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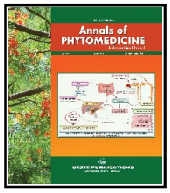

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Exploring the effect of rosuvastatin on AGE-RAGE pathway concerning its cardioprotective potential

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Abstract

Cardiovascular diseases encompass several illnesses, including acute coronary syndrome. Advanced glycation end products (AGEs) are produced through the non-enzymatic glycation and oxidation of proteins, lipids and nucleic acids. Rosuvastatin, a drug with multiple pleiotropic characteristics, has been studied for its cardioprotective effects in isoproterenol-induced myocardial injury and the role it plays in altering AGE and contributing to cardiac damage. The research involved administering a daily dose of 10 mg/kg of rosuvastatin orally to male rats for four weeks, followed by the administration of isoproterenol (85 mg/kg, subcutaneously) on the 29th and 30th days to induce cardiac damage. On the 31st day, rats were subjected to euthanasia and samples were collected. The administration of isoproterenol resulted in an increase in the level of oxidative stress parameters. As a result of heart injury, advanced glycation end products were observed to increase. The AGE-RAGE cascade also had a detrimental effect on the echocardiogram of the rat heart. According to the study, rosuvastatin has cardioprotective effects on the experimental model, which were supported by an array of parameters.

1. Introduction

Atherosclerosis obstructs coronary arteries, causing impaired nutrient delivery to cardiomyocytes and accelerated cell death, impairing cardiac function and resulting in acute myocardial infarction (AMI). Due to the ensuing myocardial ischemia, immune cells mobilize and initiate inflammatory signaling, which produces reactive oxygen species and molecular damage. AMI advances as a result of these mechanisms (McAlindon *et al*., 2015). Complex chemicals known as advanced glycation end products (AGEs) arise when reducing sugars or other α-carbonyl compounds interact chemically with amino groups found in proteins, lipids, and nucleic acids (Stirban *et al*., 2014; Won *et al*., 2012). The Millard reaction, a reversible process of reducing sugars condensing with a free amino group, is what drives the creation of these molecules. A wide range of health problems have been connected to the formation of AGEs, which are extremely varied. To prevent or lessen the harmful effects of AGE formation, it is essential to comprehend the intricate chemistry involved in their production (Ott *et al*., 2014). Once the Schiff's base has been synthesized, a ketosamine structure can be created by Amadori rearrangement. Amadori product finally forms AGEs (Stirban *et al*., 2014). It is significant to remember that this reaction happens quickly and depends on the amount of accessible carbohydrates, which is increased in hyperglycemic circumstances. Therefore, the production of AGE may have adverse effects on a number of biological systems (Gul *et al*., 2013; Qiu *et al*., 2018). The accumulation of glycated molecules is linked to both a rise in AGE synthesis and a fall in the degradation of modified proteins. Another method that rapidly separates glycated intracellular proteins is the ubiquitin-proteasome system (Ott *et al*., 2004). The AGER-1 receptor facilitates partial proteolysis. The second generation of AGEs, soluble low-molecular-peptide products of AGE degradation, are produced in macrophages. It has been shown that AGEs have a variety of physiological effects, including ability to cause inflammation and accelerate the development of chronic illnesses. Though, research on AGEs' involvement in the etiology of various illnesses is still in process, it is becoming clearer how they affect human health (Stirban *et al*., 2014).

AGEs attach to cell surface receptors and initiate signaling pathways that produce pro-inflammatory cytokines, which in turn cause inflammation (Stirban *et al*., 2014; Maryam *et al*., 2023; Kamalakkannan *et al*., 2021). AGEs cause malfunctioning of tissues and organs because they activate specific receptors called receptor for advanced glycation end product (RAGE). Oxidative stress, inflammation, and even death may arise from this (Gul *et al*., 2013; Kiuchi *et al*., 2001). Empirical investigations have demonstrated that AGEs cause complex vascular lesions. The circulation contains AGEs, which are responsible for causing atherosclerotic lesions in the aorta (Stirban *et al*., 2014). According to research there is a relationship between the quantity of coronary lesions in ischemic heart disease patients and their AGE readings (Kiuchi *et al*., 2001; Blumenthal *et al*., 2000). Because of their effectiveness in reducing cholesterol levels, statins, also known as 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors are frequently employed in the treatment of coronary heart disease. Statins have been shown to have benefits beyond only lowering cholesterol. These include stabilizing plaque, decreasing inflammation, and preventing endothelial dysfunction. Because of this, doctors are turning more

