Galangin Resolves Cardiometabolic Disorders Through Modulation of AdipoR1/COX-2/ NF-κB Expressions in High-Fat Diet Fed Rats

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Abstract

Background: Rats fed with a high-fat diet exerts signs of cardiometabolic disorders. Galangin is a natural flavonoid mainly isolated from honey and Alpinia officinarum Hance and has various biological activities. This study evaluated whether galangin could alleviate cardiometabolic disorders, inflammation and oxidative stress in a high-fat diet fed rats.

Methods: Male Sprague-Dawley rats were fed with a high-fat diet plus 15% fructose in drinking water for 4 months to induced signs of metabolic syndrome (MS), and they were treated with galangin at a dose 25 or 50 mg/kg or metformin at a dose 100 mg/kg or vehicle for the last four weeks. All data were expressed as mean ± S.E.M. Data were analyzed by one-way analysis of variance followed by Tukey's post-hoc test for multiple comparisons analysis.

Results: Rats fed with a high-fat diet had impaired glucose tolerance, insulin resistance, hyperglycemia, hypertrophy of adipocytes, impaired liver function and hypertension. These signs of MS were alleviated by galangin or metformin treatment ($p<0.05$). Galangin or metformin alleviated cardiac dysfunction and remodeling induced by a high-fat diet in rats ($p<0.05$). Tumor necrosis factor-α and interleukin-6 concentrations and expression were high in plasma and cardiac tissue in MS rats, and these inflammatory markers were suppressed by galangin or metformin treatment ($p<0.05$). Galangin alleviated a high-fat diet induced low levels of adiponectin in rats. Galangin or metformin decreased oxidative stress biomarkers, aortic superoxide generation and plasma and cardiac MDA levels, and raised endogenous antioxidant enzume activities, catalase, *and superoxide dismutase,* in MS rats ($p<0.05$). Downregulation of adiponectin receptor1 (AdipoR1) and cyclooxygenase-2 (COX-2) as well as upregulation of nuclear factor kappa B (NF-κB) expression were observed in MS rats. These alterations of protein expressions were recovered in MS rats treated with galangin or metformin.

Conclusions: Galangin reduced cardiometabolic disorders in high-fat diet induced MS rats. The underlying mechanisms might be relevant to suppression of inflammation and oxidative stress and restoration of AdipoR1/COX-2/NF-κB expression.

Introduction

Metabolic syndrome (MS) composes of a set of cardiometabolic risk factors including central obesity, insulin resistance, hypertension, and dyslipidemia. The presence of three or more specific factors is indicative of MS that contributes to the development of type-2 diabetes and cardiometabolic disease (1). It is well documented that excessive caloric intake is the major cause of obesity and metabolic syndrome in human (2). Epidemiological studies provide substantial evidence linking dietary consumption patterns that develop obesity and lead to MS (3). Obesity has been proposed to be the initiation of adverse effects on metabolic system, dyslipidemia, and hyperglycemia. In an animal model of MS, high-fat diet induced metabolic disturbances has been developed to mimic to signs of MS and cardiovascular alterations in human (4). Several studies showed that rats received a high-fat diet had high fasting blood glucose,

impaired oral glucose tolerance test (OGTT), hyperinsulinemia, dyslipidemia, visceral fat pad accumulation and hypertension (5, 6). Additionally, fructose supplementation in rats can facilitate signs of MS that has been documented (7, 8). Several lines of evidence indicate that diet-induced MS in animals also shows the characteristic of cardiac alterations including impairment of cardiac function and morphology. For example, Ouwens and coworkers demonstrated changes in cardiac phenotype in rats fed a high-fat diet supporting by cardiac hypertrophy and myocardial contractile dysfunction associated with myocardial triacylglycerol accumulation (9). A previous study showed that rats fed with a high-fat diet plus fructose in drinking water had impaired glucose tolerance, dyslipidemia, visceral fat accumulation and hypertension associated with left ventricular (LV) dysfunction and hypertrophy (10).

Chronic-low-grade inflammation has been occurred in MS associated with hypertrophy of adipocytes or visceral fat accumulation which secrete several proinflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), Interleukin-8 (IL-8) and leptin etc. (1, 11). This local inflammation in adipocytes can produce systemic inflammation and a progression of cardiovascular and metabolic disease (12, 13). On the contrary, reduction of adiponectin levels were observed in obesity and this adipokine exhibits inhibitory inflammatory processes (14). Adiponectin has been recently described to be an inti-inflammatory and cardioprotective cytokine since low levels of adiponectin are revealed in patients severe coronary artery disease and left ventricular hypertrophy with diastolic dysfunction (15–17). Cardioprotective effects of adiponectin are associated with suppressing reactive oxygen species-induced cardiac remodeling in rats (18). Additionally, adiponectin/adiponectin receptor1 (AdipoR1) signaling pathway has a crucial role in regulation of mitochondrial function, oxidative stress, lipid, and glucose metabolism in muscle of mice (19). AdipoR1 is also mainly expressed in heart and mediates a protective action of adiponectin against myocardial ischemia-reperfusion(I/R) injury via enhancing cyclooxygenase-2 (COX-2) (20). It is recognized that COX-2 is detrimental and plays a role in inflammatory processes. However, cardioprotective effects and anti-inflammatory of COX-2 has been described (21, 22). The Nuclear factor kappa B (NF-κB) is a key transcription factor that is suppressed by a adiponectin/AdipoR1 signaling pathway (23). NF-κB has the deleterious effect on heart because blockade of NF-κB can alleviate cardiac remodeling and failure in knockout mice after myocardial infarction (24). Furthermore, oxidative stress has been characterized and contributed to the development of cardiometabolic disease in diet induced MS rats (25–27). There is growing evidence that MS rats have high levels of local and systemic oxidative stress markers, malondialdehyde (MDA) and low activities of endogenous antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) (28, 29). Adipose tissue has been suggested to be a major source of reactive oxygen species in obese mice (30). In contrast, adiponectin signaling has been proposed to reduce reactive oxygen species (23).

It is well suggested that the initial management of MS involves lifestyle modifications, including changes in diet and exercise habits. Metformin is a biguanide family member and firstly recommended for type 2 diabetes treatment. Hypoglycemic effects of metformin are relevant with improving insulin sensitivity of liver and peripheral tissue as well as reducing the hepatic glucose production (31). It also improves the lipid profiles in a rat model of diet-induced MS (32). Metformin has been reported to reduce inflammatory markers in high fructose fed diabetic rats (33). Moreover, the beneficial effects of metformin in patients

with coronary artery disease has been documented since it reduces left ventricular hypertrophy, left ventricular mass indexed, systolic blood pressure (SBP), body weight (BW), and oxidative stress (34). This study, metformin was used as a positive control agent to mitigate cardiometabolic disorders in a high-fat diet induced MS rats. In recent years, several studies have focused on the beneficial of flavonoids for reducing the severity of unhealthiness together with drugs used. Galangin (3,5,7-Trihydroxyflavone or 3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one) is a natural flavonoid that mostly found in honey, Alpinia officinarum Hance (Zingiberaceae) and the rhizome of Alpinia galanga. Several biological activities of galangin have been revealed including, anti-microbial (35), antitumor (36), anti-apoptotic (37), antifibrotic (38) and anti-inflammatory activities (39). In an animal model of type I diabetes mellites, streptozotocin (STZ) -induced diabetic rats, galangin alleviated oxidative stress by increasing activities of endogenous antioxidant enzymes such as SOD, CAT, glutathione peroxidase, and glutathione-S-transferase (40). It also ameliorated hyperglycemia, hyperinsulinemia and dyslipidemia in rats treated with STZ (41). Recently, galangin exhibited hepatoprotective effects via activation of nuclear factor erythroid 2-related factor 2 and heme oxygenase 1 signaling pathway in cyclophosphamide-administered rats (42). However, little information regarding the effect of galangin on cardiometabolic disorders in model of high-fat diet induced-MS rats has been shown. This study evaluated whether galangin could alleviate signs of MS, cardiac alterations, inflammation and oxidative induced by a high-fat diet in rats.

Materials And Methods

Animals and Diets

Male Sprague-Dawley rats aged 6 weeks weighing 200-220 g were purchased from Nomura Siam International Co., Ltd., Bangkok, Thailand. All rats were housed in standard cages under temperature-controlled room (23 ± 2 °C) with a relative humidity of 30 – 60 % and light/dark cycle of 12 hours. All procedures were performed in accordance with the rules of ethical guideline for the Care and Use of Laboratory Animals, which was approved by animal ethics committee of Khon Kaen University (IACUC-KKU- 74/62), based on the ethic animal experimentation of national research council of Thailand. The animals had free access to diet and water. Standard chow diet and high-fat diet were used for feeding control rats and MS rats, respectively. The standard chow diet composed of 57.81% carbohydrates, 22.9% protein and 5.72% fat while a high-fat diet composed of 46.3% carbohydrates, 13.25% protein and 24.29% fat. The composition of the standard chow and a high-fat diet were analyzed by Central Lab Thai (Central Laboratory (Thailand) Company Limited, Khon Kaen, Thailand). The control rats were given tap water while the MS rats were supplement with 15% fructose in drinking water during night-time to facilitate sings of MS.

Research Designs

After acclimatization, control rats were fed with standard chow diet and tap water for sixteen weeks (n=8). MS rats were fed with high-fat diet and 15% fructose drinking water for sixteen weeks. At the end of twelve weeks of experiment, MS rats were subdivided into 4 groups (n = 8/group), MS rats received

vehicle, MS rats treated with galangin (25 mg/kg), MS rats treated with galangin (50 mg/kg), and MS rats treated with metformin (100 mg/kg). Galangin (purity ≥ 98%) was purchased from Aktin Chemicals, Inc. (Mianyang City, Sichuan, China). Metformin was obtained from Siam pharmaceutical Company Ltd. (Bangkok, Thailand). All treatments were administered orally using intragastric tube daily for the final four weeks an experiment period. Blood samples were collected via lateral rat tail vein at 12^{th} weeks for fasting blood glucose and lipid profile measurement to confirm the characteristic of MS.

