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ORIGINAL ARTICLE

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Omega-6 fatty acids down-regulate matrix metalloproteinase expression in a coronary heart disease-induced rat model

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Summary

The present study investigated the therapeutic potential of omega-6 fatty acids, according to their effects on antioxidant markers and matrix metalloproteinases (MMPs), in coronary heart disease-induced rats. Rats were grouped into group I (sham control), group II (control), group III (0.5 g/kg bwt of omega-6 fatty acids) and group IV (1 g/kg bwt of omega-6 fatty acids). Reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), catalase, glutathione peroxidase (Gpx) and acetylcholinesterase (AChE) enzyme activities were determined. ROS and MDA were substantially reduced, whereas SOD, catalase, Gpx and AChE were significantly increased, following supplementation with omega-6 fatty acids. MMP-2 mRNA expression was drastically increased by 95% in group II. Treatment significantly reduced MMP-2 mRNA expression by 12.3% and 26.7% in groups III and IV respectively. MMP-9 mRNA expression drastically increased, by 121%, in group II. Treatment significantly reduced MMP-9 mRNA expression by 22.6% and 29.4% in groups III and IV respectively. MMP-2 protein expression was drastically increased, by 81%, in group II. Treatment significantly reduced MMP-2 protein expression by 9.4% and 26% in groups III and IV respectively. MMP-9 protein expression was drastically increased, by 100%, in group II. Treatment significantly reduced MMP-9 protein expression by 18.9% and 26.9% in groups III and IV respectively. In summary, the consumption of omega-6 fatty acids significantly decreased MDA and ROS, while SOD, catalase, GHS, Gpx and AChE were increased. Furthermore, omega-6 fatty acids significantly downregulated MMP-2 and MMP-9 expression in our coronary heart disease-induced rat model.

KEYWORDS

antioxidant, enzymes, matrix metalloproteinases, omega-6 fatty acids, rats

1 | INTRODUCTION

Reactive oxygen species (ROS) are products of oxidative stress.[1] Several researchers have reported that dietary supplementation with fish oil containing omega-6 fatty acids exerts a protective effect against coronary heart disease and cancer.[2,3] Popović et al[4] reported that supplementation with fish oil containing omega-6 fatty acids exerts a beneficial effect on total cholesterol, uric acid and triglycerides. Verschuren et al[5] reported that a diet containing anti-inflammatory agents is effective against atherosclerosis in mice. Essential fatty acids not synthesized in humans and animals are due to a lack of the required enzymes.[6]

Matrix metalloproteinases (MMPs), which are well-known proteinases in animals and humans, are zinc-containing enzymes that participate in extracellular matrix degradation.

Several researchers have reported that MMPs play a crucial role in heart physiology, function and pathology. In addition, several researchers have reported the functional impact of essential fatty acids and fish oil on membrane fluidity, neural membrane oxidation and lipid peroxidation in neural tissues.[7,8] Research indicates a negative role for MMPs in vascular smooth muscle cell migration, cardiovascular remodelling and the formation of atherosclerotic plaque, which can lead to the development of coronary heart disease and heart failure.[9] Furthermore elevated MMP-2 and MMP-9 levels may be associated with atherosclerotic plaques. Hence, investigation of MMPs in a coronary heart disease model would be helpful to understand the pathophysiology of heart disease and have already led to attempts to modulate MMP function in coronary heart disease for therapeutic interventions.

Omega-6 fatty acids are essential and polyunsaturated fatty acids that provide several biological and therapeutic effects.[10] A preliminary investigation was carried out at different concentrations of an omega-6 fatty acid over the range 0.1-1 g/kg bwt. An optimum and significant impact was observed from 0.5 to 1 g/kg bwt of omega-6 fatty acid. Therefore, we selected 0.5-1 g/kg bwt for this study. Thus, our study evaluated the protective effects of omega-6 fatty acids on anti-oxidants and MMPs in Sprague Dawley rats.

