RESEARCH ARTICLE

Dose-Dependent Effect of Cardioprotective Properties of Azilsartan in Experimental Model of Myocardial Infracted Diabetic Rats

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ABSTRACT

This investigation aimed to investigate whether azilsartan, a drug that acts as both an angiotensin II receptor antagonist and a limited PPAR-γ inhibitor, can prevent AMI in rats that were administered isoproterenol. The study aimed to determine if azilsartan treatment could reverse the hemodynamic, biochemical, and histopathological variations observed in the rat myocardium. The damaging effects of isoproterenol on the heart were evident from the significant reduction in SAP, DAP, and MAP, in addition to in indicators of MI contraction as well as relaxation (± LVdP/dtmax) together through a surge in LVEDP, an indicator of pre-load. Additionally, the actions of important enzymes like CK-MB, LDH, along with antioxidant enzymes for example, superoxide dismutase (SOD) and catalase, in adding to the stage of glutathione (GSH), were notably reduced. An elevation in malondialdehyde (MDA) content, a marker of oxidative stress, accompanied this. In this study, rats were pre-treated with different doses of azilsartan (1, 5, and 10 mg/kg bw) orally for 14 days before being induced through isoproterenol-induced myocardial injury. The outcomes of the examination presented that azilsartan had a protecting outcome on the myocardium in this experimentally induced model of myocardial infarction (MI). The treatment with azilsartan improved various parameters, positively impacting the damaging effects caused by isoproterenol (ISO).

Keywords: Cardioprotective, ARB, Myocardial infraction, Hemodynamic, Biochemical parameters.

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INTRODUCTION

Cardiac ailments, comprising myocardial infarction (MI), continue in the direction of be a principal reason of morbidity and mortality globally, with diabetes mellitus being a major risk factor that exacerbates the severity and outcomes of these conditions.[1] The complex interplay between diabetes and myocardial infarction presents a unique challenge in the management and treatment of affected individuals. As a result, researchers and clinicians are actively investigating novel therapeutic strategies that target both diabetic and cardiac pathophysiological pathways.[1]

Among the various approaches, the use of angiotensin receptor blockers (ARBs) has gained prominence due to their potential to address diabetes-related complications and cardiovascular protection simultaneously.[1] Azilsartan, a relatively newer member of the ARB class, has garnered attention for its distinctive pharmacological properties, including potent angiotensin II receptor blockade and potential pleiotropic effects beyond the renin-angiotensin-aldosterone system.[2] Studies have demonstrated its capability in the direction of improving endothelial function, decrease oxidative stress, and modulate inflammatory responses, making it an intriguing candidate for mitigating the adverse outcomes of myocardial infarction in diabetic patients.[2]

Azilsartan medoxomil, previously known as TAK-491, is an oral prodrug that has been authorized through the Food and Drug Administration (FDA) as the eighth angiotensin receptor blocker (ARB) for hypertension treatment. Once taken orally, azilsartan medoxomil transforms into azilsartan (TAK-536) through hydrolysis in both the GIT and plasma. Azilsartan, functioning as a selective antagonist of the AT1 receptor, obstructs the binding of angiotensin II in particular regions such as vascular smooth muscles and the adrenal gland. This action leads to vasodilation and diminished effects of aldosterone.[3,4]

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The majority of therapies accessible for myocardial infarction concentrate on reinstating blood flow to oxygen-deprived tissue and mitigating the harm caused during the initial injury. Both experimental and clinical investigations have indicated that oxidative stress, stemming from the creation of free radicals, expressively contributes to the development of myocardial infarction.[5]

Isoproterenol, a synthetic agent that stimulates β-adrenoceptors without specificity, has been recognized for its ability to trigger MI in rats. This is because of the disruption in the natural equilibrium between the generation of free radicals as well as the body's protective antioxidant system. This results in an acute state of myocardial cell death, leading to impaired heart function, heightened lipid peroxidation, changes in the functioning of markers for cardiac injury, and alterations in antioxidant enzyme activity.[1,5]

