ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology

www.iajlb.com
Email : editor@iajlb.com or iajlb.editor@gamil.com

ISSN2347-2243www.iajlb.com
Indo-Am. J.ofLifeSc& Bt.,2020- Vol.13,Issue.4,Oct2020

Indo-American Journal of Life Sciences and Biotechnology

Cardioprotective Effects of Ginsenoside Rg1 in Patients Suffering from Acute Myocardial Infarction

Mr. Raj Kumar Marikanti[1] , R. Raghuveer[2], N. Swapna[3]

Abstract

Ginsenoside Rg1 (Rg1), the active component of Panaxnotoginseng, has a cardioprotective effect. However, Rg1's effectiveness against acute myocardial infarction (MI) remains unclear. The purpose of this research was to learn how Rg1 provides cardio-protection. The aim of this study was to produce myocardial infarction in wistar rats by occluding their left anterior descending coronary artery. Immunohistochemical staining was used to assess neutrophil and macrophage infiltration, and the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assay was used to detect apoptosis. 2,3,5-triphenyltetrazolium chloride (TTC) staining was used to detect infarct size. Principal Results: Superoxide dismutase (SOD) activity was considerably increased and myocardial infarct size was decreased after treatment with Extract Rg1 (P 0.01) or Ferment Rg1 (P 0.01). Then, HE and PTAH stain showed that Extract Rg1 and Ferment Rg1 protected heart structure, particularly sarcomere integrity. Extract Rg1 (P 0.001) and Ferment Rg1 (P 0.001) both decreased the amount of apoptotic cells and blocked neutrophil infiltration into the cardiac infarct site.

Keywords: MyocardialInfarction;Extract;Ferment; GinsenosideRg1

Introduction

A heart attack, also known as a myocardial infarction (MI), occurs when blood supply to the heart is suddenly reduced or even stopped due to coronary blockage, causing severe and prolonged acute ischemia and hypoxia in the associated myocardium. In 2017, ischemic heart disease was a major contributor to mortality rates in China. More than 20 million people die every year from MI across the globe [1]. There is a lack of cardioprotective drugs for MI at the moment. More potential medications to develop for MI patients are needed immediately.Panaxnotoginseng is a popular Chinese herbal remedy because of its well-documented ability to improve blood flow and prevent or treat pathological clotting [2-4]. The bioactive component ginsenoside Rg1 (Rg1) is mostly found in Panaxnotoginseng. Recent literature has shown a variety of clinical and physiological benefits of Rg1, including its ability to preserve myocardial shape and cardiac function by boosting angiogenesis and to reduce left ventricular myocardial fibrosis in a rat model of myocardial ischemia-reperfusion (MI) [5-7]. In addition to its stated cardioprotective effects, our prior animal experiment research also demonstrated that Rg1 inhibited vascular remodeling in both large conductance and small resistance arteries [8].

Associate Professor[1,2],Assistant Professor[3]
Dept. of Pharmaceutics[1], Pharmacology[2] ,Pharmaceutical Analysis[3]
Mother Theresa Institute of Pharmaceutical Education and Research, Kurnool, Andhra Pradesh

There is no data on acute protection of Rg1 against MI, although previous research has shown that Rg1 promotes angiogenesis and reduces the amount of myocardial fibrosis in a chronic cardiac damage model. Therefore, we determined Rg1's effectiveness using a rat model of acute MI.

Getting Rg1 of high purity is now hampered by two technological roadblocks. First, the soil becomes unsustainable due to the repetitive cultivation of Panax plants, which might lead to decreased yield [9]. High rates of seedling mortality suggest that replanting may be unsuccessful among Panax species [10]. Arable soils for Panaxnotoginseng cultivation are increasingly uncommon [11], and soil conditions must be addressed by several years of crop rotations before the plant can be replanted. Second, even though Panaxnotoginseng is readily available, the same structure of ginsenosides adds difficulties in the purification of each component. Due to their similar structure, Rg1 monomer and Rg2 monomer present unique challenges throughout the purification process.

Hydrophobic interactions between ginsenosides made it difficult to separate and purify the compound [12, 13]. Unlike the conventional extraction approach, fermentation requires less culture and yields Rg1 of a high purity. Because of the scarcity of plant resources and the difficulty in extracting and purifying the active components, fermentation is the preferred approach for commercial use. We have also been dedicated to fermenting ginsenosides to address the aforementioned issues [9, 14]. The effectiveness of both extracted Rg1 and fermented Rg1 were compared utilizing the MI rat model in the current research. The groundwork for Rg1's eventual clinical and commercial introduction has been created thanks to this study.