Indirect blood pressure measurements

Conscious rats were evaluated SBP changes in monthly during three month of the experimental period and weekly during the final four weeks of treatment. SBP were measured using the tail-cuff plethysmograph method (IITC/Life Science Instrument model 229 and model 179 amplifier; Woodland Hills, CA, USA). The average SBP value of three-time measurement were present.

Measurements of fasting blood glucose, serum insulin level and oral glucose tolerance test (OGTT)

Rats were fasted for 12 hours with free access to drinking water. Blood samples were collected from lateral tail veins to measure a basal glycaemic (at time 0 min (T0)) and serum insulin levels. Then, rats were fed with glucose solutions using gavage tube at a dose of 2g/kg BW. Blood glucose concentrations at 30, 60, 120 and 180 min after gavage was assessed using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany). Insulin levels in serum was assessed after 12-h fasted overnight. Serum sample were obtained upon spontaneous coagulation and centrifugation (3000 g, 4°C, 30 min). Serum insulin levels were assessed by enzyme-linked immunosorbent assay (ELISA) kits (Millipore Corporation, Billerica, MA, USA). Insulin resistance was determined from the relative-value of homeostasis model (HOMA-IR) (43). HOMA-IR score was calculated using the formula as following:

$$\text{HOMA-IR} = \frac{(\text{fasting blood glucose (mmol/L)}) \times (\text{fasting insulin (}\mu\text{IU/mL)})}{22.5}$$

Echocardiography

On the final day of experiment, all rats were anesthetized by 3% isoflurane. Echocardiography was performed to measure cardiac function using a commercially available echocardiography system (Model LOGIQ S7), equipped with a 10-MHz linear transducer (GE Healthcare, WI, USA). Each rat was shaved around their chest and applied a warmed resonance gel to the hairless chest. The ultrasound transducer was placed slightly left of chest then optimized for the left ventricle and aorta. Two-dimensional-guided M-mode images were recorded in accordance with the American Society of Echocardiography guideline. Three consecutive beats were measured at the five min after anesthesia, and the average of these measurements was taken for analysis. M-mode tracings to record interventricular septal end diastole and end systole (IVSd and IVSs), left ventricular internal diameter end diastole and end systole (LVIDd and LVIDs), left ventricular posterior wall end diastole and end systole (LVPWd and LVPWs) end-diastolic and

systole volumes (EDV and ESV), stroke volume (SV) and ejection fraction (%EF) from three consecutive cardiac cycle were performed. LV shortening fraction (%SF) was calculated using equation: %SF= [(LVIDd-LVIDs)/LVIDd] x 100.

Direct blood pressure measurements

After cardiac function measurement, the left femoral artery was cannulated. SBP, diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were monitored and recorded by pressure transducer using the Acknowledge Data Acquisition and Analysis Software (BIOPAC Systems Inc., California, USA).

Assessment of biochemical profiles

Following indirect blood pressure measurements, rats were euthanized by overdose of anesthesia and then blood samples were collected from the abdominal aorta and plasma was separated immediately using centrifugation at a speed of 3,000 g at 4°C for 30 min. Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c) levels in plasma were determined spectrophotometrically using specific commercial kits (Human Gesellschaft fuer Biochemica and Diagnostica mbH, Wiesbaden, Germany). Additionally, liver tissue was homogenized in lysis buffer for measure TC and TG using specific commercial kits as plasma. Levels of aspartate transaminase (AST) and alanine transaminase (ALT) were measured by Clinical Chemistry Laboratory Unit of Faculty of Associated Medical Sciences, Khon Kaen University, Thailand.

Tissue harvesting

After collecting blood samples, heart, liver, and visceral fat (including epididymal and retroperitoneal fats) were immediately dissected. All tissues were weighed to compare regional tissue weight (mg)/ body weight (g). A portion of the liver, heart and visceral fat was frozen at −20°C for biochemical analysis and fixed in 4% formaldehyde for histomorphology analysis.

Hematoxylin and eosin staining of cardiac and fat tissue

Myocardial tissue and epididymal fat pads were fixed in 4% paraformaldehyde for 24 hours, routinely processed, and embedded in paraffin. Briefly, all tissue paraffin blocks were cut at 5 mm thickness using a microtome. The paraffin sections (5 μm) were dewaxed and rehydrated through gradient alcohol into water. The sections were then washed with tap water, distilled water, and then stained with hematoxylin and eosin (H&E) (Bio-Optica Milano SpA., Milano, Italy). For microscopic assessment, the images of heart sections were captured by stereoscope (Nikon SMZ745T with NIS-elements D 3.2) at 1x objective lens to evaluate the LV wall thickness; cross-sectional area (CSA); the LV luminal area and wall to lumen ratio. These parameters were quantified using Image J software (National Institutes of Health, Bethesda, MD, USA).

Measurement of myocardium cell size, area of cardiomyocyte was performed for 300 myocytes per group at 40x objective lens, via Digital sight DS-2MV light microscope (Nikon, Tokyo, Japan). Mean values were obtained from 300 cells/group.

Epididymal fat sections were observed using a Digital sight DS-2MV light microscope (Nikon, Tokyo, Japan) at 40x objective lens. Adipocyte was quantitated as cell sizes area (300 cells/group) using a NIS-Elements software.

Immunohistochemical stainingof myocardial sections

An immunohistochemical technique was used to evaluate TNF-α and IL-6 expression in left ventricle. The myocardial sections were deparaffinized in xylene and rehydrated through an ethanol series. Antigen retrieval was performed by tris-ethylenediaminetetraacetic acid (EDTA) buffer and used high temperature heating method to recover the antigenicity of tissue sections. The myocardial sections were incubated with hydrogen peroxide (H_2O_2) for blocking endogenous enzymes and then incubated with 5% bovine serum albumin in PBS for blocking nonspecific protein. Thereafter, the sections were incubated with primary antibody, mouse anti-TNF-α IgG (dilution 1:500) or mouse anti-IL-6 IgG (dilution 1:500), in moistening chamber for 4 hours at room temperature (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Goat anti-mouse IgG (HRP) dilution 1:1000 (Abcam Plc, Cam-bridge, UK) was used as secondary antibody. The brown color of 3,3'-Diaminobenzidine (DAB) was visualized as a positive control and the tissues were counterstained with hematoxylin. The myocardial sections were observed using a Digital sight DS-2MV light microscope (Nikon, Tokyo, Japan) at 40x objective lens. TNF-α and IL-6 expression were quantified using Image-Pro plus 6 software (Media Cybernetics, Inc., Rockville, MD, USA).

Assays of cytokines levels

Plasma adiponectin level was assessed using the adiponectin enzyme-linked immunosorbent assay (ELISA) kits (Millipore Corporation, Billerica, MA, USA). The serum levels of TNF-α and IL-6 were measured with ELISA kits according to the manufacturer's instructions (Sigma-Aldrich, Saint Louis, MO, USA).

Oxidative stress markers assessment

Aorta was rapidly excised for the analysis of superoxide production which determined by lucigenin enhanced chemiluminescence as described previously (44). The aorta was quickly dissected. The adherent fat and connective tissue were cleaned on ice. The vessel segments (3–5mm) were placed in Krebs-KCl buffer and allowed to equilibrate at 37°C for 30 min. Lucigenin was added to the sample tube and placed in a luminometer (Turner Biosystems, Sunnyvale, CA, USA). The photon counts were integrated every 30 sec for 5 min. The vessels were dried at the room temperature for 24 hours to determine a dry weight. Superoxide production in aorta was expressed as relative light unit counts per minute per milligram of dry tissue weight. Malondialdehyde (MDA) is an end-product of lipid peroxidation and can be as a biomarker of oxidative damage. MDA was estimated in plasma and heart tissue by using a colorimetric assay or thiobarbituric acid reactive substances (TBARS) assay as described in a previous

report (45). MDA level was assessed by quantifying thiobarbituric acid (TBA) reactivity as MDA in a spectrophotometer. The resulting chromogen absorbance was determined at the wavelength of 532 nm against blank reference. The concentration of MDA was read from standard calibration curve plotted using 1, 1, 3, 3’ tetra-ethoxy propane (TEP) as a µM/L unit.

Antioxidant endogenous enzyme activity assessment

The CAT activity in plasma and heart tissue were determined using a colorimetric method. CAT is a ubiquitous enzyme that destroys hydrogen peroxides (H_2O_2) formed during oxidative stress. The level of CAT activity depends on monitoring the change of 405 nm absorbance at high levels of hydrogen peroxide solution. In Brief, samples were incubated with substrate (65 µmol/mL of H_2O_2 in 60 mmol/L sodium potassium phosphate buffer pH 7.4) in 96-well plate at 37°C for 1 min. Next step, add 32.4 mmol/L ammonium molybdate for stop reaction. The yellowish molybdate and H_2O_2 complex absorbance was determined at the wavelength of 405 nm and leading to calculate the CAT activity level.

SOD activities in heart tissue were measured via colorimetric analysis using a spectrophotometer with the corresponding detection kits (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocols.

Western-blotting analysis

LV tissue were homogenized in ice-cold lysis buffer. Processed samples, containing 50 µg protein, were heat-denatured in laemmli buffer and separated on 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). Separated proteins were electro-transferred onto polyvinylidene difluoride (PVDF) membrane (MilliporeSigma, Merck KGaA, Darmstadt, Germany) at 90 V for 90 min. After completion of the transfer, the PVDF membranes were blocked with 5% BSA in tris-buffered saline with 0.1% Tween-20 (TBS-T) for 2 hours at room temperature. After that membranes were incubated overnight at 4°C with specific primary antibodies against AdipoR1(dilution 1:1000), COX-2 (dilution 1:500) (Abcam Plc, Cam-bridge, UK), p-NF-κB (dilution 1:1000) (Cell Signaling Technology, Inc., Danvers, USA). This was followed by incubation with appropriate secondary antibody for 2 hours at room temperature. b-actin was used was used as loading control (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Bands were detected using ECL™ Prime western blotting reagents (Amersham Biosciences Corp., Piscataway, NJ, USA). The intensities of the bands were quantified using an ImageQuant™ 600 imager (GE Healthcare Life Science, Piscataway, NJ, USA) and were normalized to that of b-actin.