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and more to statins as the recommended course of treatment for a variety of cardiovascular conditions. Because of all of their advantages, statins are now often prescribed by doctors to treat and manage a variety of cardiovascular diseases (Stalker *et al*., 2001; Yang *et al*., 2010). Recent research has revealed that statins protect endothelial cells from damage induced by ischemia-reperfusion HMGB1/TLR4 pathway (Han *et al*., 2015; Ma *et al*., 2016; Gaurav *et al*., 2023). To properly understand whether statins can affect the HMGB1/RAGE/NF-' B cascade and the creation of inflammatory molecules, more research is needed. Along with inhibiting HMG CoA reductase, rosuvastatin, the newest statin, also has pleiotropic benefits, such as increased endothelial function, antioxidant, anti-inflammatory, and anti-thrombotic properties, as well as a decreased risk of cardiovascular events and death. A number of cellular processes are greatly impacted by rosuvastatin, which also mitigates the detrimental effects of AGE-RAGE.

In order to fill the research gap, the current study explores how the AGE-RAGE axis contributes to cardiac damage and suggests ways to improve it.

2. Materials and Methods

2.1 Animal

The research involved 24 adult male Sprague Dawley rats weighing between 150-180 g. These animals were housed in the Animal house at the Faculty of Pharmacy, Integral University Lucknow, under optimal conditions. This included being placed in polypropylene cages, maintaining a constant temperature of 24 ± 2°C, and alternating light and dark cycles every 12 h. Additionally, the animals were regularly examined and their cages cleaned daily while being provided with unrestricted access to food and water. The animals underwent a fasting period of 12 h and their handling and treatment were following the guidelines set forth by the Institutional Animal Ethics Committee before their sacrifice.

2.2 Ethical approval

The experimental procedures were carried out according to the guidelines laid down by the Committee for the Control and Supervision of Experiments on Animals (CCSEA). The research protocol has been duly ratified by the Institutional Animal Ethical Committee (IAEC) with an approval number of IU/IAEC/21/09, and registered under the reference number 1213/PO/Re/S/08/CPCSEA dated 5 June 2008. These ethical and procedural clearances were obtained from the faculty of pharmacy at Integral University, Lucknow, Uttar Pradesh, India.

2.3 Treatment protocol

Before the dosing process, a total of 24 rats were randomly divided into four groups, each consisting of six rats. The rats were first weighed, and their weight ranged from 150 to 180 g. Before the initiation of study, the rats were housed individually in separate cages for seven days to allow them to adapt to the study facility's conditions.

Group I: Normal control group (NCG): Rats were administered with normal saline 10 ml/kg p.o for 28 days.

Group II: Isoproterenol group (ISG): Isoproterenol (85 mg/kg/day, s.c.) twice at an interim of 24 h on the 29th and 30th day.

Group III: Treatment group (TG): Rosuvastatin (10 mg/kg b.w.) dissolved in normal saline and administered per orally (p.o.) by intubation method once a day for 28 days and isoproterenol (85 mg/kg/day, s.c.) twice at an interim of 24 h on 29th and 30th day.

Group IV: *Per se* group (PSG): Rosuvastatin (10 mg/kg b.w.) dissolved in normal saline and administered per orally (p.o.) by intubation method once a day for 28 days.

2.4 Serum preparation

After obtaining the blood samples, they were placed in a dry test tube and left to clot for 35 min at room temperature. Blood samples were centrifuged for 10 min at 5000 rpm in order to extract serum. After centrifugation, serum was carefully extracted from the tube using a micropipette. The cardiac markers assay was then performed using the serum samples.

2.5 Tissue homogenate preparation

Three millilitres of 0.25 M sucrose buffer (pH 7.4) washed on ice was used to homogenize the 0.3 g of heart tissue. The resultant homogenate was centrifuged after being treated with 30 L of Triton X-100 and allowed to cool for 30 min. After 10 min of spinning at 3000 rpm and 4°C, the glucose and AGE levels in myocardial homogenate were determined. Before being analysed, the supernatant was kept at -40°C. Test tubes with drawn blood samples were left to clot. The serum that was produced was then kept in Eppendorfs at -40°C until analysis, after which they were centrifuged for 15 min at 1500 rpm.

2.6 Measurement of heart: Body weight ratio

The animals' weight was determined and documented following their euthanasia. Laying on their back, the rats had their limbs spread out and were pinned to the board. The rats were cleaned or soaked with 70% ethanol in order to get rid of fur. The procedure to remove the heart involved dissecting the aortic root just above the aortic valves and superior vena cava above the atria. Pushing it against a Kim swab, the heart was extracted (Kaiserova *et al*., 2006).