MethodsMaterials

The Shanghai Center for Experimental Animals, Chinese Academy of Sciences, supplied us with healthy male Wistar rats. Alfasan International B.V. supplied the intraperitoneal pentobarbital sodium, and Shanghai Yousi Bio-Tech Co.,Ltd. supplied the extract Rg1. The Chinese Academy of Sciences' Institute of Plant Physiology and Ecology has given the green light to the use of the Rg1 ferment. Sigma Aldrich Co., Ltd. supplied the TTC used in this study. The Nanjing Jiancheng Bioengineering Research Institute Co., Ltd. was the source for the SOD ELISA kits. Shanghai YiFanBlological Technology Co., Ltd. was where I got my eosin and haematoxylin. Beijing Solebo Technology Co., Ltd. supplied the PTAH staining Kit. Kit for detecting apoptosis using the TUNEL method was obtained from Shanghai Yeasen Biotechnology Co., Ltd. Boster Biological Technology Co., Ltd. supplied the CD44 antibody, CD68 antibody, Goat anti-rabbit antibody, DAB kit, and DAPI. Solvents and reagents were acquired from Sinopharm Chemical Reagent Co., Ltd. unless otherwise specified.

Treatment with Rg1 in a MI model

In rooms with a regulated temperature and humidity and a 12-hour dark/light cycle, 32 adult male Wistar rats (200-230 g) were acclimated to their new environment. Both food and drink were available at will. The Shanghai Institute of MateriaMedica's Institutional Animal Care and Use Committee gave its stamp of approval to the whole protocol (IACUC number: 2019-03-GDA-62). Anesthetized rats were attached to an ALC-V9 ventilator (Alcott, Shanghai, China) after receiving an intraperitoneal injection of 40 mg/kg pentobarbital sodium. A 1:1 inspiration-to-expiration ratio was selected, along with a tidal volume of 18 mL and a respiratory rate of 80 breaths per minute. The primary pulmonary artery was ligated in order to induce MI, which blocked blood flow to the left anterior descending coronary artery. All MI rats were randomly split into three groups after surgery: the MI group (n=8) received normal saline, the Ferment group (n=8) received 15 mg/kg Rg1 produced by fermentation, and the Extract group (n=8) received 15 mg/kg Rg1 by extraction. In the sham group (Sham, n=8), the same surgery was carried out, but the suture was not ligated before passing beneath the coronary artery. Twenty-three hours following surgery, rats received a single intravenous injection of 15 mg/kg Rg1 (Supplementary Figure 1). At the same time, rats in both the Sham and MI groups received the same amount of normal saline. After the rats were given the drug intravenously, they were kept warm using an ALC-HTP animal thermostatic system (Alcott, Shanghai, China).

Quantifying S-O-D

All rats were euthanized 24 hours after surgery by injecting them with 120 mg/kg of pentobarbital sodium. The aortic blood was centrifuged for 10 minutes at 4 degrees Celsius and 8000 rpm. Separated serum was put through a SOD test kit according the manufacturer's instructions. To sum up, 20 L of serum was put to a 96-well plate, and then diluted xanthine oxidase was administered to each well individually. Each well's absorbance was measured at 450 nm using a multimode plate reader (Perkin Elmer, Boston, USA) after a 20-minute incubation at 37°C on a shaker, and the results were converted to International Units U/mL protein.

Assessing the severity of left ventricular infarction

Each heart was frozen at -20°C for 20 minutes after the animals were put to sleep, and then a cool razor blade was used to divide it into six equal pieces. Each segment was stained for 20 minutes in a 0.5% TTC solution at 37°C. Image analysis software (Image-Pro Plus, version 6.0.) was used to morphometrically quantify the size of the infarct. The magnitude of the infarct at TTC was determined by dividing the infarct area by the total heart area. Histopathological analysis of the cardiac tissue included TTC staining, fixation in 4% neutral- buffered paraformaldehyde, paraffin embedding, and sectioning in 5 mm thick slices.
Tissues were dewaxed with xylene, hydrated with gradient alcohol, hematoxylin stained for 15 minutes, and 1% alcohol hydrochloric acid applied for 2–3 seconds to facilitate differentiation. The eosin solution was submerged for 10 minutes while the water was set to blue for 5 minutes. Gradient alcohol was then used to rehydrate the tissues before xylene and neutral balsam were used to seal the wounds. The Olympus BX51 microscope and the Olympus DP71 CCD camera (both from Olympus, Tokyo, Japan) were used to capture the micrographs.

