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Effect of extruded djulis (*Chenopodium formosanum*) snacks on the ameliorative potential against diabetic cardiomyopathy

Yu-Tsung Cheng [a,b,c], Wen-Chien Lu [d], Yung-Jia Chan [e], Ping-Hsiu Huang [f], Po-Yuan Chiang [g], Ren-Shiang Chen [a,1,*], Po-Hsien Li [c,1,*]

[a] Department of Life Science, Tunghai University, Taichung 407224, Taiwan ROC
[b] Cardiovascular Center, Taichung Veterans General Hospital, Taichung 40705, Taiwan ROC
[c] Department of Food and Nutrition, Providence University, Taichung City 43301, Taiwan ROC
[d] Department of Food and Beverage Management, Chung-Jen Junior College of Nursing, Health Sciences and Management, Chia-Yi City 60077, Taiwan ROC
[e] College of Biotechnology and Bioresources, Da-Yeh University, Changhua 51591, Taiwan ROC
[f] School of Food, Jiangsu Food and Pharmaceutical Science College, Huai'an City, Jiangsu Province 223003, China
[g] Department of Food Science and Biotechnology, National Chung Hsing University, Taichung City 40227, Taiwan ROC

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ABSTRACT

There are various functional foods available for people with diabetes mellitus (DM) to help regulate their blood glucose levels. These include meal replacements and nutritional supplements. This study looked at the physicochemical properties and antioxidant activity of djulis extruded products (DEP), and also investigated their potential to help with diabetic cardiomyopathy (DCM). The results showed that the DEP developed had high antioxidant activity and high nutritional value, making them very beneficial for promoting health. The study also found that the developed DEP could help regulate blood glucose levels in an animal model of DCM, with the effectiveness of the treatment being dependent on the dosage. Furthermore, DEP was found to be effective in preventing oxidative stress (OS) and cardiac damage caused by DM, thereby helping to prevent the further development of DCM. This study suggests that DEP may be a useful functional food for controlling DM and could be of interest as a dietary intervention for people with diabetes.

1. Introduction

Diabetes mellitus (DM) is a pervasive metabolic disorder that poses a significant public health threat globally. This condition causes metabolic disturbances in the body, affecting various organs and leading to severe complications. These complications may include kidney failure, neuropathy, blindness, and cardiovascular and cerebrovascular diseases (Al Hroob et al., 2019; Bhagani et al., 2020; Farrag et al., 2023; Hsu et al., 2018; Y. Zhou et al., 2023). Approximately two-thirds of elderly patients with DM experience myocardial dysfunction, also known as diabetic cardiomyopathy (DCM), which can lead to life-threatening myocardial infarction (Apple et al., 2017). It is widely believed that several factors such as myocardial inflammation, lipid deposition, oxidative stress (OS), apoptosis, and fibrosis could lead to the development of DCM. These multifactorial pathogenic processes play a vital role in the emergence of DCM, and are all related to blood glucose control (Bhagani et al., 2020; Byrne et al., 2021; X. Li et al., 2020; Mahalakshmi & Kurian, 2018; Zhang et al., 2023; Y. Zhou et al., 2023).

Quinoa (*Chenopodium quinoa* Willd.) is a crop that has been cultivated for over 5,000 years. It originated in Latin America and has more than 16,000 cultivars. The quinoa crops that are harvested for grains are divided into two types: American grain chenopods (88 % distributed in the Andean region, including Venezuela, Colombia, Ecuador, Peru, Bolivia, Chile, and partly Argentina) and Himalayan chenopods (which cover the central Himalayas, mainly Himachal Pradesh, India, where *C. album* was cultivated). The main species of American grain chenopods are *C. quinoa*, *C. pallidicaule*, and *C. nuttaliae* (Hlásná Cepková et al., 2022; Romano & Ferranti, 2019; Stanschewski et al., 2021). It is interesting to note that due to the wide distribution and variations in topography, temperature, and other environmental factors, there are many ecotypes and varieties of quinoa. For example, red quinoa (*Chenopodium formosanum* Koidz., also known as djulis) cultivated in the

* Corresponding authors.
E-mail addresses: rschen@thu.edu.tw (R.-S. Chen), pohsien0105@pu.edu.tw (P.-H. Li).
[1] These correspondent authors contributed equally to this work.

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mountains of Taiwan. Djulis is a highly nutritious food that contains approximately 15 % protein, essential nutrients such as lysine (12 %–19 %), vitamins (B, C, and E), and minerals (calcium, magnesium, and iron) (Lin et al., 2023; Romano & Ferranti, 2019; Stanschewski et al., 2021). The djulis plant has high levels of phytosterols, triterpenes, and flavonoids such as kaempferol, rutin, and quercetin. These compounds have various health benefits. Djulis also contain betalains, which are indole-containing pigments responsible for giving the plant a red-violet (betacyanins) or yellow-orange color (betaxanthins). Betalains also exhibit potent antioxidant activities (Li et al., 2020) (Chu et al., 2016; Chyau et al., 2015; Hsu et al., 2018; Li et al., 2021).

Djulis has become a subject of interest for scientists and the commercial world due to the benefits of a healthy diet and the ability of the plant to grow in harsh conditions typically unsuitable for other grains (Dostalíková et al., 2023). Djulis is a valuable functional food source apart from being productive and environmentally friendly (Stanschewski et al., 2021). It has been observed that the use of djulis in cooking is gradually declining as the younger generations are leaning towards different dietary habits (Chung et al., 2024). Djulis contain a high percentage of nutrients, if managed to utilize djulis in the development of sensory-accepted products, this may increase the reputation and acceptability of djulis (Jarvis et al., 2022). This innovation will help to fully harness the potential of this ingredient and make its benefits available to a larger population. Hou et al. (2022) reported that djulis yogurt processed with *Lactobacillus plantarum*, *L. delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* may have higher antioxidant capacity and bioactive substance levels. These substances include free total phenolic content, total flavonoids, and γ-aminobutyric acid. Moreover, an ethanol extract of djulis increases glucose utilization and glucose transporter expression in a co-culture model of mouse liver cells (Hou et al., 2022).

Individuals with diabetes who are taking hypoglycemic medications or injectable insulin therapy should consume appropriate amounts of carbohydrates to maintain their daily activities. Hence, the objective of this study was carried out by using djulis as the corn starch substitution to develop nutritious products alleviating DM-associated parameters which were tested by the DCM animal model.

