirmed by observing prolonged ST interval and a decreased R wave amplitude. Additionally, doxorubicin treatment led to an increase in left ventricular end-diastolic pressure (LVEDP), signifying the impaired diastolic function, along with a reduction in left ventricular systolic pressure (LVSP) and the maximum rates of pressure change (± dp/dtmax) that reflects weakened ventricular contraction and relaxation. However, pre-treatment with peonidin at 200 mg/kg and 400 mg/kg for seven days before doxorubicin administration effectively mitigated these alterations.

Rats receiving peonidin exhibited improvements in ST interval and R wave amplitude, along with a partial restoration of LVSP and ± dp/dtmax, particularly at the higher dose of 400 mg/kg. These findings suggest that peonidin confers cardio-protective effects by preserving electrical and hemodynamic parameters, thereby reducing doxorubicin-induced cardiac dysfunction. Figure 1 displays ECG recordings from four experimental groups that clearly illustrate the impact of doxorubicin-induced cardiotoxicity and the protective effects of peonidin.

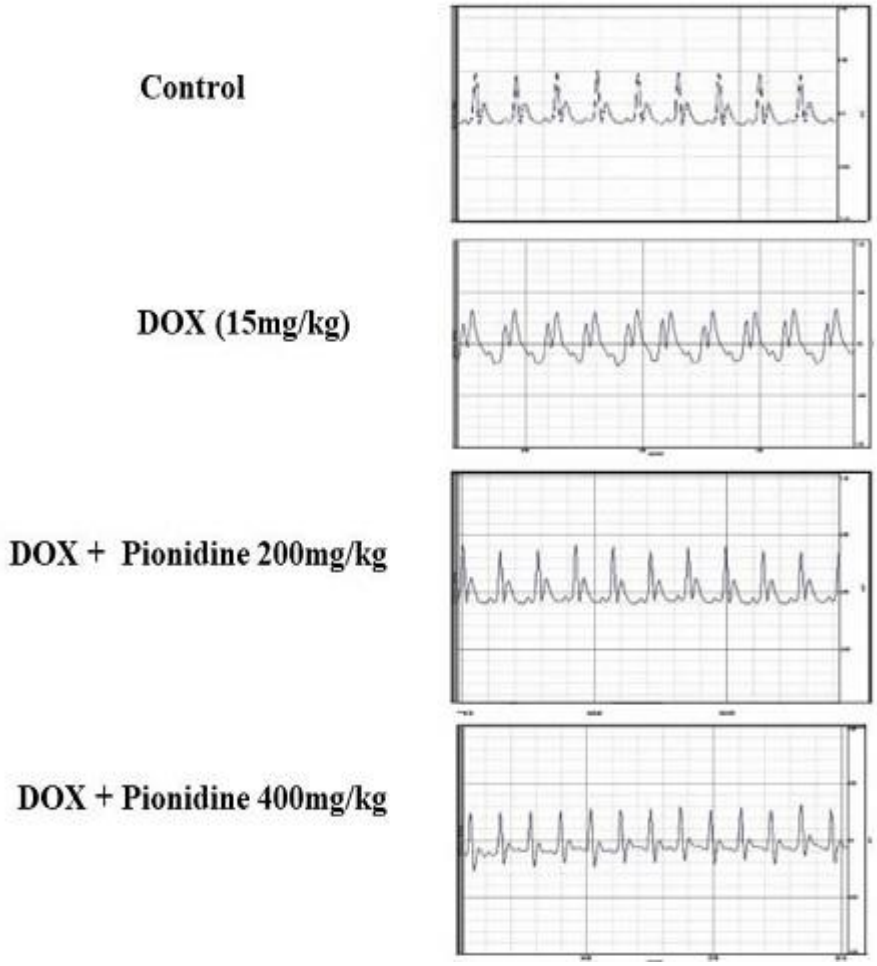
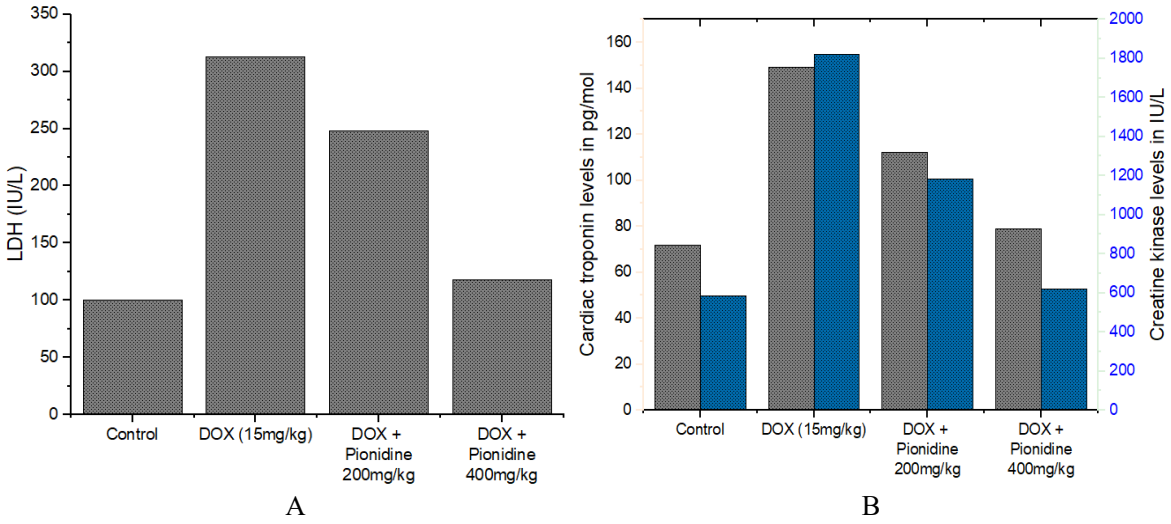


