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Histomorphological Evaluation of the Hepato-Cardiac Protective Effects of *Malus domestica* Juice in Rats Exposed to an Obesogenic Diet

Evaluación Histomorfológica de los Efectos Protectores Hepatocardíacos del Jugo de *Malus domestica* en Ratas Expuestas a una Dieta Obesogénica

Nabila Rezkallah[1]; Leila Smail[1]; Saliha Boumaza[1]; Sarra Maamri[2]; Khouloud Hemila[1]; Asma Amalou[1]; Ghouti Kacimi[3]; Nadjiba Hamlat[1]; Souhila Aouichat Bouguerra[1]

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SUMMARY: Cardiometabolic disorders, including hepatic steatosis, insulin resistance, and cardiovascular diseases, represent major public health challenges, frequently associated with Western diets high in fats, carbohydrates, and proteins. Dietary polyphenols, particularly those found in apples, have shown promising protective effects against metabolic and cardiovascular dysfunctions. This study investigated the therapeutic potential of apple juice in female Wistar rats subjected to a diet-induced model of metabolic syndrome. The animals were randomly divided into three groups: a control group (C) fed a standard chow diet; a group fed a high-carbohydrate, high-fat, and high-protein diet (HCHFP; 60 % fat, 30 % carbohydrates, and 9 % protein) for eight weeks; and a third group (HCHFP + A) receiving the same diet, supplemented orally with fresh apple juice (*Malus domestica*) (15 mL/kg/day) during the final 30 days of the experimental period. Biochemical parameters were assessed in plasma and tissues to evaluate lipid and glucose metabolism, oxidative stress (MDA, AOPP, SOD, CAT), inflammatory markers (NO, MCP-1, TNF-α), and metabolic regulators (AMPK). Histological examinations of hepatic and cardiac tissues were also performed. Apple juice supplementation significantly improved lipid and glycemic profiles, enhanced antioxidant enzyme activities, reduced inflammation, and alleviated hepatic steatosis and myocardial damage. These findings highlight the potential of apple juice as a functional nutritional intervention with antioxidant, anti-inflammatory, and metabolic regulatory properties against diet-induced cardiometabolic disorders.

KEY WORD: Apple juice; *Malus domestica*; Oxidative stress; Inflammation; Hepatic steatosis; Cardiovascular.

INTRODUCTION

Glucotoxicity and lipotoxicity resulting from insulin resistance are central to the pathogenesis of type 2 diabetes mellitus (T2DM), leading to sustained hyperglycemia and hyperlipidemia that directly impair cardiovascular tissues. Elevated plasma free fatty acids contribute to metabolic derangements, including β-cell dysfunction, dyslipidemia, and cardiac metabolic disturbances (Boden, 2011). Hypercaloric nutrition enhances reactive oxygen species (ROS) production, leading to oxidative stress, which overwhelms antioxidant defenses and damages lipids, proteins, and DNA while activating inflammatory signaling such as NF-κB. Oxidative stress is a recognized driver of diabetic cardiovascular complications (Giacco & Brownlee, 2010). AMP-activated protein kinase (AMPK) plays a central role in the regulation of cellular energy homeostasis by orchestrating the balance between catabolic and anabolic pathways in response to conditions of metabolic or cellular stress (Hardie *et al*., 2012). In cardiac tissue, AMPK is widely recognized as a key regulator of energy metabolism, modulating the transport and utilization of fatty acids and glucose under both physiological and pathological conditions, such as ischemia and cardiac hypertrophy (Zaha & Young, 2012). AMPK activation is primarily triggered by an increased AMP/ATP ratio, reflecting a state of intracellular energy deficiency that may arise from glucose deprivation, intense physical activity, hypoxia, or ischemia (Viollet *et*

[1] Laboratory of Physiology of Organisms, Team of Cellular and Molecular Physiopathology, Faculty of Biological Sciences, University of Sciences and Technology, Houari Boumediene (USTHB), Algiers, Algeria.
[2] Laboratory of Fundamental Sciences, Amar Telidji University, Laghouat, Algeria.
[3] Laboratory of Biochemistry of Central Hospital of Army, Ain Naadja, Algiers, Algeria.