Statistical Analysis

All data were expressed as mean ± standard error of the mean (S.E.M.). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test for multiple comparisons analysis. All statistical analyses were performed using PRISM software version 8.3 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant at p values < 0.05.

Results

Effects of galangin and metformin treatments on body and organ weight in high-fat diet induced metabolic syndrome rats

After sixteen weeks of experiment, there was a significant difference of final rat body weight between a control group and a MS group (p<0.05). Additionally, weight of whole heart, ventricles, retroperitoneal fat pads, epididymal fat pads and liver were significantly higher in MS rats than those of a control group (p<0.05). Moreover, MS rats showed significant increases in ratio of retroperitoneal fat pads/body weight and epididymal fat pads/body weight compared to control group (p<0.05) (Table 1.). Treatment with galangin did not reduce body and organ weight in MS rats compared to untreated MS rats. Liver weight loss has been shown in galangin-treated group at dose 50 mg/kg and metformin treated group (p<0.05). In addition, metformin treatment alleviated weight of whole heart, ventricular weight, retroperitoneal fat pads and retroperitoneal fat pads/body weight (p<0.05) as shown in Table 1.

Table 1. Effects of galangin and metformin treatments on body and organ weight in high-fat diet induced metabolic syndrome rats.

	Control	MS	MS + Galangin (25 mg/kg)	MS + Galangin (50 mg/kg)	MS + Metformin (100 mg/kg)
BW (g)	714.17 ± 22.69	837.14 ± 35.59 [a]	793.29 ± 17.75	789.50 ± 16.37	739.13 ± 28.12
HW (g)	1.47 ± 0.05	1.76 ± 0.06 [a]	1.72 ± 0.04 [a]	1.59 ± 0.04	1.54 ± 0.06 [b]
HW/ BW (mg/g)	2.06 ± 0.05	2.11 ± 0.07	2.17 ± 0.02	2.01 ± 0.06	2.09 ± 0.05
VW (g)	1.30 ± 0.04	1.52 ± 0.05 [a]	1.44 ± 0.02	1.41 ± 0.03	1.32 ± 0.05 [b]
VW/ BW (mg/g)	1.82 ± 0.03	1.84 ± 0.04	1.82 ± 0.02	1.79 ± 0.03	1.80 ± 0.04
RP pads weight (g)	18.96 ± 2.66	59.91 ± 5.23 [a]	55.62 ± 3.13 [a]	52.25 ± 1.24 [a]	40.53 ± 6.13 [a, b]
RP pads weight/ BW (mg/g)	26.43 ± 3.35	70.86 ± 3.57 [a]	69.85 ± 2.84 [a]	67.00 ± 1.00 [a]	53.54 ± 6.63 [a, b]
EP pads weight (g)	14.81 ± 0.72	29.09 ± 2.85 [a]	26.13 ± 0.76 [a]	25.52 ± 1.94 [a]	23.85 ± 2.68 [a]
EP pads weight/ BW (mg/g)	20.76 ± 0.88	34.39 ± 2.09 [a]	33.15 ± 1.15 [a]	32.62 ± 1.89 [a]	31.81 ± 3.09 [a]
LW(g)	19.01 ± 1.17	24.34 ± 1.20 [a]	21.74 ± 0.92	19.72 ± 0.70 [b]	19.79 ± 0.75 [b]
LW/ BW (mg/g)	26.81 ± 1.92	29.00 ± 1.03	27.41 ± 0.95	25.34 ± 0.51	26.85 ± 0.86

Data are presented as mean ± S.E.M. (n=8). [a]$p < 0.05$ vs control group and [b]$p < 0.05$ vs MS group. MS: Metabolic syndrome; BW: Body weight; HW: Heart weight; VW: Ventricular weight; RP: Retroperitoneal fat; EP: Epididymal fat; and LW: Liver weight

Effects of galangin on metabolic parameters

The baseline of blood glucose level (T = 0 min) was high in the MS group compared to a control group. The blood glucose levels of all rats were reached to the peak at 60 min, and thereafter the blood glucose concentration was decreased. The blood glucose levels in MS rats did not recover to the baseline at 120 min while the level of blood glucose in MS rats treated with galangin (50 mg/kg) or metformin did not differ to control group by 120 min. (Fig. 1a) ($p<0.05$). Area under the curves (AUC) of OGTT was larger in

MS rats and this was attenuated in MS treated with galangin in a dose dependent manner or metformin (Fig. 1b).

Fasting blood glucose, fasting insulin and HOMA-IR index were higher in MS group than those of in control group (p<0.05). Galangin (50 mg/kg) and metformin corrected the insulin resistance by reducing the levels of fasting glucose, fasting insulin and HOMA-IR index in MS rats (p<0.05) (Table 2.). MS rats had an apparent elevation of total cholesterol and triglyceride in plasma and liver tissue, as compared to control group. However, plasma HDL-c of was significantly low in MS group compared to a control group. The Liver enzymes, both of plasma AST and ALT levels, were significantly high in MS group compared to a control group (p<0.05). Galangin was effective in improving the levels of total cholesterol, triglyceride, HDL-c and liver enzymes in high-fat diet induced MS rats in a dose-dependent manner. Metformin also improved the disturbance of all metabolic parameters in MS rats (Table 2.).

Table 2. Effects of galangin or metformin treatment on metabolic parameters in high-fat diet induced metabolic syndrome rats.

	Control	MS	MS + Galangin (25 mg/kg)	MS + Galangin (50 mg/kg)	MS + Metformin (100 mg/kg)
Fasting blood glucose (mg/dl)	89.20 ± 3.18	120.00 ± 5.42 [a]	105.40 ± 1.33	100.40 ± 4.17 [b]	93.80 ± 4.65 [b]
Fasting serum insulin (ng/ml)	1.99 ± 0.26	4.02 ± 0.71 [a]	2.76 ± 0.25	2.06 ± 0.52 [b]	2.13 ± 0.25 [b]
HOMA-IR index	10.87 ± 1.38	30.26 ± 6.95 [a]	17.73 ± 1.38	12.49 ± 3.12 [b]	12.33 ± 1.89 [b]
Plasma total cholesterol (mmol/L)	0.92 ± 0.03	2.57 ± 0.30 [a]	1.35 ± 0.08 [b]	1.00 ± 0.10 [b]	1.17 ± 0.07 [b]
Plasma triglyceride (mmol/L)	0.41 ± 0.07	2.49 ± 0.43 [a]	0.71 ± 0.19 [b]	0.54 ± 0.09 [b]	0.45 ± 0.06 [b]
plasma HDL-c (mmol/L)	1.71 ± 0.13	0.33 ± 0.03 [a]	0.79 ± 0.09 [a,b]	1.30 ± 0.15 [b,c]	0.99 ± 0.11 [a,b]
Liver total cholesterol content (mg/g tissue)	11.17 ± 1.87	23.87 ± 2.71 [a]	17.64 ± 1.46	11.53 ± 1.57 [b]	11.58 ± 2.96 [b]
Liver triglyceride content (mg/g tissue)	13.79 ± 1.10	33.13 ± 1.58 [a]	19.42 ± 1.32 [b]	16.29 ± 2.26 [b]	15.42 ± 1.71 [b]
AST (U/L)	49.00 ± 4.63	83.55 ± 4.20 [a]	76.17 ± 4.56 [a]	52.20 ± 1.43 [b,c]	65.50 ± 3.59 [a,b]
ALT (U/L)	19.13 ± 2.88	39.60 ± 2.58 [a]	29.17 ± 3.20 [b]	22.86 ± 0.86 [b]	24.75 ± 1.66 [b]

Data are presented as mean ± S.E.M. (n=8). [a]$p < 0.05$ vs control group, [b]$p < 0.05$ vs MS group and [c]$p < 0.05$ vs MS + galangin (25 mg/kg) group. MS: Metabolic syndrome; HDL-c: High-density lipoprotein cholesterol; AST: Aspartate transaminase; and ALT: Alanine transaminase

Effects of galangin on epididymal fat pads morphology

Histological findings, as shown in Fig 2, revealed hypertrophy of adipocytes from epididymal fat pads in MS group compared with the control group. Treatment with galangin (25 or 50 mg/kg) and metformin for four weeks showed a significant decrease in the hypertrophy of adipocytes compared to untreated MS group (p<0.05) (Fig. 2a and b).

Effects of galangin on blood pressure and heart rate

Rats fed with a high-fat diet for 4 weeks significantly increased blood pressure compared to control rats ($p < 0.05$). After sixteen weeks of experiment, untreated MS rats showed hypertension compared to control group (SBP =156.10 ±0.75 vs 120.50 ± 1.10 mmHg,) (p<0.05). Galangin (25 or 50 mg/kg) administrations for four weeks significantly reduced the elevation of systolic blood pressure (SBP= 142.00 ± 0.60 or 137.57 ± 1.25 mmHg) in a dose-dependent manner in MS rats (p<0.05). Metformin restored high blood pressure in MS rats closely to the level in normal rats (SBP = 128.50 ± 0.53 mmHg, p<0.05) (Fig. 3). Furthermore, the SBP values in all rats measured by an indirect method were consistent with the result of hemodynamic parameters evaluated by a direct method as shown in (Table 4). The heart rate of MS group was significantly higher than that of control group and this was suppressed in MS rats treated with galangin (25 or 50 mg/kg) or metformin (p<0.05) (Table 3).