2 | MATERIALS AND METHODS

2.1 | Experimental rats

Sprague Dawley male rats were purchased from the animal house of Kunming General Hospital of Chengdu Military Area, Kunming Medical University, Kunming, Yunnan, China. Rats weighing 190-200 g were used in the experiment, and rats were assigned to one of four homogeneous groups. A 12-hours light and dark cycle was maintained throughout all experiments. Six rats were included in each homogenous group. All rats were provided with water and food ad libitum. All animals were kept under adaptive feeding for 7 days before experiments.

2.2 | Ethical approval

This study was approved by the ethics committee of Kunming Medical University (Approval number: TXY12341).

2.3 | Induction of coronary heart disease

Coronary heart disease was induced according to a previously described method, by knockout of the low-density lipoprotein-receptor gene and the hypercholesterolaemic apolipoprotein E gene.[11] Rats were also supplemented with an atherosclerosis-inducing diet (10% Butter, 2% cholesterol, 0.5% bile salt, 6% peanut oil and 81.5% base material). The base material composed of 48% corn, 20% wheat middlings, 15% soya bean cake, 12% rice bran and 5% fish meal. General clinical manifestations and serological indexes were detected. The results showed rats were quite susceptible to the atherosclerosis-inducing diet. Induction of coronary heart disease was confirmed by determination of total cholesterol (261 ± 21.2 mg/dL), LDL cholesterol (185.5 ± 7.3 mg/dL), HDL cholesterol (45 ± 2.6 mg/dL), triglycerides (175.4 ± 10.7 mg/dL) and fibrinogen (471.7 ± 25.8 mg/dL).

2.4 | Experimental groups and treatment

After establishing coronary heart disease, rats were grouped into group I (sham control), group II (control), group III (0.5 g/kg bwt of omega-6 fatty acids) and group IV (1 g/kg bwt of omega-6 fatty acids). Each group contained six rats. Control rats were given the same volume of normal saline. Omega-6 fatty acids (P1547, Sigma-Aldrich Shanghai China) were dissolved in normal saline. Omega-6 fatty acids were adjusted to 0.5 and 1 g in 1 mL and given for 15 consecutive days via the oral route. All laboratory experiments were monitored and approved by the ethical committee of Kunming Medical University.

2.5 | Preparation of heart tissue homogenate

At the end of 15 days, the rats were sacrificed by decapitation following anaesthesia. Blood was collected, and the heart was surgically removed and excised. Tissues were washed in normal saline and homogenized in Tris-HCl buffer (0.1 mol/L, pH 7.4) and centrifuged at 11 200 g for 10 minutes. The supernatant was collected and centrifuged again at $100\,000 \times g$ for 60 minutes, and the resulting cytosolic fractions were used to determine biochemical markers.

2.6 | Determination of antioxidant and oxidative markers

Reactive oxygen species in the heart tissue homogenate were determined using the dichlorofluorescein assay.[12] Lipid peroxidation in the heart tissue homogenate was determined in the heart tissue homogenate according to malondialdehyde (MDA) content by measuring thiobarbituric acid reactive species. The final product was measured at 534 nm.[13] Glutathione peroxidase (Gpx), superoxide dismutase (SOD), catalase and acetylcholinesterase (AChE) enzyme activities in the heart tissue homogenate were determined using spectrophotometry.[13] Reduced glutathione (GSH) content in the heart tissue homogenate was determined by measuring the final product at 405 nm.[14]

2.7 | RT-PCR

Total RNA from rat heart tissue homogenate was extracted and converted into cDNA using oligo (dt) primers. Quantitative polymerase chain reaction (qPCR) was used to quantify