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al., 2010). Once activated, AMPK rapidly modulates the activity of metabolic enzymes and, in the long term, regulates the expression of genes involved in energy metabolism. This activation promotes ATP-generating catabolic pathways, such as glycolysis and fatty acid oxidation, while simultaneously inhibiting ATP-consuming anabolic processes, including protein, fatty acid, and cholesterol synthesis (Zaha & Young, 2012). Fruits and vegetables are primarly food sources providing essential nutrients for sustaining life. They also contain a variety of phytochemicals such as phenolics and flavonoids providing important health benefits. Thus, regular consumption of fruits and vegetables is associated with reduced risks of chronic diseases, such as cancers and cardiovascular disease (Liu, 2013). Apples (*Malus domestica*) are rich in flavonoids, including phloretin and phloridzin, which exhibit antioxidant, anti-inflammatory, and cardioprotective properties through modulation of pathways such as JNK, MAPK p38, and caspase-3 (Hyson, 2011). Epidemiological studies have linked apple consumption with lower T2DM risk and improved metabolic profiles (Hyson, 2011). This study evaluated the therapeutic potential of apple juice in male Wistar rats subjected to a hypercaloric diet rich in carbohydrates, fats, and proteins to induce glucolipotoxicity. Apple-derived polyphenols were investigated for their ability to attenuate metabolic and cardiovascular abnormalities associated with insulin resistance.

MATERIAL AND METHOD

Preparation of Apple Juice *Malus domestica*

Fresh apples (*Malus domestica*) of uniform size, color, and physiological maturity were selected and washed thoroughly under running tap water, followed by rinsing with distilled water to remove surface contaminants. The apple pieces were homogenized using a high-speed homogenizer (Ultra-Turrax, IKA-Werke, Germany) for 2–3 minutes to obtain a uniform pulp. The homogenate was filtered through double-layered sterile muslin cloth to separate the juice from solid residues. The resulting juice was collected in sterile glass containers. To preserve thermolabile phytochemicals, particularly polyphenols, no pasteurization or thermal processing was applied. The juice was used fresh the same day or stored at 4 °C for no longer than 24 hours prior to use, as previously recommended (Van der Sluis *et al*., 2005).

Phytochemical Investigation

Chemical Profiling of Bioactive Compounds

Determination of Total Phenolic Content (TPC). The total phenolic content (TPC) of the aqueous apple extract was determined using the Folin–Ciocalteu colorimetric method as described by Singleton & Rossi (1965). Briefly, 0.2 mL of the extract solution (containing 1000 µg of extract) was mixed with 1 mL of Folin–Ciocalteu reagent and 46 mL of distilled water in a volumetric flask. After incubation in the dark for 3 minutes, 3?mL of sodium carbonate solution (7.5 %) was added. The mixture was incubated at room temperature in the dark for 2 hours, and absorbance was measured at 740 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). Gallic acid was used as the reference standard, and results were expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg).

Qualitative Phytochemical Screening. A preliminary phytochemical screening of the aqueous extract was conducted to identify major classes of secondary metabolites, following the standard procedures of Harborne (1998). Tests were performed to detect tannins, flavonoids, leucoanthocyanins, anthocyanins, anthraquinones, alkaloids, and reducing compounds. Results were evaluated based on colorimetric changes or fluorescence intensity and expressed semi-quantitatively (– to +++).

Antioxidant Activity (DPPH Assay)

The radical scavenging activity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method of Brand-Williams *et al*. (1995). A 25 µL aliquot of extract at different concentrations (100–1000 µg/mL) was added to 975 µL of a 60 µM DPPH methanolic solution. After 30 minutes of incubation at 25 °C in the dark, absorbance was recorded at 517 nm. Ascorbic acid and a-tocopherol were used as positive controls. The radical scavenging activity was calculated using the equation:

$$\text{Scavenging Activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs control}}$$

Experimental Animals

Ethical Approval. The present study was approved by the Institutional Animal Care and Use Committee of the National Administration of Algerian Higher Education and Scientific Research (DGRSDT; *https://www.dgrsdt.dz*) and the University of Bab Ezzouar (Algiers, Algeria). The permit number for this research project is F00220110048. The study was conducted in accordance with Executive Decree No. 10-90, supplementing Executive Decree No. 04-82 of the Algerian government, which defines the terms and conditions for animal welfare in experimental animal facilities.

Diet. The standard laboratory diet used was formulated according to the specifications of the National Livestock

Feed Office (ONAB) (*https://www.onabnutrition.dz*). The average daily intake per rat was 20 g, corresponding to approximately 9 kilocalories.