2. Materials and methods

2.1. Materials

Djulis in a completely matured stage (red color) was purchased from the Hearty Farm (Yunlin, Taiwan). Djulis was cultivated in Yunlin by Hearty Farm and harvested in the completely matured stage. Brown rice flour (BF), corn flour (CF), and food-grade ingredients (e.g., casein, lard, and cellulose) were obtained from a local market (Taichung, Taiwan). All chemicals were purchased directly from Sigma-Aldrich® (Merck KGaA, Darmstadt, Germany).

2.2. Experimental animals

Six-week-old male Wistar rats (200 ± 20 g) were purchased from LASCO Co., Ltd. (Taipei, Taiwan). All animals were allowed 1 week of acclimatization upon arrival at the laboratory and were randomly assigned to the following groups (n = 6) after STZ induction for DCM: Control group (standard diet without induction and treatment), the DCM group (standard diet for rats with DCM), DEP10 (diabetic rats orally treated with 10 % DEP); DEP20 (diabetic rats orally treated with 20 % DEP); and DEP40 (diabetic rats orally treated with 40 % DEP) (Figure A1). During the experimental period, all rats were housed in plastic cages (30 [L] × 20 [W] × 10 [H] cm^3, two/cage), maintained at a specific temperature (25 °C ± 2 °C), humidity (65 % ± 5 %), and free of pathogens. The dark period was 07:00–19:00, and the light period was 19:00–07:00. Drinking water and the diets (the dietary formulas for each group were prepared as described in Section 2.3) were supplied ad libitum and renewed every morning.

Feeding was followed by the induction of type 2 diabetes (T2DM). Briefly, all groups, except for the control group, were fasted for 12 h the night before the streptozotocin (STZ, Millipore® Merck KGaA) injection. STZ (40 mg/kg) was prepared in ice-cold 0.01 mM citric acid buffer solution (pH 4.5) in a light-protected container, maintained in an ice bath, and injected intravenously (Ghasemi & Jeddi, 2023). The frequency of induction was seven consecutive sessions at 3-day intervals.

All experimental animals fasted for 24 h, and their body weights and blood glucose levels were measured, followed by euthanasia with sodium pentobarbital (150 mg/kg body weight) by intraperitoneal injection. Then, cardiac blood was taken by puncture and centrifuged (10 min, 4 °C, 12,000 × g); the resultant serum was used for biochemical analysis (Huang et al., 2023). Next, the cardiac tissue was excised, washed in saline (0.9 % NaCl solution), and weighed. Furthermore, one-third of the cardiac tissue was preserved in a 10 % formalin-buffered solution. The remaining cardiac tissue was homogenized in phosphate-buffered saline and centrifuged at 3,000 × g for 30 min for subsequent correlative analyses (Section 2.5).

All animal procedures were conducted according to the guidelines for the Care and Use of Experimental Animals set forth by the Committee for Control and Supervision of Experiments on Animals and the National Institutes of Health. The Committee on Animal Research at Providence University approved the protocol under code number 20,230,807 A001.

2.3. Preparation of the DEP

The formulations and extrusion processing conditions of the DEP in this study were determined in preliminary experiments (Parmar et al., (2021). The control group diet was based on 55 % corn starch (other formulations are shown in Table A1), while the experimental groups (DEP10, DEP20, and DEP40) were supplemented with 10 %, 20 %, and 40 % DEP plus 45 %, 35 %, or 15 % corn starch, respectively. All other formulations had the same quantities. These four sets of formulations were prepared for the extruded experimental animal diet. This study used a co-rotating twin-screw extruder (BC-45, Clextral©, Firminy, France). The screw shaft used in this process was 750 mm long and had three barrels. The second and third barrels were equipped with an electromagnetic induction heating and cooling system to control their respective temperatures. The third barrel was set to the desired temperature for the experiment, while the second barrel was set to half of that temperature. The first barrel was maintained at room temperature. The mold used in this process had two equally spaced holes with diameters of 6 mm. The process was performed at a barrel temperature of 150 °C, a screw speed of 175 rpm, and a moisture content of 20 %.

2.4. Physicochemical analysis of the DEP

2.4.1. Proximate components analysis

We used the AOAC (2023) Standard Method Performance Requirements (SMPRs®) and the McCleary and McLoughlin (2022) description to determine the proximate composition of the DEP; moisture (934.01), crude protein (984.13), crude fat (954.01), crude fiber (962.09), ash (942.05), and dietary fiber ([total dietary fiber, soluble dietary fiber [SDF] insoluble dietary fiber [IDF], 2017.16(12)) contents were measured. Moreover, the nitrogen-free extract (NFE) content was determined by deduction of the moisture, crude protein, crude fat, crude fiber, and ash content.

2.4.2. Expansion ratio (ER)

The ER was determined for the DD extruded products in this study following Sahu et al. (2022) with minor modifications. Ten DEP sections were chosen randomly, and the diameters of the two ends and the middle end were measured with Vernier calipers. The average value was calculated and divided by the 6-mm diameter of the mold. Thus, the obtained value was the ER, and the formula was as follows:

$$\text{Expansion ratio (ER)} = \frac{Diameterofthetwoendsandthemiddleendaverage}{6mmdiameterofthemold} \quad (1)$$

2.4.3. Longitudinal expansion ratio (Long. ER)

The DEP product Long.ER was determined in this study as described by Kantrong et al. (2018) with slight modifications. Ten sections of 1.5–2.5 cm DEP were randomly selected; while cutting the two flats, their lengths were measured with Vernier calipers, and they were divided by the dry weight to calculate Long.ER with the following formula.

$$\text{Longitudinal expansion ratio (Long. ER)} = \frac{LengthofDEP}{DryweightofDEP} \quad (2)$$

2.4.4. Specific volume (SV)

The SV of the DEP was determined using the rapeseed method described by (Manger, 1966) with modifications. Millet was substituted for rapeseed and was added to a 50 mL glass cylinder, poured out, and used for the measurements. Next, some of the millet was added to a glass cylinder containing about 5 g of DEP. The millet was added into the cylinder until its volume matched that of DEP; the final volume of the millet was 50 mL. The SV of the DEP was calculated using the following formula.

$$\text{Specific volume (SV)} = \frac{VolumeofDEP}{WeightofDEP} \quad (3)$$

2.4.5. Water solubility index (WSI) and water absorption index (WAI)

The WSI and WAI of the DEP were measured following Huang et al. (2022) and Sahu et al. (2022), respectively. All DEP was milled into powder and passed through a 60-mesh sieve. Then, 2.5 g of the powder was added to 30 mL of distilled water, stirring with a glass rod to avoid caking, and the reaction was placed in a 30 °C oscillating (120 rpm) water bath for 30 min (occasional stirring with a glass rod to prevent caking). Following with centrifugation (1,000 × g, 10 min), the supernatant was transferred to a beaker (W1), which was then weighed and dried at 105 °C to constant weight (W2); then, the precipitate was weighed (W3). The following equations (the meaning of all codes described above) were used to calculate the WSI and WAI, respectively.