MMP-2 (forward: 5′-GGCCCTGTCACTCCTGAGAT-3′, reverse: 5′-GGCATCCAGGTTATC GGGGA-3′) and MMP-9 (forward: 5′-CGGAGCACGGAGACGGGTAT-3′, reverse: 5′-TGAAGGGGAAGACGCACAGC-3′) mRNA expression. GAPDH (forward: 5′-GGTCACCAGGGCTGCTTTT-3′, reverse: 5′-ATCTCGCTCCTGGAAGATGGT-3′) was used as an internal control for the quantification of caspase-3 expression. mRNA expression was calculated according to a previously described method.[15]

2.8 | Western blot analysis

Protein levels in the heart tissue homogenate were estimated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Proteins were then transferred to polyvinylidene membranes and incubated with a primary antibody against MMP-2 (ab37150; Abcam, Cambridge, UK) or MMP-9 (ab38898; Abcam) for 12 hours. Membranes were incubated with horseradish peroxidase (HRP)-IgG (goat anti-rabbit, A0545-1ML; Sigma-Aldrich, St. Louis, MO, USA) for 1 hour. MMP-2 and MMP-9 were determined by enhanced chemiluminescence.[16] The bands were quantified by densitometry using Li-Cor Odyssey software.

2.9 | Immunohistochemistry

A microtome was used to cut rat heart tissue sections, which were stored at 4°C for further use. Hydrogen peroxide was used to abolish endogenous peroxidase activity, and sections were incubated with bovine serum albumin for 30 minutes to abolish non-specific binding. Next, sections were treated with primary antibodies against MMP-2 (ab37150; Abcam) or MMP-9 (ab38898; Abcam) for 12 hours. Sections were treated with FITC Polyclonal Antibody, HRP (AB_930865, ThermoFischer Scientific) following repeated washes.[17] Sections were then washed with phosphate-buffered saline and mounted using the aqueous mounting medium. Images were obtained under a fluorescent microscope. The integrated pixel intensities were measured for the traced areas using image analysis software (Image-Pro, Media Cybernetics, Silver Spring, MD). The intensity was normalized for potential alteration in nuclear size by dividing the integrated pixel intensity by the nuclear area. Relative signal intensities provided MMP-2 and MMP-9 protein concentrations.

2.10 | Statistical analysis

Values are given as mean with standard error of the mean (SEM). Differences between the control and taurine groups were evaluated using the unpaired Student's *t* test. One-way ANOVA (SPSS 17, IBM China/Hong Kong Limited, Hong Kong) was applied for statistical analysis of data, and *post hoc* Tukey's test was used for multiple comparisons. *P*-value <0.05 was considered statistically significant.

3 | RESULTS

Our study investigated the therapeutic potential of omega-6 fatty acids, according to their effects on antioxidant markers and MMPs, in a coronary heart disease-induced rat model. ROS were measured and are expressed in relative fluorescence units: the intracellular ROS level was 37.66 RFU in group I, which increased by 461.65% in group II. Supplementation with omega-6 fatty acids significantly reduced the intracellular

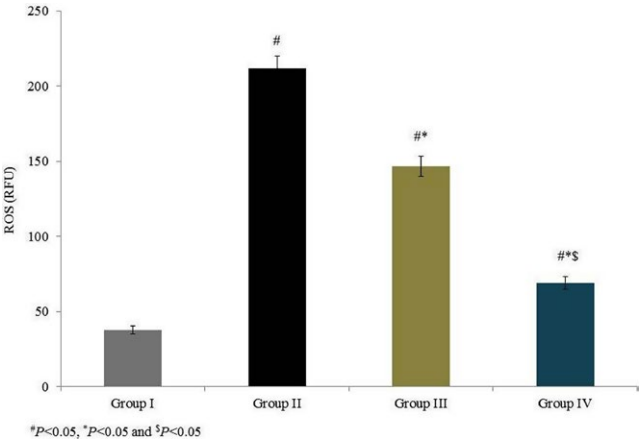

FIGURE 1 Effects of omega-6 fatty acids on intracellular reactive oxygen species (ROS) in the heart tissue homogenate of coronary heart disease-induced rat model. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