High carbohydrat high fat succhrose diet (HCHFP): high carbohydrat high fat succhrose diet was formulated to induce features of metabolic syndrome in rats. This diet was composed of a standard chow base supplemented daily with 30 g of margarine (≈270 kcal, mainly from lipids), 20 g of sucrose (≈80 kcal, carbohydrates), and one hard-boiled egg (≈92 kcal, including lipids and proteins). The estimated total caloric intake was approximately 442 kcal/day/rat.

Animals. Twenty-one adult female albino Wistar rats (weighing 125–130 g) were housed in a temperature-controlled room (22 ±2 °C) with a 12-hour light/dark cycle. The animals had free access to standard chow and water *ad libitum*. Prior to the initiation of the experimental procedures, all rats were acclimatized to laboratory conditions for one week.

The animals were then randomly assigned into three experimental groups (n = 7 per group):

- Group 1 (Control): Received a standard diet and served as the healthy control group.
- Group 2 (HCHFP) : Fed a high-carbohydrate, high-fat, protein enriched diet for 8 weeks to induce metabolic disturbances.
- Group 3 (HCHFP + A) : Received the same HCHFP diet for 8 weeks, and from day 30 onward, were orally administered freshly prepared apple juice (A) at a dose of 15 mL/kg/day for the remaining 30 days.

Biological Analyses

Biochemical Parameters. At the end of the treatment period, animals were fasted overnight and anesthetized with light ether. Blood was collected via retro-orbital sinus puncture into plain tubes, allowed to clot at room temperature, and centrifuged at 3000 rpm for 10 min. Serum was aliquoted and stored at -20 °C until analysis. Serum glucose, total cholesterol, and total protein concentrations were measured using commercial enzymatic colorimetric kits (Biosystems, Spain) following the manufacturer's protocols. Insulin concentrations were assessed using a radioimmunoassay (RIA) kit (CIS Bio International, France). Creatine kinase-MB (CK-MB) activity and uric acid levels were determined using an automated clinical chemistry analyzer (CKL 0-323).

Tissue Collection and Preparation. After euthanasia, liver tissue and heart were rapidly excised, rinsed in cold saline, blotted dry, and snap-frozen in liquid nitrogen. Samples were stored at -80 °C until further analysis. Tissue homogenates were prepared in ice-cold lysis buffer [20 mM HEPES, 8 mM EDTA, 0.2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 2.5 mM PMSF, 1 mg/mL aprotinin, 2.5 mg/mL each of benzamidine, pepstatin, and leupeptin, 160 mM NaF, 2 mM dichloroacetate, and 1 % Triton X-100; pH 7.4] using a Potter–Elvehjem homogenizer. Homogenates were incubated on ice for 20 min, centrifuged at 20,000 × g for 30 min at 4 °C, and the supernatants were stored at -80 °C.

The total lipid. Content was determined according to the method of Folch *et al.* (1957).

Hepatic Glycogen Determination. Hepatic glycogen content was quantified using the anthrone method as previously described by Van Handel (1965). Liver tissue was homogenized in 30 % KOH, boiled, and glycogen was precipitated using absolute ethanol. After centrifugation, the pellet was dissolved in distilled water, and reacted with anthrone reagent. Absorbance was measured at 620 nm. Results were expressed as mg of glycogen per gram of liver tissue.

Redox Status Markers

Malondialdehyde (MDA). Lipid peroxidation was assessed by measuring MDA levels using the thiobarbituric acid reactive substances (TBARS) assay, as described by Ohkawa *et al*. (1979). Samples were reacted with thiobarbituric acid (TBA) at 90 °C for 15 min, and absorbance was read at 530 nm. Results were expressed as μmol MDA/mg protein.

Advanced Oxidation Protein Products (AOPPs). AOPP levels were quantified spectrophotometrically at 340 nm according to the method of Witko-Sarsat *et al*. (1996). Results were expressed as nmol/mg protein.

SOD Activity Assay. Superoxide dismutase (SOD) activity was measured by the NBT photoreduction method as described by Beauchamp & Fridovich (1971). The reaction mixture contained phosphate buffer (50 mM, pH 7.8), methionine (13 mM), NBT (75 μM), EDTA (0.1 mM), and riboflavin (2 μM). After 10 min of light exposure, absorbance was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme inhibiting NBT reduction by 50 % and expressed as U/mg protein.

