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ORIGINAL ARTICLE

Effect of Indian gooseberry extract on improving methylglyoxal-associated leptin resistance in peripheral tissues of high-fat diet-fed rats

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Abstract

Increased leptin resistance and methylglyoxal (MG) levels are observed in obese patients. However, whether MG deposits contribute to leptin resistance, oxidative stress, and inflammation in peripheral tissues remains unclear. In addition, the edible fruit of Indian gooseberry (*Phyllanthus emblica* L.) contains abundant bioactive components such as vitamin C, β-glucogallin (β-glu), gallic acid (GA), and ellagic acid (EA). Water extract of Indian gooseberry fruit (WEIG) and GA has been shown to improve cognitive decline by suppressing brain MG-induced insulin resistance in rats administered a high-fat diet (HFD). Accordingly, this study investigated the functions of WEIG and GA in inhibiting MG-induced leptin resistance, oxidative stress, and inflammation in the peripheral tissues of HFD-fed rats. The results showed that MG, advanced glycation end products (AGEs), and leptin resistance accumulation in the liver, kidney, and perinephric fat were effectively restored by elevated glyoxalase-1 (Glo-1) activity after WEIG and GA administration comparable to that of alagebrium chloride (positive control) treatment in HFD-fed rats. Furthermore, WEIG and GA supplementation increased adiponectin and antioxidant enzymes (glutathione peroxidase, superoxide dismutase, catalase) and decreased inflammatory cytokines (IL-6, IL-1β, TNF-α) in the peripheral tissues of HFD-fed rats. In conclusion, these findings demonstrated that MG may trigger leptin resistance, oxidative stress, and inflammation in peripheral tissues, which could be abolished by WEIG and GA treatment. These results show the potential of *P. emblica* for functional food development and improving obesity-associated metabolic disorders.

Keywords: Adiponectin, Gallic acid, Glyoxalase-1, Methylglyoxal, WEIG

1. Introduction

Leptin is a hormone that plays important roles in inhibiting appetite, promoting energy expenditure, and regulating glycolipid metabolism; however, leptin resistance occurs frequently in obese individuals, which results in leptin malfunction [1]. In addition, the underlying mechanism that causes leptin resistance still needs to be defined. Several possibilities have been postulated including that: (1) circulating leptin fails to cross the blood−brain barrier (BBB) and cannot play a role in appetite inhibition [2], (2) leptin signaling cascade transduction is restricted [3], (3) low expression of leptin receptors, (4) desensitization of cellular activation in the central and peripheral nervous systems, and (5) oxidative stress and inflammatory responses may all contribute to leptin resistance [4]. Although the cause of leptin resistance is hypothesized, obesity cannot be overcome by targeting those assumptions, indicating that a dominating mechanism of leptin resistance should be revealed.

Both advanced glycation end products (AGEs) and their precursor methylglyoxal (MG) are involved in lipid and carbohydrate metabolism disorders [5]. In addition, MG is a well-known dicarbonyl that easily

Abbreviations: AGEs, Advanced glycation end products; ALA, Alagebrium chloride; GA, Gallic acid; Glo-1, Glyoxalase-1; MG, Methylglyoxal; WEIG, Water extract from Indian gooseberry fruit.

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glycates proteins and consequently impairs their functions, such as insulin resistance [6]. Thus, we hypothesized that leptin resistance might be directly triggered by MG-glycated leptin. A current report showed that MG enhances leptin resistance, the lipogenesis-associated signaling cascade, and lipid accumulation via the MG-glycation process [7].

Indian gooseberry (*Phyllanthus emblica* L.) is cultivated in subtropical and tropical areas including China, India, Turkey, and Taiwan. The edible fruit contains abundant bioactive components, such as vitamin C, β-glucogallin (β-glu), gallic acid (GA), ellagic acid (EA), quercetin, geraniin, chebulagic acid, syringic acid, and esculetin [8]. Several food products such as Indian gooseberry powder, juice, murabba, and jam are sold in markets. Moreover, Indian gooseberry is a traditional medicinal used against common cold, fever, diabetes, dyspepsia, peptic ulcer, inflammation, and skin diseases [9]. Several pharmacological studies have elucidated that the extract and its active compounds display antioxidative, anti-inflammatory, hepatoprotective, hypolipidemic, antitumor, and hypoglycemic abilities [9,10]. A previous study substantiated that supplementation with water extract of Indian gooseberry fruit (WEIG) and its bioactive compound GA significantly improved cognitive decline by increasing the relative abundances of *Bacteroidetes*, *Gammaproteobacteria*, and *Parasutterella*, which negatively correlated with brain MG and AGE levels, inflammatory cytokines and Alzheimer's disease-related protein expression in high-fat diet (HFD)-fed rats [11]. Moreover, a current report revealed that WEIG and its phenolic compounds (GA and EA) effectively prevent MG glycation-induced leptin resistance, which results in inhibiting suppressor of cytokine signaling 3 (SOCS3) and lipogenesis-related signaling (SREBP-1/ACC/FAS) gene expression while triggering the JAK2/STAT3/AMPK/PPAR-α/CPT-1 pathway in free fatty acid-treated human HepG2 liver cells [7]. The above evidence demonstrates the potential of WEIG and GA in repressing MG-induced leptin resistance.

Inflammation and oxidative stress in peripheral organs such as the liver, kidney, and perinephric fat act as a negative feedback loop regulating energy and metabolic homeostasis [12]. MG has been demonstrated to contribute to the development of obesity-associated metabolic syndromes such as nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus, chronic kidney diseases, cancers, cardiovascular diseases, and Alzheimer's disease [13]. Accordingly, suppressing the accumulation of peripheral MG may reduce inflammation and oxidative stress, which may terminate the increased incidence of obesity and related metabolic disorders. To our knowledge, no research exists exploring MG-triggered leptin resistance in peripheral tissues. This study aimed to investigate WEIG and GA in preventing leptin resistance in peripheral tissues by regulating MG, inflammation, and oxidative stress in HFD-fed rats.

