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RESEARCH ARTICLE

Evaluating the Cardioprotective Efficacy of Diosmin against LPS-Induced Cardiac Dysfunction: In Silico Docking and Experimental Investigations

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ABSTRACT

Sepsis presents a significant global health threat, characterized by systemic inflammatory responses and cardiac sepsis infections, often triggered by gram-negative bacteria, resulting in severe inflammatory reactions, multiorgan failure, and high mortality rates. Diosmin, known for its phlebotonic properties enhancing vein health, possesses notable anti-inflammatory, antioxidant, and anti-mutagenic attributes. This study examines Diosmin's cardioprotective potential in Lipopolysaccharide (LPS)-induced cardiac dysfunction using both in- silico docking and experimental approaches. In-silico docking studies revealed Diosmin's interactions with target proteins, demonstrating higher docking scores and unique interactions compared to Imatinib, suggesting its potential to mitigate Imatinib effects. Experimental protocols employed albino Wistar rats (weighing 180-200g, with n=5-6 per group), wherein cardiac dysfunction was induced by administering LPS (10mg/kg i.p) commencing on the 14th day of the study. Diosmin (50 and 100 mg/kg, p.o.) and Imatinib mesylate (30mg/kg, p.o.) were administered as pretreatment for two weeks. Furthermore, Diosmin exhibited comparable interactions with Montelukast, highlighting its versatile therapeutic potential. Throughout the study, Diosmin significantly attenuated the aberrant metabolic alterations in LPS-treated rats. Treatment showed notable benefits in alleviating LPS-induced cardiac dysfunction, as evidenced by hemodynamic studies, biochemical assays, tissue estimations, and histopathological examinations, revealing dose-dependent improvements in cardiac biomarkers and functional parameters. However, experimental validation remains crucial despite promising in-silico predictions. In conclusion, Diosmin shows promise in ameliorating LPS-induced cardiac dysfunction due to its anti-inflammatory and antioxidant properties. Synergistic effects with Imatinib and in-silico findings underscore Diosmin's potential, urging rigorous experimental validation for addressing sepsis-related cardiac complications.

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INTRODUCTION

Sepsis, a global affliction characterized by its high incidence and mortality rates, manifests as a systemic inflammatory response. Bacterial endotoxin, particularly lipopolysaccharide (LPS), plays a central role in this pathology, contributing significantly to organ impairment (Wang *et al*., 2023). Recent research involving dogs, calves, and horses has demonstrated that sepsis, severe sepsis, and septic shock in these animals can lead to left and right ventricular systolic dysfunction, left ventricular diastolic dysfunction, and circulatory disturbances (Naseri *et al*., 2023). The high prevalence of myocardial depression in sepsis and septic shock underscores its significance as an indicator of cardiac dysfunction (Habimana *et al*., 2020). Recent research emphasizes the importance of timely detection and intervention for severe infection-induced organ dysfunction, including conditions like pneumothorax, toxemias, and nosocomial infections (Landelle *et al*., 2010).

Traditionally rooted in germ theory, sepsis was initially attributed solely to microorganisms, overlooking other contributing factors (Wang *et al.,* 2023). However, a paradigm shift occurred with the recognition of the significance of systemic inflammation in sepsis, highlighting the inadequacy of antibiotic-centric approaches. Septicemia, or blood poisoning, involves self-directed immune assaults against invasive microbes, leading to early immune suppression and increasing the risks of organ failure, shock, and fatality due to bloodstream infiltration (Nakahira *et al.,* 2011; Huang *et al.,* 2019).

LPS, a principal component of bacterial endotoxin, triggers the tyrosine kinase pathway, leading to phosphorylation, signaling, cell growth, and cytokine release, which fuel inflammation, tissue damage, and sepsis (Gabarin *et al.,* 2021). Sepsis also heightens the risk of myocardial infarction in individuals with prior events (Wu *et al.,* 2019). Cardiovascular Disease (CVD) encompasses various heart conditions, including congenital and factors-influenced ones like heart failure, each unfolding through distinct mechanisms (Gabarin *et al.,* 2021). Initial treatments for Congestive Heart Failure (CHF) involve various drugs, with Nebivolol and carvedilol showing promise in hypertension and CHF management. The high cost of sepsis therapy underscores the importance of managing inflammation and infection in treatment.

Diosmin, a phlebotonic compound found in citrus fruits, is widely used in human medicine for treating venous insufficiency. However, its application in veterinary medicine remains under-researched. While it shows potential due to its anti-inflammatory and vasoprotective properties, more studies are needed to confirm its safety and effectiveness in animals. Additionally, diosmin is known for its versatility in treating venous disorders and supporting blood vessel health (Feldo *et al*., 2018). With its array of bioactivities, including anti-lipid peroxidation, anti-inflammatory, antioxidant, and antimutagenic traits, Diosmin stands out as a notable flavonoid (Mustafa *et al.,* 2022). While some studies highlight Diosmin's efficacy against oxidative stress and inflammation, research on its involvement in lipopolysaccharide-induced cardiac dysfunction is lacking. This study investigates the cardioprotective effects of Diosmin in LPS-induced cardiac dysfunction using both in-silico docking and experimental methodologies. By exploring the molecular interactions between Imatinib, Diosmin, and their respective protein targets, the research aims to fill the existing gap in understanding Diosmin's mechanism of action in sepsis-related cardiac impairments in rats. Bridging these challenges with a molecular understanding is crucial for navigating the complexities of sepsis and related disorders.