Table 3. Effects of galangin or metformin treatment on blood pressure obtained from a direct method of blood pressure measurement in high-fat diet induced metabolic syndrome rats.

	Control	MS	MS + Galangin (25 mg/kg)	MS + Galangin (50 mg/kg)	MS + Metformin (100 mg/kg)
Systolic blood pressure (mmHg)	118.49 ± 2.50	156.78 ± 1.96 [a]	146.71 ± 2.24 [a]	131.41 ± 2.75 [a,b,c]	120.86 ± 3.04 [b,c,d]
Diastolic blood pressure (mmHg)	80.28 ± 3.06	107.32 ± 1.11 [a]	97.46 ± 3.12 [a]	85.72 ± 2.00 [b,c]	75.24 ± 3.43 [b,c]
Mean arterial pressure (mmHg)	93.58 ± 2.82	123.90 ± 1.19 [a]	113.73 ± 2.55 [a]	100.95 ± 2.05 [a,b,c]	90.48 ± 2.78 [b,c]
Pulse pressure	39.89 ± 1.67	49.73 ± 2.11	48.81 ± 2.99	45.68 ± 2.10	45.72 ± 3.81
Heart rate (bmp)	331.86 ± 6.06	378.57 ± 9.84 [a]	336.15 ± 11.29 [b]	336.09 ± 9.99 [b]	314.95 ± 4.68 [b]

Data are presented as mean ± S.E.M. (n=8). [a]$p < 0.05$ vs control group, [b]$p < 0.05$ vs MS group, [c]$p < 0.05$ vs MS + galangin (25 mg/kg) group and [d]$p < 0.05$ vs MS + galangin (50 mg/kg) group. MS: Metabolic syndrome

Effects of galangin on cardiac function parameters

Echocardiography revealed increased LVIDd and LVPWd in MS group compared to control group (p<0.05). The value of EDV, SV, EF and FS were significantly lower in MS rats than those of control rats (p<0.05). These impairments of cardiac function were alleviated in galangin and metformin administrations (p<0.05) in MS rats compared to control rats (Table 4).

Table 4. Effects of galangin or metformin treatment on transthoracic echocardiographic parameters in high-fat diet induced metabolic syndrome rats.

	Control	MS	MS + Galangin (25 mg/kg)	MS + Galangin (50 mg/kg)	MS + Metformin (100 mg/kg)
IVSd (cm)	0.178 ± 0.005	0.200 ± 0.008	0.177 ± 0.008	0.174 ± 0.012	0.180 ± 0.008
IVSs (cm)	0.273 ± 0.010	0.272 ± 0.015	0.271 ± 0.10	0.283 ± 0.010	0.281 ± 0.012
LVIDd (cm)	0.759 ± 0.018	0.626 ± 0.030 [a]	0.793 ± 0.018 [b]	0.776 ± 0.026 [b]	0.747 ± 0.008 [b]
LVIDs (cm)	0.463 ± 0.023	0.434 ± 0.024	0.501 ± 0.020	0.463 ± 0.014	0.459 ± 0.008
LVPWd (cm)	0.197 ± 0.010	0.244 ± 0.013 [a]	0.191 ± 0.011 [b]	0.190 ± 0.010 [b]	0.198 ±0.006 [b]
LVPWs (cm)	0.278 ± 0.009	0.307 ± 0.014	0.279 ± 0.008	0.277 ± 0.009	0.285 ± 0.009
EDV (ml)	0.969 ± 0.061	0.586 ± 0.077 [a]	1.096 ± 0.070 [b]	1.036 ± 0.092 [b]	0.930 ± 0.029 [b]
ESV (ml)	0.252 ± 0.031	0.212 ± 0.030	0.314 ± 0.037	0.249 ± 0.020	0.238 ± 0.013
EF (%)	74.480 ± 1.880	63.591 ± 3.185 [a]	71.683 ± 1.417	75.839 ± 0.928 [b]	74.093 ± 1.416 [b]
SV (ml)	0.717 ± 0.033	0.374 ± 0.058 [a]	0.780 ± 0.034 [b]	0.790 ± 0.074 [b]	0.689 ± 0.029 [b]
FS (%)	38.840 ± 1.680	30.518 ± 2.137 [a]	36.854 ± 0.972 [b]	39.859 ± 0.851 [b]	38.369 ± 1.204 [b]

Data are presented as mean ± S.E.M. (n=8). [a]p < 0.05 vs control group and [b]p < 0.05 vs MS group. MS: Metabolic syndrome; IVSd; Interventricular septal at end diastole; IVSs: Interventricular septal at end systole; LVIDd: Left ventricular internal dimension at end-diastole; LVIDs: Left ventricular internal dimension at end-systole; LVPWd: Left ventricular posterior wall at end diastole; LVPWs: Left ventricular

posterior wall at end systole; EDV: End-diastolic volumes; ESV: End-systolic volumes; EF: Ejection fraction; SV: Stroke volume; and FS Fractional shortening

Effects of galangin on cardiac morphology

Structural changes in left ventricles were found in MS rats induced by a high-fat diet. Significant increases in LV wall thickness, CSA and wall/lumen ratio and a reduction of LV luminal areas were observed in MS group (p<0.05). This cardiac hypertrophy was consistent with appearance of cardiomyocyte since the cell size of cardiomyocyte was significantly increased in MS group. Galangin (25 or 50 mg/kg) and metformin treatments significantly reduced cardiac hypertrophy and cell size in MS rats compared to untreated group (p<0.05) (Fig. 4c, d, e, f and g).

Effects of galangin on myocardial inflammation

The expression of inflammatory mediator protein TNF-α and IL-6 were enhanced as shown in Fig. 5a and b. Notably, the immunohistochemical staining revealed that galangin (25 or 50 mg/kg) and metformin treatment suppressed the expression of TNF-α and IL-6 in a dose-dependent manner (p<0.05) (Fig. 5c and d). The plasma level of TNF-α and IL-6 were significantly increased in MS group compared with control group (p<0.05). In turn, galangin and metformin treatment alleviated the high levels of plasma inflammatory cytokines in MS rats as a compared to untreated rats (p<0.05) (Fig. 5e and f).

Effects of galangin on plasma adiponectin levels

There was a significant decrease in adiponectin concentration in MS group comparing to control group (p<0.05). Rats received galangin (25 or 50 mg/kg) or metformin, however, reversed adiponectin concentration in MS rats (Fig. 6).

Effects of galangin on oxidative stress markers and endogenous antioxidant enzymes

The results showed that superoxide production in aorta was significantly elevated in MS group compared with the control group (p<0.05) (Fig. 7a). The level of MDA in plasma and heart tissue were increased in MS group and these were significantly attenuated by galangin (25 or 50 mg/kg) and metformin administrations compared with untreated MS group (p<0.05) (Fig. 7b and c). CAT activities were decreased in plasma and heart tissue in MS group compared those of control group (p<0.05) (Fig. 7e and f). The activity of SOD was lower in MS rats than those of control rats. Galangin (25 or 50 mg/kg) and metformin treatment in MS rats significantly enhanced CAT and SOD activities in a dose-dependent manner in MS rats (p<0.05) (Fig. 7d, e and f).

Effects of galangin on AdipoR1, COX-2 and p-NF-κB expression in cardiac tissue

The expression of AdipoR1 and COX-2 in cardiac tissue in MS group were significantly lower than those of in control group, however, the expression of p-NF-κB was significantly higher in MS group than those of

control group (p<0.05). Meanwhile, the MS rats treated with galangin at dose 50 mg/kg and metformin exhibited a significantly improved the expression of AdipoR1, COX-2 and p-NF-κB (p<0.05) (Fig. 8).

Discussion

The results of this study found that galangin alleviated signs of MS, dyslipidemia, hyperglycemia, insulin resistance, visceral fat accumulation and hypertension in a high-fat diet plus 15% fructose induced MS in rats. Cardiac dysfunction and remodeling were shown in MS rats and were regressed in galangin treated group. High levels of pro-inflammatory cytokines, TNF-α and IL-6 in plasma and myocardium and low levels of plasma adiponectin were observed in MS rats and these were alleviated in galangin treated group. Galangin had antioxidant activity by decreasing plasma and cardiac MDA and aortic superoxide production and increasing in endogenous antioxidant enzyme activities in MS rats. Galangin also recovered the alterations of AdipoR1/COX-2/NF-κB protein expression in cardiac tissue of MS rats. Metformin ameliorated signs of MS, cardiac abnormalities, inflammation, and oxidative stress in a high-fat diet plus 15% fructose fed rats.

Many studies presented the animal model of MS induced by a high-fat diet that was characterized by an augmented body weight gain, hyperglycemia, hyperinsulinemia, hypertension, and dyslipidemia (46). This study showed that rats received a high-fat diet plus 15 % fructose developed MS together with enlargement of adipocytes and high levels of liver enzymes. Impairment of liver function has been described in a high-fat diet induced MS in rats that resulted from fat accumulation, insulin resistance, oxidative stress, and inflammation (46). Fructose is an important factor to facilitate the development of MS since hepatic fructose uptake is independent of energy status leading to increased lipogenesis (47). High blood pressure was the consequence of insulin resistance induced by a high-fat diet and fructose that has been noted (48). Furthermore, impairment of cardiac function including decreased EDV, SV, FS and FS were observed in a high-fat diet fed rats. These results were supported by previous studies (9, 27). It is possible that cardiac dysfunction in MS rats in the present study was the consequence of cardiac remodeling evidenced by increases in LV wall thickness, cross-sectional areas, wall/lumen ratio and cardiomyocyte area as well as a decrease in LV luminal areas. Increased LVPWd and decreased LVIDd were consistent with the results of LV hypertrophy. Generally, there are at least two factors, mechanical or pressure overload and humoral agents that stimulate cardiac remodeling (49). Furthermore, inflammation has been established as the main process of myocardial remodeling response to adaptive responses from heart to the stress (50). Numerous reports illustrated that cardiac changes were associated with obesity, hyperinsulinemia, impaired glycaemic control, dyslipidemia, inflammation, and oxidative stress (51). The results of this study showed local and systemic inflammation, indicated by increasing cardiac and plasma pro-inflammatory cytokines, IL-6 and TNF-α. This study, oxidative stress was occurred in a high-fat diet fed rats as the consequence of increasing aortic superoxide production and cardiac and plasma MDA levels as well as decreasing endogenous antioxidant enzyme activities. These results were supported by the evidence that long-term consumption of a high-fat diet promoted oxidative stress, inflammation that activate stimulate fibrotic remodeling in heart (52).