2. Materials and methods

2.1. Chemicals and reagents

Alagebrium chloride (ALA), MG, and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). A BCA protein assay kit was purchased from BD Biosciences (Taipei, Taiwan). TNF-α (DY510), IL-1β (DY501), IL-6 (DY506-05), leptin (MOB00), and adiponectin (DY3100-05) ELISA kits were purchased from R&D System Inc. (Minneapolis, Minnesota, USA). Glutathione peroxidase (GPx, 703102), superoxide dismutase (SOD, 706002), and catalase (707002) assay kits were purchased from Cayman Chemicals (Ann Arbor, MI, USA). A glyoxalase I assay kit was purchased from Biovision Incorporated (Milpitas, CA, USA). LabDiet 5001 (Laboratory rodent diet) was purchased from Newco Distributors Corporation (Rancho Cucamonga, CA, USA).

2.2. Preparation of WEIG

Indian gooseberry fruits were kindly provided by the Miaoli District Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan (Miaoli, Taiwan) in October 2017. Fresh Indian gooseberry fruits were hot-air dried at 60 °C and then powdered using a high-speed grinder machine (RT-N04, Rong Tsong, Taiwan). The dry powder of Indian gooseberry fruits was extracted with deionized water at a ratio of 1:40 and soaked overnight. Then, the extract was filtered and lyophilized to obtain WEIG. Major bioactive compounds such as GA, EA, and β-Glu were identified in a recent report [11].

2.3. Animal treatment

Five-week-old male Sprague–Dawley (SD) rats were purchased from BioLASCO Experimental Animal Center, Taiwan. Rats were housed in the feed room (temperatures of 22 ± 5 °C with 65 ± 5% humidity and 12-h light/dark cycle) and fed a normal chow diet (LabDiet 5001) *ad libitum* to acclimate to the environment for one week. The animal experiment was approved by the Laboratory

Animal Care and Use Committee of National Chung Hsing University (approval no: 108-095).

After one week of adaptation, thirty rats were randomly allocated to six groups (n = 5). Except for the control (LabDiet 5001), rats were fed an HFD (LabDiet 5001 + 40% butter, total 60% kcal). The experimental grouping included Control, HFD, L-WEIG (low dose of WEIG, 250 mg kg/b.w./daily), H-WEIG (high dose of WEIG, 500 mg kg/b.w./daily), ALA (ALA recognized as an MG scavenger and AGEs crosslink breaker, was utilized as a positive control at a dosage of 1 mg kg/b.w./daily), and GA (pure GA, 100 mg kg/b.w./daily) groups, which were administered daily by oral gavage for 112 consecutive days [11]. In our previous report, fat accumulates in perinephric adipose tissue more than in epididymal and mesenteric tissues [11]. At the end of the experiments, rats were sacrificed using isoflurane anesthesia, and samples (serum, liver, kidney, and perinephric fat) were collected. Then, tissues were homogenized with PBS using a tissue homogenizer (SH-100, KURABO, Japan) at 4 °C and centrifuged at 10000×*g* at 4 °C for 15 min. The supernatant was stored in a freezer at −80 °C for further analysis. The animal study was conducted according to the National Research Council's Guide for the Care and Use of Laboratory Animals.

2.4. Hematoxylin-eosin (H&E) staining

The H&E staining of the liver slice and the hepatic pathology were commissioned by Professor Jiunn-Wang Liao (Graduate Institute of Veterinary Pathobiology, National Chung Hsing University). The H&E-stained liver tissue was scored from 1 to 5 according to the lesion evaluation standard [14].

2.5. Assay of tissue MG level

The level of MG in peripheral tissues was determined using HPLC [15]. Briefly, the supernatants of homogenized peripheral tissue were derivatized with 10 mM o-phenylenediamine (OPD) and 100 μg/mL 5 MQ (as an internal standard) in the dark for 24 h. After the incubation, the derivatization was filtered with a 0.22 μm filter and then analyzed by HPLC. The HPLC analysis conditions by HPLC (Hitachi, Tokyo, Japan) consisted of a Chromaster 5110 Pump, a Chromaster 5210 autosampler, a Chromaster 5310 column oven, a Chromaster 5430 diode array detector, and LiChrospher® 100 RP-18 Column (5 μm, 4 nm × 250 nm) (Merck KGaA, Darmstadt, Germany). Mobile phase: Solution A was 5 mM NaH_2PO_4 in water, and solution B was acetonitrile. Gradient: B solution maintained 17.6% at 0–21 min, 50% at 21–22 min, 50% at 22–23 min, 17.6% at 23–24 min, and 17.6% at 24–35 min. The flow rate was 1 mL/min. The temperature was controlled at 30 °C, the sample injection volume was 20 μL, and the UV–Vis detector absorbed at 317 nm. The integrated area of the relevant signal generated was compared with the MG concentration made into a standard curve to determine the amount of MG in the tissue.

2.6. Assay of malondialdehyde (MDA) level

The tissue homogenate solution was incubated with TCA-TBA-HCl reagent [trichloroacetic acid (15%, w/v in 0.25 N HCl) and thiobituric acid (0.375%, w/v in 0.25 N HCl)] and heated in a water bath at 100 °C for 15 min. After cooling, butanol was added to the mixture, mixed well, and then centrifuged. The pink supernatant was measured at a wavelength of 535 nm (BMG Labtech, Ortenberg, Germany). 1,1,3,3-tetramethoxypropane was a standard for MDA calculation [16].

2.7. Assay of oxidative enzymes and inflammatory cytokine levels

The levels of oxidative enzymes (SOD, catalase, GPx) and inflammatory cytokines (IL-1β, IL-6, TNF-α) were analyzed using commercial kits according to the manufacturer's instructions.

2.8. Assay of leptin, adiponectin, and Glo-1 levels

The levels of leptin (Rat Leptin Quantikine ELISA kit), adiponectin (Rat Adiponectin/Acrp30 Duoset ELISA kit), and Glo-1 (Glyoxalase I activity assay kit) were measured using ELISA kits according to the manufacturer's instructions.