MATERIALS AND METHODS

In-silico study (Docking study)

Protein target preparation: The three-dimensional structures of tyrosine kinase inhibitor, and Cysteinyl leukotrienes receptor 1 were retrieved from the RCSB protein data bank with PDB ID: 5EWL, 1QCF, and 6RZ5 (Berman, 2000; Choudhary *et al.,* 2023).

Ligand generation: The two-dimensional structures ofImatinib, Diosmin, and Montelukast were drawn from ACD/Chem sketch software. The drawn structures were then saved in MDL molfile format and ligands were then imported in MVD software and optimized to 3D format (Choudhary *et al.,* 2023).

Protein-ligand docking: The prepared ligands were screened to check their ability to bind with selected proteins pocket using the software Molegro Virtual Docker (MVD 2010.4.1.0), an average of 10 trial runs for each structure were performed by software, and the best five poses were then determined. The post-docking tool was then studied to attain the best docking pose and energy values (Choudhary *et al.,* 2023).

Chemicals: Lipopolysaccharide, Montelukast, Diosmin, and Imatinib mesylate were obtained from Merck, Yarrowchem, HiMedia, and Hetero Lab Limited respectively. Additionally, potassium phosphate (K_3PO_4), potassium sulfate (K_2SO_4), Thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), butanol ($C_4H_{10}O$), pyridine (C_5H_5N), and sodium hydroxide (NaOH) were sourced from LobaChemie, while 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) was acquired from Sisco Research Laboratories, and acetic acid ($C_2H_4O_2$) from SD Finechem Limited. LDH assay kit, CK-MB assay kit, alkaline phosphatase (ALP) activity assay kit, and Serum Calcium assay kit were purchased from ENZOPAK, AGD Clinipak, Oscar Medicare, and Proton Biologicals India respectively.

Animals and experimental design: The research procedure for this investigation was authorized by the Ethics Committee of Nanchong Vocational and Technical College. All animal experiments were conducted in strict accordance with animal ethical guidelines. Adult Albino Wistar rats of either sex weighing approximately 180-200g were obtained from a licensed breeder. The animals were quarantined until their overall health was monitored and then transported to the housing area. Prior to the studies, the animals were acclimatized for seven days to the suitable housing conditions at the institute's animal house facilities. They were housed in polypropylene cages with dust-free rice husk bedding under conventional laboratory settings, including controlled temperature (23±2°C), humidity (55±10%), and a natural light-dark cycle (12-12 hour daily). The animals were provided with a conventional pellet diet and had free access to water.

Cardiac dysfunction was induced by administering lipopolysaccharide at a dose of 10mg/kg intraperitoneally from day 14 of the study. The test drugs Diosmin (at doses of 50 and 100mg/kg, orally) and Imatinib mesylate (30mg/kg, orally), along with the optimal dose of Diosmin, were administered as pretreatment for 2 weeks. At the end of the study, the animals underwent assessment for normal metabolic parameters and hemodynamic studies including blood pressure (systolic/diastolic); mean arterial pressure, and heart rate. Blood samples were collected for biochemical studies (lactate dehydrogenase - LDH, creatine kinase-MB - CK-MB, alkaline phosphatase - ALP, and serum calcium test), after which the animals were sacrificed for tissue estimation study

parameters (lipid peroxidation - LPO, glutathione - GSH estimation), and histopathological studies.

Measurement of hemodynamic parameters: The rats were trained to familiarize themselves with the restrainer for 15–30 minutes per day for at least 5 days before the measurement of hemodynamic parameters. Hemodynamic parameter measurements were conducted using the non-invasive volume-pressure recording (VPR) tail-cuff plethysmography method, employing the CODA® Monitor. Briefly, the rats were allowed to move freely into an acrylic rodent restrainer equipped with a darkened nose cone to minimize stress. The restrained rats were pre-warmed using a warming pad wrapped around the restrainer, with the temperature set to 35°C. The body temperature of the rats was monitored using a rectal infrared temperature probe, while the tail temperature was monitored with an infrared thermometer. Blood pressure measurements were taken at a temperature of 33–35°C.

Assessment of haematological parameters: After the trial was completed on the 15th day, blood samples were collected from the rats. Under anesthesia, the retro-orbital plexus was punctured to obtain blood, which was then collected into blood collection tubes. Subsequently, serum was separated for the measurement of various hematological markers using diagnostic kit-based methods, including serum calcium, lactate dehydrogenase, alkaline phosphatase, and creatine kinase-MB.

Tissue biochemical estimation studies in rat heart: After performingthehematologicalparameters, therats were sacrificed, andtheirhearts were excised, rinsed, dried, andweighed in ice-cold normal saline. A 10% w/v homogenatewasprepared in 0.05M ice-cold phosphate buffer. After centrifugation, the supernatant was used for the estimation of antioxidant enzyme levels using TBARS (Ohkawa *et al.*, 1979) and reduced glutathione (GSH) (Ellman, 1959).