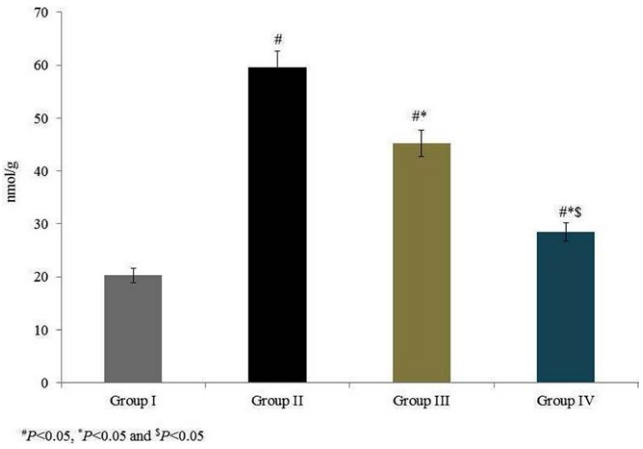

FIGURE 2 Effects of omega-6 fatty acids on lipid peroxidation in the heart tissue homogenate of coronary heart disease-induced rat model. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

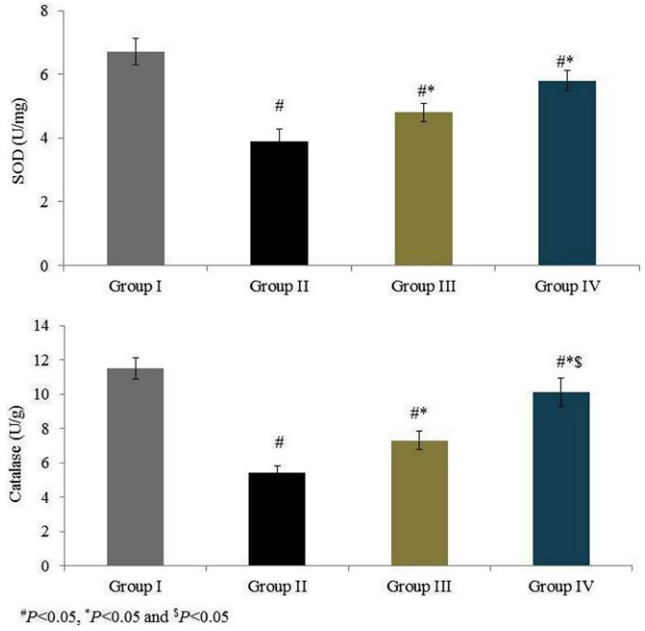

FIGURE 3 Effects of omega-6 fatty acids on superoxide dismutase (SOD) and catalase activities in the heart tissue homogenate of coronary heart disease-induced rat model. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

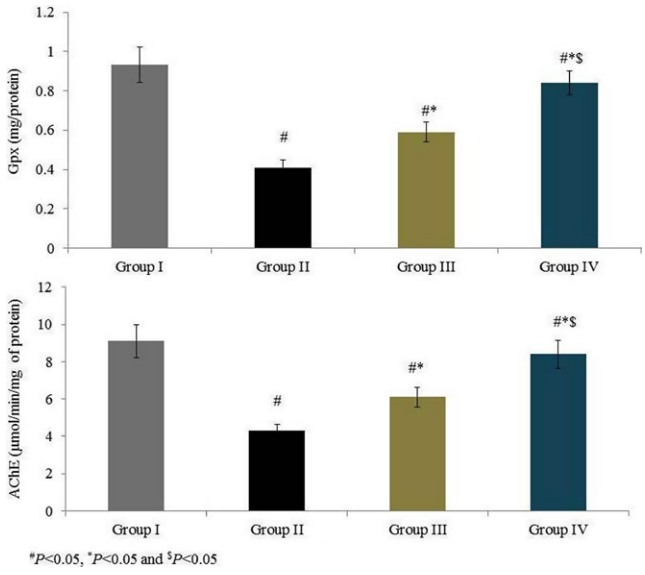

FIGURE 4 Effects of omega-6 fatty acids on glutathione peroxidase (Gpx) and acetylcholinesterase (AChE) activities in the heart tissue homogenate of coronary heart disease-induced rat model. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