Figure 1: The ECG recordings from four experimental groups in the study

Effect of Peonidin on Heart biomarkers: The impact of peonidin on cardiac biomarkers was assessed by determining LDH, cardiac troponin and CK levels in various experimental groups. The experimental results were summarized in figure 2. In the control group, biomarker levels were within the normal level, with LDH level of 100.43 ± 0.82 IU/L, cardiac troponin of 71.81 ± 1.63 pg/mol and CK level of 585.58 ± 0.15 IU/L. The treatment with DOX at 15 mg/kg markedly increased these markers and cause serious cardiac injury. In DOX treated animal group, LDH level was noticed to be 312.69 ± 0.74 IU/L, cardiac troponin was observed as 149.32 ± 0.47 pg/mol and CK level as 1820.66 ± 0.78 IU/L. The co-administration of peonidin at 200 mg/kg elicited a significant decline in levels of biomarkers with evidence of partial cardio-protection where LDH came down to 248.19 ± 0.68 IU/L, cardiac troponin to 112.28 ± 0.47 pg/mol and CK to 1182.81 ± 0.24 IU/L.

A greater protective effect was noted at a higher dose of peonidin (400 mg/kg), where LDH, cardiac troponin and CK levels were 118.33 ± 0.74 IU/L, 78.90 ± 0.39 pg/mol and 620.93 ± 0.40 IU/L respectively. These findings suggest that peonidin has a dose-dependent cardioprotective effect by significantly inhibiting DOX-induced myocardial damage, possibly through mechanisms involving the reduction of oxidative stress and membrane stabilization.

Effect of Peonidin on inflammatory mediators: The impact of peonidin on inflammatory mediators was assessed through the measurement of TNF-α and IL-6 levels, which are central markers of inflammation and cardiac injury. TNF-α and IL-6 levels in the control group were 52.29±0.45 pg/mg of protein and 68.21±1.45 pg/mol respectively, reflecting normal inflammatory status. Nevertheless, treatment with doxorubicin (DOX) at 15 mg/kg substantially elevated these inflammatory markers, with TNF-α reaching 168.44±0.35 pg/mg of protein and IL-6 reaching 187.36±0.45 pg/mol, indicating extreme DOX-induced inflammatory response and cardiac stress.