2.7 Echocardiography measurement

Rats were anesthetized with an intraperitoneal injection of chloral hydrate (5%, 0.7 ml/100 g, or 350 mg/kg) in order to evaluate the cardiac function of the rats using echocardiography. After that, images were captured with a 12-MHz linear transducer and a Vivid 7 echocardiography instrument (GE Healthcare Life Sciences, Chalfont, UK). The level of the papillary muscle was used to acquire two-dimensional targeted M-mode tracings and a two-dimensional short-axis picture of the left ventricle. The identifying markers were diastolic left ventricular internal dimension (LVIDd), systolic left ventricular internal dimension (LVIDs), fractional shortening of the left ventricle (LVFS), left ventricular ejection fraction (LVEF), and left ventricular end-diastolic pressure (LVEDP).

2.8 Measurement of aldose reductase activity

Two intraperitoneal infusions of ketamine (200 mg/kg) and xylazine (40 mg/kg) were used for euthanasia. Spectrophotometric analysis was performed on the aortic homogenates to determine the activity of aldose reductase (Mclellan *et al*., 1993). Bradford test was used to determine the protein content.

2.9 Measurement of glyoxalase 1 (GLO-1) activity

According to McLellan and Thornalley's approach (Mclellan *et al*., 1993), spectrophotometry was used to evaluate GLO-1 activity. At a temperature of 25°C, it entailed tracking the rise in absorbance at 240 nm over the course of 10 min. The production of S-D-lactoylglutathione was the cause of this rise in absorbance (Untereiner *et al*., 2011).

2.10 Measurement of methylglyoxal (MG)

Using HPLC techniques on neutralized perchloric acid extracts of cardiac tissue, MG, a key precursor in the development of AGE was identified (Untereiner *et al*., 2011).

2.11 Measurement of AGE

1 × 9 PBS solution was used to wash the cardiac tissues, and then 1 ml of 1 × 9 PBS was used for homogenization. 20°C was the overnight storage temperature for the resultant samples. The homogenates were then centrifuged for 5 min at 5000 rpm after two freeze-thaw cycles were used to break down the cell membranes. For additional analysis, the supernatant was gathered. Using a rat-specific ELISA kit (ABIN368041, antibodies-online GmbH, Aachen, Germany) and following the manufacturer's instructions, homogenates and serum were analyzed to measure the levels of AGE.

2.12 Measurement of HMGB1

The HMGB1 ELISA kit A76696, by Sigma-Aldrich, was a commercially available test used to assess HMGB1 levels.

2.13 Measurement of receptor for advanced glycation end product (RAGE)

Measurements of RAGE levels were made using a commercial Rat RAGE/AGER ELISA kit (product number #RAB009, Sigma-Aldrich).

2.14 Histopathological findings

Histopathological slides were made from heart tissue and protocol-compliant photos were obtained. The hearts and lipids were removed with care after the animals were euthanized. Using a microtome, they were sectioned into 5-6 µm slices after being quickly fixed in 10% buffered formalin for 48 h, dried by progressive immersion in different water-ethanol concentrations, cleaned in xylene and then embedded in paraffin once again. Hematoxylin and eosin were utilized to stain the sections (Yang *et al*., 2013).

2.15 Statistical analysis

The analysis was expressed as mean ± SD (Standard deviation). Statistical analysis was performed by using one-way ANOVA, followed by Dunnett's test: Compare all Vs control (Graph Pad Instat, USA).

3. Results

3.1 Heart: Body weight ratio

Heart: Body weight ratio is determined by dividing the entire weight of the heart by the complete weight of the body. This ratio is computed mathematically. In the case of rats that were a part of the isoproterenol group, it was discovered that the heart: body weight ratio was statistically highly significant (p<0.001) when compared to the normal control group. The group that was administered with rosuvastatin displayed a statistically very significant (p<0.01) decrease in the heart: Body weight ratio when compared to the isoproterenol group. However, the *per se* group did not show any significant (p>0.05) change in terms of heart: body weight ratio when compared to the normal control group. Heart: Body weight ratio is shown in Figure 1.

