ORIGINAL ARTICLE

A NOVEL DEPSIDE SEKIKAIC ACID IS PROTECTIVE AGAINST CYCLOPHOSPHAMIDE INDUCED CARDIOTOXICITY IN RATS

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Background: Cyclophosphamide is a widely prescribed chemotherapeutic agent. Its toxic metabolites induce oxidative stress which burdens the cardiac tissue. With recent advancements in gaining deeper insight in natural antioxidants, Sekikaic acid (SA) is gaining popularity as a novel depside compound with significant organ protective potential. This study seeks to explore and assess the cardio-protective and antioxidant effects of sekikaic acid against anti-cancer drug toxicity. **Methods:** Experimental animals were divided into three groups: normal untreated control, cyclophosphamide toxic control, and cyclophosphamide with sekikaic acid group where sekikaic acid was administered for 14 continuous days followed by a single dose of cyclophosphamide. For assessing myocardial injury serum cardiac biomarkers (creatine kinase, lactate dehydrogenase, troponin I, C-reactive proteins) and tissue oxidative stress markers (glutathione, super-oxide dismutases and malondialdehyde) were evaluated. **Results:** The control group demonstrated normal physiologic values of serum and tissue biomarkers. The toxic control demonstrated signs of cardiac injury with a single dose of cyclophosphamide, with raised levels of serum and tissue oxidative stress markers alongside receding natural antioxidant pools of glutathione and super-oxide dismutases. On the contrary the oxidative burden on myocardium was significantly reduced ($p \leq 0.05$), and cellular antioxidants were preserved with co-administration of sekikaic acid validating its potential as a cardioprotective agent. **Conclusion:** The antioxidant potential of sekikaic acid can effectively ameliorate cyclophosphamide induced cardiotoxicity and can be incorporated in cyclophosphamide containing chemotherapeutic and immunosuppressive regimens.

Keywords: Cardiotoxicity, Cyclophosphamide (CP), Oxidative stress, Sekikaic acid (SA)

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INTRODUCTION

Cyclophosphamide (CP), is a classic alkylating agent with a global utilization as an immunosuppressant and cancer chemotherapeutic. It is a prodrug which undergoes biotransformation by CYP450 isozymes. Its therapeutic end-product is phosphoramide mustard which produces its cytotoxic action by formation of deoxyribonucleic acid (DNA), adducts and immunosuppression by activation of T-lymphocytes and myeloid derived suppressor cells. The dose-limiting factor is the concurrent production of the toxic byproduct acrolein.[1]

Acrolein can cause cardiac damage by inducing nitrative and oxidative stress raising concern about rocketing prevalence of post chemotherapy cardiovascular events including myocardial ischemia/ infarction; ventricular arrythmias and hypertension. The various aetiological pathways identified so far include direct injury to vascular endothelium, lipid peroxidation raising serum malondialdehyde levels, exhaustion of nicotinamide dinucleotide (NAD^+) and adenosine triphosphate (ATP) leading to altered mitochondrial respiration and electron transport chain, and formation of acrolein-protein ducts with glutathione pools.[2]

Sekikaic acid (SA) gained its explorative potential after Ramalina species became renowned for their biopharmaceutical potential. SA is a carbonyl compound extracted from lichens particularly Ramalina species.[3] Sekikaic acid was discovered around 2007 as a bioactive compound.[4] It has a complex depside structure with benzoic acid derived polyphenolic aromatic rings bonded with ester linkages. This unique structure has the capability to scavenge and neutralize reactive oxygen species (ROS) including superoxide anions (O_2^-), nitric oxide (NO), peroxyl radicals (ROO•), and hydroxyl radicals (OH•) by donation of a hydrogen atom from its hydroxyl groups. It further inactivates lipid peroxidation helping conserve the integrity of cellular and organelle membrane.[5]

Sekikaic acid and its analogues are under research regarding their anti-oxidant potential. While there is no direct evidence linking SA to cardioprotective effects, its antioxidant and enzyme-inhibitory properties suggest it could have a role in reducing cardiovascular risk factors. Since the cardiotoxicity by CP attains its pathology via oxidative stress we have tried to explore the protective potential of SA against CP since the scavenging ability of this compound against 2,2-diphenyl-1-picrylhydrazyl (DPPH) which is one of the most crucial oxidative biomarkers involved in cardiac injury was established by fellow researchers.[6–8]

This study, using a mouse model, aimed at assessing the cardio-protective and antioxidant effects of Sekikaic acid against anti-cancer drug toxicity. The longitudinal objective was to optimize the CP containing anticancer and immunosuppressants regimen.

MATERIAL AND METHODS

This laboratory-based experimental study was conducted in the Department of Pharmacology, Army Medical College, Rawalpindi, after obtaining approval from the Ethical Review Committee (ERC ID No. 11/2024/435).

Commercial preparations of injectable CP were procured from Pharmedic Laboratories, Pakistan. SA was collected and further processed via extraction by Soxhelt method by Global Pharmaceuticals Islamabad on request.[7] One mg/mL solution was formulated with 0.5% dimethyl sulfoxide (DMSO) to ensure safe oral consumption.

