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Cardioprotective study of *Parmelia perlata* extract in albino Wistar rats

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Abstract: **Background**: Indigenous lichens have been used in the treatment of cardiovascular ailments for decades in humans. **Objectives**: To confirm their therapeutic value, current study aims to explore the prophylactic as well as cardioprotective effects of *Parmelia perlata*, a lichen used traditionally. **Methods**: The study was conducted by treating wistar albino rats with ethanolic extract (70/30) solubilized in 10% DMSO of *Parmelia perlata* (Pp.Cr), intoxicated with doxorubicin (DOX) (25mg/kg; i.p.) at 14th day and evaluated for blood parameters like lactate dehydrogenase (LDH), creatinine kinase myocardial band (CK-MB), aspartate transaminase (AST) and alanine transaminase (ALT). Hearts were excised; heart and body weight ratios were found and hearts were assessed for histopathology. **Results**: Treatment of animals with Pp.Cr cause significant decrease in serum markers (CK-MB, LDH, AST, ALT) (p-value<0.05) with insignificant effect on heart to bodyweight ratio. Histological results also showed marked reduction in the cardiac inflammatory lesions when compared with the intoxicated group. **Conclusion**: Thus this study concludes that crude extract of *Parmelia perlata* (Pp.Cr) can be claimed as cardioprotective.

Keywords: *Parmelia perlata;* Cardioprotective; Doxorubicin; CK-MB; Albino Wister Rats

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INTRODUCTION

Myocardial cell death is generally associated with myocyte injury that pathologically separates myocardial infarction (MI) from other forms of myocardial dysfunctions. MI has taken attention of researchers in recent years. According to World Health Organization survey, cardiovascular ailments are one of the most jeopardized diseases and will claim 23.6 million lives by 2030 (Gill, 1991). Diagnosis of MI is made on the basis of physical assessment , ECG; i.e. prevalence of arrhythmic attacks-wave flattening and QT prolongation interval, low voltage serial echocardiography with stress, positron emission tomography, biopsy of endomyocardium, measurement of humoral factors like ANP, BNP, endothelin, troponin T and various other biomarkers such as CK-MB, LDH, AST, ALT, ALP etc (Takemura and Fujiwara,2007). Modifications in myocardial damage, inflammation, apoptosis, and oxidative stress indicators such as troponin, TNF-α, caspase-3, SOD, catalase, and glutathione are indicative of doxorubicin-induced cardiotoxicity (Radeva and Yoncheva, 2025). Experimental studies have demonstrated that doxorubicin administration significantly alters cardiac injury, inflammatory, apoptotic, and oxidative stress biomarkers in myocardial tissues (Al-Salam *et al*., 2022). These changes coincide with enhanced inflammatory and apoptotic signalling pathways during chemotherapy-induced cardiac injury, as well as disruption of antioxidant defence mechanisms (Li *et al*., 2021). As a result, tracking oxidative stress and biochemical markers is frequently employed to assess the severity and course of doxorubicin-induced cardiomyopathy in experimental mice (Kim *et al*., 2025). Among the treatments of this disease, phytomedicine is always a matter of concern due to its cardiotonic action as they act as antagonists of platelet activating factor, free-radical scavengers, improve functioning of the blood vessels and cerebrovascular circulation (Briskin, 2000). Ibn sina; author of *Kitab al-Adwiyah al-Qalbiyah* has written one of the remarkable reference books for the use of cardioprotective plants which comprehended almost all aspects of cardiac diseases Comprising Pharmaceuticals and their biological actions covering over 63 cardiac remedies. Drugs such as Abresham (*Bombyx mori*), Darunaj aqrabi (*Doronicum hookeri*), Badranjboya (*Melissa officinalis*) are cited in the book have been found efficacious in the cure of heart diseases (Briskin, 2000). *Parmelia perlata* (A lichen belonging to family Parmeliaceae), have been used traditionally for various cardioprotective ailments as it possess antioxidant potential that makes them interesting to researchers for their evaluation as test plant (Naseer *et al*.,2016). The researchers have been using Doxorubicin (DOX) for induction of MI. DOX has a dose-dependent cardiotoxic effect that results from elevated generation of cellular oxidants such as ROS and is coupled with depletion of concentration of oxidative inhibitors like superoxide

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dismutase (SOD), catalases and reduced glutathione (GSH) (Singh *et al*., 2008). Thus, this model can be used extensively in research for the induction of cardiac infarction.