$$\text{Water solubility index (WSI)} = \frac{W2 - W1}{WeightofDEP} \quad (4)$$

$$\text{Water absorption index (WAI)} = \frac{W3}{WeightofDEP - (W2 - W1)} \quad (5)$$

2.4.6. Identification and analysis of the phenolic compounds

Phenolic compounds were identified as described by Liu et al. (2023) using high-performance liquid chromatography (HPLC) with minor modifications. Specifically, the conditions for the HPLC analysis were a Mightysil RP-18 column (250 × 4.6 mm, 5 μm, Kanto Co., Tokyo, Japan), temperature of 25 °C, UV–VIS detection at 300–360 nm using the HPLC system (L-7100, Hitachi, Ltd., Tokyo, Japan). A 20 μL aliquot was injected. The mobile phases were 5 % acetic acid (mobile phase A) and 100 % acetonitrile (mobile phase B) at a flow rate of 1 mL/min. The gradient elution program was 0 min [100:0 (v/v)], 20 min [87:13 (v/v)], 45 min [76:24 (v/v)], and 60 min [72:28 (v/v)] of 5 % acetic acid/acetonitrile. External standards (rutin, quercetin, flavonoids, gallic acid, and hydroxycinnamic acid) were used to quantify the phenolic compounds. The results are expressed as mg/100 g DEP.

2.5. Serum biochemical analysis and determination of cardiac oxido-inflammatory factors

2.5.1. Serum biochemical analysis

The blood biochemical analysis of the samples was performed as described by Huang et al. (2023). The serum obtained previously was analyzed for triglycerides (TG, mg/dL), total cholesterol (TC, mg/dL), aspartate aminotransferase (AST, U/L), and lactate dehydrogenase (LDH, U/L), using a Synchroh system (LX-20, Beckman Coulter Inc., Brea, CA, USA).

2.5.2. Myocardial infarction (MI) index determination

Cardiac Troponin I ELISA Kit (ab200016, Sensitivity: 4.4 pg/mL, Range: 31.3 pg/mL – 4000 pg/mL, Abcam Plc., Cambridge, UK) and Creatine Kinase MB ELISA Kit (ab285231, Sensitivity: 46.825 pg/mL, Range: 78.125 pg/mL – 5000 pg/mL, Abcam Plc., Cambridge, UK) was used to measuring the C cardiac troponin (TnT) and creatine kinase-MB (CK-MB) levels with enzyme-linked immunoassay (ELISA) by following the standard operating procedures provided by the manufacturer.

2.5.3. Cardiac OS and inflammation

The supernatant obtained from the cardiac homogenate (Section 2.2) was used to determine the cardiac OS parameters, including malondialdehyde (MDA ELISA kit, CSB-E08558r, Sensitivity: 7.81 pmol/mL, Range: 31.25 pmol/mL – 2000 pmol/mL, Shanghai Gaochuang Chemical Technology Co., Ltd.), catalase (CAT Activity Assay Kit, ab118184, Range: 1 μg/mL – 1000 μg/mL, Abcam Plc., Cambridge, UK), glutathione peroxidase (GPx Assay Kit, ab102530, Sensitivity: 0.5 mU/mL, Abcam Plc., Cambridge, UK), superoxide dismutase (SOD Activity Assay Kit, ab65354, Abcam Plc., Cambridge, UK), and caspase-3 (ab281085, Reference range value: 23—–1500 pg/mL, Abcam Plc., Cambridge, UK). These parameters were determined based on Chou et al. (2023) with modifications using specific biochemical kits. Furthermore, immune-related factors were determined, including interleukin (IL)-6 (IL-6 ELISA Kit, ab234570, Sensitivity: 43 pg/mL, Range: 125 pg/mL – 8000 pg/mL, Abcam Plc., Cambridge, UK), IL-1β (IL-1β ELISA Kit, ab255730, Sensitivity: 26.58 pg/mL, Range: 54.69 pg/mL – 3500 pg/mL, Abcam Plc., Cambridge, UK), tumor necrosis factor (TNF-α ELISA Kit, ab236712, Sensitivity: 1.04 pg/mL, Range: 18.75 pg/mL – 1200 pg/mL, Abcam Plc., Cambridge, UK), and nuclear factor (NF-κB ELISA Kit, ab100785, Sensitivity: 25 pg/mL, Range: 82.3 pg/mL – 20000 pg/mL, Abcam Plc., Cambridge, UK). These factors were determined in cardiac homogenates using ELISA kits according to the manufacturer's instructions.

2.6. Statistical analysis

All data are expressed as mean ± standard deviation (SD). The developed DEP were measured in triplicate (n = 3), and the animal experiments were performed with 6 replicates per group (n = 6). Furthermore, all data were plotted using Graphpad Prism software (9, Graphpad Software, LLC, Boston, MA, USA) and analyzed by one-way analysis of variance with Newman–Keuls post-hoc analyses. A p-value < 0.05 was considered significant.

3. Results and discussion

3.1. Proximate components and physicochemical properties of the DEP

Four groups of experimental animals were given the DEP diet (Table A1). Briefly, the control group received 10 %, 20 %, and 40 % djulis, indicating that the control group had the highest water content ($p < 0.05$) (Table A2), which declined as djulis was increased. Conversely, crude protein was positively correlated with the amount of djulis added, and the 40 % DEP group had the highest value ($p < 0.05$). This result was attributed to the protein richness of djulis. For the dietary fiber, SDF and IDF were dependent on the amount of djulis added. This result was also attributed to the enrichment of djulis with dietary fiber, which changed the composition of the DEP. Multiplication in nutritional composition was obtained in the ratios of the complexes (djulis, CF, and BF) used and compared to the control group (CF and BF). Therefore, this result

indicates satisfactory performance in terms of protein, fat, and dietary fiber (SDF and IDF) while enhancing the original nutritional composition of the djulis (Hlásná Cepková et al., 2022; X. Zhou et al., 2023).