ROS levels by 30.63% and 67.46% in groups III and IV respectively ($P < 0.05$; Figure 1). MDA content, measured as the end product of lipid peroxidation, was 20.27 nmol/g in normal control rats (group I) and drastically increased by 193.8% in group

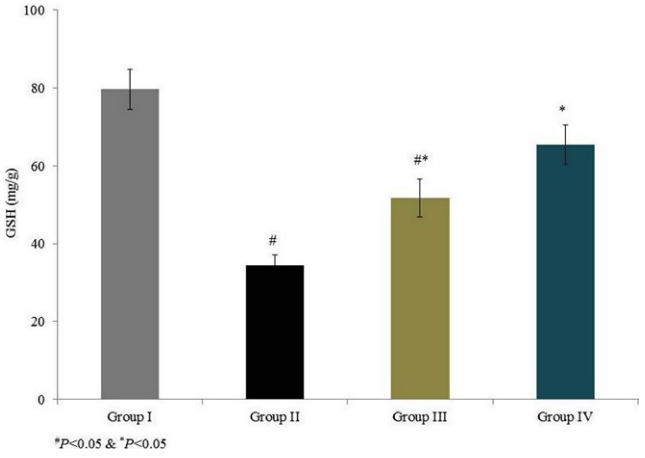

FIGURE 5 Effects of omega-6 fatty acids on reduced glutathione (GSH) content in the heart tissue homogenate of coronary heart disease-induced rat model. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

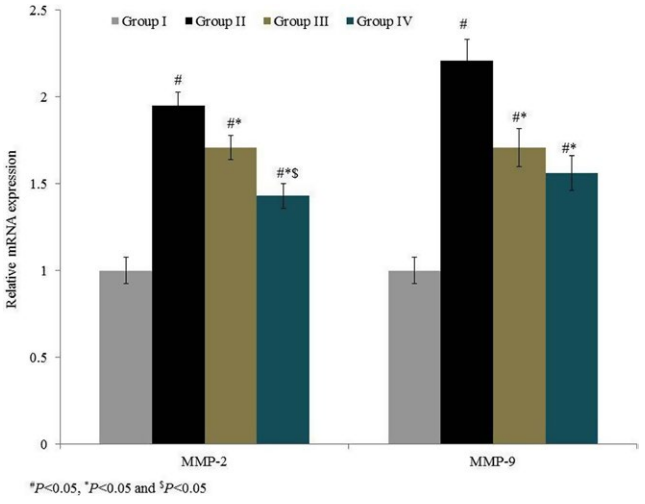

FIGURE 6 Effects of omega-6 fatty acids on the mRNA expression of matrix metalloproteinase (MMP)-2 and MMP-9 in the heart tissue homogenate of coronary heart disease-induced rat model. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

II. Compared to the untreated control (group II), omega-6 fatty acid treatment significantly reduced MDA content 24.14% and 52.23% in groups III and IV respectively (Figure 2, $P < 0.05$).

Superoxide dismutase activity was 6.7 U/mg in group I and was substantially reduced, by 41.8%, in group II. Rats supplemented with omega-6 fatty acids showed a significant increase in SOD activity (by 23% and 48.2% in groups III and IV, respectively, $P < 0.05$; Figure 3). Catalase activity was 11.5 U/g in group I and was substantially reduced, by 53%, in group II. Rats supplemented with omega-6 fatty acids showed a significant increase in catalase activity (by 35.2% and 87% in groups