2.9. Assay of AGE level

The AGE content in tissues was measured according to a previous report [11]. In brief, tissues were homogenized with 0.1 N NaOH and modulated the pH to 8.5 by 0.2 M boric acid solution. The supernatants were measured at 355 nm (excitation) and 405 nm (emission) using a FLUOstar galaxy spectrophotometer (BMG Labtech, Offenburg, Germany) by detecting the autofluorescence characteristic of AGEs. The relative fluorescence intensity of AGEs was measured at a protein concentration of 1 mg/mL and normalized against a BSA solution (1 mg/mL BSA was defined as 1 AU).

2.10. Statistical analysis

The results are presented as the mean ± SEM. Statistical analysis was performed using the SPSS software package. One-way ANOVA with Duncan's test was used to evaluate the statistical significance significant between groups (p-value < 0.05).

3. Results

3.1. Effect of WEIG on MG metabolism in the peripheral tissues of HFD-induced rats

An obesity rat model was established by HFD administration to evaluate MG metabolism in peripheral tissues. Administration of H-WEIG and GA significantly reduced the body weight in rats subjected to HFD treatment (Fig. 1A). The appearance of large and small vacuoles in the liver tissue indicated that NAFLD was successfully established with lipid deposition (Fig. 1B). Histopathological alteration of general fatty change, fatty infiltration with microvesicles (multifocal), fatty infiltration with macrovesicles (multifocal), and fat inflammatory infiltration (infiltration, mononuclear cell, multifocal) and total scoring were applied to assess the pathological degree of fatty liver among groups. All indices were significantly increased in the HFD group compared with the control group (Table 1).

To validate whether NAFLD contributed to MG accumulation in peripheral tissues, the MG levels in the liver, kidney, and perinephric fat were determined using HPLC. As shown in Fig. 2, MG levels were effectively increased in the liver, kidney, and perinephric fat of HFD-induced rats compared to those in the control group. The MG accumulation in peripheral tissues was significantly repressed in H-WEIG and ALA-administered obese rats (Fig. 2). Glo-1 is a crucial enzyme in regulating MG metabolism [13]. HFD intake caused the lowest activity of Glo-1 in peripheral tissues, which was lifted by administration of H-WEIG, ALA, and GA in liver and perinephric tissues of rats subjected to HFD treatment (Fig. 3).

MG glycates proteins, lipids, and DNA to form AGEs, which leads to cell toxicity and impairs physiological functions in the body [6]. Here, the level of AGEs in peripheral tissues was increased in the HFD group compared with the control group (Fig. 4). WEIG, ALA, and GA administration significantly reduced AGE activity in the liver and kidney tissues of HFD-induced rats (Fig. 4A–B). AGE activity in the perinephric fat tissue was also suppressed by H-WEIG and GA supplementation (Fig. 4C). The above evidence indicated

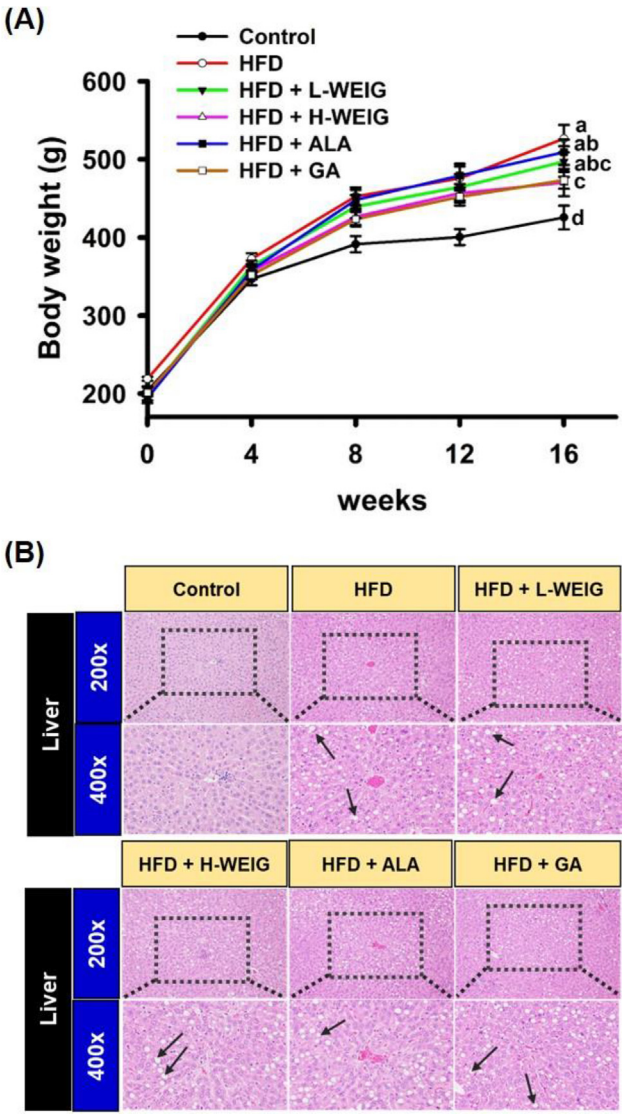

Fig. 1. The effects of WEIG, ALA, and GA administration on body weight and hepatic lipid accumulation in HFD-induced SD rats. (A) Body weight. The results are shown as the mean ± SEM ($n = 5$). Different letters indicate statistically significant differences ($p < 0.05$). (B) Histological changes in liver tissues were evaluated by H&E staining at 200X magnification and 400X magnification, respectively. Arrow: fat vesicles.

HFD-induced MG and AGE accumulation in peripheral tissues.