However, while the cardioprotective potential of azilsartan is promising, the dose-dependent effects of this compound remain an area of interest requiring further exploration. The optimal dosage that elicits the most significant benefits while avoiding potential adverse effects needs to be determined, especially in the context of diabetic myocardial infarction. Animal models serve as invaluable tools for studying such interactions, as they allow for controlled experimentation and investigation of mechanistic pathways that might be challenging to assess in clinical studies.[2-4]

Until now, no research has been conducted to examine how azilsartan impacts cardiac function, markers of cardiac injury, natural antioxidant levels, and tissue structure. As a result, our present research was crafted to explore the latent protecting impacts of azilsartan in rats with isoproterenol-induced myocardial infarction. We aim to gain insights into the underlying mechanisms of these cardioprotective effects.[6]

In this context, the present study holds substantial clinical relevance, as it could potentially provide valuable insights into optimizing azilsartan dosing regimens for managing MI in the diabetic population. Furthermore, a comprehensive understanding of azilsartan's dose-dependent effects and mechanisms could pave the way for personalized treatment strategies tailored to individual patient profiles, thereby enhancing therapeutic outcomes and improving the quality of life for those afflicted by the intricate interplay between diabetes and cardiovascular diseases.

MATERIAL AND METHODS

Materials

Animals

Male wistar albino rats weighing 150 to 200 g were sourced from the National Institute of Bioscience, Pune, with approval from IAEC team with No: (1091/GO/Bt/S/07/CPCSEA). All procedures followed ethical guidelines outlined by the Indian National Science Academy. Rats were housed in controlled conditions (25 ± 2°C (T), 60 ± 5% RH, 12:12 hours. light-dark cycle), delivered with unlimited accessing towards water and food pellets from Nutrivet Life Sciences, Pune, in cages designed for up to four rats.

Drugs and chemicals

Azilsartan, provided as a complimentary sample by Mankind Pharma Limited, India, was mixed with a solution containing 0.5% hydroxyethyl cellulose (HEC). ISO hemisulfate was liquefied in 0.9% saline and utilized in 10 minutes. The CK-MB isoenzyme recognition kit was sourced Logotech, India. All analytical grades chemicals procured from Loba Chemical, Mumbai.

Methods

Induction of experimental myocardial infraction

Rats received subcutaneous (SC) injections of ISO (85 mg/kg) on dual sequential days, specifically on the 13th–14th days, with a 24-hour interval between doses, to induce experimental MI.[7,8]

Experimental groups

This research involved an overall of 128 animals, which were distributed randomly across eight groups, every encompassing 16 rats.

- *Group 1 (vehicle-treated)*

Rats received oral doses of a 0.5% HEC solution (3 mL/kg/day) for 14 days. On the 13–14th days, they were given SC injections of saline (0.3 mL) with a 24-hour gap between doses.

- *Groups 2 to 4 (Azilsartan per se)*

Rats were orally administered azilsartan at dosages of 1, 5, and 10 mg/kg/day for 14 days. On the 13–14th days, they received subcutaneous injections of saline (0.3 mL) with a 24-hour gap between doses.

- *Group 5 (vehicle+ ISO)*

Rats received oral doses of a 0.5% HEC solution (3 mL/kg/day) for 14 days. Additionally, they were given ISO (85 mg/kg) via SC injections on the 13th–14th days, with a 24-hour interval.

- *Groups 6 to 8 (Azilsartan + ISO)*

Rats were orally administered by azilsartan at dosages of 1, 5, and 10 mg/kg/day for 14 days. Simultaneously, they received ISO (85 mg/kg) via subcutaneous injections on the 13th–14th days, with a 24-hour interval.[7,8]

Operation for Record Hemodynamics Factors

Following the operating protocol via Loh et al. (2007), rats underwent anesthesia with sodium pentobarbitone (60 mg/kg, i.p.) and atropine (4 mg/kg, i.p.) in the direction of reducing tracheobronchial excretions. Body temperature was sustained at 37°C. A tracheostomy was executed, as well as rats were ventilated through room air. A polyethylene tube implanted into the left jugular vein enabled constant infusion of 0.9% saline, while another tube in the right carotid artery, associated in the direction of a pressure transducer, measured arterial parameters including SAP, DAP, MAP, and HR.