Inflammatory markers

Nitric Oxide (NO). Total nitrite and nitrate concentrations, indicative of NO production, were determined following cadmium-mediated nitrate reduction as previously described by Grand *et al*. (2001). Absorbance was read at 540 nm.

Tumor necrosis factor-a (TNF-α) and Monocyte Chemoattractant Protein-1 (MCP-1). TNF-α and MCP-1 levels were quantified using a commercially available ELISA kit (Invitrogen, USA) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (BioTek Instruments, USA).

Metabolic markers

p38 Mitogen-Activated Protein Kinase (p38 MAPK) and AMP-Activated Protein Kinase (AMPK). Phosphorylated p38 MAPK and AMPK levels were evaluated by sandwich ELISA (ENZO Life Sciences, USA). Absorbance readings were recorded at 450 nm.

Histological Study

Samples of heart and liver tissues from both control and treated rats were immediately fixed in 10 % neutral buffered formalin at room temperature for 24 hours. Tissues were then dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of approximately 3 µm thickness were prepared using a microtome and stained with Periodic Acid-Schiff (PAS) and Masson's Trichrome for the assessment of glycogen content and collagen deposition, respectively. Histological examination was performed using a light microscope.

Statistical analysis. Statistical analyses were conducted using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). Data are expressed as mean±standard deviation (SD). Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test as a post hoc analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

Chemical Test Results

Total Phenolic Content and Antioxidant Properties of *Malus domestica* juice. The juice of *Malus domestica* showed a high total phenolic content (114.89 µg GAE/ mg), reflecting a substantial polyphenol composition. Nevertheless, its DPPH radical scavenging activity (IC_{50} = 37.83 ± 1.41 µg/mL) was weaker than that of synthetic antioxidants BHA and BHT, indicating moderate in vitro antioxidant capacity. The lack of reducing power data limits comprehensive assessment. Overall, despite its phenolic richness, the antioxidant potential of the apple extract appears less effective than that of reference synthetic compounds (Table I).

Phytochemical Screening. Phytochemical screening of *Malus domestica* juice revealed the presence of major classes of phenolic secondary metabolites, including tannins, flavonoids (flavones, flavonols, leucoanthocyanins, and anthocyanins), anthracenic

Table I. Total Phenolic Content and Antioxidant Properties of *Malus domestica* juice and Synthetic Antioxidants.

Extract/Standard	Total Phenolic Content (µg GAE/mg extract)	DPPH Radical Scavenging Activity (IC_{50}, µg/mL)	Reducing Power (EC_{50}, µg/mL)
Aqueous extract (80 % ethanol, 20 % water)	114.89	37.83 ± 1.41	—
BHA	—	21.28 ± 0.12	18.44 ± 0.14
BHT	—	12.76 ± 0.08	16.11 ± 0.11

Each value is expressed as the means ± standard deviations. GAE: Gallic acid equivalents, BHA : Butylated Hydroxyanisole, BHT : Butylated Hydroxytoluene

Table II. Qualitative phytochemical screening of *Malus domestica* juice.

Phytochemical Class	Test/Reaction Performed	Result
Tannins (total)	Ferric chloride test	(+) Presence confirmed
Catechic tannins	Butanol-HCl test	(+) Presence confirmed
Flavonoids	—	—
– Flavones/Flavonols	Cyanidin reaction	(++) Moderate positive
– Leucoanthocyanins	Potassium hydroxide coloration	(+++) Strong positive
– Anthocyanins	Fluorescence with AlCl_	(+) Presence confirmed
Anthracenic derivatives	Bornträger reaction (or similar)	(+++) Strong positive
Alkaloids	Bouchardat, Dragendorff, Mayer	(-) Not detected
Reducing compounds	Standard reduction test	(+) Presence confirmed

(+): Positive reaction (presence); (++/+++): Moderate to strong presence; (-): No detectable reaction. Phytochemical screening was conducted using standard qualitative tests based on colorimetric and precipitation reactions, as commonly described in plant pharmacognosy protocols.

derivatives, and reducing compounds. No reaction was observed with classicalalkaloid reagents (Bouchardat, Dragendorff, Mayer), suggesting the absence of nitrogenous alkaloids. These findings suggest that the phenolic-rich profile of the juice may contribute to its observed antioxidant and cardiometabolic activities (Table II).