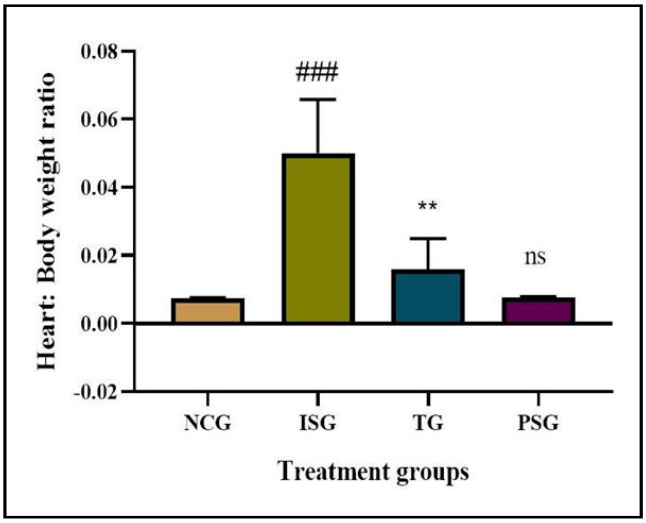


Figure 1: Heart: Body weight ratio in different groups. All values are expressed as mean ± SD; (n=6) in each group. Data was subjected to one-way ANOVA, followed by Dunnett's test when normal control group (NCG) was compared to isoproterenol control group (ISG), treatment group (TG) was compared to isoproterenol control group (ISG), while per se (PSG) was compared to normal control group (NCG). ^{ns}p>0.05, $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001 when compared to normal control group (NCG), $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001 when compared to isoproterenol control group (ISG).

3.2 Echocardiography measurement

Isoproterenol-induced hearts suffered considerable damage after the experiment. The ISO group exhibited worse LVEF and FS than the normal control group, indicating systolic dysfunction. We observed a gradual rise in LV diameters over time in the ISG group in the same manner. The isoproterenol-induced hearts had a reduced infarcted area in the treatment group. After 28 days of rosuvastatin treatment, the echocardiography markers were found to be at normal levels. The findings were effectively reversed by the rosuvastatin-treated group (10 mg/kg). The PSG group did not show any significant difference when compared to the NCG group. Echocardiography measurement is shown in Table 1.

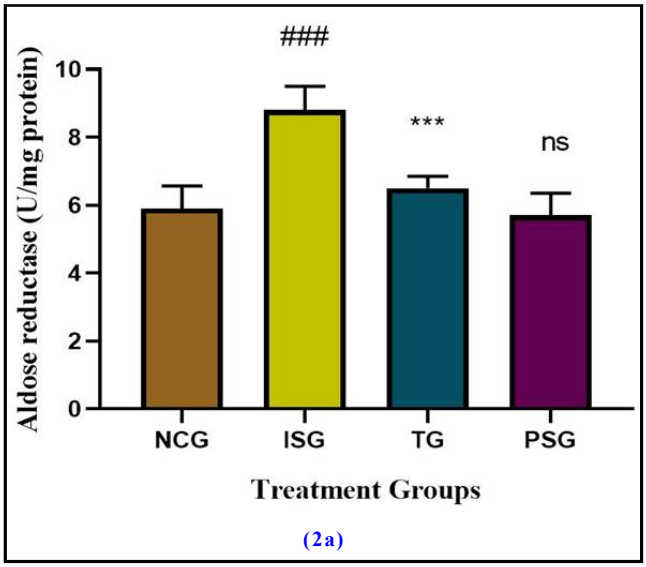
Table 1: Ejection fraction, fractional shortening, LV systolic diameter, LV diastolic diameter, infarct size of treatment group

	NCG	ISG	TG	PSG
Ejection fraction (%)	64.6	$32.3^{###}$	49.1^{***}	63.9^{ns}
Fractional shortening (%)	37.2	$19.7^{###}$	31.5^{***}	38.6^{ns}
LV systolic diameter (mm)	3.7	$7.9^{###}$	6.8^{***}	3.9^{ns}
LV diastolic diameter (mm)	7.8	$12.7^{###}$	11.4^{***}	12.4^{ns}
Infarct size (%)	"	42±1.7###	12±0.8***	"