The physicochemical properties of the DEP were examined after different amounts of djulis were added (Table 1). The results showed that ER, Long.ER and SV were highest in the control group, which decreased as more djulis was added, and significant differences ($p < 0.05$) were detected for each group. This result indicates that the higher amount of djulis added to the extruded product induced more swelling due to the high protein content. However, the same trend was evident for the WSI and WAI, as both decreased with increasing djulis in the DEP groups. These comparisons were significantly different compared to the control group ($p < 0.05$). Moreover, the water content affected the DEP groups, which were retarded by longer retention in the screw rolls resulting in poorer expansion and a slightly different appearance compared to the control group. These findings were similar to those reported by Li et al. (2015); they indicated that substituting djulis flour in the dough decreased the rheological properties of the dough but increased the maximum resistance to elongation and that cooking loss and hardness were positively correlated with the amount of substituted djulis. Thus, no change in the overall sensory acceptability of the finished product was observed.

Polyphenols and flavones, which are natural plant compounds, mitigate DM and DCM-related cardiovascular complications and have the potential to act as natural drug sources (Byrne et al., 2021; Y. Zhou et al., 2023). Moreover, these compounds regulate intracellular signaling pathways and gene expression levels to exert anti-glycemic, antioxidant, anti-inflammatory, antiapoptotic, and hyperlipidemic effects (Bhagani et al., 2020; Kim et al., 2019; Lee et al., 2019; Li et al., 2022). Dietary acquired flavonoids (quercetin) accumulate in the mitochondria (the prominent reactive oxygen species [ROS]-producing organelle, as well as a primary SOD target) and are beneficial for reducing OS in the cardiac system (Byrne et al., 2021; Pazarín-Villaseñor et al., 2023). Castillo et al. (2018) reported that OS in rats with high cholesterol/hyperglycemia can be treated with quercetin. These results were consistent with those reported by Lee et al. (2019) for a djulis methanol extract (rich in free total phenolic and flavonoid components), which protected rats against OS. Notably, Chen et al. (2019) reported that a djulis water extract enriched with rutin, betanin, and phenolic compounds inhibits angiotensin-converting enzyme activity and alleviates hypertension in spontaneously hypertensive rats.

Moreover, the changes in individual phenolic components (including rutin, quercetin, flavonoids, gallic acid, and hydroxycinnamic acids) in the DEP were positively correlated ($p < 0.05$) depending on the amount of djulis added and were highest in the DEP40 group (Table 2). This study showed that djulis enriched with phenolic components showed similar results to those of Hsu et al. (2017). This indicated that djulis is a good source of polyphenolic compounds that can withstand high-temperature processing without significant loss. However, hydroxycinnamic acid was the most abundant among the individual phenolic components in each group ($p < 0.05$). It has been hypothesized that the phenolic and flavonoid compounds contained in DEP contribute to the decreased fasting blood glucose levels in DM rats (Hsu et al., 2018). This effect can also increase insulin sensitivity and protect pancreatic β-cells, thereby reducing blood glucose levels (Hsu et al., 2018).

3.2. Evaluation of growth performance in the experimental animals

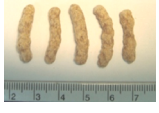
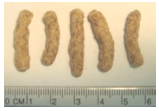
We used DEP (djulis contents of 10 %, 20 %, and 40 %, respectively) to improve the growth performances of the DM rats. The body and heart weights of the rats in the DM group were significantly lower than those in the control group (Figs. 1A and B), whereas the DEP groups exhibited a dose dependence that was best in the DEP40 group, but lower than the control group ($p < 0.01$). However, the heart and body weight ratio of

Table 2
Changes in the individual phenolic compounds in djulis (*Chenopodium formosanum* Koidz.) extruded products (DEP).

Compound (mg/100 g DEP)	Control	Djulis extruded products (DEP)		
		10	20	40
Rutin	0.13 ± 0.24^{d}	1.38 ± 0.12^{c}	3.24 ± 0.67^{b}	7.11 ± 1.31^{a}
Quercetin	0.13 ± 0.02^{d}	1.09 ± 0.03^{c}	2.39 ± 0.25^{b}	6.05 ± 0.53^{a}
Flavonoids	1.12 ± 0.12^{d}	4.56 ± 0.63^{c}	5.16 ± 0.44^{b}	11.29 ± 1.03^{a}
Gallic acid	0.16 ± 0.03^{d}	3.29 ± 0.55^{c}	6.83 ± 0.25^{b}	14.35 ± 0.99^{a}
Hydroxycinnamic acids	15.29 ± 1.23^{d}	36.25 ± 2.65^{c}	67.62 ± 6.56^{b}	136.58 ± 7.12^{a}

Different lowercase letters on the same row represent significant differences ($p < 0.05$).

Table 1
Physicochemical properties of djulis (*Chenopodium formosanum* Koidz.) extruded products (DEP).

Group		ER	Long. ER	SV (cm^3/g)	WSI	WAI	Appearance
Control		2.14 ± 0.21^{a}	16.51 ± 2.53^{a}	3.50 ± 0.15^{a}	23.33 ± 1.98^{a}	4.27 ± 0.05^{a}	
Djulis extruded products (DEP)	10	1.86 ± 0.18^{b}	15.52 ± 1.72^{ab}	2.54 ± 0.12^{b}	20.85 ± 1.19^{ab}	4.17 ± 0.08^{b}	
	20	1.57 ± 0.16^{c}	14.23 ± 2.02^{b}	2.04 ± 0.21^{c}	19.93 ± 1.23^{b}	4.03 ± 0.11^{c}	
	40	1.42 ± 0.19^{c}	13.52 ± 1.71^{b}	1.78 ± 0.05^{d}	18.35 ± 1.09^{c}	3.83 ± 0.05^{d}	

ER: expansion ratio, Long ER: longitudinal expansion ratio, SV: specific volume, WSI: water solubility index, WAI: water absorption index. Different lowercase letters on the same column represent significant differences ($p < 0.05$).