Biochemical Results

Serum Metabolic Parameters. Rats subjected to the high-carbohydrate, high-fat, high-protein (HCHFP) diet displayed a significant increase in serum glucose, insulin, total protein, and total cholesterol levels at both T1 and T2 compared to the control group (Fig. 1), reflecting pronounced metabolic dysregulation. Interestingly, co-treatment with *Malus domestica* juice (HCHFP+A) significantly mitigated these alterations at T2, as evidenced by lower concentrations of all four parameters relative to the untreated HCHFP group. These results suggest that *Malus domestica* juice may exert antihyperglycemic, insulin-sensitizing, and hypocholesterolemic effects in the context of diet-induced metabolic disturbances. No significant differences were detected between HCHFP and HCHFP+A groups at baseline (T0), confirming equivalent metabolic profiles prior to intervention.

Cardiac biomarkers (CK-MB). Exposure to a high-carbohydrate, high-fat, high-protein (HCHFP) diet induced a significant increase in serum CK-MB levels at both T1 and T2 compared to the control group, indicating possible myocardial damage secondary to the obesogenic stress (Fig. 2). Interestingly, co-administration of *Malus domestica* juice (HCHFP+A) significantly attenuated CK-MB concentrations at T2 relative to the untreated HCHFP group, suggesting a cardioprotective potential of the intervention. No significant differences were observed between the HCHFP and HCHFP+A groups at baseline (T0), confirming a comparable pre-intervention cardiac biomarker profile.