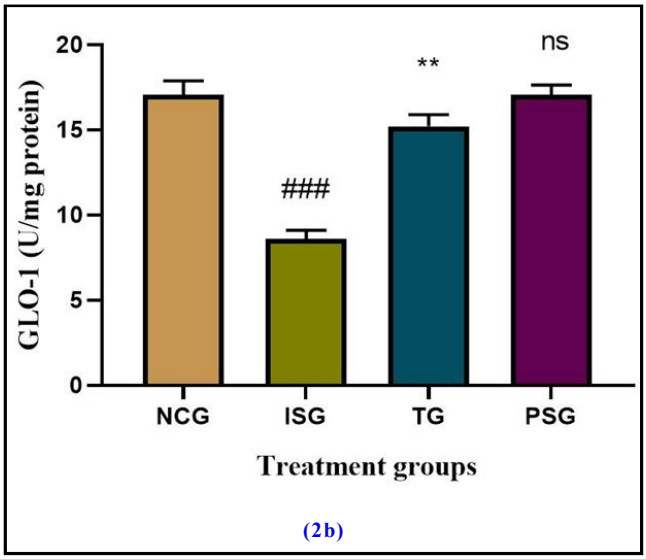
All values are expressed as mean ± SD; (n=6) in each group. Data was subjected to one-way ANOVA, followed by Dunnett's test when normal control group (NCG) was compared to isoproterenol control group (ISG), treatment group (TG) was compared to isoproterenol control group (ISG), while per se (PSG) was compared to normal control group (NCG). $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group (NCG), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to isoproterenol control group (ISG).

3.3 Measurement of AGE

ISG group showed an increase in the activity of aldose reductase and a decrease in the activity of Glo-1 and higher levels of MG and AGE. Additionally, the serum RAGE level was upregulated, which suggested that the AGE-RAGE pathway was activated in the ISG group. The results showed that the presence of AGEs level in the ISG induced group was significantly higher when compared to the NCG group. However, treatment with rosuvastatin improved the serum AGE level when compared to the ISO group. The PSG group did not show any significant changes in AGE levels when compared to the NCG group. Measurement of AGE is shown in Figure 2.



(2a)



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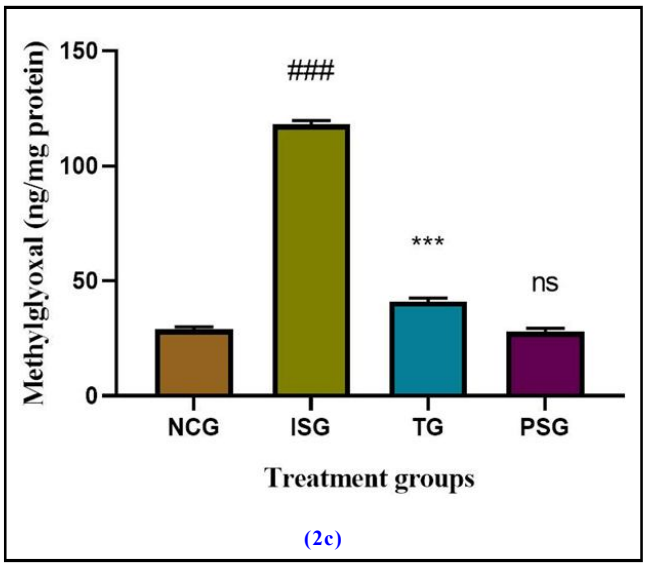

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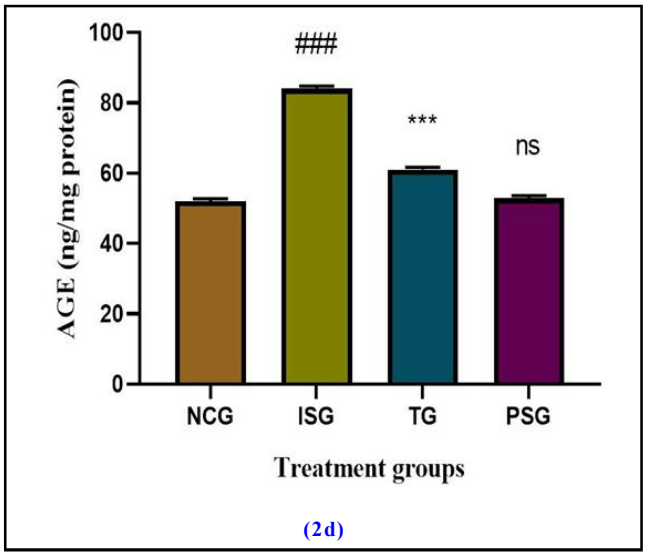

(2d)

Figure 2: Measurement of AGE pathway in the different groups. All values are expressed as mean ± SD; (n=6) in each group. Data were subjected to one-way ANOVA, followed by Dunnett's test when normal control group (NCG) was compared to isoproterenol control group (ISG), treatment group (TG) was compared to isoproterenol control group (ISG), while per se (PSG) was compared to normal control group (NCG). $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group (NCG), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to isoproterenol control group (ISG).