Histopathological studies of rat heart: The animals were sacrificed, and their hearts were harvested. Following dewaxing, the excised hearts were preserved in a 10% buffered formalin solution, and slices were produced using paraffin blocks. These slices were then stained with Hematoxylin and Eosin.

Statistical analysis: Using the GraphPad Prism software package, the data were presented as Mean ± SEM and were analyzed using one-way ANOVA with Tukey's multiple comparison tests and two-way ANOVA with Bonferroni's post hoc analysis within the GraphPad Prism software program. A p-value <0.05 was considered to be significant.

RESULTS AND DISCUSSION

In-silico study (Docking study): This study embarked on *in-silico* docking investigations to unravel the interactions of Imatinib and Diosmin with target proteins. Imatinib showed a docking score of 5131.17 and interactions with Thr338 and Asp404 in Tyrosine Kinase Inhibitor (PDB ID: 1QCF). In contrast, Diosmin exhibited a higher score of 9186.43, engaging Gln277, Phe278, Lys295, and Asn391. Exploring Cysteinyl Leukotrienes Receptor 1 (PDB ID: 6RZ5), Montelukasthad a score of 5194.76 with Ser193, while Diosmin scored 9166.68 and engaged Thr280, Pro176, Phe174, Glu175, and Tyr104. These insights underscored Diosmin's potential to mitigate Imatinib Mesylate and Montelukast effects. However, experimental validation remains essential despite *in-silico* predictions.

In- silico study for Tyrosine kinase the PDB ID 1QCF: In the *in-silico* study for Tyrosine kinase the PDB ID 1QCF with a cavity volume of 221.184 were selected to carry out the docking protocol (Fig. 1 A and Fig.1B). The results indicated that Imatinib has dock score 5131.17 and 2 interactions, while Diosmin has dock score 9186.43 and 8 interactions with the protein (Table 1).

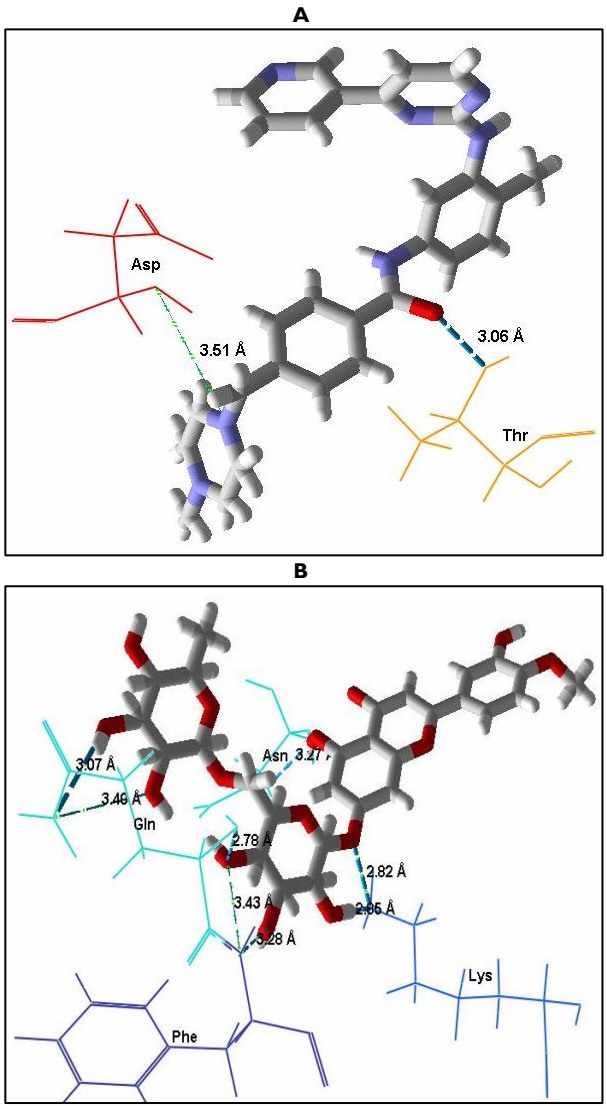

Fig. 1A: Docking interaction of Imatinib with PDB ID 1QCF; Fig.1B Docking interaction poses Diosmin with PDB ID 1QCF.

In- silico study for Cysteinyl leukotrienes the PDB ID 6RZ5: In the *in-silico* study for Cysteinyl leukotrienes the PDB ID 6RZ5 with a cavity volume of 43.472 were selected to carry out the docking protocol (Fig. 2A and Fig. 2B). The results indicated that montelukast has dock score 5194.76 and interaction, while Diosmin have dock score 9166.68 and 6 interactions with protein (Table 2).