3.2. Effect of WEIG on leptin and adiponectin expression in peripheral tissues

Studies have shown that high food intake and energy metabolism obstacles in obese individuals lead to leptin resistance and hyperleptinemia [17]. A report has demonstrated that MG-glycated leptin consequently impairs its function [7]. Accordingly, the next step was to examine whether MG

Table 1. Effect of WEIG on administration hepatic pathology in HFD-induced SD rats.[a]

Histopathological findings	Group					
	Control	HFD	HFD + L-WEIG	HFD + H-WEIG	HFD + ALA	HFD + GA
1. General fatty change	0.00 ± 0.00[b]	2.60 ± 0.24[a]	2.20 ± 0.37[a]	2.00 ± 0.45[a]	1.80 ± 0.58[a]	2.00 ± 0.45[a]
2. Fatty infiltration with micro-vesicles, multifocal	0.00 ± 0.00[b]	2.60 ± 0.24[a]	2.17 ± 0.37[a]	2.00 ± 0.45[a]	1.80 ± 0.58[a]	2.00 ± 0.45[a]
3. Fatty infiltration with macro-vesicles, multifocal	0.00 ± 0.00[b]	1.80 ± 0.37[a]	2.00 ± 0.45[a]	1.40 ± 0.24[a]	1.20 ± 0.37[a]	1.60 ± 0.24[a]
4. Infiltration, mononuclear cell, multifocal	0.00 ± 0.00[b]	0.80 ± 0.20[a]	0.60 ± 0.24[ab]	0.40 ± 0.24[ab]	0.40 ± 0.24[ab]	0.20 ± 0.20[ab]
Total score	0.00 ± 0.00[b]	7.80 ± 0.89[a]	7.00 ± 1.38[a]	5.80 ± 1.36[a]	5.20 ± 1.50[a]	5.80 ± 1.24[a]

[a] Results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

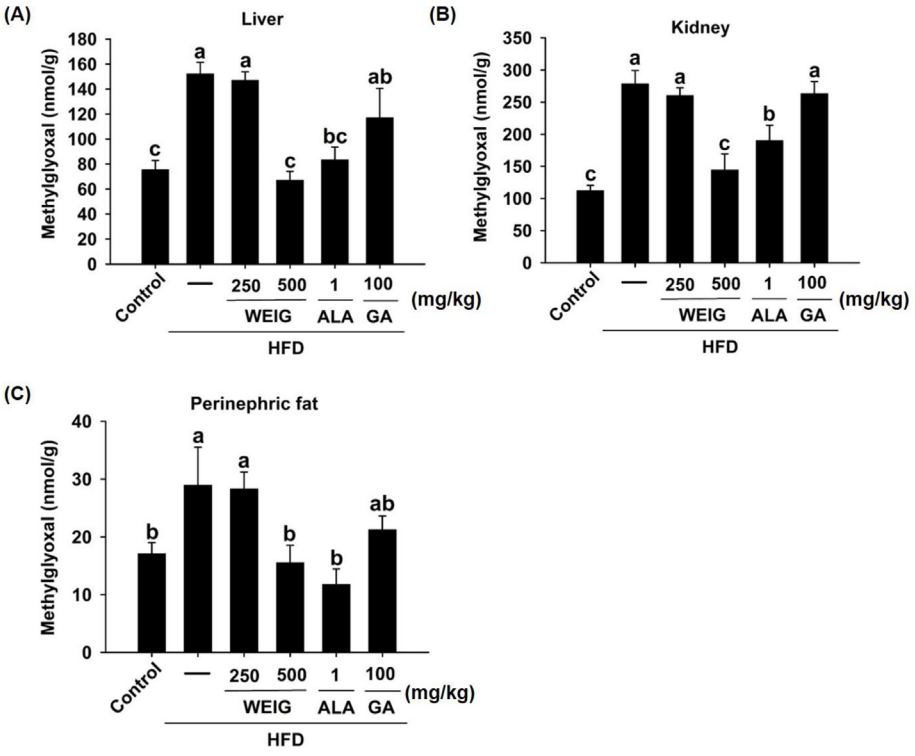

Fig. 2. The effects of WEIG, ALA, and GA administration on the levels of tissue methylglyoxal in HFD-induced SD rats. The levels of methylglyoxal in (A) liver, (B) kidney, and (C) perinephric fat. The results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

contributed to leptin resistance in sera and peripheral tissues. Fig. 5 shows that HFD induced high leptin content in sera and peripheral tissues of rats compared to those in the control group (Fig. 5). Moreover, HFD intake dramatically increased serum leptin compared to the control group (Fig. 5A), indicating that leptin resistance occurred in HFD-induced rats. Furthermore, H-WEIG, ALA, and GA supplementation significantly inhibited leptin expression in the serum and liver of HFD-induced rats (Fig. 5A–B). Although the administration of H-WEIG seemed to reduce leptin levels in the kidney and perinephric fat, there were no statistically significant differences between the HFD and the H-WEIG groups (Fig. 5C–D).

Adiponectin is an adipocyte-derived hormone with anti-inflammatory properties and can reduce the incidence of obesity-associated metabolic diseases [18]. As shown in Fig. 6B, the hepato-adiponectin content in the HFD group was significantly decreased compared to the control group. Additionally, adiponectin levels showed a slight reduction in the perinephric fat tissue of the HFD group in contrast to the control group (Fig. 6D). L-WEIG, and H-WEIG could significantly promote the increase in adiponectin