3.4 Measurement of RAGE

To further investigate the protective role of rosuvastatin in preventing cellular death, we conducted an analysis of its target genes. The total RAGE levels in serum increased by 50% in the ISO group when compared with the NCG group. However, treatment with rosuvastatin effectively ameliorated the serum RAGE level when compared with the ISO group. It is noteworthy that the PSG group did not show any changes in RAGE levels when compared to the NCG group. Measurement of RAGE is shown in Figure 3.

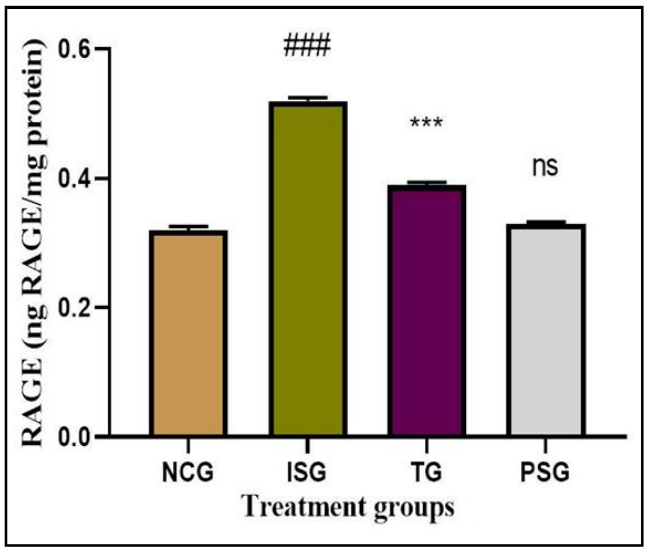

Figure 3: RAGE levels in the different groups. All values are expressed as mean ± SD; (n=6) in each group. Data were subjected to one-way ANOVA, followed by Dunnett's test when normal control group (NCG) was compared to isoproterenol control group (ISG), treatment group (TG) was compared to isoproterenol control group (ISG), while per se (PSG) was compared to normal control group (NCG). $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group (NCG), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to isoproterenol control group (ISG).

3.5 Measurement of high-mobility group box-1 (HMGB1)

HMGB1 has been identified as a ligand for the receptor for advanced glycation end-products (RAGE), with the HMGB1-RAGE interaction being linked to cardiac dysfunction. In comparison to the normal control group, the administration of Isoproterenol significantly increased the levels of HMGB1 in the heart. However, pre-treatment with rosuvastatin was found to decrease the ISO-induced elevation of serum HMGB1. Notably, the PSG group did not exhibit any changes in HMGB1 levels when compared to the NCG group. Measurement of HMGB1 is shown in Figure 4.

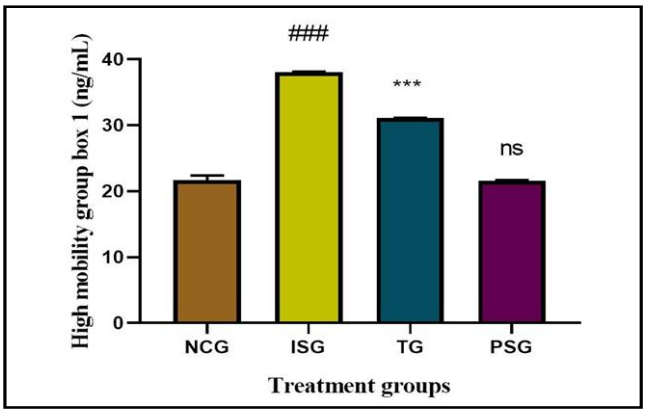

Figure 4: High mobility group box 1 in the different groups. All values are expressed as mean ± SD; (n=6) in each group. Data were subjected to one-way ANOVA, followed by Dunnett's test when normal control group (NCG) was compared to isoproterenol control group (ISG), treatment group (TG) was compared to isoproterenol control group (ISG), while per se (PSG) was compared to normal control group (NCG). $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to normal control group (NCG), $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to isoproterenol control group (ISG).