The heart was visible via a left fifth intercostal space incision. A sterile metal cannula was introduced into the LV, associated in the direction of a pressure transducer for

recording LVP dynamics. Parameters including LVEDP as well as extreme rates of increase besides reduction of LVP were measured. Following hemodynamic measurements, animals were euthanized through anesthesia overdose. Hearts were eliminated for biochemical as well as histopathological investigation, with heart tissue frozen for biochemical studies and tissue fixed in formalin for light microscopic examination.[7]

Biochemical Assessment

Treating of heart tissue

Heart tissue was retrieved from liquid nitrogen storage, weighed, and homogenized in ice-chilled buffer pH 7.4 to create a 10% mixture. This homogeneity was utilized to measure the levels of MDA and GSH. Enzyme activity (CK-MB, LDH, SOD, and catalase) and protein amount were assessed after centrifuging the homogenate at 5000 rpm for 20 minutes at 4°C, yielding a supernatant for further examination.[8]

MDA assessment

MDA levels, indicating lipid peroxidation in cardiac tissue, were assessed. A 0.2 mL specimen was blended by thiobarbituric acid, CH_3COOH, and sodium dodecyl sulfate, heated, besides then cooled. After adding n-butanol:pyridine and water, the mixture was centrifuged. The pink color's absorption in the organic layer was estimated at 532 nm. Standard MDA was utilized for comparison, with results conveyed as nmol/g tissue.[9]

Reduced GSH assessment

Myocardial GSH levels were calculated. In brief, 100 µL of tissue homogenate was blended and centrifuged with an equivalent quantity of 10% TCA. Then, 0.05 mL of the resulting supernatant was combined with a reaction blend encompassing phosphate buffer and DTNB. Absorbance at 412 nm was measured within 10 minutes using a spectrophotometer. GSH levels were quantified using a standard curve generated with GSH standards and conveyed as µg/g tissue.[10]

CK-MB assessment

CK-MB isoenzyme quantification was performed with the Logotech Kit (Delhi, India). Absorbance at 340 nm was recorded every 60 seconds over 3 minutes. Enzyme concentration was expressed as IU per milligram of protein.[8]

LDH assessment

MI LDH estimation followed the Cabaud and Wroblewski (1958) method. The reaction blend included tris-buffer, tissue supernatant, sodium pyruvate, and NADH. Absorbance change at 340 nm was examined for 2 minutes at 30 sec intermissions. LDH content was determined by the reduction of NADH, with enzyme action communicated as IU/mg of protein, referenced against a standard curve established using LDH standards.[11]

Catalase estimation

Catalase activity was assessed following Aebi's (1974) method. Tissue supernatant (50 µL) was blend utilizing phosphate buffer and hydrogen peroxide. Absorbance at 240 nm was supervised each 5 for 30 seconds. Catalase levels were quantified as units mg/protein.[12]

SOD estimation

SOD activity was determined following Marklund and Marklund's (1974) method. Tissue supernatant (100 µL) was blend utilizing phosphate buffer and pyrogallol. Absorbance at 420 nm was recorded for 3 minutes at 30-second breaks. Enzyme stages were quantified as units per milligram of protein.[12]

Protein estimation

Protein content was determined using Bradford's (1976) method. Tissue supernatant (10 µL) was mixed with NaOH and Bradford reagent, and absorbance was recorded at 595 nm. Protein levels were quantified through equating outcomes to a standard curve formed with identified concentrations of BSA.[13]

Histopathological Studies

Light microscope studies

Tissue samples were processed, sliced, and stained with H&E. An examination was conducted using a light microscope, and pictures were taken through a digital camera and investigated utilizing software. A minimum of 4 hearts per group were evaluated, with at least 10arenas/slide categorized for modifications. Importantly, the pathologist conducting the analysis was blinded to the treatment allocation.[8]

Data examination

Mean values along with their standard deviations (SD) were provided. Differences in hemodynamic and biochemical data among groups were assessed using One-Way ANOVA, monitored through a Scheffe Post-hoc test. A level of $p < 0.05$ determined statistical significance.