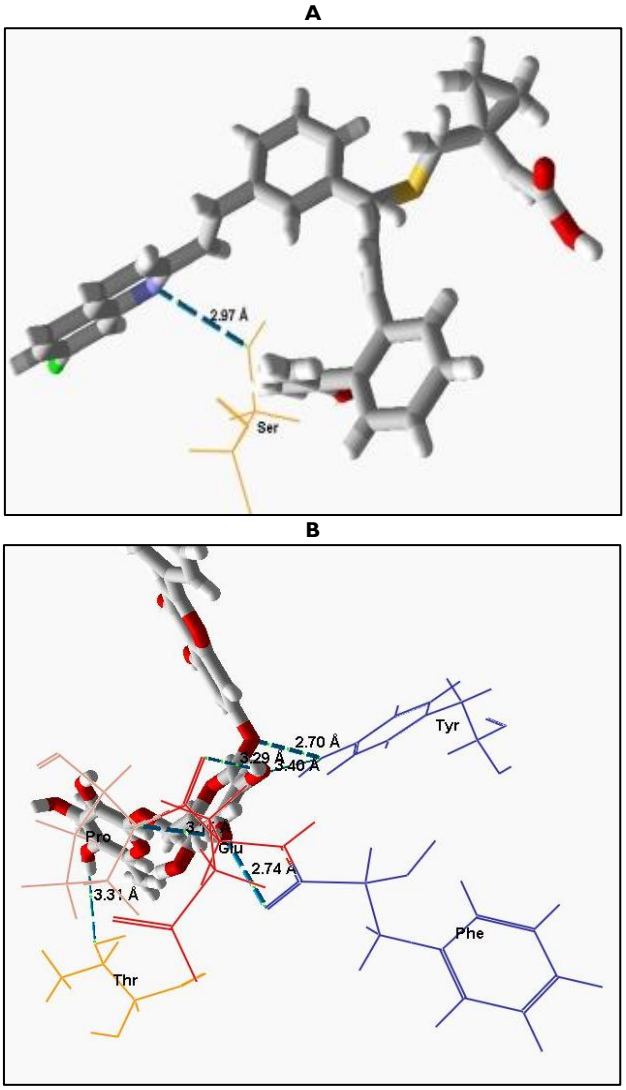

Fig. 2A: Docking interaction of Montelukast with PDB ID 6RZ5; Fig.2B Docking interaction poses Diosmin with PDB ID 6RZ5.

The *in-silico* docking study conducted in this research aimed to elucidate the interactions between Imatinib, Diosmin, and their respective target proteins, as well as Montelukast and its target protein. Imatinib demonstrated a docking score of 5131.17 with interactions primarily involving Thr338 and Asp404 in the Tyrosine Kinase Inhibitor (PDB ID: 1QCF). In contrast, Diosmin exhibited a higher docking score of 9186.43, engaging multiple amino acids such as Gln277, Phe278, Lys295, and Asn391. Additionally, Montelukast displayed a docking score of 5194.76 with Ser193 in the Cysteinyl Leukotrienes Receptor 1 (PDB ID: 6RZ5), while Diosmin showed a higher score of 9166.68 and interacted with several amino acids including Thr280, Pro176, Phe174, Glu175, and Tyr104 (Table 1 and 2).

These findings suggest potential differences in the binding affinities and interactions of Imatinib, Diosmin, and Montelukast with their respective target proteins. Diosmin, in particular, demonstrated strong interactions with both target proteins, indicating its potential as a modulator of the effects of Imatinib and Montelukast. The higher docking scores and multiple interactions observed with Diosmin suggest a robust binding affinity and potential therapeutic efficacy (Lai *et al.*, 2014; Vologzhanina *et al.*, 2020).

However, it's crucial to acknowledge the limitations of *in-silico* studies, as they provide theoretical insights that require experimental validation. While the computational predictions presented here offer valuable preliminary information, further experimental studies, such as *in-vitro* assays and animal models, are necessary to confirm the actual binding affinities and therapeutic effects of Diosmin, Imatinib, and Montelukast. Additionally, future investigations could explore the mechanisms underlying the observed interactions and potential synergistic effects of these compounds in modulating the target proteins (Fig. 1A, Fig. 1B, Fig. 2A and Fig. 2B).

Effect of Diosmin on hemodynamic parameters: The study examined the impact of Diosmin on hemodynamic parameters in response to lipopolysaccharide (LPS) administration compared to control and other treatment groups. Measurements included average systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), and heart rate (HR) at different time points.

In the control group (CNTRL), minimal changes in hemodynamic parameters were observed throughout the study. Conversely, the LPS group exhibited significant reductions in SBP, DBP, MBP, and HR, indicating compromised cardiovascular function induced by LPS.

Treatment with Diosmin at doses of 50mg/kg and 100mg/kg (DS50+LPS, DS100+LPS) significantly improved hemodynamic parameters compared to the LPS group. Both doses attenuated the decrease in SBP, DBP, MBP, and HR induced by LPS. For example, on day -14 to -15, SBP was restored in the DS50+LPS and DS100+LPS group, while DBP was respectively ($P<0.001$ compared to LPS group).

Interestingly, treatment with Diosmin in combination with montelukast (MNT+LPS) or imatinib mesylate (IMT+LPS) also improved hemodynamic parameters compared to the LPS group, though to a lesser extent than Diosmin alone.

These findings suggest that Diosmin has a protective effect on hemodynamic parameters in LPS-induced cardiovascular dysfunction, likely mediated by its antioxidant and anti-inflammatory properties (Senthamizhselvan *et al.*, 2014; Feldo*et al.*, 2018). Diosmin'svasoprotective and vasodilatory effects may counteract the hypotensive effects induced by LPS, while its antioxidant and anti-inflammatory actions may reduce oxidative stress and inflammation associated with cardiovascular dysfunction (Tong *et al.*, 2013; Feldo *et al.*, 2018) (Table.3).