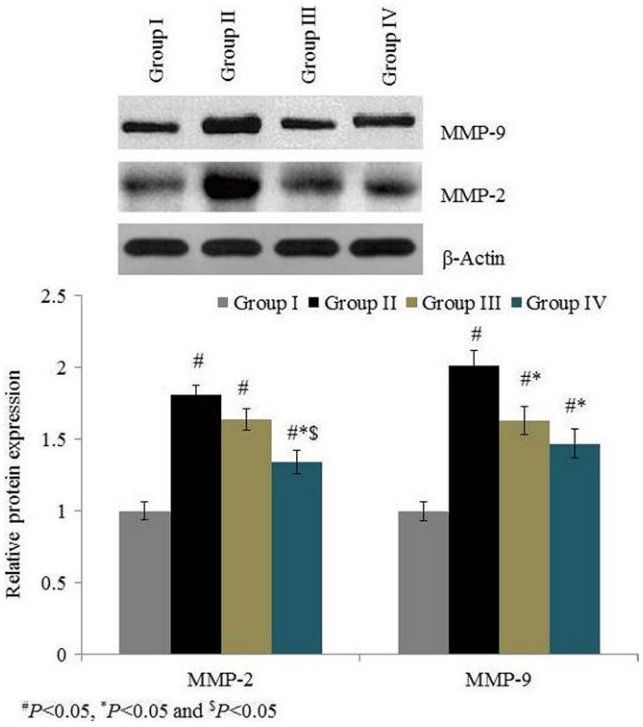

FIGURE 7 Effects of omega-6 fatty acids on the protein expression of MMP-2 and MMP-9 in the heart tissue homogenate of coronary heart disease-induced rat model. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

III and IV, respectively, $P < 0.05$; Figure 3). GPx activity was 0.93 (mg/protein) in group I and was reduced by 55.9% in group II. GPx activity was increased by 43.9% and 104.5% in groups III and IV respectively ($P < 0.05$; Figure 4). AChE activity was 9.1 (μmol/min/mg of protein) in group I and was reduced by 52.7% in group II. Rats supplemented with omega-6 fatty acids showed a significant increase in AChE activity (by 41.9% and 95.3% in groups III and IV, respectively, $P < 0.05$; Figure 4). GSH content was 79.67 mg/g in group I and was drastically reduced, by 56.7%, in group II. Treatment significantly increased the GSH content by 49.5% and 89.6% in groups III and IV respectively ($P < 0.05$ Figure 5).

MMP-2 mRNA expression was drastically increased, by 95%, in group II. Treatment significantly reduced MMP-2 mRNA expression by 12.3% and 26.7% in groups III and IV respectively ($P < 0.05$ Figure 6). MMP-9 mRNA expression drastically increased, by 121%, in group II. Treatment significantly reduced MMP-9 mRNA expression by 22.6% and 29.4% in groups III and IV respectively ($P < 0.05$; Figure 6). MMP-2 protein expression was drastically increased, by 81%, in group II. Treatment significantly reduced MMP-2 protein expression by 9.4% and 26% in groups III and IV respectively ($P < 0.05$; Figure 7). MMP-9 protein expression was drastically increased, by 100%, in group II. Treatment significantly reduced MMP-9 protein expression by 18.9% and 26.9% in groups III and IV respectively ($P < 0.05$; Figure 7). Immunohistochemical