The purpose of this research is to provide the community with better options such as naturally sourced lichen *Parmelia Perlata* to combat with DOX-induced cardiotoxicity.

MATERIALS AND METHODS

Crude extract preparation

The lichens were obtained from the local supplier identified and drived under shade. After drying sample was subjected to thick crushing and milling. Aqueous methanolic (70/30) extract *Parmelia perlata* (Pp.Cr) was developed by maceration of roughly ground lichen for three days thrice with occasional stirring and shaking. After the vaporization of filterate under vaccum using Heidolph rotary evaporatoion unit at 40-50^{o}C temperature. Semi-solid extract of *Parmelia perlata* (Pp.Cr) was obtained and weighed and percentage yield was calculated.

Acute toxicity tests

The acute toxicity of Pp.Cr was carried out as per OECD instructions (Turnheim, 1994).

Experimental protocol

To carry out this research, the guidelines of Pharmacy Research Ethics committee (PREC) were followed under registration no 52/S-2018-/PREC. All the experimental protocol was performed on Wistar albino rats of both sexes having weight about 150-250 g, picked on their normal serum LDH values. The animals housing was done under specified lab environment (temperature: 25± 2^{o}C and humidity 60±5%), 12 hrs light and dark rhythm was maintained, feed and water ad libitum (Alkreathy *et al*., 2010).

Rats were randomly divided as follows and treated for 14 days keeping N=6

Group I (Normal Control): Received 5mL/kg of 10% DMSO orally

Group II (Intoxicated Control): Received doxorubicin (25mg/kg, i.p.) on day 12 (Alkreathy *et al*., 2010)

Group III (Low Dose Treatment): Received Pp.Cr 30mg/kg (10% DMSO, p.o.) for 14 days + doxorubicin on day 12.

Group IV (Medium Dose Treatment): Received Pp.Cr 100mg/kg (10% DMSO, p.o.) for 14 days + doxorubicin on day 12.

Group V (High Dose Treatment): Received Pp.Cr 300mg/kg (10% DMSO, p.o.) for 14 days + doxorubicin on day 12.

Group VI (Standard Control): Received Atenolol (100mg/kg, p.o.) for 14 days + doxorubicin on day 12.

Upon completion of the experimental period; i.e. 72 hours of doxorubicin administration (El-shitany *et al*.,2008), ketamine/xylaxine was used to anesthetized animals, blood was drawn by technique of cardiac puncture and sera isolated at 4500 rpm for 15 minutes by the process of centrifugation (Takahashi *et al*., 2014). The hearts were excised immediately from the animals, heart to body weight ratio was analysed (Srivastav *et al*., 2013). Hearts were stored in 10% formalin and histopathology was done (Fiette and Slaoui, 2010).

Estimation of serum cardiac marker enzymes; i.e. creatinine kinase-MB (CK-MB), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALP) was done with the aid of commercially accessible enzymatic kits.

The data manifested as mean ± S.E.M. and significance level between different experimental groups was assessed by using One-way ANOVA following post hoc bornferroni test via Graph Pad Prism version 5. $P<0.05$ regarded as statistically valid.

RESULTS

Effect on biochemical parameters

DOX not only halts general energy production, but also leads to specific impairment in release of creatine kinase (CK) isoenzymes Our study reveals that serum CK-MB,LDH, AST, ALT values are significantly increased ($^{###}$ $p<0.001$) in intoxicated group at single bolus of DOX (25mg/kg, i.p.), in comparison with the normal control, indicated in Table 1.

Heart to Bodyweight Ratio

The results shown in Table 1 are in coherence with the expected results; i.e. heart/body weight is 0.371 for intoxicated group, whereas the treatment groups (30, 100 and 300 mg/kg) showed values of 0.376, 0.379, 0.385 respectively. Tissues sections from intoxicated groups showed defined structural disruption of striated cardiac muscles which was shown by vascular edema, disorganized myocytes with vacuolation and necrotic cells as shown in Figure 1 whereas, control groups showed normal histopathology.

Histopathological Interventions

Histopathological evaluation of heart muscles under light microscope, showed defined structural disruption of striated heart muscles which was shown by vascular edema, disorganized myocytes with vacuolation and necrotic cells as shown in Figure 1 whereas, control groups showed normal histopathology.