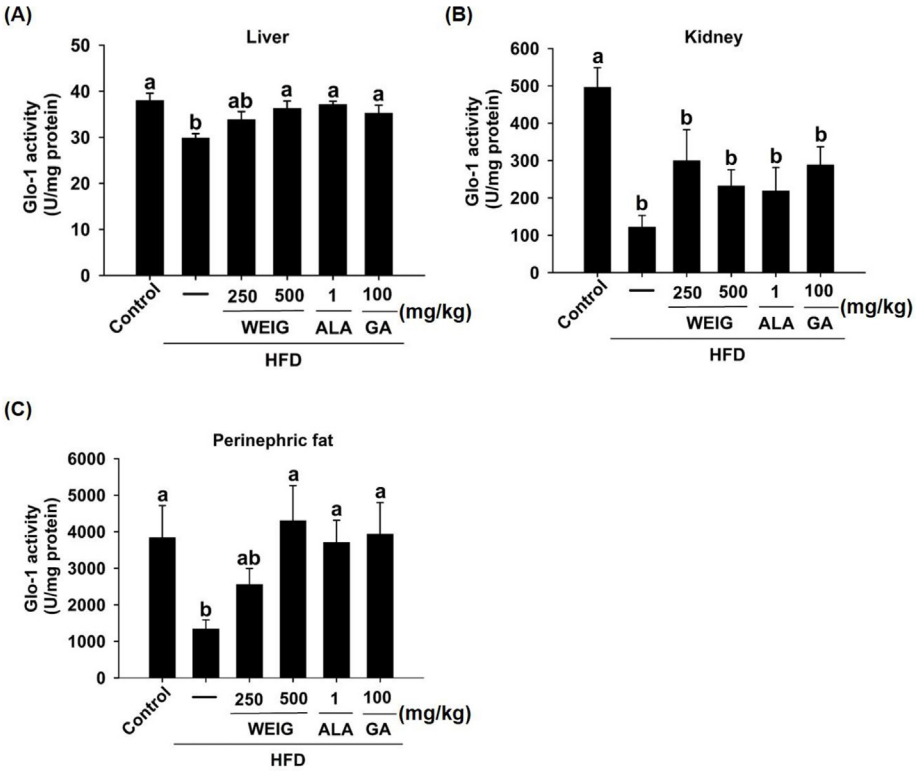

Fig. 3. The effects of WEIG, ALA, and GA administration on the activities of tissue Glo-1 in HFD-induced SD rats. The activities of Glo-1 in (A) liver, (B) kidney, and (C) perinephric fat. The results are shown as the mean ± SEM ($n = 5$). Different letters indicate statistically significant differences ($p < 0.05$).

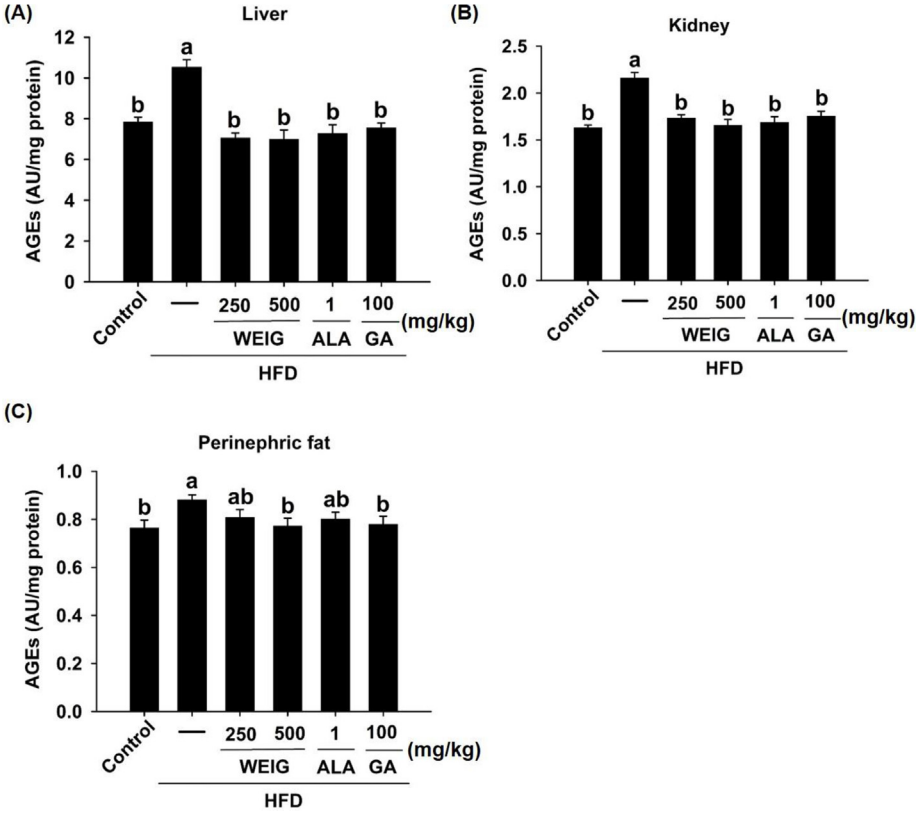

Fig. 4. The effects of WEIG, ALA, and GA administration on the levels of tissue advanced glycation end products in HFD-induced SD rats. The levels of advanced glycation end products in (A) liver, (B) kidney, and (C) perinephric fat. The results are shown as the mean ± SEM ($n = 5$). Different letters indicate statistically significant differences ($p < 0.05$).

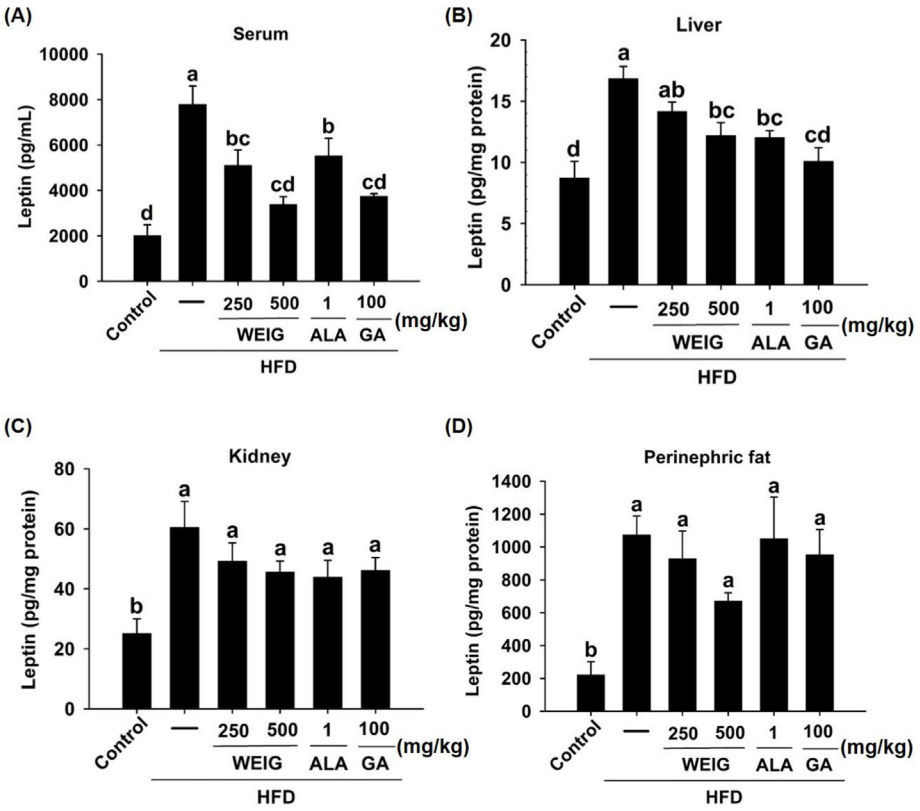

Fig. 5. The effects of WEIG, ALA, and GA administration on serum and tissue leptin levels in HFD-induced SD rats. The levels of leptin in (A) serum, (B) liver, (C) kidney, and (D) perinephric fat. The results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