Adiponectin is an anti-inflammatory cytokine and exerts cardioprotective effects (16). In preclinical pig and mouse models of ischemia/reperfusion (I/R) injury, adiponectin treatment reduced infarct size and improved cardiac function that was associated with reducing inflammation, apoptosis, and oxidative stress (20, 53). This study found that the serum levels of adiponectin were low in a high-fat diet fed rats. This result was consistent with other previous studies that adiponectin plays an important role in regulation of metabolic parameters to increase insulin sensitivity (54-56). In cardiac tissue of a high-fat diet fed rats, the expression of AdipoR1 and COX-2 protein was decreased in while upregulation of expression of NF-κB was observed. The protective action of adiponectin against myocardial I/R injury appears to be mediated by its ability to activate COX-2 in cardiac cells (20). Shibata and coworker demonstrated that adiponectin also increased the expression of COX-2 which is inhibits TNF-α production in myocytes. The protein expression of NF-κB in the present study was consistent with the expression of TNF-α and IL-6 in cardiac tissue as well as their high levels in plasma. It is possible that cardiac remodeling occurred in a high-fat diet fed rats could be relevant to the protein expressions of AdipoR1, COX-2 and NF-κB.

Galangin alleviated cardiometabolic disorders in a high-fat diet induced MS rats, evidenced by resolving hyperglycemia, impaired glucose tolerance, insulin resistance, dyslipidemia, visceral fats deposition and hypertension. Galangin reduced the enlargement of adipocytes and levels of hepatic enzymes. It also improved cardiac function and regressed cardiac remodeling in MS rats. These findings are accordance with previous studies that galangin decreased adipose tissue and liver weight in cafeteria diet fed female rats (57). It has been reported to improve blood glucose, total cholesterol, triglyceride and HDL-c in plasma and liver of STZ-induced hyperglycemia and cafeteria diet fed female rats (41, 57). The effect of galangin on cardiometabolic disorders in the present study might be related with reducing oxidative stress biomarkers in systemic, vessels and heart and raising endogenous antioxidant activities. An anti-oxidative effect of galangin has been strongly recommended (40). Additionally, anti-inflammatory effects of galangin in the present study, supported by reducing heart and systemic level of TNF-α and IL-6, might enhance its beneficial effects on heart. Serum adiponectin levels were raised by galangin treatment in a high-fat diet fed rats and this might suppress inflammation in heart and circulation. Galangin also increased the expression of AdipoR1/COX-2 and suppressed the expression of NF-κB expressions in cardiac tissue. It is well established that NF-κB associate inflammatory responses (58). In contrast, COX-2 has been reported to preserve heart failure in late ischemic preconditioning (59, 60). These data could suggest that galangin alleviated cardiometabolic disorders associated with oxidative stress and inflammation via restoration of AdipoR1/COX-2/NF-κB protein expressions in heart. Metformin suppressed signs of MS and alleviated cardiac changes in a high-fat diet fed rats. It also reduced oxidative stress and inflammation relevant to modulation of AdipoR1/COX-2/NF-κB protein expressions in heart. These results confirmed the fact that metformin is a hypoglycemic agent (31). Cardioprotective effects of metformin has been reported in patients and experimental rats (61, 62). It was reported to have anti-inflammatory and antioxidant effects (33, 34). The level of adiponectin was raised by metformin in a high-fat diet fed rats that was consistent with the study that metformin treatment increased plasma adiponectin levels in obese and type 2 diabetes (63, 64).

Conclusions

In conclusion, these data suggest that galangin resolved cardiometabolic disorders induced by a high-fat diet plus fructose in rats associated with reducing inflammation and oxidative stress. Its regressed LV dysfunction and remodeling via restoration of AdipoR1/COX-2/NF-κB protein expression in cardiac tissue of MS rats.

Abbreviations

AdipoR1: Adiponectin receptor1

ALT: Alanine transaminase

ANOVA: One-way analysis of variance

AST: Aspartate transaminase

BW: Body weight

CAT: Catalase

COX-2: Cyclooxygenase-2

CSA: Cross-sectional area

DAB: 3,3'-Diaminobenzidine

DBP: Diastolic blood pressure

EDTA: Tris-ethylenediaminetetraacetic acid

EDV: End-diastolic volumes

EF: Ejection fraction

ELISA: Enzyme-linked immunosorbent assay

EP: Epididymal fat

ESV: End-systole volumes

H&E: Hematoxylin and eosin

H2O2: Hydrogen peroxide

HDL-c: High-density lipoprotein cholesterol

HOMA-IR: Homeostatic model assessment for insulin resistance

HR: Heart rate

HRP: Horseradish peroxidase

HW: Heart weight

I/R: Ischemia-reperfusion

IL-6: Interleukin-6

IL-8: Interleukin-8

IVSd: Interventricular septal at end diastole

IVSs: Interventricular septal at end systole

LV: left ventricular

LVIDd: Left ventricular internal diameter at end diastole

LVIDs: Left ventricular internal diameter at end systole

LVPWd: Left ventricular posterior wall at end diastole

LVPWs: Left ventricular posterior wall at end systole

LW: Liver weight

MAP: Mean arterial pressure

MDA: Malondialdehyde

MS: Metabolic syndrome

NF-κB: Nuclear factor kappa B

OGTT: Oral glucose tolerance test

PVDF: Polyvinylidene difluoride

RP: Retroperitoneal fat

S.E.M.: Standard error of the mean

SBP: Systolic blood pressure

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel

SF: Left ventricular shortening fraction

SOD: Superoxide dismutase

STZ: Streptozotocin

SV: Stroke volume

TBA: Thiobarbituric acid

TBARS: Thiobarbituric acid reactive substances

TBS-T: Tris-buffered saline with 0.1% Tween-20

TC: Total cholesterol

TEP: 1, 1, 3, 3’ tetra-ethoxy propane

TG: Triglycerides

TNF-α: Tumor necrosis factor-α

VW: Ventricular weight

Declarations

Ethics approval and consent to participate.

All procedures were performed in accordance with the rules of ethical guideline for the Care and Use of Laboratory Animals, which was approved by animal ethics committee of Khon Kaen University (IACUC-KKU- 74/62), based on the ethic animal experimentation of national research council of Thailand.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

All authors have no competing interests.

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Authors' contributions

Conceptualization, P.P.; methodology and formal analysis, P.Pr., S.M., S.B., PM; Pa.Pr. writing, review and editing manuscript, P.P. All authors have read and agreed to the published version of the manuscript.

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Figures

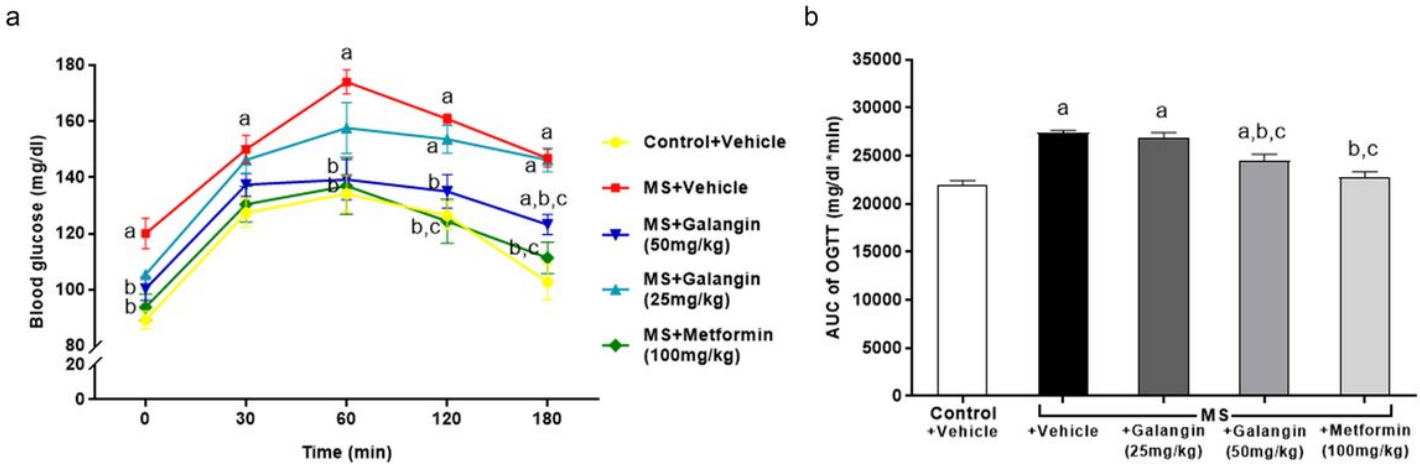

Figure 1

Effects of galangin and metformin treatments on blood glucose concentrations (a) OGTT and (b) and area under the curves of OGTT (c). Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group, bp < 0.05 vs MS group and cp < 0.05 vs MS+ Galangin (25 mg/kg) group. MS: Metabolic syndrome; OGTT: Oral glucose tolerance test; and AUC: area under the curves