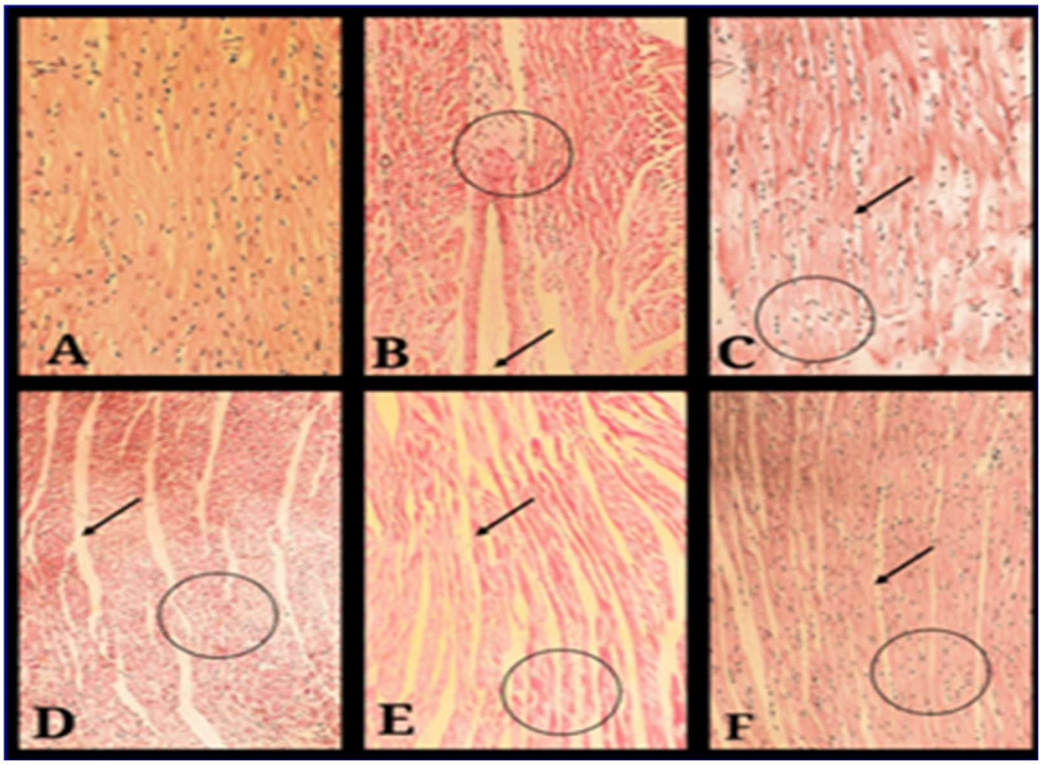


Fig. 1: Histopathological parameters:
The effects of pretreatment with different doses of the crude extract of *Parmelia perlata* (Pp.Cr) on histopathological alterations in cardiac tissue; (A) control, (B) intoxicated (DOX 25 mg/kg once at 12th day of pretreatment) (C) Atenolol (100 mg/kg)+ intoxication (D) Pp.Cr (30 mg/kg)+intoxication, (E) Pp.Cr (100 mg/kg)+intoxication and (F) Pp.Cr (300 mg/kg)+intoxication in DOX-induced MI in rats. Arrows (→) show interstitial edema, with vacuolation whereas, area encircled (O) shows scattered cardiomyocytes and subendocardial cells necrosis. Heart tissues were stained with H & E and visualized under light microscope at 10× magnification.

Table 1: Haematological Analysis

		CK-MB (IU/l)	LDH (IU/l)	AST (IU/l)	ALT (IU/l)	AST/ ALT	Body weight	Heart weight	Heart/Body weight ratio (×100)
Control (10%DMSO 5ml/kg)		14.98±1.603	18.23±4.53	23.38±1.35	45.56±4.66	0.513	191±11.46	0.693±0.04	0.334
DMSO Intoxicated (DOX 25mg/kg)		976.7±15.78$^{###}$	1071±8.06$^{###}$	231.7±4.75$^{###}$	208.9±3.78$^{###}$	1.109	175.7±11.52	0.652±0.04	0.371
Treatment+ DOX	Pp.Cr (30mg/kg)	898.0±25.63^{ns}	740.0±17.98***	201.5±2.71**	1.179	170.8±4**	0.545±0.04	0.376	0.376
	Pp.Cr (100mg/kg)	343.6±11.10***	491.3±18.17***	166.1±4.71**	1.12	148.0±5.97**	0.578±0.05	0.578±0.05	0.379
	Pp.Cr (300mg/kg)	261.3±12.93***	393.9±5.50***	150.8±4.36***	1.43	105.4±5.24***	0.580±0.02	0.580±0.02	0.385
Standard + DOX	10%DMSO Atenolol (100mg/kg)	232.2±12.57***	259.9±13.04***	165.0±3.62***	1.20	136.7±3.20***	0.605±0.03	0.605±0.03	0.340

The effects of the crude extract of *Parmelia perlata* (Pp.Cr) on serum cardiac biomarker levels; Creatinine Kinase (CK-MB), Lactate dehydrogenase (LDH), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and AST/ALT Ratio body weight, heart weight, heart / body weight ratio (* p<0.05: significant, **p<0.01: more significant and *** p<0.001: highly significant).