RESULT

Impact of Azilsartan on Cardiac Functionality

Table 1 illustrates the effect of azilsartan on pressure of artery. ISO-control rats indicated a noteworthy decline in SAP, DAP, and MAP equated towards the vehicle-treated group ($p < 0.001$). Azilsartan management at doses of 1, 5, and 10 mg/kg for 14 days also lowered AP, which equated to the vehicle-treated group, with no notable difference in heart rate between the drug therapy and control groups.

Figures 1 and 2 demonstrate the detrimental effects of ISO on LVEDP and LVdP/dtmax, correspondingly, in addition to the protective effects of azilsartan in restoring these parameters. Isoproterenol-treated rats exhibited increased LVEDP ($p < 0.0001$), and declined +LVdP/dtmax ($p < 0.01$), besides decreased LVdP/dtmax paralleled towards vehicle-treated rats. Azilsartan dosage dependent mitigated the upsurge in LVEDP as well as enhanced LVdP/dtmax compared towards the ISO-control group. At dosages of 5 as well as 10 mg/kg, azilsartan suggestively prohibited increases in LVEDP and enhanced +LVdP/dtmax ($p < 0.01$) and LVdP/dtmax ($p < 0.0001$) paralleled towards ISO-control rats.

Table 1: Result of azilsartan on ABP in ISO-induced MI in rats

Treated groups	*SAP (mm Hg)*	*DAP (mm Hg)*	*MAP (mm Hg)*	*HR (Per Min)*
Vehicle Treated	130.73 ± 13.88	105.21.20 ± 5.02	115 ± 5.28	397.85 ± 12.30
Azilsartan (1-mg/kg)	126.22 ± 12.03	98.20 ± 11.20	1.2.97. ± 12.03	383.22 ± 12.03
Azilsartan (5 mg/kg)	132.20 ± 12.03	95.23 ± 8.20	105.23 ± 5.21	380.22 ± 12.15
Azilsartan (10 mg/kg)	118.23 ± 6.23	95.23 ± 6.21	102.20 ± 5.32	376.5 ± 14.33
Isoproterenol-control	84.32 ± 11.32	76.32 ± 12.36	75.32 ± 10.32	405.23 ± 13.65
Azilsartan(1-mg/kg)+ISO	132.22 ± 7.23	90.33 ± 12.55	102.32 ± 12.33	409.11 ± 12.32
Azilsartan(5 mg/kg)+ISO	115.23 ± 12.35	65.32 ± 3.21	86.23 ± 5.32	423.21 ± 7.23
Azilsartan(10 mg/kg)+ISO	100.23 ± 2.35	88.23 ± 3.21	86.22 ± 7.23	403.00 ± 2.15

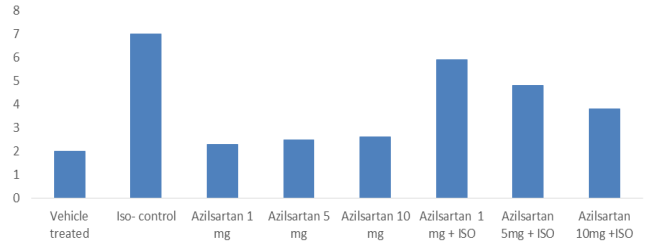

Figure 1: Result of azilsartan on LVEDP induced MI in rats

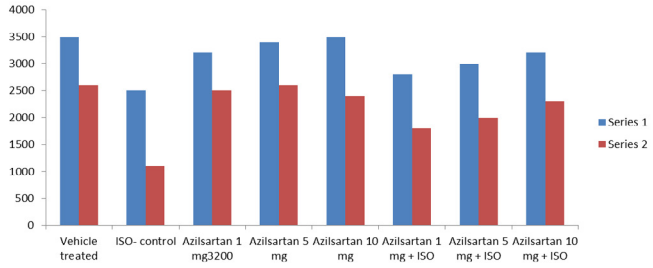

Figure 2: Result of azilsartan on CK-MB and LDH actions in ISO induced MI in rats