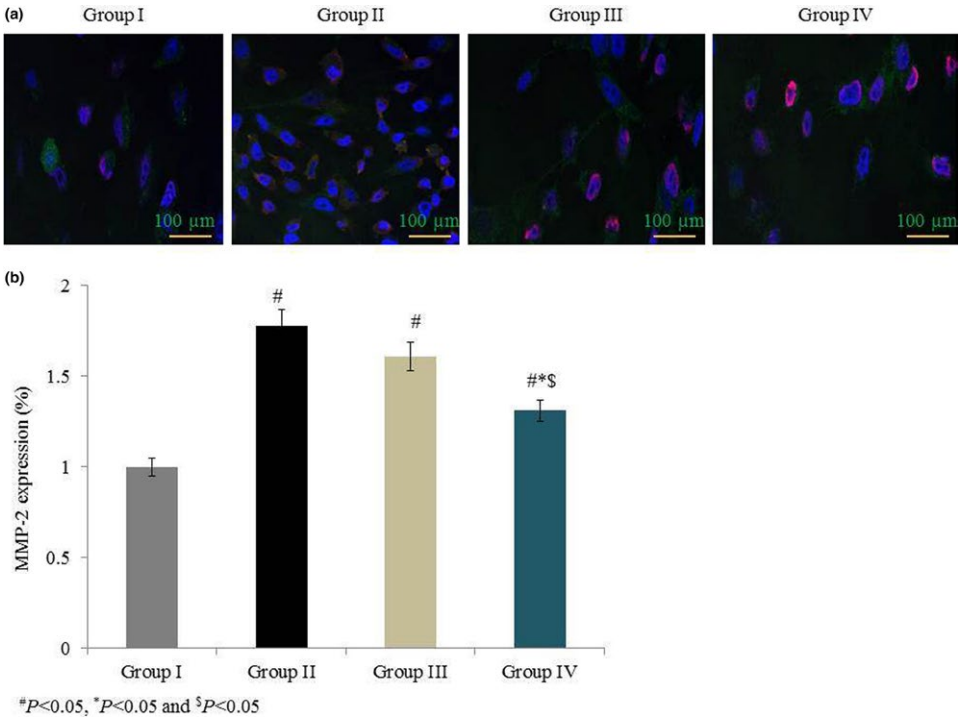

FIGURE 8 Effects of omega-6 fatty acids on MMP-2 protein expression in the heart tissue of coronary heart disease-induced rat model. In A, immunohistochemistry labelling shows the MMP-2 expression in green colour. MMP-2 expression (green), DAPI staining (blue) and AO staining (purple). In B, quantitative analysis shows the MMP-2 protein expression. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