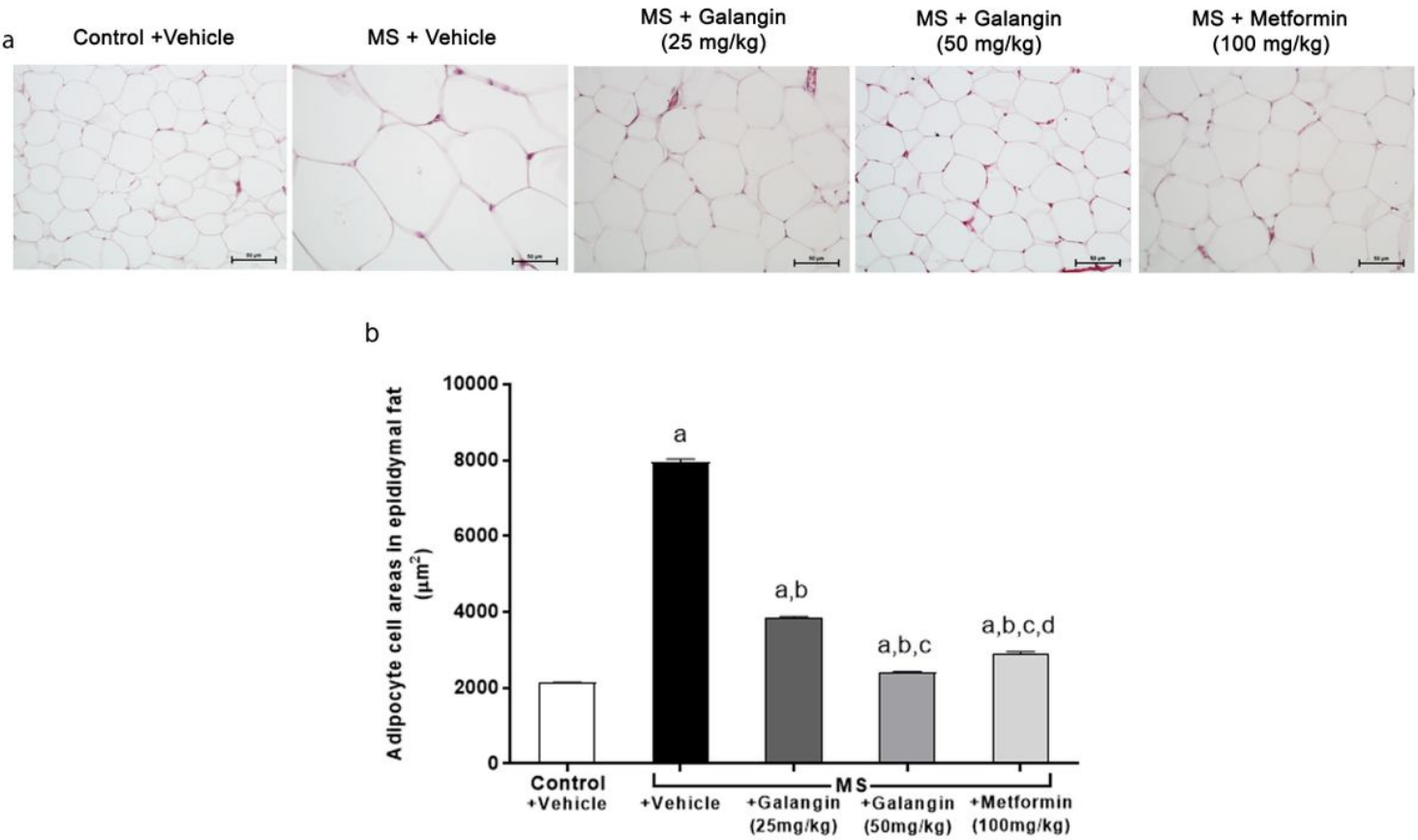

Figure 2

Morphology of epididymal fat pads. (a) Representative photographs of epididymal fat sections stained with H&E (magnification ×200) (scale bar = 50 μm). (b) Effects of galangin and metformin treatments on adipocyte cell areas. Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group, bp < 0.05 vs MS group, cp < 0.05 vs MS + galangin (25 mg/kg) group and dp < 0.05 vs MS + galangin (50 mg/kg) group. MS: Metabolic syndrome

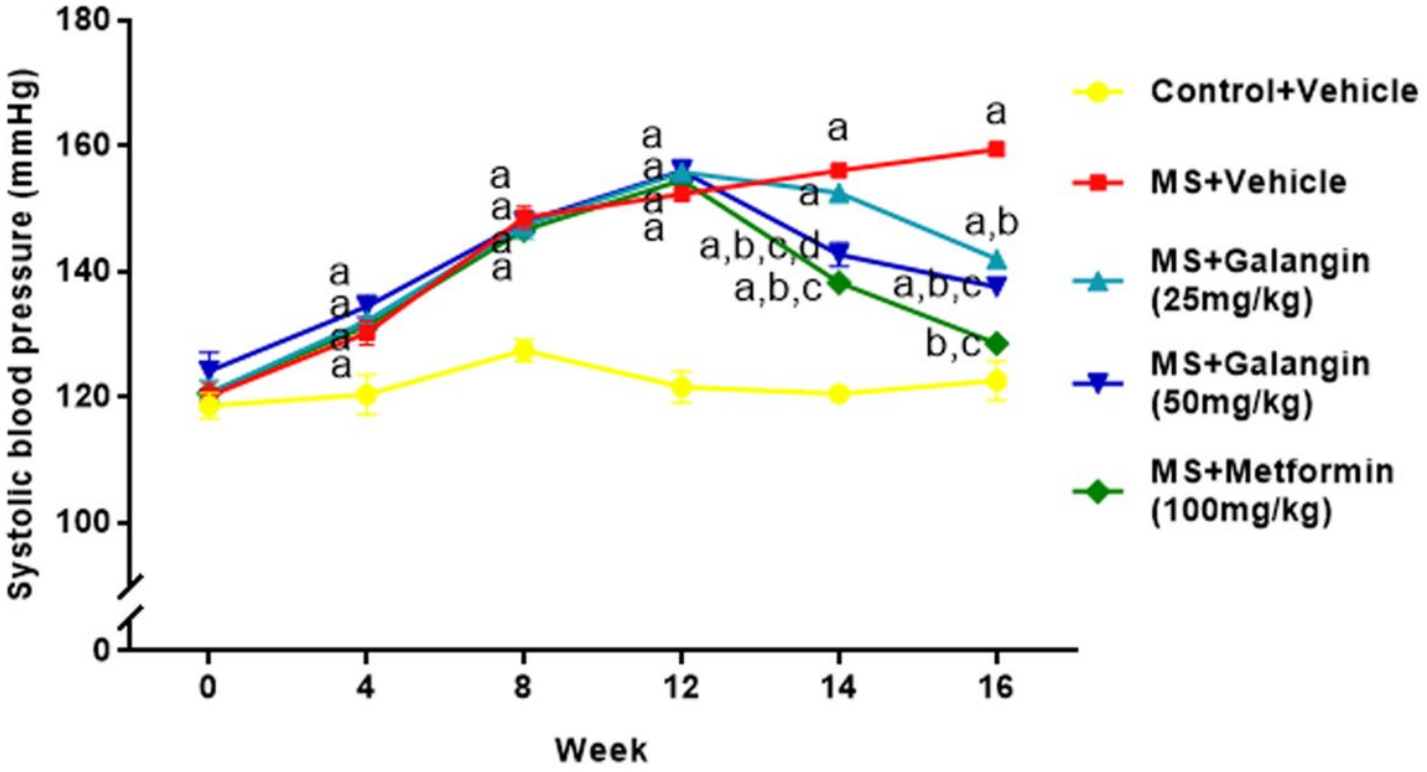

Figure 3

Effects of galangin and metformin treatments on systolic blood pressure. Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group, bp < 0.05 vs MS group, cp < 0.05 vs MS + galangin (25 mg/kg) group and dp < 0.05 vs MS + galangin (50 mg/kg) group. MS: Metabolic syndrome