Staining with an antibody

To perform antigen retrieval, slides were deparaffinized, rehydrated, and microwaved in sodium citrate buffer (pH 6.0) for 20 minutes. After inhibiting endogenous peroxidase with 3% hydrogen peroxide and preincubating with 10% normal goat serum, sections were treated with CD44 or CD68 antibody diluted 1:100 in 1% PBS at 4°C overnight. Sections were incubated with goat anti-rabbit antibody diluted 1:200 in 1% PBS at 37 °C for 1 hour after the primary antibodies had been removed with three 5-minute washes in PBS. The sections were first washed for a total of 15 minutes in PBS before being developed using a DAB kit and then rinsed in double-distilled water. Hematoxylin staining was repeated on the sections. The Olympus BX51 microscope and the Olympus DP71 CCD camera (both from Olympus, Tokyo, Japan) were used to capture the micrographs.

Analysis of apoptosis by TUNEL

The TUNEL Apoptosis Detection Kit was used as directed by the manufacturer to analyze apoptosis in the rat heart infarct region. Incubation in proteinase K solution deparaffinized, rehydrated, and permeabilized the sections. The DAPI solution was immersed the ventricular specimens after the equilibration and labeling reaction. An Olympus Fluoview1000 confocal laser scanning microscope from Tokyo, Japan was utilized to observe the fluorescence staining. At 330-380 nm, all cells could be seen.

DAPI labeling was done at 465-495 nm, and apoptotic cells were seen by FITC staining. The apoptosis index was calculated as the percentage of dead cells relative to the total number of cells.

Examining PTAH

For this procedure, we used xylene to remove wax, gradient alcohol to hydrate the slides, PTAH oxidizer for 5 minutes, oxalic acid solution for 90 seconds, and Mallory PTAH stain for 24 hours. After 24 hours, the slides were dehydrated with xylene, sealed with neutral balsam, and the surplus colours were washed away with 95% ethanol. The Olympus BX51 microscope and the Olympus DP71 CCD camera (both from Olympus, Tokyo, Japan) were used to capture the micrographs.

Mathematical dissection

GraphPad Prism 5.0 (GraphPad software, LA Jolla, CA, USA) was used to analyze the data. All data were presented as means +/- standard deviations, and homogeneity of variance was determined using one-way ANOVA. If the sample sizes (n) were the same, we utilized the Tukey test; if they weren't, we applied the Bonferroni correction (P0.05 indicated statistical significance).

Results

TTC stain is one of the primary procedures used to quantify infarct size in animal models, which is an essential marker for assessing cardiac injury. Figure 1A displays typical pictures of TTC stain, where red areas represent non-infarct tissue and white areas represent infarct tissue. Figure 1B displayed the results of a quantitative analysis of the TTC stain. Myocardial infarct size was substantially larger in the MI group than in the Sham group (39.1% 8.0% vs. 0.0 0.0%, P 0.001). Myocardial infarct size was considerably decreased by both Extract Rg1 (25.6 3.4% vs. 39.1% 8.0%, P 0.01) and Ferment Rg1 (24.9 9.0 % vs. 39.1% 8.0%, P 0.01). Myocardial edema may be assessed in many ways, one of which is using the heart index. Figure 1C demonstrates that the heart index in the MI group

increased more rapidly than in the Sham group, whereas neither the Extract Rg1 nor the Ferment Rg1 affected the heart index in any way. Another biomarker that represents the severity of ischemia damage is superoxide dismutase (SOD) activity. Figure 1D demonstrated a statistically significant decrease in SOD activity between the MI and Sham groups (119.911.7 U/mL vs. 164.514.9 U/mL, P 0.001). SOD activity was considerably increased in the Extract Rg1 (166.4 16.2 U/mL vs. MI group's 119.9 11.7 U/mL, P 0.001) and Ferment Rg1 (151.9 12.6 U/mL vs. MI group's 119.9 11.7 U/mL, P 0.01) groups.