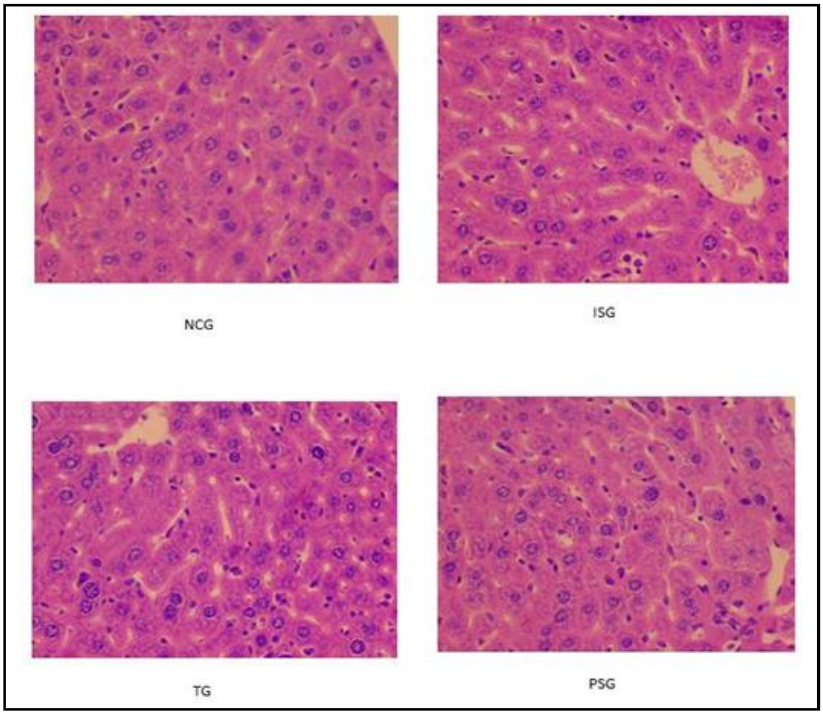

Figure 5: Histopathological images of the heart in different treatment groups.

3.6 Histopathology

The histological investigation revealed that the myocardium, endocardium, epicardium, papillary muscle fibres and vasculature were all regularly aligned in the normal control (NC) rats that were given a daily dose of saline. The isoproterenol group (ISG) showed thrombi, sporadic acute aneurysms, wall lesions, and substantial infarction in sharp contrast to this. It is interesting to note that the rosuvastatin group (TG) showed focal lesions devoid of any lymphocytic infiltration, myonecrosis, or myophagocytosis symptoms. Lastly, the *per se* group (PSG) displayed a consistent pattern of myofiber striae with nuclei in the center and a systemized pattern showing no vacuolation. Histopathology of different treatment groups is shown in Figure 5.

4. Discussion

The development of cardiovascular ailments is significantly influenced by the activation of the AGE/RAGE pathway. Lowering AGE-RAGE activation is beneficial, as demonstrated by multiple animal models of cardiovascular disorders. In addition to its well-known benefits of decreasing cholesterol, rosuvastatin, a highly efficient statin, has also been shown to have anti-inflammatory and endothelium-protective properties (Greque *et al*., 2016; Bonsu *et al*., 2015; Okamoto *et al*., 2002).

Furthermore, growing body of research indicates that rosuvastatin may be able to lower blood levels of AGEs and the expression of AGE receptors (RAGE) in heart damage. It appears that this novel molecular mechanism explains rosuvastatin's pleiotropic effects (Calkin *et al*., 2008; Cuccurullo *et al*., 2006). The results of this study indicate that rosuvastatin improved AGE-RAGE. Intracellular oxidative stress is produced by AGE/RAGE activation and then triggers the redox-sensitive transcription factor NF-κB (Fei *et al*., 2016). The results of this investigation indicate that by focusing on the AGE-RAGE axis, rosuvastatin can provide therapeutic effects. Rosuvastatin can also stop the long-term negative effects of ADR on left ventricular function (Kim *et al*., 2012). Pre- and post-conditioning with rosuvastatin can reduce ischemia/reperfusion myocardial injury by decreasing HMGB1 (Ke *et al*., 2013; Du *et al*., 2014). Furthermore, statins primarily lower blood cholesterol levels, but they also have pleiotropic effects on the AGE-RAGE axis that are regarded as secondary effects. Many studies have used isoproterenol-induced myocardial damage to examine how it affects AGE-induced myocardial infarction. Thus, this study's findings contribute to the body of knowledge on the therapeutic advantages of rosuvastatin and its function in reducing the negative effects of the AGE-RAGE axis (Abbas *et al*., 2016; Kilhovd *et al*., 2005).

In the current investigation, AGE induction dramatically raised serum AGE accumulation. Previous study has demonstrated a clear association between the degree of coronary artery disease and the amounts of circulating AGEs, leading to unfavourable clinical outcomes (Yamagishi *et al*., 2005). However, the exact mechanisms driving this association remain unclear. However, it has been discovered that rosuvastatin lowers the serum level of AGEs in a way that is independent of lowering cholesterol and time-dependent. Oxidative stress, a cause of free-radical superoxide production, is what forms AGEs (Nishikawa *et al*., 2000; Aviram *et al*., 1998). Furthermore, it has been shown that rosuvastatin's hydroxyl metabolites have anti-oxidative properties (Takemoto *et al*., 2000).