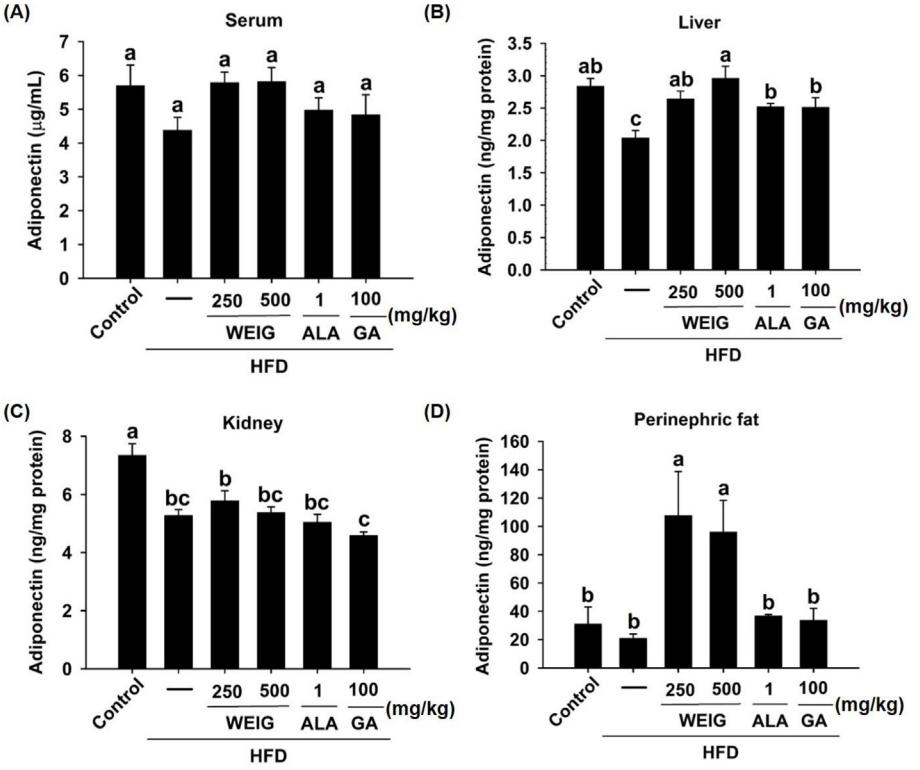

Fig. 6. The effects of WEIG, ALA, and GA administration on serum and tissue adiponectin levels in HFD-induced SD rats. The levels of adiponectin in (A) serum, (B) liver, (C) kidney, and (D) perinephric fat. The results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

content in the liver and perirenal fat (Fig. 6B and D). However, serum and renal adiponectin levels were not significantly changed in the HFD group compared with the WEIG, ALA, and GA groups (Fig. 6 A, C).

3.3. Suppression of leptin resistance reduced oxidative stress and inflammation

MG and AGEs trigger inflammation or oxidative stress processes that may contribute to leptin resistance [19]. The levels of MDA and antioxidant enzymes (catalase, SOD, GPx) were assessed. In liver tissue, the administration of HFD increased MDA and decreased antioxidant enzyme (catalase, GPx) levels compared to the control group (Table 2). Feeding of H-WEIG and ALA effectively reduced MDA expression and induced the activity of SOD and GPx (Table 2). Moreover, upregulation of MDA and downregulation of catalase and GPx levels were observed in the kidney tissue of HFD rats compared with the control group. The H-WEIG, ALA, and GA supplementation effectively increased catalase and SOD activities in rats subjected to HFD treatment (Table 3). Similarly, WEIG, ALA, and GA administration inhibited MDA production and enhanced antioxidant enzyme (catalase, SOD, GPx) production in perinephric fat tissues (Table 4).

In addition, obesity causes chronic inflammation and metabolic disorders [20,21]. Tables 5–7 show that inflammatory cytokine (IL-6, IL-1β, TNF-α) expression in the HFD group was significantly higher in the liver, kidney, and perinephric fat than in the control group. Feeding WEIG, ALA, and GA significantly reduced the secretion of inflammatory

Table 2. Effect of WEIG on liver oxidative stress and antioxidant enzyme levels in HFD-induced SD rats.[a]

Liver	Group					
	Control	HFD	HFD + L-WEIG	HFD + H-WEIG	HFD + ALA	HFD + GA
MDA (nmole/mg protein)	1.40 ± 0.28^{b}	2.31 ± 0.13^{a}	1.67 ± 0.25^{ab}	1.34 ± 0.34^{b}	1.34 ± 0.14^{b}	1.94 ± 0.19^{ab}
Catalase (mM)	12.82 ± 1.13^{c}	8.54 ± 0.46^{a}	11.05 ± 0.40^{ab}	11.18 ± 0.65^{ab}	9.98 ± 0.35^{bc}	10.99 ± 0.70^{ab}
SOD (U/mg protein)	3.12 ± 0.20^{cd}	2.35 ± 0.25^{d}	3.17 ± 0.17^{bcd}	4.87 ± 0.67^{a}	4.41 ± 0.39^{ab}	3.96 ± 0.50^{abc}
GPx (nmole/min/mg protein)	892.7 ± 61.0^{a}	588.5 ± 68.3^{b}	750.9 ± 62.1^{a}	807.1 ± 38.0^{a}	848.9 ± 27.9^{a}	793.2 ± 48.6^{a}

[a] Results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

Table 3. Effect of WEIG on kidney oxidative stress and antioxidant enzyme levels in HFD-induced SD rats.[a]