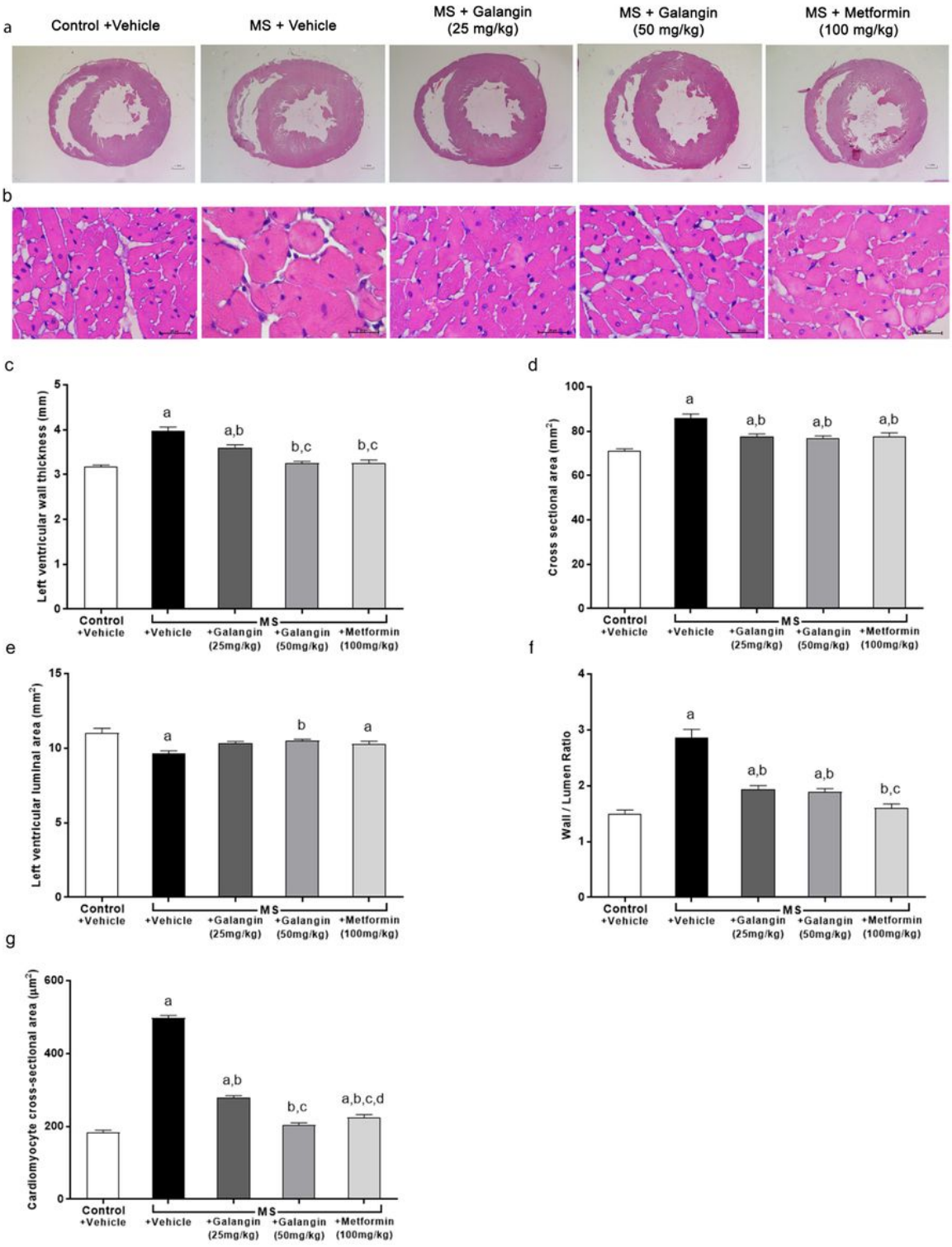

Figure 4

Morphology of heart. (a) Representative photographs of heart sections stained with H&E (magnification ×10) (scale bar = 5 mm). (b) Representative cross-sections of cardiomyocytes stained with H&E (magnification ×400) (scale bar = 20 μm). (c) Effects of galangin and metformin treatments on left ventricular wall thickness, (d) cross-sectional areas, (e) left ventricular luminal areas, (f) wall/lumen ratio and (g) cardiomyocyte area. Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group, bp <

0.05 vs MS group, cp < 0.05 vs MS + galangin (25 mg/kg) group and dp < 0.05 vs MS + galangin (50 mg/kg) group. MS: Metabolic syndrome

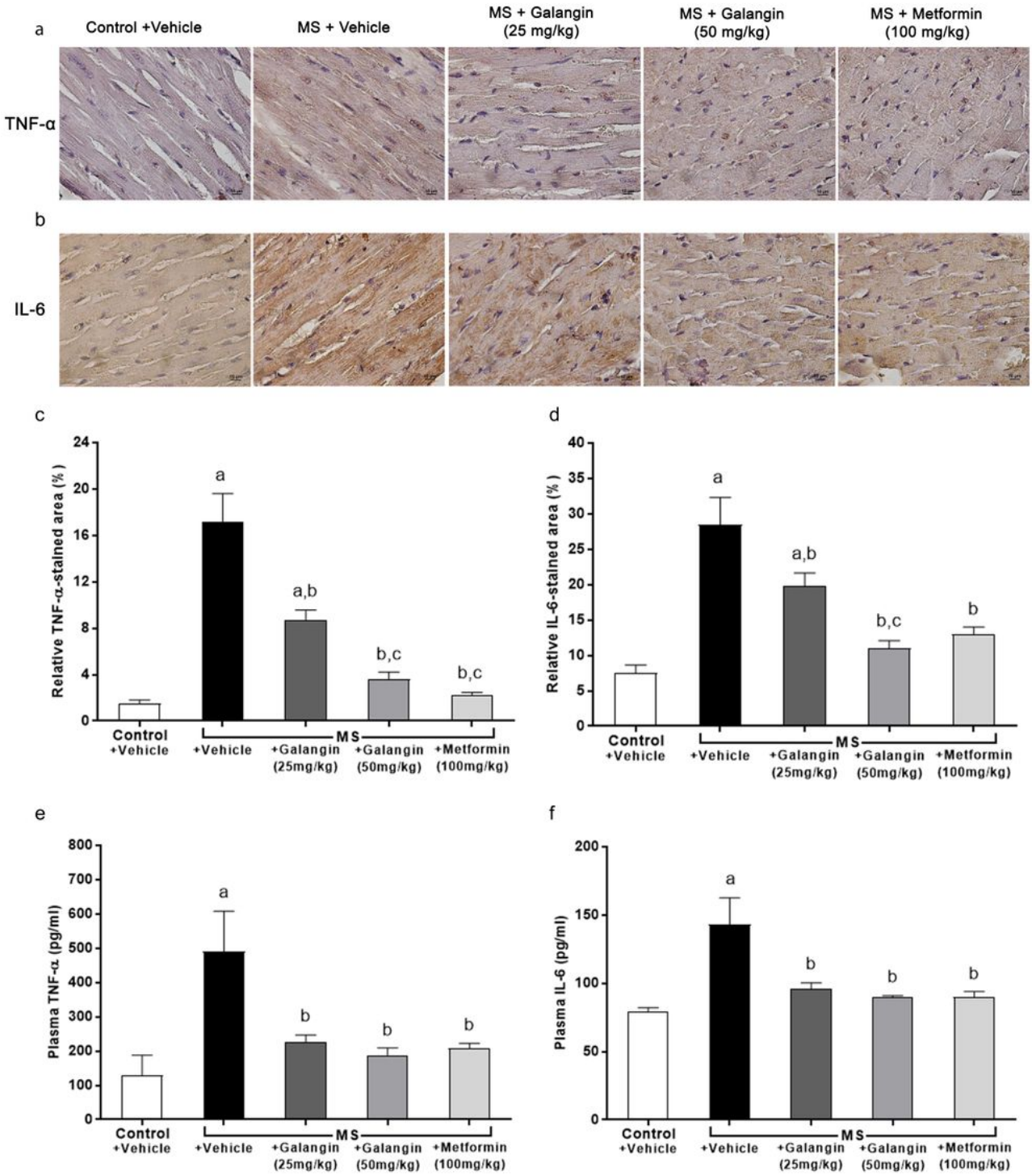

Figure 5

Effects of galangin and metformin treatments on TNF-α and IL-6 immunohistochemical staining in myocardial and TNF-α and IL-6 levels. (a) TNF-α, (b) IL-6 (Brown Chromogen) immunohistochemical staining in myocardial (magnification×400) (scale bar = 10 μm), (c) relative TNF-α-stained area (%), (d)

relative IL-6-stained area (%), (e) plasma TNF-α and (f) plasma IL-6. Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group, bp < 0.05 vs MS group and cp < 0.05 vs MS + galangin (25 mg/kg) group. MS: Metabolic syndrome; TNF-α: Tumor necrosis factor alpha; and IL-6: Interleukin-6

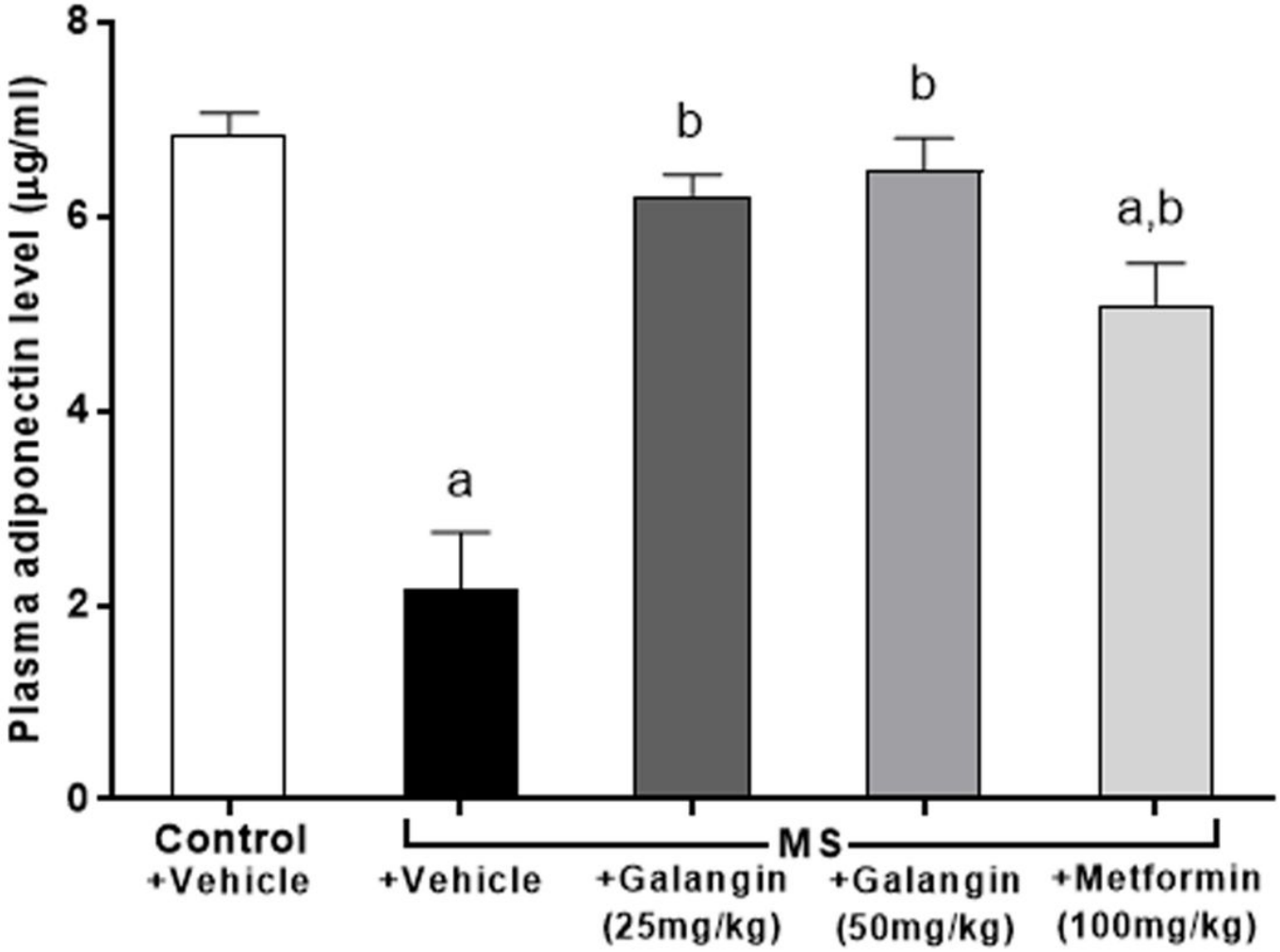

Figure 6

Effects of galangin and metformin treatments on plasma adiponectin levels. Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group and bp < 0.05 vs MS group. MS: Metabolic syndrome