Outcome of Azilsartan on Cardiac Injury Markers

Figure 3 illustrates CK-MB isoenzyme as well as LDH activity in rats treated with vehicle or ISO. ISO treatment significantly reduced the activity of these myocardial impairment indicators associated towards vehicle-treated rats (p <0.001). CK-MB and LDH activity decreased from 140.17 ± 3.42-102.10 ± 9.03 and 90.02 ± 3.86-52.18 ± 11.42 IU/mg, respectively, compared to vehicle treatment. Pre-treatment with azilsartan at 5 and 10 mg/kg for 14 days significantly inhibited the normalization of these enzymes induced by isoproterenol (p <0.01)

Impact of Azilsartan on the Actions of Catalase, SOD and GSH

Equated in the direction of vehicle-control rats, isoproterenol-induced cardiotoxicity significantly decreased catalase activity (p <0.001) from 43.53 ± 5.6-25.49 ± 4.81 U/mg protein, SOD activity from 11.72 ± 2.06-6.43 ± 0.82 U/mg protein, then GSH amount from 3.28 ± 0.27 to 1.10 ± 0.20 µ/g tissue. Azilsartan dose-dependently counteracted this effect by increasing antioxidant levels. Notably, a dosage of 10 mg/kg considerably raised up SOD as well as catalase levels (p <0.001). Furthermore, related in the direction of ISO-control rats, animals treated through azilsartan at dosages of 5 and 10

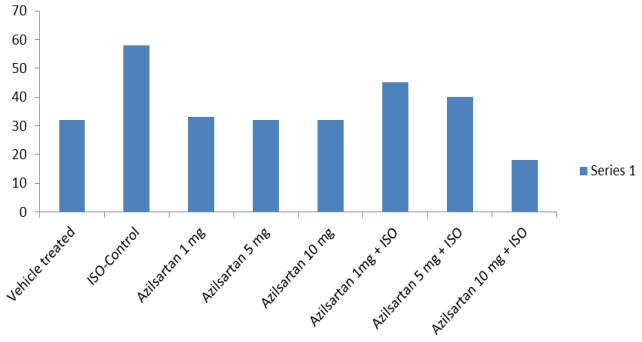

Figure 3: Outcome of azilsartan on level of MAO in ISO induced MI

mg/kg suggestively increased their GSH content (p <0.01 and p <0.001, correspondingly) (Table 2).

Histopathological Investigation

Table 3 illustrates the impact of azilsartan on histological variations in cardiac tissues of rats treated through whichever the vehicle or the drug. A light micrograph of a vehicle-treated heart shows regular structure with no signs of damage. Rats administered with 1, 5, and 10 mg/kg of azilsartan exhibited intact cardiac muscle bundles. Conversely, the isoproterenol-control group displayed severe damage including red blood cell leakage, muscle fiber necrosis, inflammation, edema, and myophagocytosis. Treatment with 1-mg/kg of azilsartan in isoproterenol-treated rats caused related myocardial damage as the control group. However, rats treated with 5 mg/kg of azilsartan showed reduced edema, inflammatory cells, and myonecrosis. Those treated with 10 mg/kg of azilsartan exhibited minimal edema and significantly decreased infarction, indicating preserved myocardial architecture.

Isoproterenol Induced MI in Rats

Microscopic analysis depicted in Figure 4 reveals the normal architecture of MI fibers in a longitudinal subdivision, characterized by central nuclei and a syncytial arrangement of fibers, some of which exhibit pale intercalated disks upon H&E sectioning. Figure 5 illustrates the presence of mononuclear cell infiltration and hemorrhagic myocardium, indicative of myocardial infarction. In Figure 6, degenerative changes and mononuclear infiltration within the myocardium are observed. Finally, Figure 7 demonstrates moderate degenerative changes within the myocardium based on H&E sectioning.