Kidney	Group					
	Control	HFD	HFD + L-WEIG	HFD + H-WEIG	HFD + ALA	HFD + GA
MDA (nmole/mg protein)	2.07 ± 0.16^{b}	2.73 ± 0.15^{a}	2.48 ± 0.19^{ab}	2.27 ± 0.28^{ab}	2.04 ± 0.22^{b}	2.36 ± 0.18^{ab}
Catalase (mM)	2.41 ± 0.19^{a}	1.62 ± 0.04^{b}	1.94 ± 0.25^{ab}	2.43 ± 0.20^{a}	2.50 ± 0.28^{a}	2.44 ± 0.20^{a}
SOD (U/mg protein)	37.40 ± 3.79^{ab}	32.43 ± 0.93^{b}	42.77 ± 1.62^{a}	45.53 ± 4.15^{a}	46.67 ± 3.11^{a}	45.65 ± 3.01^{a}
GPx (nmole/min/mg protein)	452.4 ± 33.5^{a}	367.3 ± 7.7^{b}	385.1 ± 22.7^{b}	394.8 ± 8.1^{b}	411.7 ± 10.1^{ab}	417.5 ± 10.8^{ab}

[a] Results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

Table 4. Effect of WEIG on the levels of perinephric fat oxidative stress and antioxidant enzymes in HFD-induced SD rats.[a]

Perinephric fat	Group					
	Control	HFD	HFD + L-WEIG	HFD + H-WEIG	HFD + ALA	HFD + GA
MDA (nmole/mg protein)	1.30 ± 0.17^{a}	1.40 ± 0.33^{a}	0.47 ± 0.22^{b}	0.33 ± 0.08^{b}	0.79 ± 0.17^{ab}	0.88 ± 0.13^{ab}
Catalase (μM)	83.34 ± 7.56^{a}	58.27 ± 6.54^{b}	85.34 ± 7.80^{a}	68.76 ± 3.30^{ab}	78.40 ± 2.45^{a}	93.28 ± 6.90^{a}
SOD (U/mg protein)	672.6 ± 81.0^{a}	373.7 ± 41.0^{b}	420.1 ± 98.2^{b}	455.3 ± 99.9^{ab}	477.7 ± 40.1^{ab}	403.8 ± 28.8^{b}
GPx (nmole/min/mg protein)	792.4 ± 118.4^{a}	280.5 ± 45.1^{c}	459.6 ± 42.8^{bc}	522.2 ± 65.2^{abc}	618.1 ± 61.6^{ab}	631.0 ± 142.6^{ab}

[a] Results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

Table 5. Effect of WEIG on the levels of liver proinflammatory cytokines in HFD-induced SD rats.[a]

Liver	Group					
	Control	HFD	HFD + L-WEIG	HFD + H-WEIG	HFD + ALA	HFD + GA
IL-6 (ng/mg)	1.30 ± 0.11^{c}	2.05 ± 0.14^{a}	1.90 ± 0.05^{ab}	1.71 ± 0.15^{b}	1.87 ± 0.07^{ab}	1.63 ± 0.08^{b}
IL-1β (pg/mg)	48.60 ± 6.89^{c}	112.81 ± 9.40^{a}	80.86 ± 9.83^{b}	65.57 ± 5.96^{bc}	68.24 ± 3.64^{bc}	74.18 ± 3.15^{b}
TNF-α (pg/mg)	30.19 ± 3.43^{c}	61.14 ± 5.33^{a}	48.29 ± 7.20^{ab}	40.47 ± 3.48^{bc}	41.45 ± 4.65^{bc}	38.98 ± 4.96^{bc}

[a] Results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

Table 6. Effect of WEIG on the levels of kidney proinflammatory cytokines in HFD-induced SD rats.[a]

Kidney	Group					
	Control	HFD	HFD + L-WEIG	HFD + H-WEIG	HFD + ALA	HFD + GA
IL-6 (ng/mg)	2.29 ± 0.09^{b}	3.24 ± 0.26^{a}	2.28 ± 0.13^{b}	1.90 ± 0.08^{bc}	1.90 ± 0.21^{bc}1	1.63 ± 0.09^{c}
IL-1β (ng/mg)	0.87 ± 0.06^{b}	1.15 ± 0.10^{a}	0.86 ± 0.06^{bc}	0.67 ± 0.05^{bc}	0.64 ± 0.09^{bc}	0.63 ± 0.07^{c}
TNF-α (pg/mg)	103.5 ± 7.3^{b}	141.9 ± 9.6^{a}	99.5 ± 11.1^{b}	88.9 ± 5.1^{b}	96.6 ± 5.6^{b}	82.9 ± 3.0^{b}

[a] Results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

Table 7. Effect of WEIG on the levels of perinephric fat proinflammatory cytokines in HFD-induced SD rats.[a]

Perinephric fat	Group					
	Control	HFD	HFD + L-WEIG	HFD + H-WEIG	HFD + ALA	HFD + GA
IL-6 (pg/mg)	234.3 ± 24.2^{bc}	420.8 ± 83.6^{a}	131.0 ± 16.9^{c}	137.7 ± 35.5^{c}	297.4 ± 46.0^{ab}	144.4 ± 51.3^{c}
IL-1β (pg/mg)	250.1 ± 31.1^{ab}	369.6 ± 66.4^{a}	198.1 ± 33.3^{b}	194.9 ± 17.6^{b}	232.0 ± 47.8^{ab}	311.5 ± 48.5^{ab}

[a] Results are shown as the mean ± SEM (n = 5). Different letters indicate statistically significant differences ($p < 0.05$). The expression of TNF-α was not detected.

cytokines (IL-6, IL-1β, TNF-α) in liver and kidney tissues compared to the HFD group (Tables 5–6). Supplementation with WEIG effectively repressed IL-6 and IL-1β production in the perinephric fat of HFD-induced rats (Table 7).

4. Discussion

The increasing global prevalence of obesity dramatically increases its medical and economic burden [22]. Oxidative stress and inflammation are key factors in obesity and obesity-related metabolic disorder diseases [21]. Recently, a report revealed that peripheral T cells secrete TNF-α to contribute to peripheral inflammation and consequently trigger obesity-associated diabetes [23], indicating that peripheral inflammation dominates the evolution of obesity-related metabolic diseases. Diet and obesity-induced MG production have been documented to be linked to metabolic disease without a deep investigation of the underlying mechanism [13,24]. A previous report demonstrated that trapping brain MG effectively reduced oxidative stress, inflammatory response, insulin resistance, and obesity-associated cognitive decline through WEIG and GA supplementation in HFD-fed rats [11]. However, the contributions of WEIG and GA to the suppression of MG-triggered leptin resistance in peripheral tissues remains unclear. This study found that WEIG and GA significantly prevented leptin resistance by inhibiting MG- and MG-induced inflammation and oxidative stress in HFD-fed rats.