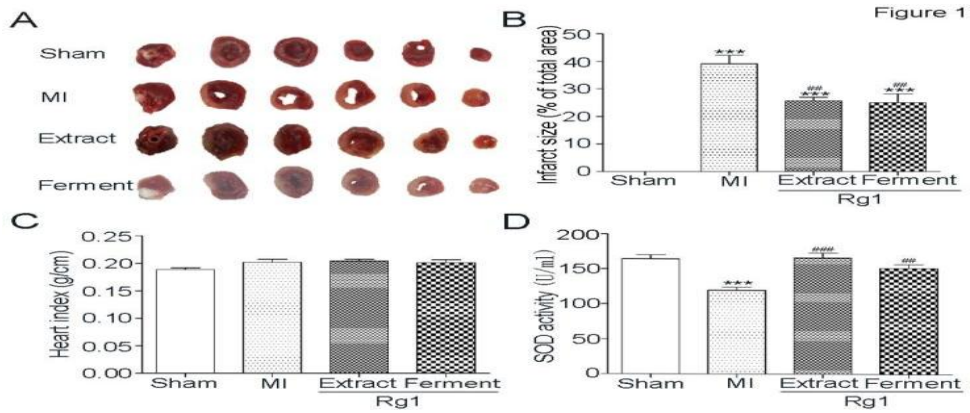


Figure 1: Ferment Rg1 decreased infarct size and up-regulated SOD activity. **A.** The representative images of TTCstain. **B.** The quantification of TTC stain. **C.** Heart index of each group. **D.** The activity of SOD from serum wasdetermined by ELISA. Data are presented as mean ± SE. ***p<0.001 comparedwith Sham group; ##p<0.01,###p<0.001 comparedwithMIgroup.n=5-8.

After we confirmed the protective effect of both Ferment Rg1 and Extract Rg1 against myocardial infarction basedon TTC stain, we further evaluated the effects of Rg1 on myocardial structure in infarct, border and remote area,respectively. Representative photomicrographs of HE stain were shown in **Figure 2**. In MI group, the myocardialstructure was destroyed seriously at the infarct area,with the characteristics of inflammatory cells infiltration,cellular degeneration, coagulation necrosis, interstitial edema, nuclei loss. Comparedwith MIgroup, Extract Rg1and Ferment Rg1 improved the above-mentioned damages. In the border area of MI group, inflammatory cellsinfiltrationwasalsoobvious,andthisphenomenonhadbeenimprovedbyExtractRg1andFermentRg1. Cardiomyocytesaligned inanorderlyarrangementwithoutobviousdifferencei nremoteareaforeachgroup.

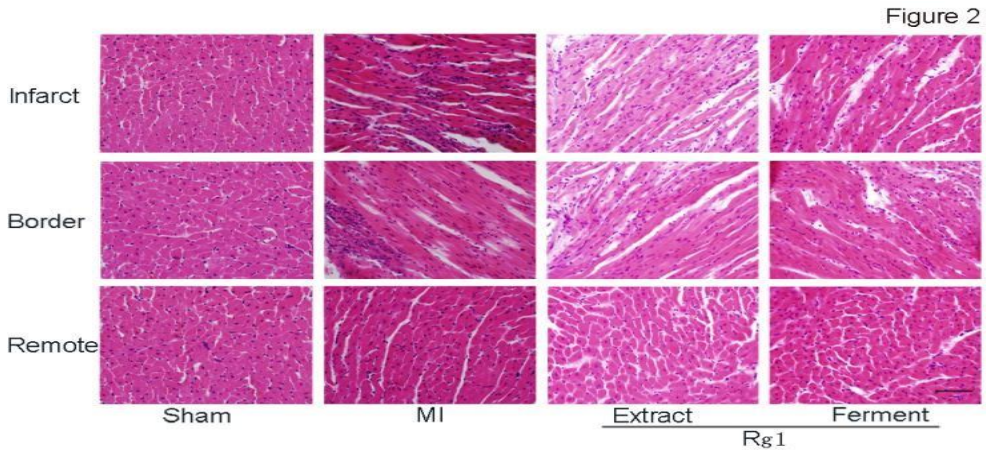
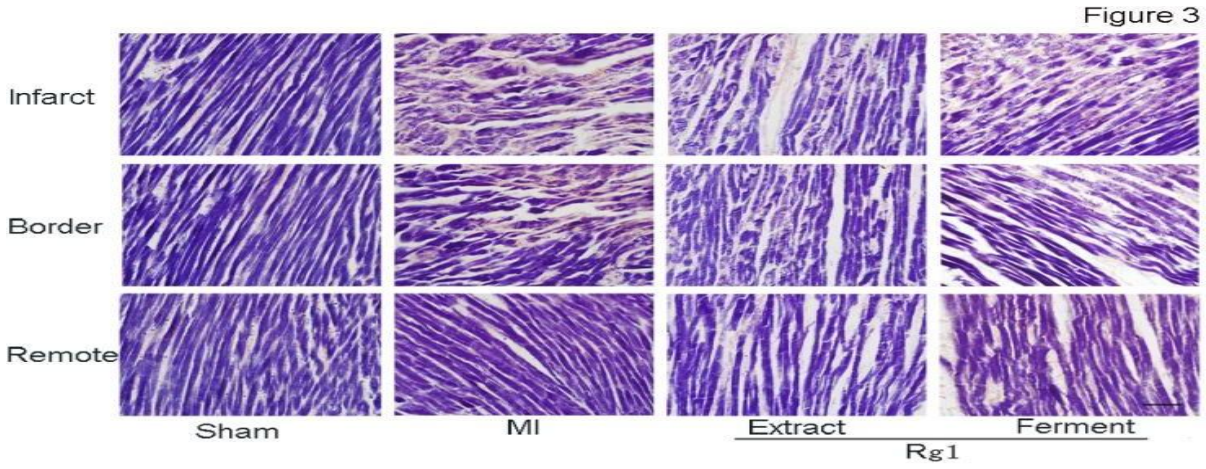