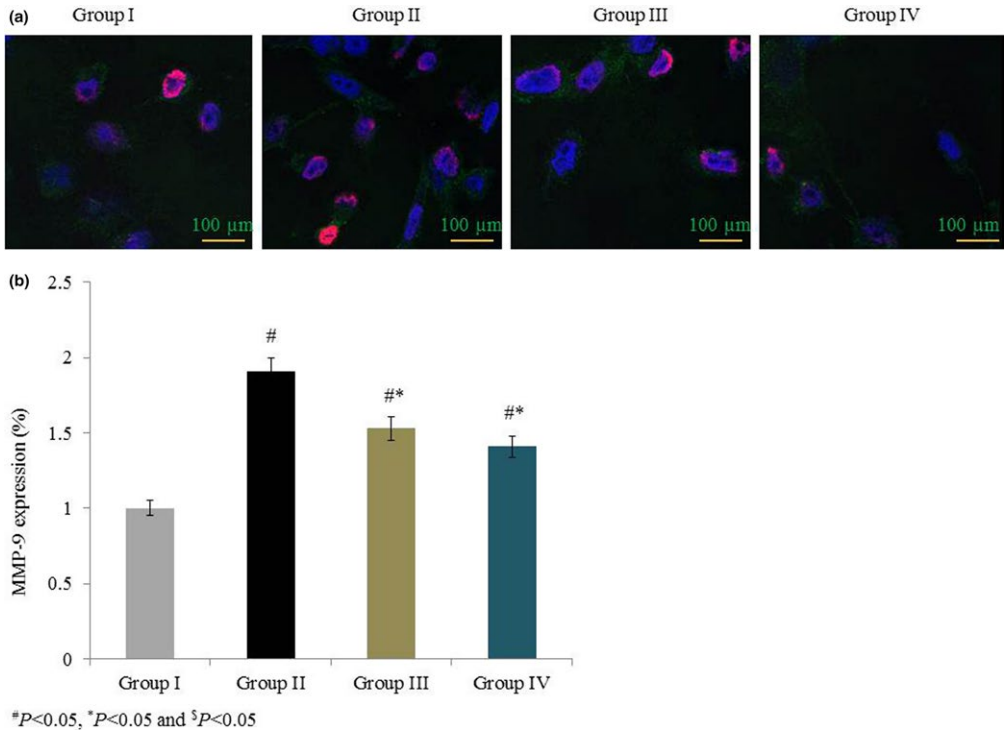

FIGURE 9 Effects of omega-6 fatty acids on MMP-9 protein expression in the heart tissue of coronary heart disease-induced rat model. In A, immunohistochemistry labelling shows the MMP-9 expression in green colour. MMP-9 expression (green), DAPI staining (blue) and AO staining (purple). In B, quantitative analysis shows the MMP-9 protein expression. $^{\#}P < 0.05$ vs group I, $^{*}P < 0.05$ vs group II and $^{\$}P < 0.05$ vs group III. n = 6. Values are shown as means ± standard error of the mean (SEM) [Colour figure can be viewed at wileyonlinelibrary.com]

analysis of MMP-2 and MMP-9 showed similar results to Western blot analysis (Figures 8 and 9).

4 | DISCUSSION

Our study investigated the therapeutic potential of omega-6 fatty acids, according to their effects on antioxidant markers and MMPs, in a coronary heart disease-induced rat model. Several researchers have reported beneficial effects of omega-6 fatty acids. Merendino et al[18] have reported the consumption of polyunsaturated fatty acids and fish oil associated with anti-proliferative, anti-inflammatory and anti-apoptotic effects. The balanced ratio of omega-3 and omega-6 fatty acids was important in male rat reproduction system.[19] Compare to omega-3 fatty acids rich diet, the diet rich in omega-6 fatty acids, docosahexaenoic and eicosapentaenoic fatty acids improved the circulating lipid profile.[20]

Salvati et al[8] reported the functional impact of essential fatty acids on membrane fluidity, inflammatory eicosanoids and neural membrane oxidation. Bas et al[7] reported the beneficial effects of fish oil on lipid peroxidation in neural tissues. Infarction size was reduced in ischaemia/reperfusion-induced rats following supplementation with polyunsaturated fatty acids.[21] Other researchers have reported that polyunsaturated fatty acids are incorporated into neuron membranes following uptake by the brain.[22] Oxidative burden and neurological deficits are reduced in ischaemia/reperfusion-induced rats following essential fatty acid supplementation.[23]

In this study, we noted increased levels of SOD, catalase, GSH, Gpx and AChE, whereas MDA and ROS were reduced, following supplementation with omega-6 fatty acids. Supplementation with fish oil significantly reduced MDA content in ischaemia/reperfusion-induced rats.[24] Atherosclerotic plaque can be identified by elevated MMP-2 and MMP-9 in the blood (2014). Several researchers have attempted to modulate MMP function in coronary heart disease for therapeutic interventions.

Omega-6 fatty acids are essential polyunsaturated fatty acids that provide several biological and therapeutic effects. Several researchers have reported that MMPs are reduced following supplementation with omega-3 fatty acids.[25,26] Renier et al[27] reported that dietary factors play an important role in the prevention and treatment of coronary heart diseases. Protective effects of polyunsaturated fatty acids against coronary heart diseases have been reported. Research has indicated a negative role for MMPs in vascular smooth muscle cell migration, cardiovascular remodelling and the formation of atherosclerotic plaque, which can lead to the development of coronary heart disease and heart failure (2014).

5 | CONCLUSION

In summary, we conclude that consumption of omega-6 fatty acids significantly decreases MDA and ROS levels, while those of SOD, catalase, GHS, Gpx and AChE increase. Furthermore, omega-6 fatty acids significantly downregulated MMP-2 and MMP-9 in our coronary heart disease-induced rat model.

CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

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