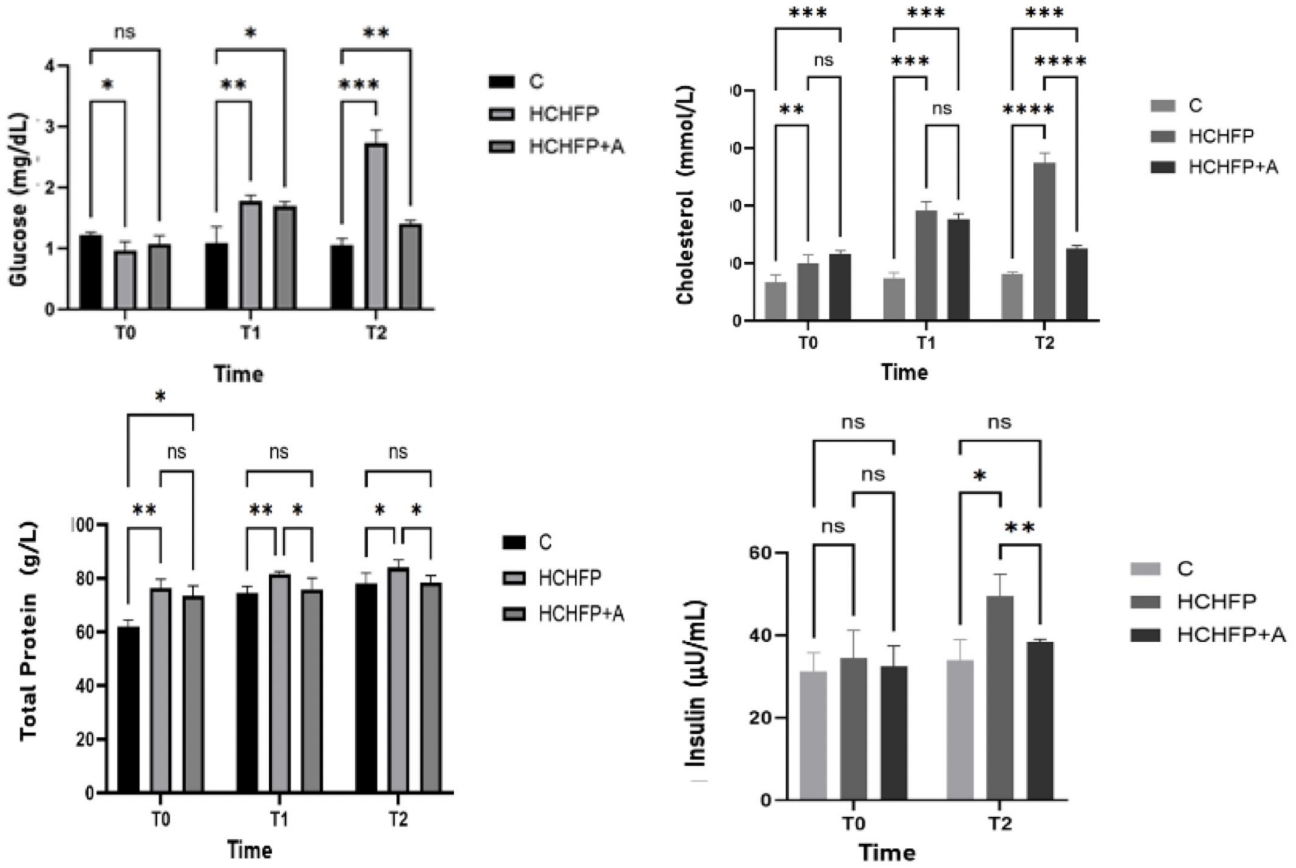

Fig. 1. Effect of *Malus domestica* juice on serum glucose, insulin, total cholesterol, and total protein levels in rats fed a high-carbohydrate, high-fat, high-protein (HCHFP) diet at different time points (T0, T1, and T2). Data are expressed as mean ± SEM (n = 7 per group). T0 (baseline); T1 (after 1 month of HCHFP diet) and T2 (end of the experimental period (after 2 months of HCHFP diet, including 30 days of *Malus domestica* juice administration at 15 mL/kg/day). *ns* (not significant), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****) significant differences between groups.

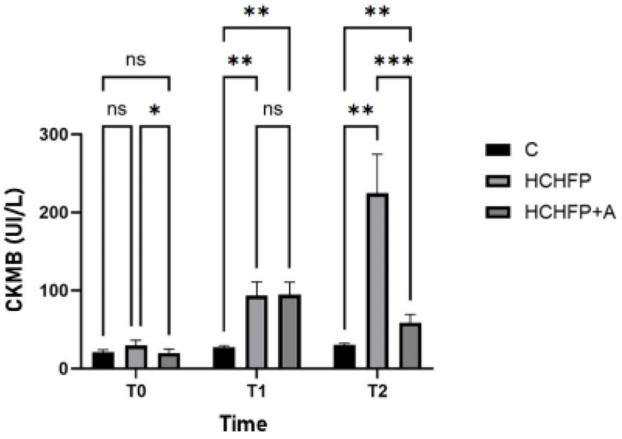

Fig. 2. Effect of *Malus domestica* juice on serum CK-MB levels in rats fed a high-carbohydrate, high-fat, high-protein (HCHFP) diet at different time points (T0, T1, and T2). Data are expressed as mean ± SEM (n = 7 per group). T0 (baseline); T1 (after 1 month of HCHFP diet) and T2 (end of the experimental period (after 2 months of HCHFP diet, including 30 days of *Malus domestica* juice administration at 15 mL/kg/day). ns (not significant), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***),significant differences between groups.

Cardiac Findings

Cardiac Biochemical Profile. Biochemical analysis of cardiac tissue demonstrated that the HCHFP diet significantly elevated oxidative stress markers (MDA and AOPP) and reduced SOD activity, reflecting a marked redox imbalance. Treatment with *Malus domestica* juice effectively attenuated these alterations, restoring oxidative homeostasis. Similarly, levels of pro-inflammatory mediators NO, TNF-a, and MCP-1 were significantly increased in the HCHFP group, while juice administration markedly reduced their concentrations, indicating a robust anti-inflammatory effect. Moreover, the diet-induced upregulation of AMPK and p38 MAPK expression was significantly reversed by *Malus domestica* supplementation, underscoring its cardioprotective and modulatory properties (Fig. 3).

Myocardial histopathology. Histological examination of cardiac tissue stained with Masson's trichrome (Fig.4) revealed substantial structural alterations between experimental groups. In the control group (Fig.4A, 4B), normal myocardial architecture was preserved, with intact and well-aligned cardiomyocyte fibers. In contrast, the HCHFP group (Fig. 4C, 4D) exhibited severe myocardial damage, including cardiomyocyte disarray, fiber disruption, trabecular splitting, inflammatory cell infiltration, and overall structural disorganization hallmarks of cardiac remodeling and inflammation. Remarkably, myocardial sections from rats treated with *Malus domestica* juice (15 mL/kg/day for 30 days) (Fig. 4E) showed notable histological improvement, with reduced inflammatory infiltration and partial restoration of myocardial fiber organization. These findings support the cardioprotective potential of apple juice, likely mediated by its antioxidant and anti-inflammatory properties.