Figure 2: Ferment Rg1 protected the structure detected by HE stain. Representative photomicrographs of HE stain.n=7-8 for eachgroup.Scale bar:100 μm.

Each sarcomere is formed from a miniature, precised ordered array of parallel and partly overlapping thin and thickfilaments. After we confirmed the protective effect of both Extract Rg1 and Ferment Rg1 on myocardial structurethrough HE stain, we further evaluated the effects of Rg1 on sarcomere with PTAH stain in infarct, border andremote area (**Figure 3**). In MI group, cardiac muscle cells in shortened and widened shape not only lost co-directional arrangement, but also the light bands and dark bands of sarcomere were ruptured seriously at the infarctarea. In the border area of MI group, the sarcomere also showed a certain extent rupture. Both Extract Rg1 andFerment Rg1 protected the structure of sarcomere; maintain the cardiac muscle cells in striated arrangement at theinfarct and border area.Therewasno differenceonsarcomere structureinremote areaforeachgroup.

Figure 3: Ferment Rg1 protected the sarcomere detected by PTAH stain.Representative photomicrographs ofPTAHstain. n=6-8.Scale bar:100μm.



HE stain and PTAH stain indicated the cardio-protection of Extract Rg1 and Ferment Rg1,and then TUNELanalysis was conducted to elucidate the underlying mechanism. Representative photomicrographs of TUNEL stainwere shown in **Figure 4A**, and TUNEL-positive cells stain green. In **Figure 4B**, the percentages of TUNEL-positivecells in MI group was higher than Sham group (43.9±10.5% vs. 0.0%±0.0, P<0.001). Both Extract Rg1 (16.0±2.7%vs.43.9±10.5%,P < 0.001)andFermentRg1(15.7±4.7%vs.43.9±10.5%,P < 0.001)down-regulatedthepercentagesofTUNEL-positivecells