A B

A) graph showsing LDH levels; B) dual Y-axis graph showing Cardiac troponin levels and creatine kinase levels
Figure 2: Effect of Peonidin on Heart biomarkers of DOX induced cardiotoxicity

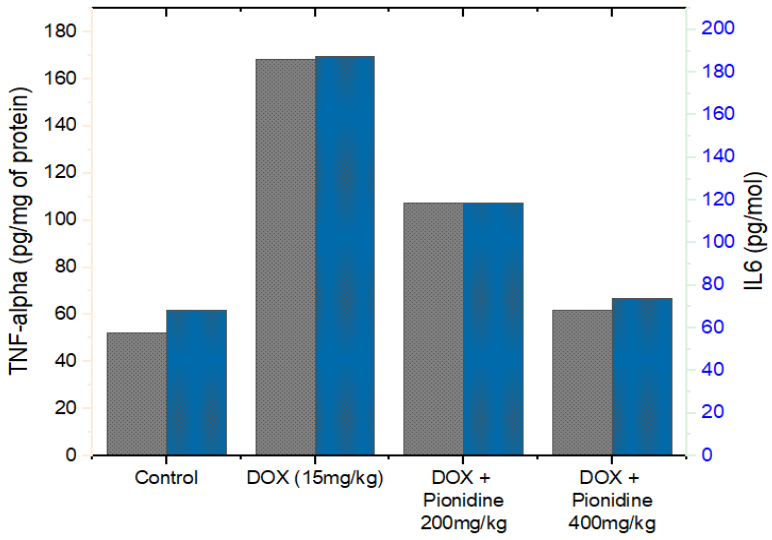


Figure 3: Dual Y-axis graph showing the effect of Peonidin on inflammatory mediators such as TNF-α and IL-6 levels

Co-treatment with peonidin at 200 mg/kg resulted in a substantial decrease in TNF-α (107.57±0.96 pg/mg of protein) and IL-6 (118.52±0.21 pg/mol), reflecting a moderate anti-inflammatory effect. A greater reduction was noted at a higher dose of peonidin (400 mg/kg), with TNF-α and IL-6 values of 61.80±0.74 pg/mg of protein and 74.01±2.35 pg/mol respectively, reaching near-control levels. These data as visualized in figure 3 indicate that peonidin has a dose-dependent anti-inflammatory action which can prevent DOX-induced cardiotoxicity by inhibiting the release of pro-inflammatory cytokines and myocardial inflammation.

Effect of Peonidin on liver profile: The effect of peonidin on liver function was evaluated through the quantification of SGOT, SGPT and alkaline phosphatase ALP, which are important indicators of hepatic viability and damage. At control levels, SGOT, SGPT and ALP were 24.28±0.45 IU/L, 23.91±0.69 IU/L and 6.18±0.73 KA/100 mL respectively, reflecting normal liver function. But doxorubicin (DOX) administration at 15 mg/kg led to a noteworthy rise of these enzymes, with SGOT rising to 76.63±0.32 IU/L, SGPT to 82.55±1.58 IU/L and ALP to 11.22±0.91 KA/100 mL, which indicates hepatic toxicity and DOX-induced liver damage. Co-treatment with peonidin at 200 mg/kg reduced the enzyme levels, SGOT to 41.85±0.14 IU/L, SGPT to 64.03±0.42 IU/L and ALP to 7.09±0.86 KA/100 mL, suggesting a moderate hepatoprotective activity. More severe protection was evidenced at 400 mg/kg of peonidin, when levels of SGOT, SGPT and ALP were 26.01±0.75 IU/L, 24.15±0.37 IU/L and 4.44±0.14 KA/100 mL respectively, reaching almost control values. These findings visualized in figure 4 suggest that peonidin has a dose-dependent hepatoprotective effect, presumably through its antioxidant and anti-inflammatory activities, which ameliorate DOX-induced liver injury and preserve hepatic function.