Effect of Diosmin on hematological Cardiotoxicity biomarkers level and Antioxidants level in heart: The investigation revealed significant alterations in hematological cardiotoxicity biomarkers and antioxidants in response to Lipopolysaccharide (LPS) administration, indicative of cardiotoxicity and oxidative stress. In the LPS group, lactate dehydrogenase (LDH) levels were markedly increased (479±3.71 U/L, $P<0.001$), suggesting myocardial injury. Similarly, creatine kinase-MB (CK-MB) levels showed a significant elevation (124.4±1.20 U/L, $P<0.001$),

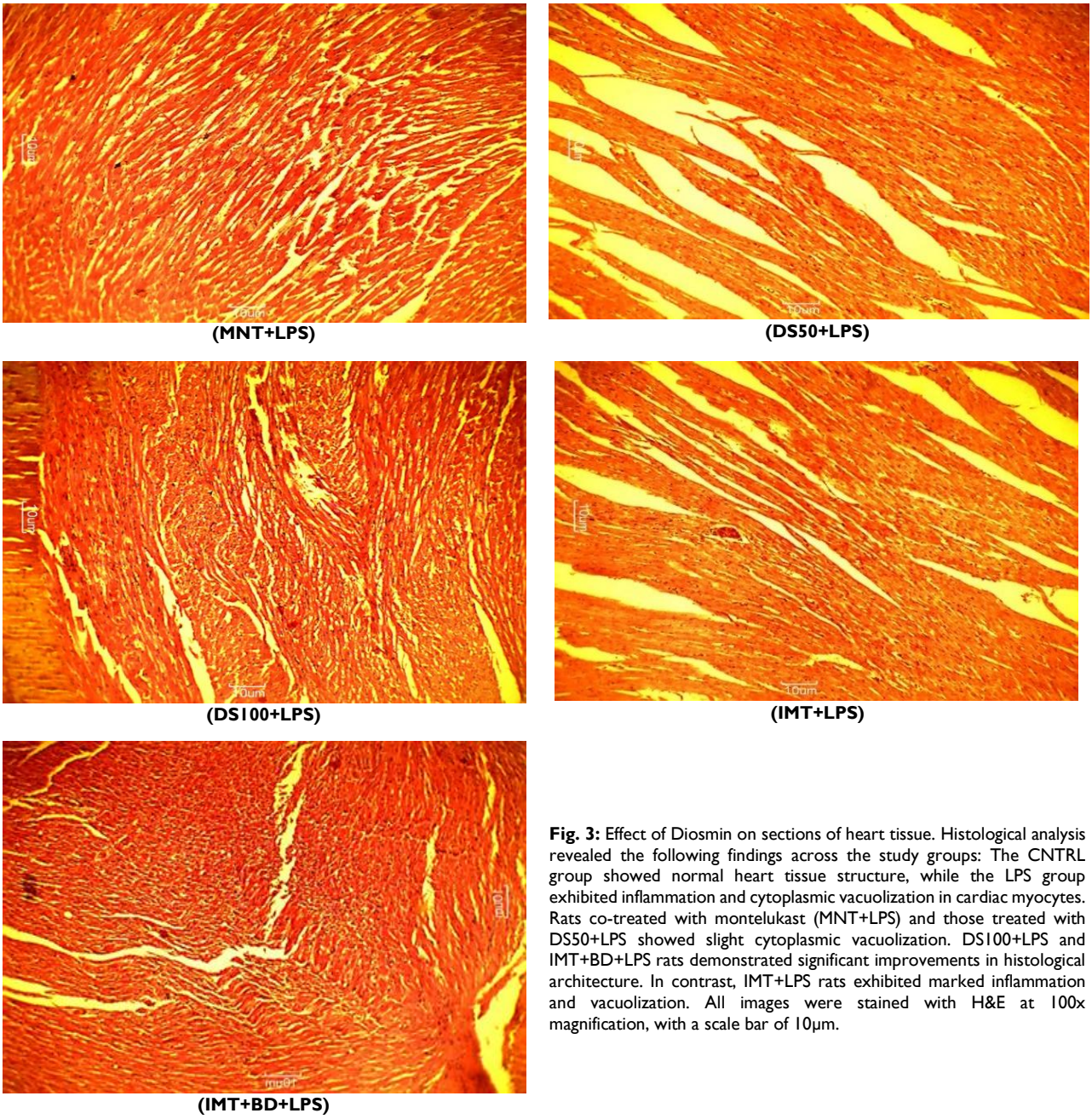

Fig. 3: Effect of Diosmin on sections of heart tissue. Histological analysis revealed the following findings across the study groups: The CNTRL group showed normal heart tissue structure, while the LPS group exhibited inflammation and cytoplasmic vacuolization in cardiac myocytes. Rats co-treated with montelukast (MNT+LPS) and those treated with DS50+LPS showed slight cytoplasmic vacuolization. DS100+LPS and IMT+BD+LPS rats demonstrated significant improvements in histological architecture. In contrast, IMT+LPS rats exhibited marked inflammation and vacuolization. All images were stained with H&E at 100x magnification, with a scale bar of 10µm.

further indicating cardiotoxicity. Alkaline phosphatase (ALP) levels were notably higher in the LPS group (336±5.07 U/L, P<0.001), reflecting cellular damage. Calcium levels were significantly decreased (39.06±0.63 mg/dL, P<0.001), suggesting disturbances in calcium homeostasis. Glutathione (GSH) levels were reduced (66.03±1.81 µmol/mL, P<0.001), indicating oxidative stress. Thiobarbituric acid reactive substances (TBARS) levels were markedly elevated (85.4±0.59 nmol/mL, P<0.001), reflecting lipid peroxidation (Table 4).