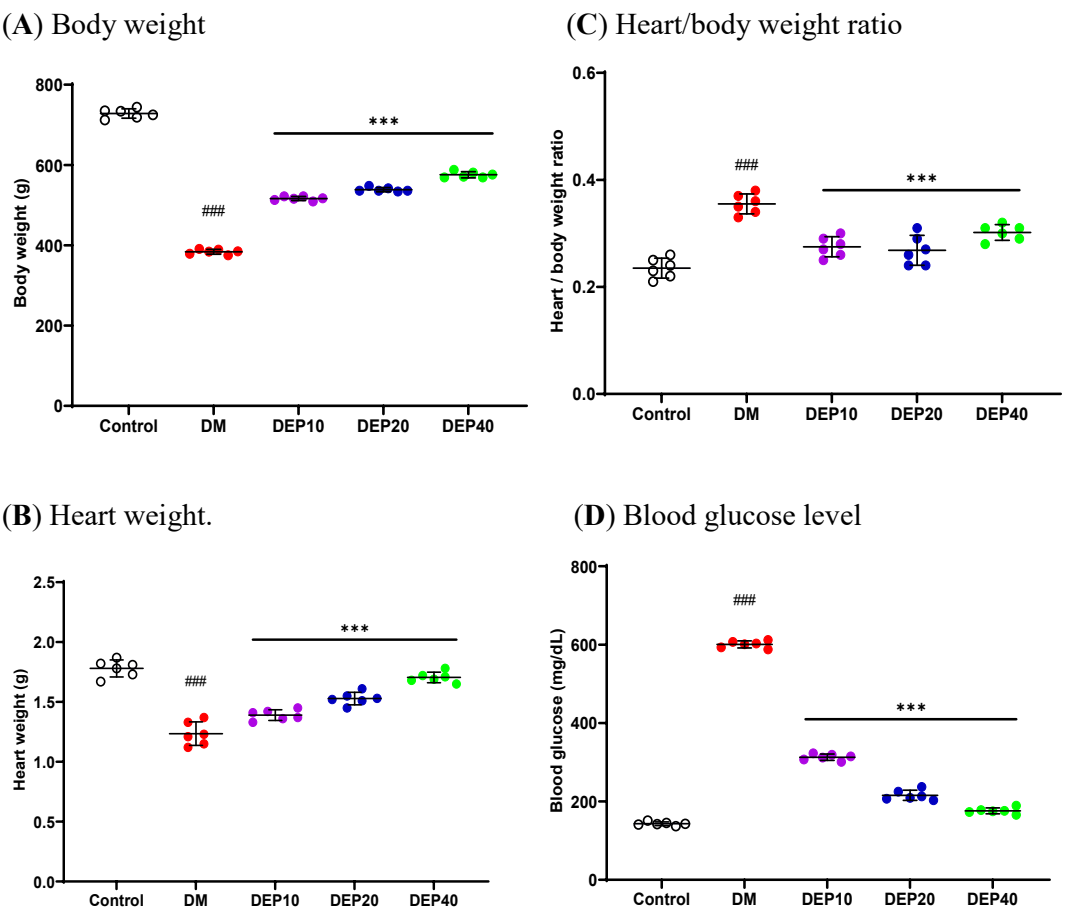


Fig. 1. Effect of the developed DEP treatment on the studied rat groups (A) Body weight, (B) heart weight, (C) heart/body weight ratio, and (D) blood glucose level. Control group: normal diet with no treatment; diabetes (DM) group: Normal diet of DM rats; DEP10: diabetic rats orally treated with 10% DEP; DEP20: diabetic rats orally treated with 20% DEP; DEP40: diabetic rats orally treated with 40% DEP. Data presented as mean ± SD, n = 6. $^{***}p < 0.01$ vs. control group. $^{\#\#\#}p < 0.01$ vs. DM group.

the DM group rats was significantly higher than that of the control group (Fig. 1C). Notably, the DEP treatment significantly improved this change in the ratio, whereas the highest therapy dose had values closer to those of the control group ($p < 0.01$). These results demonstrated that higher dose treatment effectively lowers the heart/body weight ratio, which suggests a potential reversal or attenuation of cardiac inflammation (Wen et al., 2022). The treatment might have improved cardiac function, resulting in a more efficient pumping action of the heart. Consequently, the heart may undergo remodeling towards a more normal size and shape, reducing the hypertrophic response (Zou et al., 2021). The DEP40 group performed satisfactorily. DEP had a beneficial effect on body weight in the DM rats. This finding is consistent with Lin et al. (2019), who demonstrated improved body weight loss in BALB/c mice with OS after CCl4-induced liver injury following administration of a djulis extract.

Furthermore, a three-fold increase in blood glucose levels was observed in the DM group compared to the control group (Fig. 1D). In contrast, all DEP treatment groups significantly suppressed blood glucose levels in a dose-dependent manner ($p < 0.01$). Consuming djulis hulls 30 or 60 min before a meal decreased blood glucose levels in patients with T2DM, suggesting their potential benefits related to T2DM (Li et al., 2021). X. Li et al. (2020) reported that clinically obese and nonobese T2DM patients showed signs of serious cardiac hypertrophy and myocardial fibrosis, whereas obese patients with T2DM exhibited more advanced cardiac re-modelling, along with a substantial amount of glycogen deposition in their hearts.

3.3. Changes in the serum biochemical parameters

The DM group had the highest TG level (Fig. 2A). In contrast, the DEP treatment groups exhibited dose-dependently lower TG levels, whereas the DEP40 group exhibited a better effect; however, the values of all three DEP groups were close to the control group ($p < 0.01$). These results were consistent with clinical evidence that TG levels in the serum of patients with DM increase during periods of poor glycemic control with atherogenesis (Yahaya et al., 2023).

Furthermore, the serum biochemical parameters, such as TC, AST, LDH, and troponin in the DM group, were compared to those in the DEP-treated groups (Fig. 2B–D). The results indicated that the DM group had the highest values for all these parameters. However, the DEP treatment reduced all parameters ($p < 0.01$). Chu et al. (2016) discovered a decrease in CCl4-induced hepatotoxicity (suppression of elevated serum ALT levels) in rats fed a djulis water extract (2.5 mg/kg body weight dosage), indicating no harmful effects on the liver or kidneys. The dose dependence of DEP was more evident in these parameters, and a slight difference remained with the control group. We hypothesized that increasing the djulis dosage without affecting the DEP textural profile could lead to more satisfactory results, whereas the DEP contains djulis, a cereal grain with no adverse side effects. The composition of the corn starch was modified proportionally in the design of the product. Therefore, these findings suggest that the DEP treatment may help lower elevated serum biochemical parameters in patients with DM.