Homogeneous population of 36 Sprague Dawley rats, aged 6–8 week and weighing 200–250 grams were included via non-probability convenient sampling. Pregnant, diseased and younger animals were excluded. The rats were kept under standard housing conditions. The animals were divided into three equal groups, (n=12 each) via simple randomization technique. Each group received following respective protocol for 14 days.[6] (Table-1).

Table-1: Experimental groups and treatment protocol

Group	Description	Treatment	Time point of Sample Collection
Control Group	Normal baseline group	No Treatment	14th Day of study
CP Group	Toxic Control	Single dose of 200 mg/Kg CP (IP) on 14th day	24 hours post CP administration on 15th day of study
CP+SA Group	Treatment group, receives SA and CP	2 mg/Kg SA was administered orally once daily for consecutive 14 days. One hour after the last dose of SA on day 14, a single injection of 200 mg/Kg of CP was administered IP	24 hours post CP administration on 15th day of study

The procedure for sample collection was performed in a bio-safety cabinet ensuring standard safety protocols. Euthanasia was done with drop jar method using 30% v/v isoflurane in propylene glycol. The blood was drawn through cardiac puncture and exsanguination for facilitating tissue sampling via mid-line sternotomy. The serum was separated by centrifugation. The tissue homogenate was prepared in isotonic phosphate saline buffer and centrifuged to obtain the supernatants.

Biochemical markers including creatine kinase-myocardial band [CK-MB], lactate dehydrogenase (LDH) and C-Reactive Proteins (CRP) were calculated by automated high-throughput chemistry analyzer (Selectra XL) effectively within 1–1.5 mL of the blood sample and Troponin-I was estimated by with commercial Cardiac Troponin I (cTnI) Rapid Test Kit utilizing a blood volume of 75–90 µL. Markers of oxidative stress including glutathione [GSH], malondialdehyde [MDA] and superoxide dismutases (SOD) were assessed by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with commercial kits.

The data were recorded on Microsoft Excel Sheets as Mean±Standard Error of Mean and analysed using SPSS-22. Quantitative variables were compared through ANOVA followed by Post Hoc Tuckey test, and $p \leq 0.05$ was considered significant.

RESULTS

To assess the cardiotoxicity each parameter with CP alone was compared with baselines. There was high statistical significance as CP markedly increased the tissue injury indicators, i.e., CK-MB (765.33±39.89 IU/L), LDH (2790.83±89.3 IU/L), Troponin-I (21.54±3.44 pg/mL), CRP (72.8±6.28 mg/mL) and MDA (2.87±0.006 U/mL) ($p<0.05$). Simultaneously there was reduction in endogenous antioxidant GSH (0.5±0.03 ng/mL) and SOD (100.43±5.22 U/mL) ($p<0.05$ and $p<0.01$ respectively).

The SA+CP group helped to gauge antioxidant potential of SA by reducing the deranged CK-MB (365.66±15.39 IU/L), LDH (1,529±10.23 IU/L), Troponin-I (15.43±0.97 pg/mL), CRP (54.48±4.99 mg/mL) with a remarkable statistical significance ($p<0.001$). Our values validated the potential of SA against cardiotoxicity caused by CP. SA actively reduced the deranged values of MDA (2.02±0.05 U/mL) ($p<0.001$) and restoration of SOD (122±3.11 U/mL) reserves ($p<0.01$). The glutathione levels with SA were numerically but not significantly raised compared to toxic controls ($p=0.12$). (Table-2).

Table-2: Comparison of quantitative values of serum and tissue oxidative stress markers indicative of cardiac injury

Parameters (Units)	Control Group	CP Group	SA+CP Group	*p*
CK-MB (IU/L)	75.16±19.69	765.33±39.89	365.66±15.39	<0.001
LDH (IU/L)	900.89±8.24	2790.83±89.3	1529±10.23	<0.001
Troponin-I (pg/mL)	5.96±0.92	21.54±3.44	15.43±0.97	<0.001
CRP (mg/mL)	20.09±0.9	72.8±6.28	54.48±4.99	<0.05
GSH (ng/mL)	0.98±0.12	0.5±0.03	0.847±0.32	0.12
MDA (U/mL)	1.75±0.02	2.87±0.006	2.02±0.05	<0.001
SOD (U/mL)	152.58±2.32	100.43±5.22	122±3.11	<0.01

ANOVA and post Hoc Tuckey Test applied

DISCUSSION

Cyclophosphamide (CP) has a dose-dependent cardiotoxicity mediated by its metabolite acrolein. Acrolein's affinity for thiol groups leads to glutathione depletion, lipid peroxidation, and irreversible damage to cellular membranes.[1] Recent data suggests upregulation of inflammatory cytokines (e.g., TNF-α, IL-6), activation of NF-κB, and disruption of mitochondrial membrane potential, releasing cytochrome c and activating caspase-mediated apoptosis.[9] This cascade is also substantiated in our study with a significant rise in serum CRP, aligning our findings with the pro-inflammatory milieu reported earlier.[1,2,9] This suggests that CP toxicity model not only validates the sustained oxidative stress burden but also shows its translational potential in mimicking the clinical signs of CP induced cardiotoxicity.