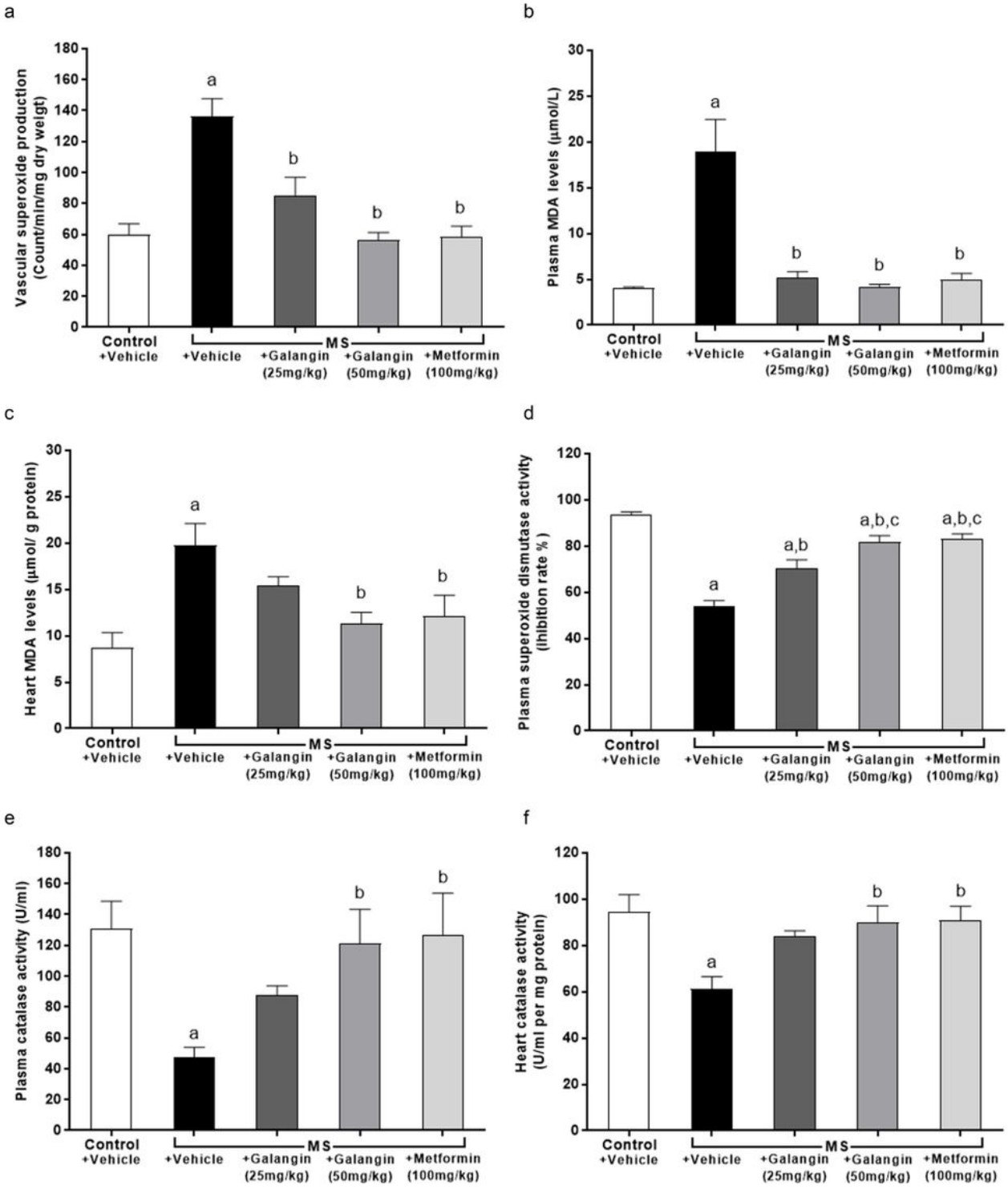

Figure 7

Effects of galangin and metformin treatments on oxidative stress markers and endogenous antioxidant enzymes. (a) aortic superoxide production, (b) plasma MDA level, (c) heart MDA level (d) plasma SOD activity, (e) plasma CAT activity and (f) heart CAT activity. Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group, bp < 0.05 vs MS group and cp < 0.05 vs MS + Galangin (25ml/kg) group. MS: Metabolic syndrome; MDA: Malondialdehyde; SOD: Superoxide dismutase; and CAT: Catalase

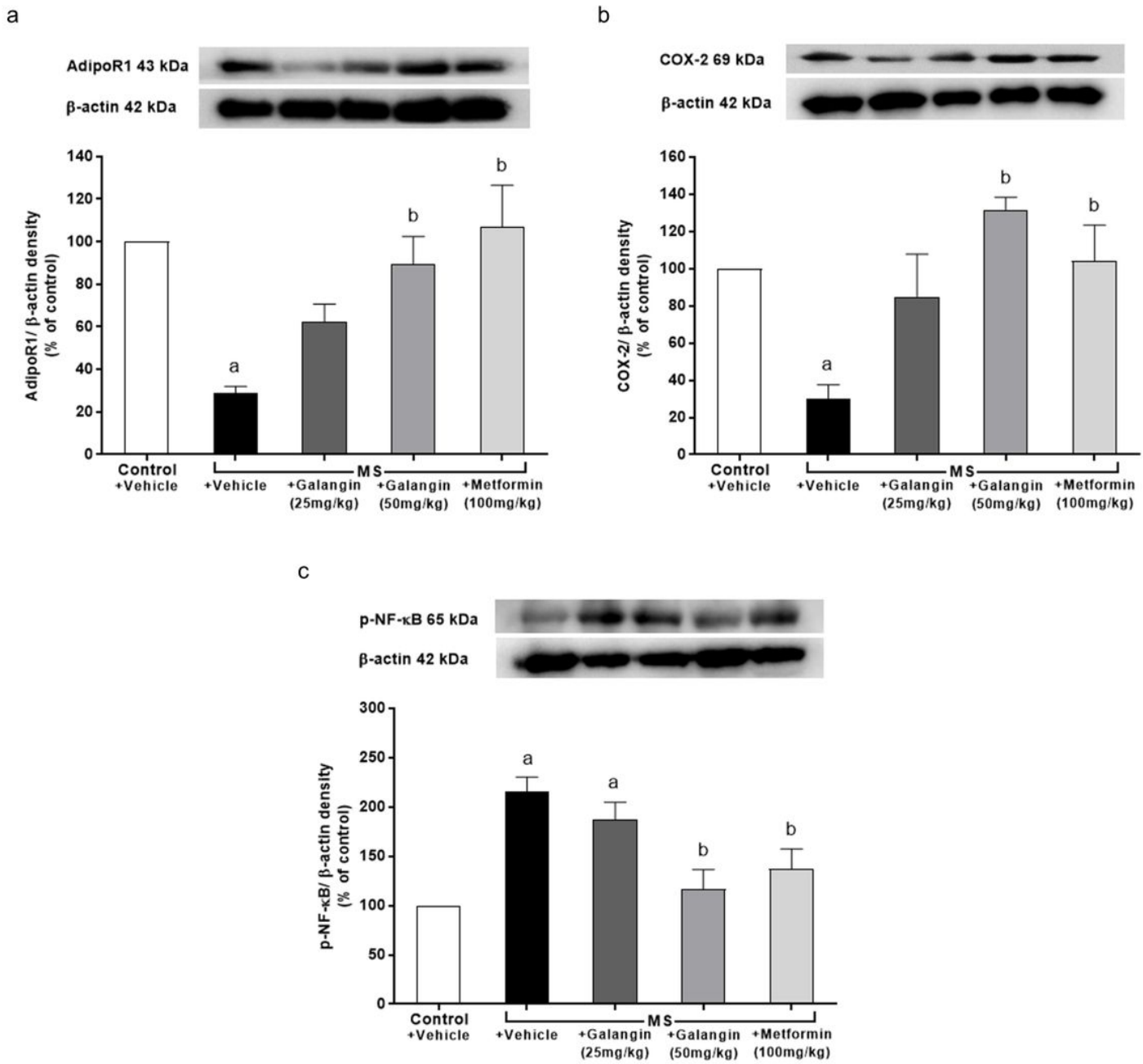

Figure 8

Effects of galangin and metformin treatments on protein expression in heart (a) AdipoR1, (b) COX-2 (c) p-NF-κB. Data are presented as mean ± S.E.M. (n=8). ap < 0.05 vs control group and bp < 0.05 vs MS group. MS: Metabolic syndrome; AdipoR1: Adiponectin receptor1; COX-2: Cyclooxygenase-2; and p-NF-κB: Phospho nuclear factor kappa B