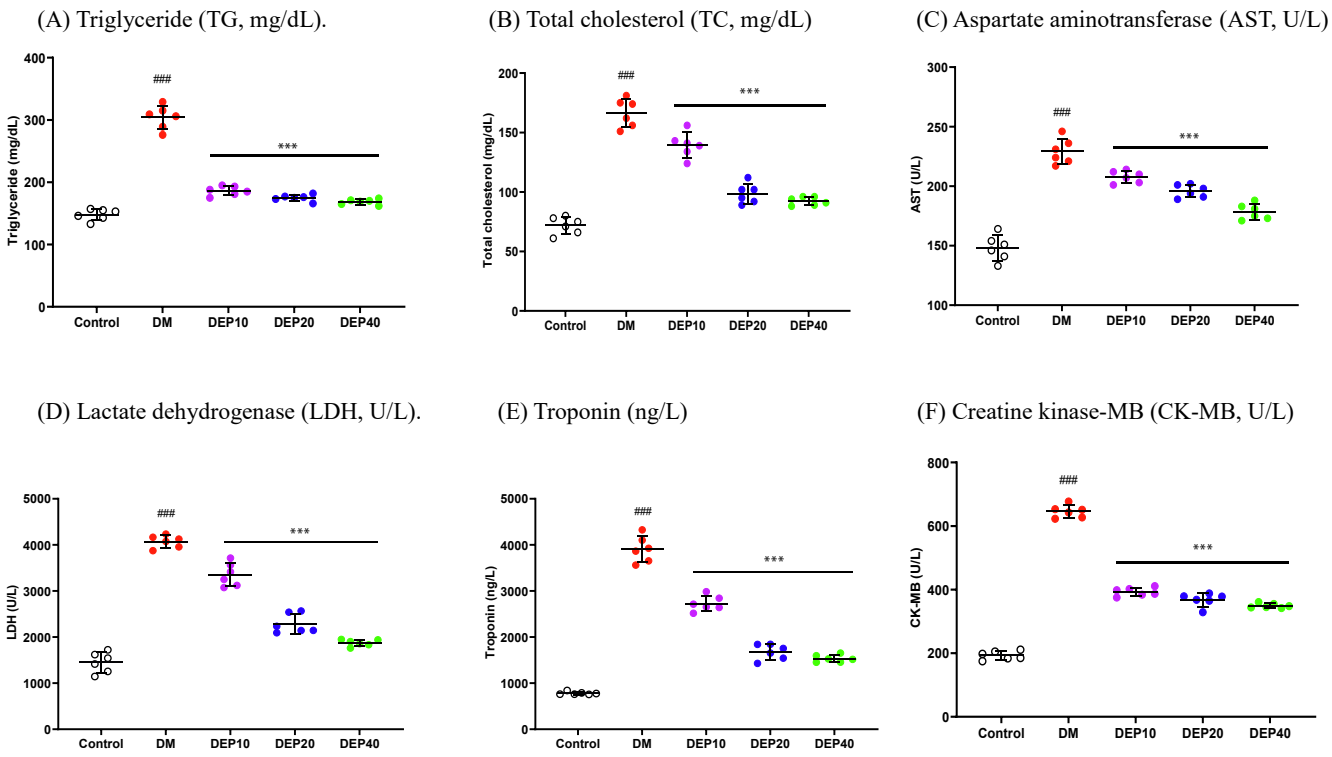


Fig. 2. Effect of the developed DEP treatment on the studied rat groups. (A) triglycerides (TG), (B) total cholesterol (TC), (C) aspartate aminotransferase (AST), (D) lactate dehydrogenase (LDH), (E) troponin, and (F) creatine kinase-MB (CK-MB). Control group: normal diet without treatment; diabetes (DM) group: Normal diet of DM rats; DEP10: diabetic rats orally treated with 10 % DEP; DEP20: diabetic rats orally treated with 20 % DEP; DEP40: diabetic rats orally treated with 40 % DEP. Data presented as mean ± SD, $n = 6$. $^{***}p < 0.01$ vs. control group. $^{\#\#\#}p < 0.01$ vs. DM group.

3.4. Changes in the MI indices

Clinical evidence indicates that DM can affect the metabolic pathways of the heart (Mahalakshmi & Kurian, 2018). Moreover, DM affects the balance between the flow of blood to the heart and the metabolic demands of the heart while increasing both microvascular and macrovascular systems, leading to left ventricular dysfunction and an increased risk of MI, which is associated with heart failure (Mahalakshmi & Kurian, 2018; Nishikawa et al., 2000). The high levels of TnT and CK-MB indicate irreversible myocardial injury and are commonly used as a diagnostic tool for early detection, risk stratification, outcome assessments, and identifying patients with acute coronary syndrome who may benefit from therapeutic interventions to mitigate myocardial damage (Apple et al., 2017).

Significant increases in the levels of TnT and CK-MB were observed in the rats with T2DM (Fig. 2E and F), indicating irreversible damage to the heart. However, dietary therapy with DEP effectively mitigated the damage in a dose-dependent manner.

3.5. Changes in the cardiac oxido-inflammatory parameters

Patients with DM have elevated plasma glucose levels due to insufficient insulin-dependent glucose intake and improper glycogenolysis and gluconeogenesis. This contributes to increased plasma glucose levels, promoting the synthesis of cardiac ROS (Faria & Persaud, 2017; Nishikawa et al., 2000). Moreover, DM patients are highly vulnerable to OS, which leads to severe cellular damage (including mitochondrial DNA damage, myocardial lipotoxicity, glucose toxicity, post-translational modification of proteins, and apoptosis autophagy, necroptosis, pyroptosis, ferroptosis, and cardiac dysfunction) (Byrne et al., 2021; X. Li et al., 2020; Zhang et al., 2023; Zheng et al., 2023). Mahalakshmi and Kurian (2018) revealed that DM rats suffering from concomitant DCM experience a significant decrease in GSH in heart muscle, which decreases the damage caused by ROS but intensifies the cellular damage. However, a normal heart has a cellular antioxidant defense network (SOD, CAT, and GPx) that catalyzes the conversion of superoxide radicals to hydrogen peroxide, followed by conversion to water by hydrogen peroxide lyase or GPx (Mahalakshmi & Kurian, 2018).

The results revealed that the DM group had the highest MDA level, four-fold higher than the control group. In contrast, the DEP-treated groups exhibited the same dose dependence as described above, most favorably in the DEP40 group ($p < 0.01$) (Fig. 3A). These findings agree with those of Chu et al. (2016), who reported that a djulis water extract effectively reduces serum MDA content and inhibits lipid peroxidation in CCl4-intoxicated rats.

In contrast, the DEP treatment groups improved the parameters significantly compared to the DM group and enhanced the antioxidant activities of CAT, GPx, GSH, and SOD ($p < 0.01$) (Fig. 3B–E), with more satisfactory results observed at higher DEP doses. Similar to the results of the present study, the antioxidant effect of the djulis water extract contributes to enhancing CAT and GPx activities in vivo (Chu et al., (2016). Ye et al. (2004) reported that overexpressing CAT in DM mice has a similar effect to that of SOD, which leads to inhibiting MDA expression and serves as an adaptive response to myocardial overexpression, which helps safeguard against the negative impact of myocardial injury in DM mice, and effectively prevents injury by protecting against OS (Byrne et al., 2021). The pathomechanism of DCM is the development of lipotoxicity in myocardial cells, followed by OS and apoptosis, which emphasizes the importance of understanding the link between metabolic dysregulation and heart function (X. Li et al., 2020).