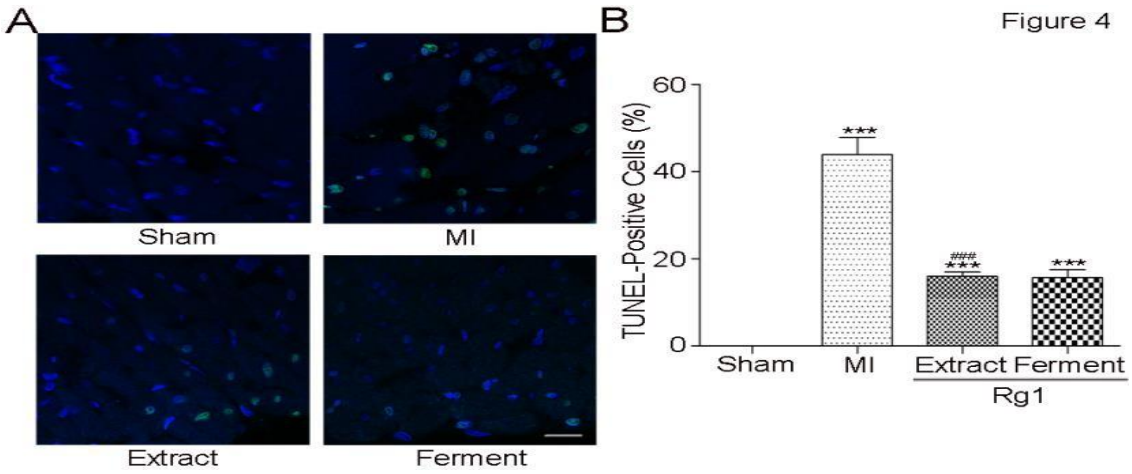


Figure 4

Figure 4: Ferment Rg1 inhibited cardiomyocyte apoptosis. **A.** Representative photomicrographs of TUNEL stain. **B.**The quantification of TUNEL stain. Data are presented as mean ± SE. **p<0.01, ***p<0.001 compared with Shamgroup;$^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001comparedwithMIgroup. n=7-8.Scale bar:50μm.

Because inflammatory infiltration was observed by HE stain, we want to further detect which type of inflammatorycells involved. Representative photomicrographs of immune histochemical stain of CD44 positive cells (neutrophils,**Figure 5A**) and CD68 positive cells (macrophages, **Figure 5B**) were shown. In MI group, abundant CD44 positivecells infiltrated into infarct area, while the infiltration of CD68 positive cells was not so obvious. At the border areaof MIgroup,sporadicdistribution of CD44positivecellswas observed.Both ExtractRg1andFerment Rg1inhibited neutrophilsinfiltrationintheinfarct andborderarea.

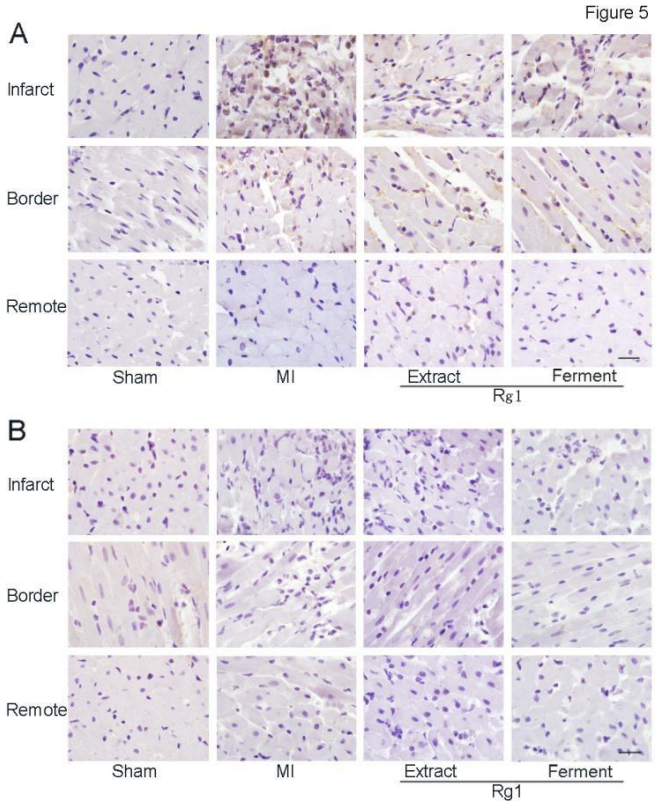


Figure 5: Ferment Rg1 inhibited the neutrophils infiltration. **A.** Representative photomicrographs of neutrophilsimmunohistochemistrystain.**B.**Representativephotomicrographsofmacrophageimmunohistochemistrystain.n=6-8. Scale bar: 50μm.

Discussion

In this work, we showed that Rg1 has a cardio-protective impact by decreasing the size of myocardial infarcts, increasing SOD activity, preserving sarcomere integrity, blocking apoptosis in cardiomyocytes, and decreasing neutrophil infiltration.

Worldwide, heart attacks are among the main causes of death and disability. There is a dearth of reliable and effective medications for MI at the moment. Acute protection of Rg1 against MI has not been observed, despite the fact that earlier research has shown that Rg1 promotes angiogenesis and reduces the amount of myocardial fibrosis in a chronic cardiac damage model [6, 7]. Chronic protection against myocardial fibrosis was shown with a decrease in infarct size and neutrophil infiltration in rats with acute MI, as we reported for the first time.