These lethal effects warrant the pursuit of compounds with dual antioxidant and anti-inflammatory potential. In the present study, Sekikaic acid (SA), a lichen-derived polyphenolic depside demonstrated a significant amelioration of CP-induced cardiotoxicity. To our knowledge, this is the first *in vivo* report exploring the cardioprotective effects of SA in a CP-induced toxicity model. The novel findings highlight the candidature of SA as an adjuvant therapy in CP based chemotherapeutic regimens.

The observed improvements in serum biomarkers, i.e., CK-MB, LDH, Troponin-I, and CRP alongside favourable regulation in oxidative stress markers such as GSH, SOD, and MDA, strongly support the hypothesis that SA confers tissue protection via redox modulation, likely by scavenging reactive oxygen species (ROS) and up-regulating endogenous defence systems. SA is structurally rich in functional hydroxyl and carbonyl groups, which are ideal for electron donation and proton transfer, the rudimentary mechanics behind radical scavenging.[8] Various natural antioxidants quercetin, resveratrol, and curcumin, among others, have shown protective effects against CP or doxorubicin-induced cardiomyopathy through similar redox-sensitive pathways.[8] SA shares structural and functional resemblance to these compounds, yet its utility remains underexplored in cardiovascular settings. Importantly, the significant decrease in MDA and increase in SOD and GSH levels in our treatment group is consistent with other antioxidant intervention studies involving N-acetylcysteine, melatonin, and ellagic acid.[10,11]

Research has indicated that SA exhibits inhibitory effects on key metabolic enzymes α-glucosidase, which indirectly contributes to cardiovascular health by reducing hyperglycaemia induced cardiac stress.[12] SA exhibits potent antidiabetic effects in streptozotocin-induced diabetic rats, by significant reduction of blood glucose levels and promoting pancreatic β-cell regeneration, attributed to its inhibition of carbohydrate digestive enzymes.[5,13] SA is reported to have hypolipidaemic affects by reducing LDL and total triglycerides and cholesterol levels. Strengthening its cardiometabolic protective role.[13]

SA may also exert cardioprotection by modulating inflammatory and apoptotic pathways. Several *in-silico* docking studies and *in vitro* enzyme assays suggest that SA interacts with cyclooxygenase-2 (COX-2) and 5-lipoxygenase, inhibiting pro-inflammatory mediators. It also inhibits key regulatory cardiotoxic enzymes including matrix metalloproteinases (MMPs) and nitric oxide synthase (NOS).[14]

While not directly studied for SA, related compounds like caffeic acid have demonstrated vasorelaxant effects through the endothelial nitric oxide (NO) pathway. This involves the activation of endothelial NOS, leading to increased NO production, which is crucial for vascular relaxation and overall cardiovascular health.[4] It is plausible that SA may exert similar effects given its structural properties. Some studies on related compounds suggest that they may interact with adrenergic receptors, which play a significant role in regulating heart rate and vascular tone. The potential for SA to influence these receptors remains an area for further exploration.[4,14]

SA reduced the C-reactive protein levels to a significant extent when administered with CP. This C-reactive protein reduction was also demonstrated by the compound and its analogue in an arthritic murine model where CRP levels were reduced significantly in murine model of rheumatoid arthritis.[15] The significant reduction in CRP levels observed in our study aligns with murine models of arthritis and colitis treated with SA, where it dampened systemic inflammation and modulated immune responses. This dual antioxidant and anti-inflammatory capacity makes it an ideal candidate for further exploration in cardio-oncology.

These findings encourage incorporation of SA as a complementary agent in CP-containing regimens, especially considering the lack of approved drugs specifically targeting CP-induced cardiotoxicity. As in comparison to the synthetic agents like dexrazoxane, which interferes with anticancer efficacy or have own toxicities, natural agents like SA offer a safer multi-oriented approach.[16]

While SA shows potential through its antioxidant and enzyme-inhibitory activities, more targeted research is needed to fully understand its cardioprotective properties and mechanisms of action. This study opens avenues to evaluate the molecular mechanisms of SA in myocardial tissue, particularly its impact on Nrf2/ARE, NF-κB, and PI3K/Akt signalling pathways.[8,10] Studies for evaluation of its efficacy with other established antioxidants in CP cardiotoxicity models can bring validation to its potential.

CONCLUSION

The study highlights the protective role of SA against CP induced cardiotoxicity by its antioxidant, anti-inflammatory and enzyme inhibitory pathways. Sekikaic acid successfully restored both serum and tissue oxidative stress markers against CP induced oxidative burden. While these results are encouraging, they warrant further research towards exploration of molecular landscapes and clinical trials to explore the feasibility of adding SA in existing chemotherapeutic regimens.

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