Table 2: Result azilsartan on antioxidant factors of the heart of ISO-induces MI in rats

Treated groups	*Catalase w(U/mg protein)*	*SOD (U/mg protein)*	*GSH (µ/g tissue)*
Vehicle treated	43.53 ± 5.6	12.55 ± 1.98	3.40 ± 028
Azilsartan (1-mg/kg)	40.12 ± 6.12	13.01 ± 1.66	3.45 ± 035
Azilsartan (5 mg/kg)	40.82 ± 2.48	12.55 ± 3.02	3.25 ± 0.28
Azilsartan (10 mg/kg)	40.23 ± 1.98	12.44 ± 3.02	3.15 ± 0.34
ISO-Control	25.33 ± 3.52[a]	5.87 ± 0.78[a]	1.20 ± 0.30[a]
Azilsartan (1-mg/kg) + ISO	30.25 ± 5.82	6.55 ± 064	1.70 ± 0.23
Azilsartan (5 mg/kg) + ISO	34.25 ± 2.98	8.95 ± 0.65	2.36 ± 0.39[b]
Azilsartan (10 mg/kg) + ISO	35.60 ± 3.02[c]	11.02 ± 0.05[c]	3.22 ± 0.25[c]

Table 3: Outcome of azilsartan on histopathological estimation of myocardium of rats

Treated groups	*Myonecrosis*	*Inflammatory cells*	*Edema*
Vehicle treated	-	-	-
Azilsartan (1-mg/kg)	-	-	-
Azilsartan (5 mg/kg)	-	-	-
Azilsartan (10 mg/kg)	-	-	-
ISO-Control	+++	+++	+++
Azilsartan (1-mg/kg) + ISO	++	+++	++
Azilsartan (5 mg/kg) + ISO	+	++	+
Azilsartan (10 mg/kg) + ISO	-	+	-

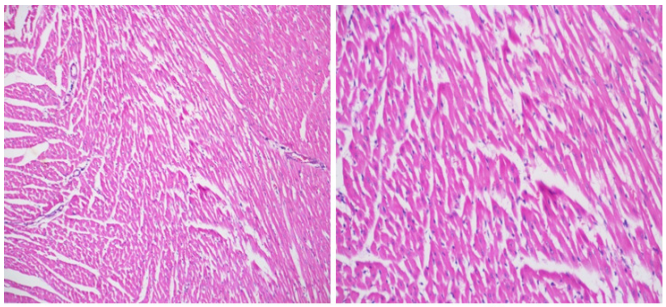

Figure 4: Histopathology examination of negative control: H_2O 10 mL/kg p.o. as well as positive control: Isoproterenol 85 mg/kg s.c

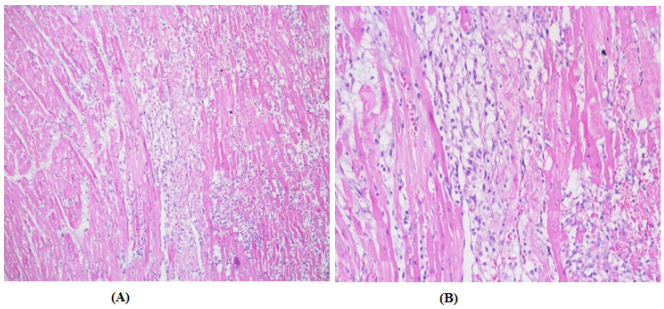

Figure 5: Histopathology examination of negative control: azilsartan 1-mg/kgp.o. and positive control: Isoproterenol 85 mg/kg s.c

DISCUSSION

ISO administration in rats mimics human MI indications, crucial for studying necrosis mechanisms and treatments[2]. Myocardial damage syndrome contains cardiac dysfunction, lipid peroxidation, and weakened antioxidant defenses, in addition to cell necrosis because of ISO-induced oxidative stress via adrenochrome generation.[2,14-18]

The research examined the impact of azilsartan, an angiotensin II receptor blocker, on cardiac functionality, and histopathological alterations, in addition to cardiac injury markers in rats with isoproterenol-induced MI. The results revealed significant improvements in several parameters following azilsartan treatment, indicating its potential therapeutic efficacy in MI. Firstly, azilsartan treatment led to a dose-dependent reduction in arterial blood pressure, with significant decreases observed in SP, DP, and MAP compared to the isoproterenol-control group. This suggests azilsartan's ability to mitigate the hypertensive response associated with MI.[19-21]

Secondly, azilsartan administration ameliorated ventricular dysfunction induced by isoproterenol, as evidenced by decreased LVEDP and improved LVdP/dtmax parameters. These improvements indicate enhanced cardiac function and contractility following azilsartan treatment, potentially attributed to its anti-inflammatory and anti-fibrotic effects.