When there has been a MI damage, the first kind of leukocytes recruited are neutrophils [15]. While these neutrophils are essential for host defense against infections, their ability to generate a variety of proinflammatory mediators and reactive oxygen species [16-18] has led to their involvement in the pathophysiology of many illnesses. The body's inflammatory immune response and damage healing process are often sent into high gear after a myocardial infarction.

cicatrices replace the necrotic myocardium [19]. Our findings demonstrated that both Ferment Rg1 and Extract Rg1 reduced neutrophil infiltration, suggesting further protection against cardiac remodeling. One of the primary mechanisms of MI is the burst of oxygen free radicals [20]. The capacity of the organism to scavenge oxygen free radicals is indirectly reflected by the activity of superoxide dismutase (SOD), a particular enzyme for doing so in vivo. Superoxide anions, which are damaging to the myocardium, are removed at a slower rate when SOD activity is low [21].

Rg1's anti-oxidative action has been linked to its cardioprotective effects during hypoxia/reoxygenation of cardiomyocytes [22]. Myocardial cells were protected from oxygen free radical damage, as was previously reported, and SOD activity was increased thanks to Ferment Rg1 and Extract Rg1. Cardiomyocyte apoptosis is another process that SOD mediates. Early stage cardiac cell death is mostly due to apoptosis [23]. Persistent apoptosis leads to substantial loss of cardiac myocytes and consequent impairment of cardiac function [24, 25]. One sign of cardiac malfunction is sarcomere rupture. Myocyte apoptosis was prevented and sarcomere integrity was maintained by both fermented and extracted Rg1, suggesting that Rg1 provides protection for cardiac contractility. Future research will assess Rg1's impact on cardiac performance.

Conclusions

In conclusion, both Extract Rg1 and Ferment Rg1 effectively reduced neutrophil infiltration and enhanced heart structure in rats with MI damage. Similar effectiveness in cardio-protection is seen between

Ferment Rg1 and Extract Rg1, demonstrating that Rg1's unambiguous biological activity and obvious mechanism support its further clinical development.

References

1. S. Sahoo& D. W. Losordo. 1. Myocardial infarction and the role of exosomes in recovery. Journal of the American Heart Association 114, no.
2. Song ZH, Dong TT, Cui XM, et al. Regional and seasonal differences in the chemical composition of Chinese ginseng (Panaxnotoginseng) roots were evaluated. Pages 4617–4623 of the Journal of Agricultural and Food Chemistry, volume 51, 2003.
3. Sanchi ginseng's (Panaxnotoginseng) pharmacological properties. Ng TB. Referenced in 58 (2006), 1007–1019. Journal of Pharmacy and Pharmacology.
4. For example: 4 Gillis CN. Panax ginseng pharmacology: a nitric oxide link?, Biochemical Pharmacology 54 (1997), 1-8.
5. Five authors: Li, Pan, Yan, and Li. The ginsenoside Rg1 reduces the damage caused by ischemia and reperfusion to the rat heart. Physiology: Frontiers 9 (2018), p. 78.
6. For intramyocardial injection into a rat model of infarcted myocardium, Wei HJ, Yang HH, Chen CH, et al. encapsulated ginsenoside Rg1 in gelatin microspheres. 120 (2007), pages 27-34, Journal of Controlled Release.
7. Reference: Wang XD, Gu TX, Shi EY, et al. Panaxoside Rg1 inhibits neovascularization in rats with myocardial infarction; effects and mechanism of action. There were 16 issues of the Chinese Journal of Integrative Medicine in 2010.
8. Protective benefits of ginsenoside Rg1 against hypertension-related organ damage in rats with spontaneous hypertension. Chen H, Yin J, Deng Y, et al. There was 53 in BMC Complementary and Alternative Medicine in 2012.
9. P. Wang, W. Wei, W. Ye, and others. High-yield synthesis of ginsenoside Rh2 using the yeast Saccharomyces cerevisiae. Research in Cell Biology 5 (2019): 1-4.
10. Influence of continuous cropping of Panax ginseng on the metabolic function of soil microbial communities. Ying YX, Ding WL, Zhou YQ, et al. 4 (2012), pages 329-334 of Chinese Herbal Medicines.
11. They are Dong LL, Jiang X, Feng G, and others. Changes in the bacterial and fungal communities of continuously cropped soil and their effect on the mortality of Panaxnotoginseng. Research Reports in Science 6: 31802.