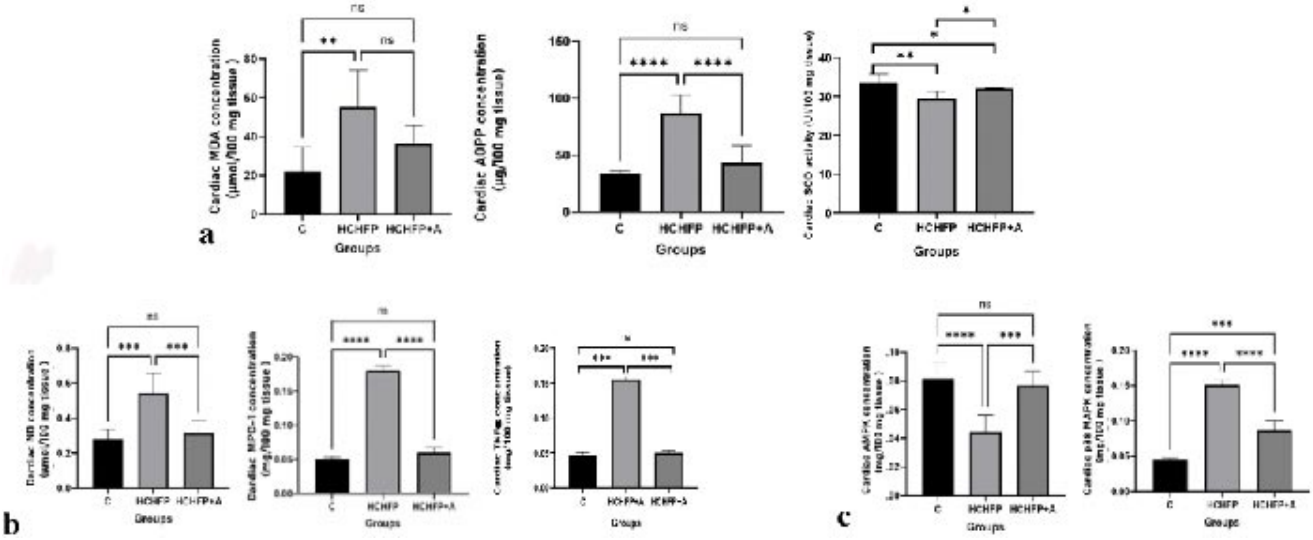

Fig. 3. Effect of *Malus domestica* juice on cardiac oxidative stress, inflammatory, and metabolic markers in rats fed a high-carbohydrate, high-fat protein (HCHFP) diet. Data are expressed as mean ± SEM (n = 7 per group). C: control ; HCHFP: rats receiving a high-carbohydrate, high-fat, high-protein diet; HCHFP+A : HCHFP-fed rats treated with *Malus domestica* juice (15 mL/kg/day) for 30 days. ns (not significant), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), significant differences between groups. (a) Oxidative stress markers; (b) Inflammatory markers; (c) Metabolic markers

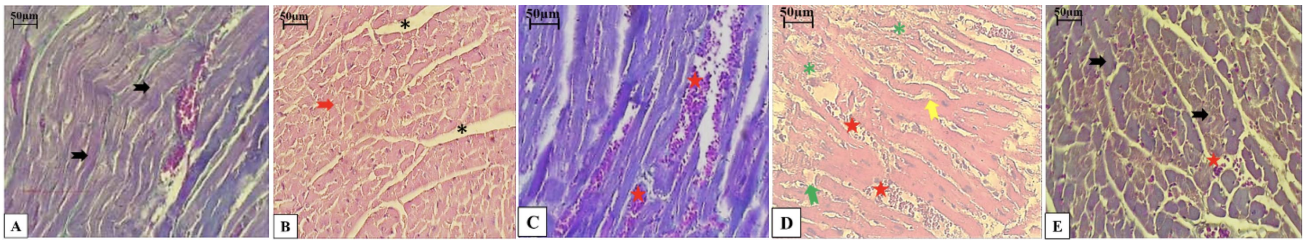

Fig. 4. Representative histological sections of cardiac tissue stained with Masson's trichrome from Rattus norvegicus (original magnification ×400; scale bar = 50 µm): (A, B) Control group showing normal myocardial architecture with intact cardiomyocyte fibers (black arrow); (C, D) HCHFP group exhibiting marked pathological alterations including inflammatory cell infiltration (red star), cardiomyocyte disarray and structural disorganization (green star), fiber disruption (green arrow), trabecular splitting, (yellow arrow); (E) HCHFP + *Malus domestica* juice (15 mL/kg/day for 30 days) showing notable histological improvement with reduced inflammation and partial restoration of myocardial organization.