Treatment with Diosmin at doses of 50 mg/kg and 100 mg/kg attenuated these adverse effects induced by LPS. Diosmin intervention significantly improved LDH levels compared to the LPS group (P<0.001), at both 50 mg/kg and 100 mg/kg. Similarly, Diosmin mitigated the increase in CK-MB levels compared to the LPS group (P<0.001), at both 50 mg/kg and 100 mg/kg. ALP levels were significantly improved with Diosmin treatment compared to the LPS group (P<0.001), at both 50 mg/kg and 100 mg/kg. Diosmin also significantly improved calcium levels compared to the LPS group (P<0.001), at both 50 mg/kg and 100 mg/kg. Additionally, Diosmin treatment significantly increased GSH levels compared to the LPS group (P<0.001), at both 50 mg/kg and 100 mg/kg. Furthermore, Diosmin attenuated the increase in TBARS levels compared to the LPS group (P<0.001), at both 50 mg/kg and 100 mg/kg (Table 4).

The findings of this study underscore the potential therapeutic efficacy of Diosmin in managing cardiovascular dysfunction induced by LPS. LDH, CK-MB, and ALP are enzymes associated with myocardial damage and cellular injury (Senthamizhselvan *et al.*, 2014). The significant reduction in their levels following Diosmin treatment suggests a protective effect against cardiotoxicity. Furthermore, restoration of calcium levels by Diosmin indicates preservation of cardiac function and calcium homeostasis disrupted by LPS administration. Additionally, incorporating findings from studies on other species, such as

Table 1: In- silico study for Tyrosine kinase the PDB ID 1QCF

Sr. No.	Targets	Tyrosine Kinase Inhibitor (PDB ID: 1QCF)			
	Compound	Dock score	Amino acid	Interaction	Distance Annotation
1	Imatinib	5131.17	Thr338	1	3.06Å
			Asp404	1	3.51 Å
	Diosmin	9186.43	Gln277	1	3.40Å
2			Gln277	2	3.07Å
			Gln277	3	2.78Å
			Phe278	3	3.43 Å
			Phe278	4	3.28 Å
			Lys295	5	2.85 Å
			Lys295	6	2.82 Å
			Asn391	7	3.27 Å

Table 2: In- silico study for Cysteinyl leukotrienes the PDB ID 6RZ5.

Sr. No.	Targets	Cysteinyl leukotrienes receptor 1(PDB ID: 6RZ5)			
	Compound	Dock score	Amino acid	Interaction	Distance Annotation
1	Montelukast	5194.76	Ser193	1	2.97Å
2	Diosmin	9166.68	Thr280	1	3.31Å
			Pro176	2	3.14Å
			Phe174	2	2.74Å
			Glu175	3	3.29 Å
			Tyr104	4	3.40 Å
			Tyr104	5	2.70 Å

Table 3: Effect of Diosmin on hemodynamic parameters.

Study Groups	CNTRL	LPS	MNT+LPS	DS50+LPS	DS100+LPS	IMT+LPS	IMT+BD+LPS
				Average Systolic Blood Pressure			
Day -0	123± 0.83	121.8±1.46	120.2±1.39	121.4±0.67	121.4±0.50	121.4±0.74	120.4±0.87
Day -7	123.4±1.36	119.6±1.56	119±1.41	113.8±2.61	116±1.73	115.4±1.28	121±1.30
Day -14-15	121.4±0.50	79±0.70 ^{c}	120.2±0.58 ^{n}	87.8±0.86 ^{n,r,z}	119.6±0.50 ^{n}	106.4±0.69 ^{n}	119.4±0.50 ^{n}
				Average Diastolic Blood Pressure			
Day -0	83±0.44	80±0.83	81.8±0.37	81.2±0.58	81±0.89	81.8±0.86	82±070
Day -7	82.8±0.66	83.2±1.06	82±0.63	76.8±0.73	78.4±0.24	77±0.44	82.2±0.89
Day -14-15	84.8±1.15	63±1.02 ^{c}	83.6±0.50 ^{n}	70.6±0.50 $^{q, y}$	80.4±0.50 ^{n}	71±0.54	82.4±0.47 ^{n}
				Average Mean Blood Pressure			
Day -0	96.4± 0.50	93.6±0.81	94.8±0.48	94.6±0.24	94.6±0.50	95±0.70	95±0.54
Day -7	96.2±0.86	95.6±1.03	94.6±0.6	89.2±0.96	91±0.44	89.6±0.50	95.2±0.37
Day -14-15	96.8±0.73	68.6±0.81 ^{c}	95.8±0.37 ^{n}	76.4±0.6 ^{n,r,z}	93.4±0.24 ^{n}	83±1.26 ^{n}	93.4±0.4 ^{n}
				Average Heart Rate			
Day -0	335.6±0.98	335.2±0.45	338±0.25	334.6±0.34	336.9±0.56	337±0.83	335.9±0.45
Day -7	341.5±0.42	334.3±0.67	339.6±0.45	335.8±0.53	335.9±0.39	338.5±0.54	336.4±0.76
Day -14-15	342.4±0.45	328.7±0.83 ^{c}	346.2±0.45 ^{n}	331.8±0.54 ^{n,r,z}	348.2±0.56 ^{n}	348.1±0.48 ^{n}	347.8±0.56 ^{n}