Effect of Peonidin on lipid profile: The impact of peonidin on lipid metabolism was evaluated by comparing the concentrations of LDL, TC and HDL, which are vital markers of cardiovascular health and lipid homeostasis (Figure 5). In the control group, LDL, total cholesterol and HDL were 19.52±0.12 mg/dL, 48.11±0.25 mg/dL and 44.63±0.56 mg/dL respectively, indicating a normal lipid profile.

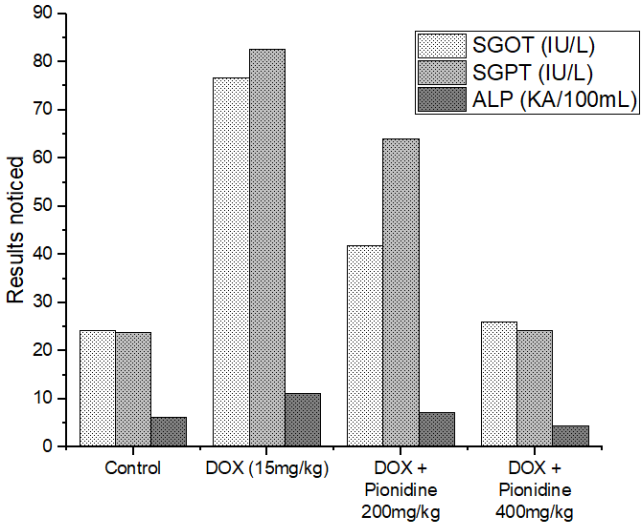


Figure 4: Effect of Peonidin on liver profile including SGOT, SGPT and ALP

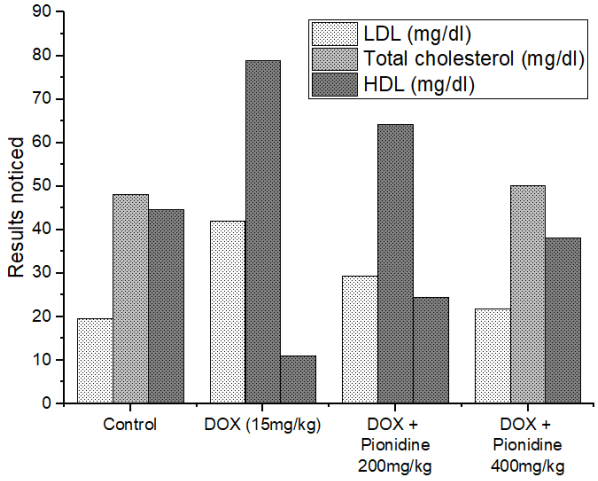


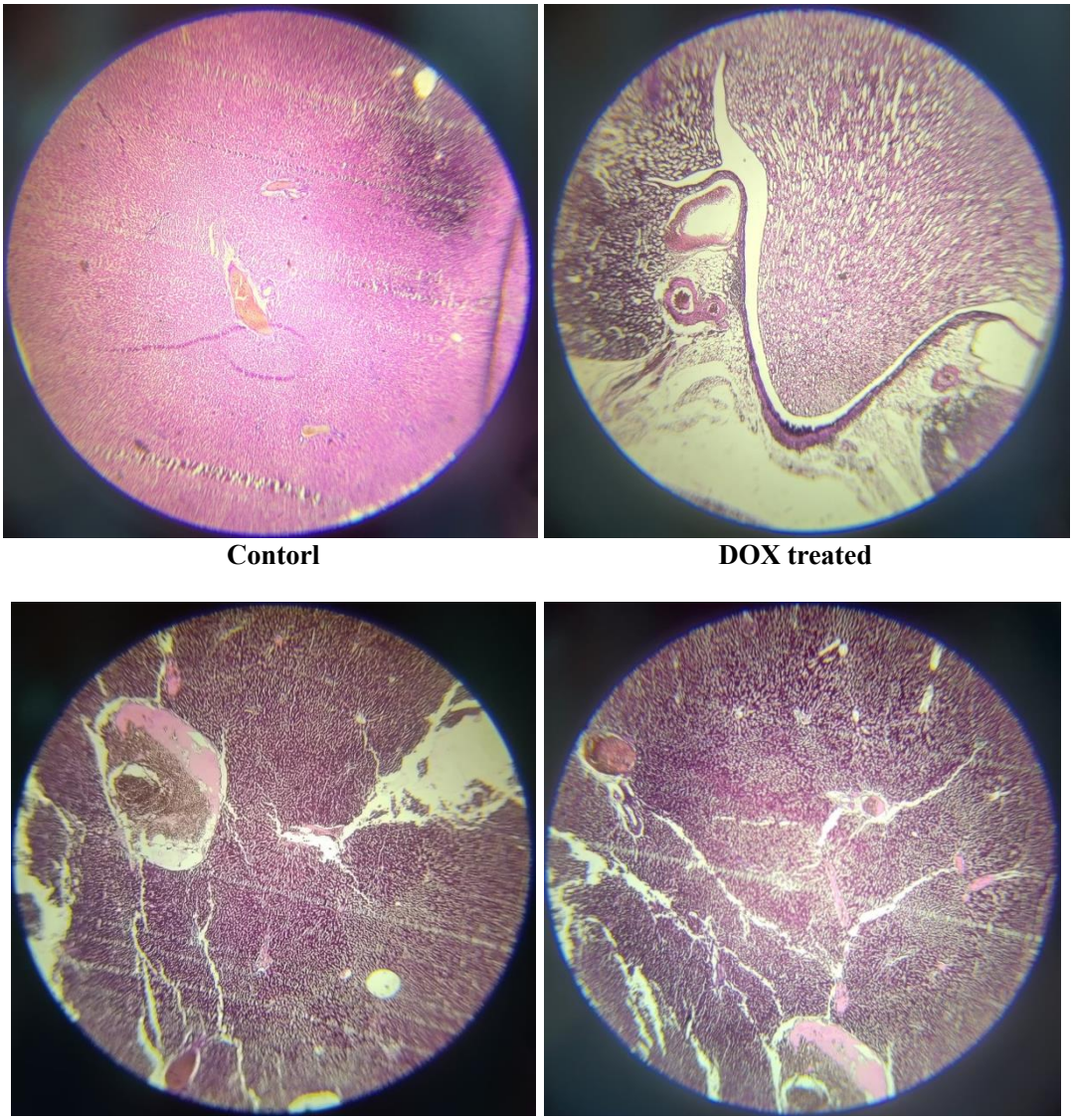
Figure 5: Effect of Peonidin on lipid profile such as LDL, total cholesterol and HDL

Administration of DOX at 15 mg/kg resulted in a severe impairment of lipid metabolism, resulting in the elevation of LDL (41.91±0.28 mg/dL) and total cholesterol (78.89±0.89 mg/dL) and the reduction of HDL to 11.07±0.69 mg/dL, suggesting DOX-induced dyslipidemia and enhanced risk of cardiovascular complications.

Co-treatment with peonidin at 200 mg/kg produces partial restoration of these values with LDL returning to 29.40±0.78 mg/dL, total cholesterol to 64.13±0.45 mg/dL and HDL improving to 24.52±0.47 mg/dL, which indicates moderate lipid-regulating action. Higher restoration was at the dose of 400 mg/kg of peonidin when levels of LDL, total cholesterol and HDL were at 21.79±0.03 mg/dL, 50.05±0.89 mg/dL and 38.14±0.16 mg/dL respectively, tending toward near-control. These results indicate that peonidin has a dose-dependent protective action against DOX-induced lipid disturbances, possibly through the modulation of lipid metabolism and the attenuation of oxidative stress, thus contributing to better cardiovascular health.