Furthermore, GPx has a similar function to CAT, which detoxifies hydrogen peroxide into oxygen and water, whereas GPx receives hydrogen peroxide from SOD and converts it to water, thus, preventing the spontaneous formation of hydroxyl groups (Byrne et al., 2021). However, GPx activity predisposes the system to interference with the

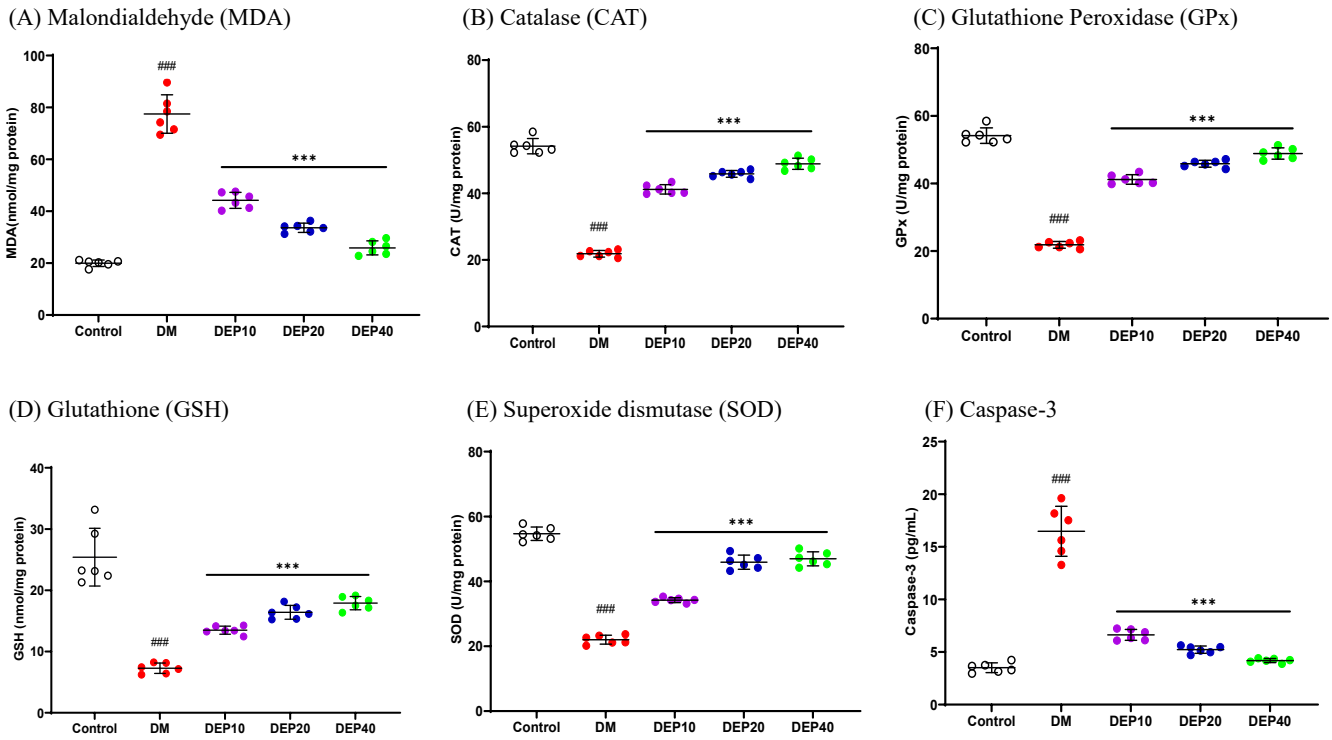


Fig. 3. Effect of the developed DEP treatment on the studied rat groups on (A) Malondialdehyde (MDA), (B) Catalase (CAT), (C) Glutathione peroxidase (GPx), (D) Glutathione (GSH), (E) Superoxide dismutase (SOD), and (F) caspase-3 levels in the cardiac homogenates. Data presented as mean ± SD, $n = 6$. $^{**}p < 0.01$ vs. control group. $^{**}p < 0.05$ and $^{\#\#\#}p < 0.01$ vs. DM group. Control group: normal diet without treatment; diabetes (DM) group: Normal diet of DM rats; DEP10: diabetic rats orally treated with 10 % DEP; DEP20: diabetic rats orally treated with 20 % DEP; DEP40: diabetic rats orally treated with 40 % DEP.

ROS required for insulin signaling, which affects insulin function (McClung et al., 2004). In particular, Ghosh et al. (2005) reported that the GSH system may be overwhelmed under conditions of excessive ROS production, leading to OS that causes apoptotic cell death in the acute diabetic heart and leads to DCM. The inhibition of OS by GPx agrees with the results of Chyau et al. (2015), who reported that a djulis water extract maintains the regular redox state of cells and eliminates oxidative damage. Moreover, Byrne et al. (2021) showed that enhancing exogenous antioxidant capacity is an approach for treating OS-induced cardiac injury in patients with T1DM, although its exact role in DCM remains to be elucidated. Nevertheless, improving endogenous antioxidant capacity as a therapeutic intervention may be more effective (van der Pol et al., 2019). Interestingly, Isnain et al. (2023) demonstrated that an ethanol extract of djulis hulls increases SOD and CAT activities in the gastric mucosa of C57BL/6J mice, whereby the indomethacin-induced generation of OS was mitigated. However, we concluded that adjusting the dietary composition by adding a DEP supplement and increasing flavonoid intake reduced the risk of OS. Furthermore, the levels of all parameters associated with antioxidant status were beneficial, and using djulis and the hulls as dietary supplements was worthwhile. Moreover, caspase-3 activity has been used to determine apoptosis, and caspase-3 levels in this study were significantly elevated in the DM group of rats (Fig. 3F), whereas caspase-3 levels decreased significantly in the DEP-treated groups ($p < 0.01$), which agrees with the results reported by Mahalakshmi and Kurian (2018). Notably, Chyau et al. (2015) also reported that a water extract inhibits caspase-3 expression, resulting in reduced apoptosis.