DISCUSSION

Our findings confirm the pathological intervention of MI in the rats due to DOX-induced toxicity which is a reason to cause CK-MB marker enzyme to leak out from ruptured or necrotic cardiac cells into blood stream leading to increased serum concentration of enzyme. The treatment groups for the crude extract Pp.Cr, along with intoxication, showed cardioprotective effects by significantly decreasing the release of CK-MB in the serum. The results also showed an increased heart to body weight ratio in intoxicated group while the effect of crude extract minimized the prevalence of this symptom in treatment groups, comparable to standard however this report was insignificant statistically. The development of thrombotic lesion is induced by inflammatory reactions in the heart. The underlying mechanism is free radical forming, peroxidation of lipids, DNA suppression and RNA and synthesis of proteins (Gomes *et al*., 2013). The current study deals with the successful development of cardiotoxic model. The intoxicated group had significant elevation of biomarkers. While the decreased cardio toxicity is shown in the treatment groups significantly. The lichen's historic usage as a cardiac tonic to improve heart muscle function has been assumed due to the strong antioxidant activity of salazinic acid and other phenolic metabolites in *Parmelia perlata*.

Among the physical parameters, cardiac dystrophy is seen in the animals with cardiotoxicity. This causes change in body and heart weight. Therefore, cardiac weight in DOX-induced cardiotoxic animals increases in a significant manner as compared to control ones. So this is being seen that geart to body weight ratio is a significant parameter to conclude cardiotoxicity. The body weight in DOX-treated animals decreases significantly as compared to the normal animals. This causes variation in heart and body weight ratio; i.e. the heart weight / body weight ratio is increased in normal animals as compared to the DOX-induced intoxicated animals (Swamy *et al*., 2012). Furthermore, this healing effect compensates the formation of scar which increases heart/body weight ratio for treated animals. Thus scarring, recovery and oedema caused by the healing of toxicity increases showing that the crude extract has potential to reverse the dox induced cardiotoxicity. However, this parameter is not considered as a sole parameter to diagnose DOX-induced MI and effect of crude extracts therapy in animals.

Furthermore, remarkable structural disorganization of cardiac tissues when intoxicated with DOX shows a pronounced pathological response such as cardiac hypertrophy, ventricular dilatation, inflammation of myocytes, and enlargement as a whole. The DOX-induced cardiotoxicity is also depicted by reduced body weight and increase in the weight of cardiac muscles (Swamy *et al*., 2012). The foliose lichen *Parmelia perlata*, sometimes known as Stone Flower, is highly prized in traditional systems, due to its potent anti-inflammatory properties (Gandhi *et al*., 2020). These therapeutic effects are mostly caused by a rich profile of bioactive secondary metabolites, including as usnic acid, salazinic acid, and atranorin, which work together to alter inflammatory pathways and reduce oxidative stress (Saha *et al*., 2021).

Author's contributions

All authors contributed equally to the conception and design of the study, data collection, data analysis and interpretation, manuscript writing, and critical revision. All authors read and approved the final version of the manuscript.

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

To carry out this research, the guidelines of Pharmacy Research Ethics committee (PREC) were followed under registration no 52/S-2018-/PREC.

Conflict of interest

Authors will include a statement about the conflict of interest. If there are no conflicts, the following statement should be included: "The authors declare no conflict of interest."

List of abbreviations

Parmelia perlata (Pp.Cr), Doxorubicin (DOX), Lactate dehydrogenase (LDH), Creatinine kinase myocardial band (CK-MB), Aspartate transaminase (AST), Alanine transaminase (ALT), Myocardial infarction (MI), B-type natriuretic peptide (BNP), Superoxide dismutase (SOD), Reduced glutathione (GSH), Reactive Oxygen Species (ROS), Organisation for Economic Co-operation and Development (OECD), Pharmacy Research Ethics committee (PREC),

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