Histopathology: The histopathological analysis (Figure 6) of myocardial tissue gave clues about the cardioprotective effects of peonidin against DOX-induced cardiac injury. In the control group, cardiac tissue showed normal histoarchitecture with well-organized myofibrils, intact nuclei and no evidence of necrosis or inflammatory infiltration. But in the DOX-treated group (15 mg/kg), extensive myocardial degeneration, cytoplasmic vacuolization, loss of striations, nuclear pyknosis and widespread necrotic changes were seen.

Further, prominent inflammatory cell infiltration and interstitial edema were noted, which signifies DOX-induced cardiotoxicity. Co-treatment with peonidin at 200 mg/kg showed partial mitigation of myocardial injury, with moderate cardiac architecture preservation, reduced necrotic myocytes and attenuation of inflammatory cell infiltration. Nevertheless, mild interstitial edema and vacuolization remained evident.



Contorl **DOX treated**

DOX + 200 mg/kg of Peonidin **DOX + 400 mg/kg of Peonidin**

Figure 6: The histopathological analysis of myocardial tissue showing cardioprotective effects of peonidin against DOX-induced cardiac injury

Of interest, in a higher dose of peonidin (400 mg/kg), there was marked histological improvement in cardiac tissue with well-maintained myofibrillar structure, little necrotic change, decreased inflammatory infiltration and restored cellular integrity similar to the control group. The results indicate that peonidin has dose-dependent cardioprotective effects probably due to its antioxidant and anti-inflammatory activities, reducing DOX-induced myocardial injury and maintaining the integrity of cardiac tissue.

Conclusion

The investigation sought to determine the cardioprotective effects of peonidin against DOX-induced cardiotoxicity through the assessment of electrophysiological parameters, cardiac biomarkers, inflammatory mediators, liver function, lipid profile and histopathological alterations. The electrophysiological examination showed DOX treatment to cause severe cardiac dysfunction manifested by prolonged ST intervals, reduced R-wave amplitude, elevated LVEDP and LVSP and impaired ventricular contraction and relaxation. However, peonidin pre-treatment, especially at 400 mg/kg, caused significant improvement in these parameters, pointing to its potential for maintaining cardiac electrical and hemodynamic function. The measurement of cardiac biomarkers confirmed that DOX induced a sudden increase in levels of LDH, cardiac troponin and CK, which were indicative of major myocardial damage.

Co-treatment with peonidin had a dose-dependent reduction in such biomarker levels, validating its cardioprotective action via mechanisms most probably involving the lowering of oxidative stress and membrane stabilization. In addition, DOX treatment led to dramatic upregulation of inflammatory mediators TNF-α and IL-6, which are involved in myocardial injury and systemic inflammation. Peonidin co-treatment successfully blunted these inflammatory mediators and the 400 mg/kg dose attained levels close to control, highlighting its powerful anti-inflammatory property. The research also assessed the impact of peonidin on liver function because DOX-induced liver toxicity is a critical issue.

Increased levels of SGOT, SGPT and ALP in DOX-exposed animals reflected liver damage, while co-administration of peonidin substantially decreased these liver enzyme levels, proving its hepatoprotective potential. The histopathological examination also supported the cardioprotective role of peonidin. DOX exposure caused extensive myocardial degeneration, necrosis, inflammatory cell infiltration and interstitial edema, treatment with peonidin largely maintained cardiac tissue integrity, diminishing inflammation and structural damage. The protective action was strongest at the higher dose of 400 mg/kg, which was very close to normal cardiac histology.

In conclusion, the findings of the present study reveal that peonidin possesses remarkable cardioprotective and lipid-regulating activities against DOX-induced toxicity in a dose-dependent manner. Future studies directed towards its molecular targets and clinical utility will be necessary to develop peonidin as a promising adjunct therapy to prevent DOX-induced cardiotoxicity.

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