This study of the effect of DEP treatment on cardiac inflammation in DM rats showed that the levels of IL-6, IL-1β, TNF-α, and NF-κB increased significantly in cardiac tissues from the DM group compared to those from the control group ($p < 0.01$) (Fig. 4A–D), whereas all of these inflammatory markers decreased significantly in the DEP-treated groups compared to the DM group. ROS and lipid peroxidation damages DNA in cells, leading to inflammation, and macrophages release proinflammatory cytokines (IL-1β, IL-6, and TNF-α), which enhance the inflammatory response. Lin et al. (2019) reported that a DD ethanol extract inhibits this inflammatory response, which was consistent with the present study. Additionally, Lin et al. (2023) showed that a djulis extract inhibits inflammation, thereby significantly improving TNF-α induced insulin resistance, while this effect was attributed to the anti-inflammatory properties of the extract, which prevented the glucose uptake, glycolysis, and gluconeogenesis caused by inflammation. Various intracellular stressors (OS and inflammatory cytokines) activate IκB-α phosphorylation through IκB kinase and subsequent IκB-α degradation by proteases while releasing NF-κB and providing anti-apoptotic and apoptotic effects (Chyau et al., 2015; Yuan et al., 2014). Djulis water extracts contain naturally active substances that inhibit NFκB activity by scavenging ROS used to activate NFκB and altering the redox potential to reduce NFκB DNA binding, thus inhibiting upstream IκB-α degradation to achieve a protective effect (Chyau et al., 2015). The above results suggest that a DEP-supplemented diet may be a promising approach to mitigate DCM.

4. Conclusions

In this study, we developed DEP products based on the bioactive properties of djulis to inhibit the progression of DM despite the lack of ideal physicochemical properties. Furthermore, DEP with 40 % djulis substitution performed satisfactorily; in particular, it improved body weight, fasting blood glucose, MI, and cardiac oxido-inflammatory parameters in rats with DM. These changes improved the damage caused by OS and alleviated the damage caused by DM to the heart of rats. However, as the current study was a preliminary validation with a classical animal model, such evidence provides warranty and confidence regarding the next stage of possible verification in human clinical trials. Therefore, this study provides the necessary information and new

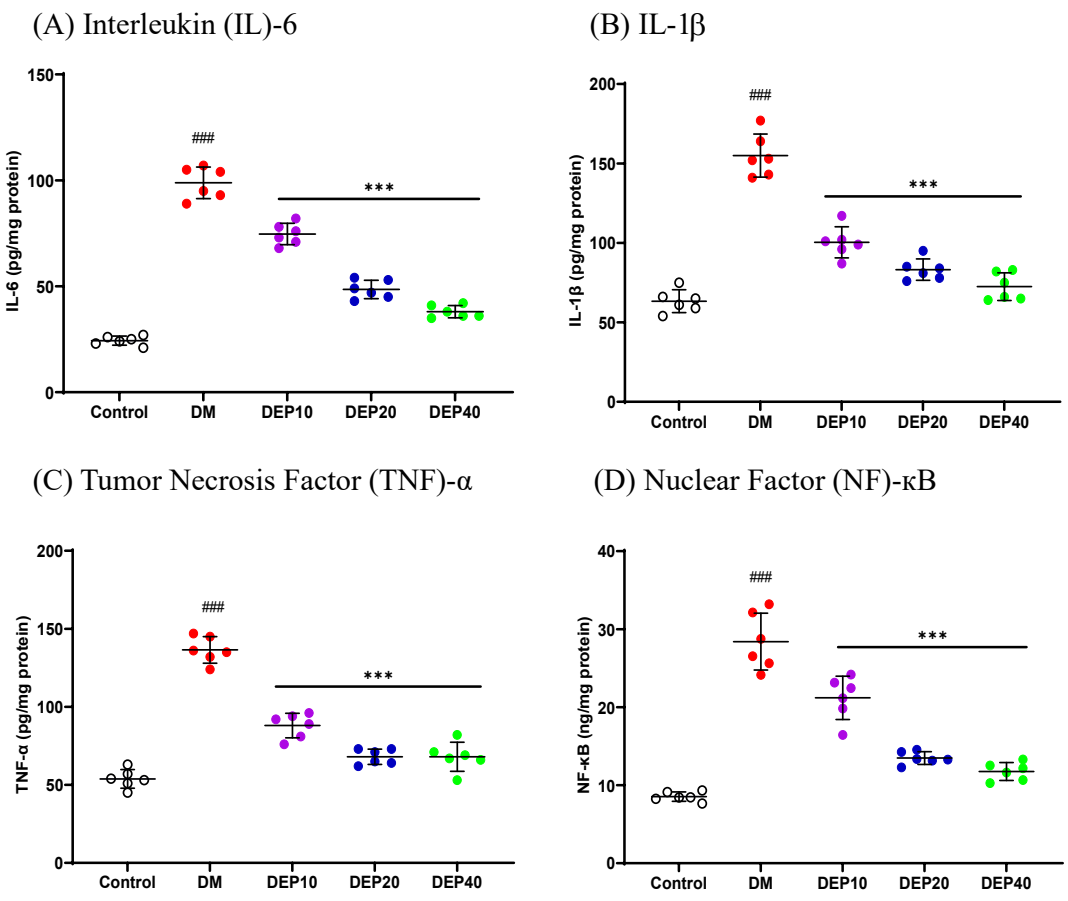


Fig. 4. Effect of the developed DEP treatment on the studied rat groups (A) Interleukin (IL)-6, (B) IL-1β, (C) tumor necrosis factor (TNF)-α, and (D) nuclear factor (NF)-κB in the cardiac homogenates. Data presented as mean ± SD, $n = 6$. $^{**}p < 0.01$ vs. control group. $^{\#\#\#}p < 0.01$ vs. DM group. Control group: normal diet without treatment; diabetes (DM) group: Normal diet of DM rats; DEP10: diabetic rats orally treated with 10 % DEP; DEP20: diabetic rats orally treated with 20 % DEP; DEP40: diabetic rats orally treated with 40 % DEP.

insight into the processing utilization, functionality, and syndication of djulis. It also promotes the development of djulis ingredients that can be used to create commercially viable, healthy foods.

Ethical Statement

Animal Welfare

All animal procedures were conducted according to the guidelines for the Care and Use of Experimental Animals set forth by the Committee for Control and Supervision of Experiments on Animals and the National Institutes of Health. The Committee on Animal Research at Providence University approved the protocol under code number 20230807 A001.

CRediT authorship contribution statement

Yu-Tsung Cheng: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wen-Chien Lu:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology. **Yung-Jia Chan:** Investigation, Formal analysis, Data curation. **Ping-Hsiu Huang:** Writing – original draft, Investigation, Formal analysis, Data curation. **Po-Yuan Chiang:** Resources, Funding acquisition. **Ren-Shiang Chen:** Resources. **Po-Hsien Li:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106154.

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