We examined in this work the effectiveness of statins in preventing the proliferation of vascular smooth muscle cells (VSMCs) by suppressing the inflammatory pathway and proliferation induced by reactive oxygen species (ROS). The generation of reactive oxygen species (ROS) is commonly recognized as occurring when NADPH oxidase is activated by the interaction of advanced glycation end products (AGE) with receptors for AGE (RAGE). We propose that the AGE-RAGE interaction could be an upstream mechanism that increases ROS generation and, in turn, VSMC proliferation (Haendeler *et al*., 2004; Giroux *et al*., 1993).

Through the S-nitrosylation of thioredoxin, rosuvastatin has been shown in recent experiments to have antioxidant qualities and the potential to reduce ROS in endothelial cells. Additionally, the results imply that rosuvastatin may function as an antioxidant in rabbit models of experimental atherosclerosis by blocking the oxidation of low-density lipoprotein (LDL) by means of activated macrophages derived from monocytes. According to our research, the activation of AGEs led to an increase in ROS generation, whereas the administration of statins had the opposite effect (Singh *et al*., 1997; Ain *et al*., 2022).

According to the current study, rosuvastatin treatment significantly lowers the high levels of intracellular oxidative stress (ROS) brought on by the interaction between AGE and RAGE as well as ROS-induced cellular signaling. Rosuvastatin is essential in reducing HMGB1 and RAGE expression, according to the results. Rosuvastatin has been shown to decrease RAGE expression in rat aortas in a number of studies (Xu *et al*., 2004; Yang *et al*., 2010). Moreover, studies by Yang *et al*. (2010) have demonstrated that rosuvastatin reduces the activation of HMGB1. Pro-inflammatory cytokines like TNF-α and IL-6 are upregulated when HMGB1 binds to RAGE (Yang *et al*., 2017). Numerous prior research (Kokkola *et al*., 2005, Joshi *et al*., 2023) and Gao *et al*. (Gao *et al*., 2017) have confirmed the validity of these findings by presenting evidence that TNF-α overproduction was caused by HMGB1 and RAGE. This suggests that the HMGB1 and RAGE signaling pathways may be connected to TNF-α overexpression.

Furthermore, after inflammatory damage, it has been noted that HMGB1 can bind to RAGE, elevating anti-inflammatory cytokines including IL 4 and IL 10 (Huang *et al*., 2009; Dumitriu *et al*., 2005; Wang *et al*., 2014). According to research by Du *et al*. (2014), rosuvastatin post-conditioning can successfully lower oxidative stress markers in rats that have had an ischemia/reperfusion damage. (Ke *et al*. 2013) also showed that rosuvastatin preconditioning can significantly reduce the accumulation of inflammatory cells in the heart, which is frequently associated with abnormal regulation of anti-inflammatory cytokines like IL-4 and IL-10 and an increase in the production of inflammatory cytokines like TNF-α and IFN-γ. In the current study, it was found that giving rosuvastatin to the ISG group increased their levels of TNF-α and IL-6 as well as their expression of HMGB1 and RAGE. According to these results, enhanced functional recovery might be facilitated by rosuvastatin's activation of HMGB1/RAGE. To determine how rosuvastatin affects inflammatory cells, more investigation is needed.

The study that was carried out produced noteworthy findings that indicate how well rosuvastatin works to enhance the left ventricle's structure and function. The length of rosuvastatin treatment is one of the elements that contribute to the drug's beneficial effect on

LVEF. According to research indicating a time-dependent relationship between rosuvastatin and improvement in LVEF (Ahsan *et al*., 2014), longer treatment periods may be more beneficial for raising LVEF. Rosuvastatin influences the expression of inflammatory cytokines and serological markers prior to having a favorable effect on heart tissue. More investigation is required to fully comprehend the mechanism underlying this drug's impact on the LVEF.

5. Conclusion

This study has shown that after taking rosuvastatin, the functional AGE/RAGE axis is decreased. An etiological hypothesis on the role of rosuvastatin therapy in cardiac injury is put forth in the study's conclusion. Notably, these results may have applications since they imply that rosuvastatin's alteration of AGE-RAGE signaling and may represent a novel therapeutic approach to reduce the occurrence and progression of various cardiovascular diseases. This study highlights the importance of comprehending the link between these parameters by demonstrating the clinical correlation between the AGE-RAGE axis and cardiac damage.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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