Furthermore, azilsartan treatment attenuated myocardial injury, as indicated by decreased levels of cardiac damage markers for example, CK-MB isoenzyme then LDH action. This protective effect suggests azilsartan's ability to preserve myocardial integrity and reduce cellular damage associated with MI.[22-24]

The histopathological examination revealed that azilsartan treatment mitigated histological alterations in cardiac tissues induced by isoproterenol, including myonecrosis, inflammatory cell infiltration, and edema. Higher dosages of azilsartan (5 and 10 mg/kg) demonstrated noteworthy reductions in infarct size and inflammatory response, indicating preserved MI and improved tissue integrity.[24,25]

Additionally, Azilsartan treatment modulated antioxidant parameters in the heart, enhancing the actions of catalase, SOD, and GSH levels. This antioxidant effect may contribute to azilsartan's cardioprotective properties by reducing oxidative stress and preventing further myocardial damage.[25-27]

Overall, the findings suggest that azilsartan exerts beneficial effects on cardiac function, histopathological alterations, and cardiac injury markers in isoproterenol-induced MI rats. These

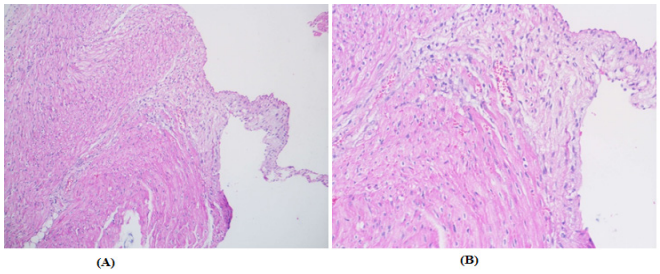

Figure 6: Histopathology examination of Negative control: azilsartan 5 mg/kgp.o. and positive control: Isoproterenol 85 mg/kg s.c

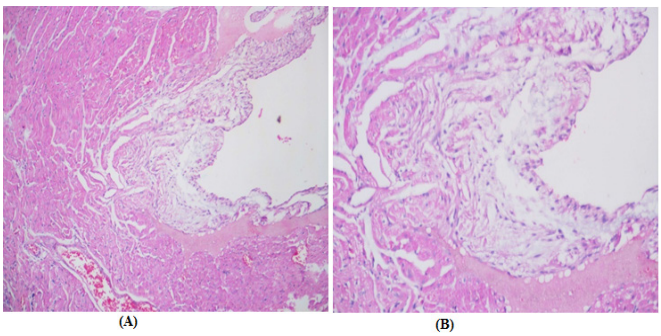

Figure 7: Histopathology examination of Negative control: azilsartan 10 mg/kgp.o. and positive control: Isoproterenol 85 mg/kg s.c

results highlight the potential therapeutic utility of azilsartan in controlling MI in addition to connected cardiac difficulties. Further clinical examinations are warranted to validate these findings and explore azilsartan's clinical efficacy and safety in human patients with MI.

CONCLUSION

In conclusion, azilsartan shows promising cardioprotective impacts in isoproterenol-induced MI in rats. Acting on both angiotensin II receptors and as a partial inhibitor of PPAR-γ, it mitigates hemodynamic, biochemical, and histopathological changes associated with myocardial injury. Improved cardiac function, enzyme activities, and antioxidant defenses, alongside reduced oxidative stress and histological damage, highlight its potential therapeutic efficacy. Azilsartan's dual mechanism of action offers novel treatment avenues, particularly in managing diabetes-related cardiovascular complications. Further research into its mechanisms and clinical effectiveness in humans is warranted, emphasizing its latent as a valuable therapeutic agent in cardiovascular disease management.

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