The elevation of circulating glucose and free fatty acid (FFA) levels have been linked to enhanced MG generation, chronic inflammation, and the development of obesity-associated complications, such as NAFLD, type 2 diabetes mellitus, chronic kidney diseases, and cardiovascular diseases [13], indicating that MG accumulation in peripheral tissues is a severe issue in obese individuals. Previous evidence has shown that kidney MG levels are increased by 38% in spontaneously hypertensive rats (SHRs) in contrast to age (20 weeks)-matched Wistar Kyoto (WKY) rats, which results in AGE formation and damage to local vasculature and tubules in the kidney [25]. Moreover, oral administration of chlorella water extract (100 mg/kg b.w.) and phenethylamine (10 μg/kg b.w.) effectively reversed HFD-induced liver damage by inhibiting MG-triggered lipid accumulation and oxidative stress in rats [26]. Additionally, increasing heat shock proteins and mechanoprotection in both young (age 12 weeks) and aged (70 weeks) mice with UCP1 ectopic expression of uncoupling protein-1 in skeletal muscle effectively improves the clearance of MG and MG-derived AGEs that subsequently exhibit a healthy aging phenotype [6]. The above evidence indicates that excessive MG accumulation in peripheral tissues is highly associated with organ damage. However, there is a knowledge gap regarding whether high concentrations of peripheral MG underlie carbonyl stress-induced leptin resistance, oxidative stress, and inflammation in obesity conditions. Interestingly, previous reports have shown that MG-glycated leptin exacerbates brain leptin resistance and cognitive decline in HFD-fed rats [7,11]. Furthermore, similar mechanisms were found in peripheral tissues which exhibited that HFD caused MG accumulation in the liver, kidney, and perinephric fat, subsequently enhancing AGE formation, lipid accumulation, leptin resistance, oxidative stress, and inflammation.

Glo-1 is essential for protecting cells from MG stress. Nevertheless, few studies have explored the Glo-1-promoting ability of natural plant extracts in obesity models. Several reports have tested the MG trapping ability of pure compounds. For example, 0.2% genistein (w/w in 45% lipid HFD) administration for 19 weeks significantly mitigates MG and AGE accumulation by upregulating Glo-1 activity in HFD-fed C57BL/6J mice [27]; supplementation with pentoxifylline (50 mg/kg b.w.) for 14 weeks increases Glo-1 levels and sequentially lowers renal glycoxidative stress, lipid deposition, and proinflammatory cytokine levels by inhibiting AGE/RAGE signaling in C57BL-6J isogenic specific pathogen-free male mice [28]. Recently, a report showed that enhancing the capacity of Glo-1 is a reliable indicator for identifying natural antioxidant compounds from plant foods [29]. This study is the first to demonstrate that WEIG and its major bioactive component GA dramatically increased Glo-1 activity in peripheral tissues of HFD-induced rats, and its effectiveness was comparable to that of ALA administration.

MG (75 mg/kg b.w./day)-treated Wistar rats exhibit triglyceride accumulation concomitant with reduced adiponectin levels that hamper the metabolism of adipocytes [30]. Clinical trials have demonstrated that impaired leptin signaling significantly contributes to lower adiponectin expression in obese subjects compared with healthy individuals [31,32], indicating that MG is not only involved in leptin resistance but also restrains adiponectin production. In agreement with this, these results showed that MG accumulation in peripheral tissues resulted in increased leptin resistance and decreased adiponectin expression. Interestingly, the literature displayed the antihyperlipidemic effect of *P. emblica* L. and GA on increasing adiponectin levels in HFD-induced rats [33]. Importantly, these results corroborated those of a previous study, which revealed that WEIG and GA significantly reduced MG deposition in peripheral tissues by restoring the homeostatic conditions of leptin and adiponectin.

Excessive MG accumulation in adipocytes contributes to oxidative stress and the inflammatory response. Clinical evidence has validated that decreasing adiponectin performance in obese subjects can cause inflammation [34,35]. Here, we found that MG accumulation in peripheral tissues triggered similar effects on impairing adiponectin expression and promoting MDA, IL-1β, IL-6, and TNF-α levels. Administration of WEIG ameliorates nonalcoholic steatohepatitis progression by inhibiting oxidative stress, lipid peroxidation, and inflammation in C57BL/6 mice subjected to methionine and choline-deficient diet (MCD diet) treatment [8]. Consistently, WEIG and GA supplementation upregulated antioxidative enzyme (SOD, catalase, GPx) activities and downregulated inflammatory cytokine (IL-1β, IL-6, TNF-α) secretion in the peripheral tissues of HFD-fed rats. Furthermore, oral supplementation of *P. emblica* L. ethanolic extract is found to be safe at the dosage of 2000 mg/kg b.w. in rats [36]. No toxic effects of *P. emblica* L. are observed through hematological parameters, biochemical indices, and behavioral examinations, which suggests that *P. emblica* fruits is safe for oral intake [9]. Likewise, GA even at a high dose of 5000 mg/kg body weight without combines any signs of toxicity [37]. Those evidences indicate that *P. emblica* L. fruit and GA are suitable for the application of functional food development.

5. Conclusion

In summary, this study showed that NAFLD-enhanced MG and AGE accumulation in peripheral tissues may subsequently cause leptin resistance, oxidative stress, and inflammatory cytokine (IL-6, IL-1β, and TNF-α) production in HFD-induced obese rats, and these phenomena were reversed by WEIG and GA administration by increasing the enzyme activity of Glo-1 and increasing adiponectin and antioxidant enzyme (GPx, SOD, and catalase) expression in the peripheral tissues of HFD-induced obese SD rats. These novel data suggest the significance of providing alternative approaches to improve the challenges in obesity and may enhance the value of *P. emblica* L. for functional food development.

Conflicts of interest

There are no conflicts to declare.

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