The study investigated six experimental groups over a 2-week period. CNTRL received saline for 14 days, while LPS received saline for 14 days with the last dose given 2 hours before lipopolysaccharide (LPS) injection (10mg/kg, i.p) on day 14. MNT+LPS were treated with montelukast (20 mg/kg p.o.) alongside LPS. DS50/DS100+LPS received Diosmin (50 and 100 mg/kg, p.o.) respectively with LPS, while IMT+LPS was administered Imatinib mesylate (30mg/kg p.o) with LPS. IMT+BD+LPS received Imatinib mesylate (30mg/kg p.o) alongside the optimal Diosmin dose plus LPS. Hemodynamic parameters were presented as Mean ± SEM (n=5-6), with statistical significance denoted as ^{c}(P<0.001) when compared with CNTRL, ^{n}(P<0.001) when compared with LPS, ^{r}(P<0.001) when compared with MNT, ^{z}(P<0.001) when compared with IMT+BD, ^{q}(P<0.01) when compared with MNT, and ^{y}(P<0.01) when compared with IMT+BD. Statistical analysis employed Two-way ANOVA followed by Bonferroni's post hoc analysis.

Table 4: Effect of Diosmin on hematological Cardiotoxicity biomarkers level and Antioxidants level in heart.

	Study Groups						
Biomarkers	CNTRL	LPS	MNT+LPS	DS50+LPS	DS100+ LPS	IMT+LPS	IMT+BD+LPS
LDH	162.8±	479±	185.4±	270±	191.4±	216.6±	190±
(U/L)	5.39	3.71 ^{c}	1.63 ^{n}	1.22 ^{n,r,z}	1.96. ^{n}	4.45 ^{n}	1.70 ^{n}
CK-MB	42.2±	124.4±	54.8±	93.6±	60±	71.8±	58.2±
(U/L)	1.15	1.20 ^{c}	1.28 ^{n}	1.86 ^{n,r,z}	0.70 ^{n}	1.39 ^{n}	0.58 ^{n}
ALP	120.6±	336±	136.2±	223.8±	143.4±	150±	140.4±
(U/L)	2.24	5.07 ^{c}	1.06 ^{n}	0.73 ^{n,r,z}	3.41 ^{n,p}	0.70 ^{n}	0.81 ^{n}
Calcium (mg/dL)	9.62±	39.06±	14.38±	25.4±	17.46±	18.58±	15.78±
	0.46	0.63 ^{c}	0.64 ^{n}	0.69 ^{n,r,z}	0.70 ^{n,x}	0.65 ^{n}	0.58 ^{n}
GSH	190.8±	66.03±	151.6±	86.08±	146.2±	122.6±	148.6±
(µmol/ml)	1.16	1.81 ^{c}	1.42 ^{n}	1.10 ^{n,r,z}	2.27 ^{n}	1.18 ^{n,z}	1.75 ^{n}
TBARS	8.22±	85.4±	14.21±	64.05±	16.66±	27.2±	15.44±
(nmol/ml)	0.43	0.59 ^{c}	0.54 ^{n}	0.87 ^{n,r,z}	1.03 ^{n}	0.0.92 ^{n,z}	0.50 ^{n}

The study consisted of several groups over a 2-week period. The CNTRL group received saline for 14 days, while the LPS group received saline for the same duration, with the last dose given 2 hours before lipopolysaccharide (LPS) injection (10mg/kg, i.p) on day 14. MNT+LPS received montelukast (20 mg/kg p.o.) for 14 days alongside LPS. The test groups DS50/DS100+LPS were administered Diosmin (50 and 100 mg/kg, p.o.) respectively with LPS, while IMT+LPS received Imatinib mesylate (30mg/kg p.o) alongside LPS. IMT+BD+LPS received Imatinib mesylate (30mg/kg p.o) alongside the optimal Diosmin dose plus LPS. Values were expressed as Mean ± SEM (n=5-6), with statistical significance indicated as ^{c}(P<0.001) when compared with CNTRL, ^{n}(P<0.001) when compared with LPS, ^{p}(P<0.05), ^{r}(P<0.001) when compared with MNT, and ^{x}(P<0.05), ^{z}(P<0.001) when compared with IMT+BD. The statistical analysis employed One-way ANOVA followed by Tukey's multiple comparison tests.

dogs, where cardiovascular effects have been observed, strengthens the study's relevance to veterinary medicine and demonstrates diosmin's potential across diverse animal populations, emphasizing the importance of species-specific responses (Naseri *et al*., 2023).

Moreover, Diosmin's antioxidant properties were evident in the restoration of GSH levels and attenuation of TBARS elevation. GSH is a crucial antioxidant involved in scavenging free radicals, while elevated TBARS levels reflect lipid peroxidation and oxidative damage. Diosmin's ability to restore GSH levels and mitigate TBARS elevation suggests its role in reducing oxidative stress and preserving cellular integrity in heart tissue (Srinivasan & Pari, 2012). Overall, these results highlight Diosmin as a potential therapeutic agent for managing cardiotoxicity and oxidative stress in cardiovascular diseases.

Effect of Diosmin on sections of heart tissue: Histological examination of heart tissue sections revealed significant differences among the study groups. In the control group (CNTRL), normal histological structure of the heart tissue was observed. Conversely, the LPS group exhibited inflammation of the cardiac wall and cytoplasmic vacuolization of the sarcoplasm of cardiac myocytes, indicative of cardiac damage.

Rats co-treated with montelukast plus LPS (MNT+LPS) showed a generally normal heart wall with slight cytoplasmic vacuolization of some focal cardiac myocytes. In contrast, rats co-treated with Diosmin plus LPS at both 50mg/kg and 100mg/kg doses (DS50+LPS, DS100+LPS) showed varying degrees of cytoplasmic vacuolization in some focal cardiac myocytes, indicating potential protective effects of Diosmin against LPS-induced cardiac damage. Notably, the DS100+LPS group showed marked improvement in histological architecture compared to the LPS group.

Rats co-treated with imatinib mesylate plus LPS (IMT+LPS) exhibited marked inflammation of the cardiac wall and pronounced cytoplasmic vacuolization of cardiac myocytes, suggesting exacerbation of cardiac damage. However, co-treatment with imatinib mesylate and a higher dose of Diosmin plus LPS (IMT+BD+LPS) showed significant improvement in histological architecture, indicating a potential synergistic effect between imatinib mesylate and Diosmin in protecting against LPS-induced cardiac damage.

The histological findings indicate that LPS administration induces cardiac damage characterized by inflammation and cytoplasmic vacuolization of cardiac myocytes. However, treatment with Diosmin, particularly at the higher dose of 100mg/kg, demonstrated protective effects against LPS-induced cardiac damage, as evidenced by the improved histological architecture.

Montelukast co-treatment showed a modest protective effect, while imatinib mesylate exacerbated cardiac damage induced by LPS (Hoxha *et al.*, 2017). Interestingly, co-treatment with imatinib mesylate and a higher dose of Diosmin exhibited significant improvement in histological architecture, suggesting a potential synergistic effect between these compounds (Baek *et al.*, 2021).

These results suggest that Diosmin has a protective role against LPS-induced cardiac damage, possibly mediated by its antioxidant and anti-inflammatory properties. Further research is warranted to elucidate the precise mechanisms underlying Diosmin's cardioprotective effects and its potential synergistic interactions with other drugs, such as imatinib mesylate, in managing cardiovascular disorders induced by inflammatory insults like LPS.

The study's culmination beckoned an exploration into the histopathological alterations within myocardial tissue. The discernment of substantial changes in myocardial tissue structure, infarct area, inflammatory responses, and myocardial fibre separation in the LPS control group underscores the deleterious consequences of LPS induction. Contrastingly, treatment regimens involving Diosmin and Imatinib Mesylate exhibited a noteworthy reduction in these adverse changes. The collective results allude to the protective attributes of Diosmin, particularly when administered in higher doses alongside Imatinib Mesylate, against LPS-induced cardiac dysfunction in rat models. This protective effect might emanate from Diosmin's antioxidant capabilities, demonstrated by its adeptness in scavenging free radicals produced during LPS induction, thereby enhancing cardiac marker activity and reducing lipid peroxidation (Srinivasan & Pari, 2012' Senthamizhselvan *et al.*, 2014). The pivotal role played by Diosmin, particularly at the 100mg/kg dose, was further underscored across all estimations—biochemical, hemodynamic, and histopathological.

Conclusions: In conclusion, this study convincingly highlights Diosmin's potential as a cardioprotective agent, unveiling avenues for safeguarding cardiac health. Through *in-silico* predictions and rigorous experimental validations, a comprehensive journey was undertaken, establishing a strong link between Imatinib Mesylate, myocardial fibrosis, and Diosmin's beneficial effects. The research underscores Diosmin's capacity to counteract Imatinib Mesylate's negative effects, suggesting promising therapeutic avenues for mitigating cardiac issues associated with tyrosine kinase inhibitors. Moreover, Diosmin's anti-inflammatory and antioxidant properties exhibited a beneficial impact on biomarkers associated with lipopolysaccharide-induced cardiac dysfunction. The synergistic effect observed with Imatinib Mesylate further accentuates Diosmin's potential in addressing sepsis-related cardiac complications. The *in-silico* docking investigations provided insights into Diosmin's interactions with target proteins, reinforcing its therapeutic potential. However, rigorous experimental validation remains crucial to further substantiate these findings. Overall, this study provides valuable insights that could pave the way for innovative cardiac interventions aimed at improving both human and animal well-being. While the research was conducted on rats, the findings hold potential relevance for veterinary medicine as well. Authors should emphasize the applicability of these results to both human and animal health, thereby justifying the study's suitability for a veterinary audience.

Ethics approval and animal guidelines: The research procedure for this investigation was authorized by the Ethics Committee of Nanchong Vocational and Technical College. All animal experiments were conducted in strict accordance with animal ethical guidelines.

Conflicts of interest: The authors declare no conflict of interest.

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