Hepatic findings

Hepatic biochemical profile. Biochemical analysis of liver tissue revealed that the high-carbohydrate, high-fat, high-protein (HCHFP) diet induced significant hepatic metabolic disturbances, as evidenced by a marked increase in total lipid content and a significant reduction in glycogen levels compared to the control group. These alterations reflect impaired hepatic lipid and glucose metabolism. Notably, treatment with *Malus domestica* juice (15 mL/kg/day for 30 days) effectively mitigated these changes, leading to a significant decrease in hepatic lipid accumulation and partial restoration of glycogen stores (Fig. 5). These findings support the hepatoprotective potential of apple juice against diet-induced hepatic steatosis and glycogen depletion.

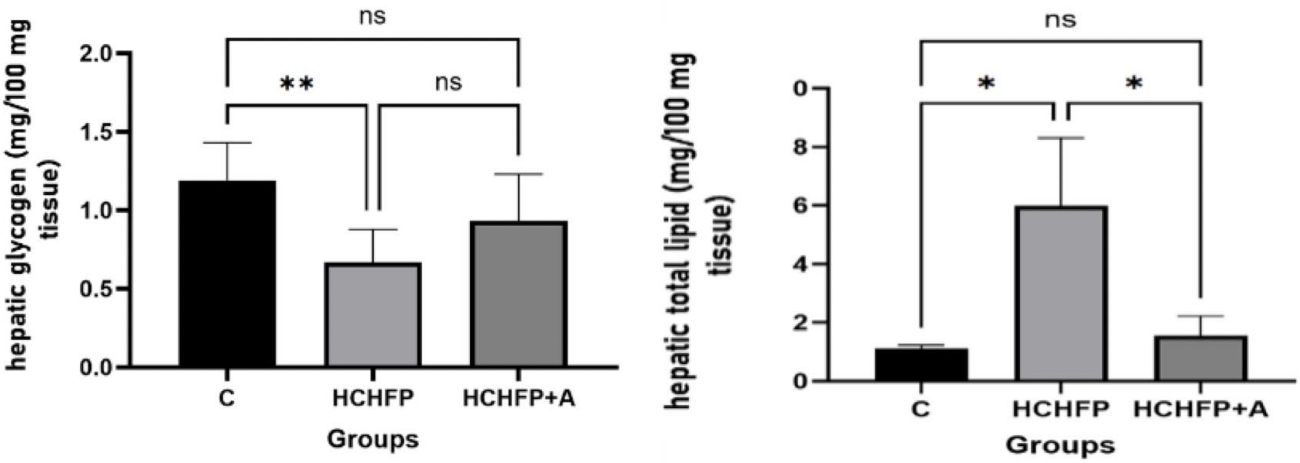

Fig. 5. Effect of *Malus domestica* juice on hepatic glycogen and total lipid in rats fed a high-carbohydrate, high-fat protein (HCHFP) diet. Data are expressed as mean ± SEM (n = 7 per group). C: control ; HCHFP : rats receiving a high-carbohydrate, high-fat, high-protein diet ; HCHFP+A : HCHFP-fed rats treated with *Malus domestica* juice (15 mL/kg/day) for 30 days. *ns* (not significant), $p < 0.05$ (*), $p < 0.01$ (**) significant differences between groups.

Hepatic histopathology. Histological analysis of liver tissue (Fig. 6) revealed marked differences between the experimental groups. Sections stained with Masson's trichrome and Periodic Acid-Schiff (PAS) demonstrated preserved hepatic architecture in the control group (Fig. 6A, 6E), characterized by well-organized hepatocytes, intact sinusoids, and a normal centrilobular vein. In contrast, liver sections from rats fed a high-carbohydrate, high-fat, high-protein (HCHFP) diet (Fig. 6B, 6C, 6F) exhibited significant histopathological alterations, including macrovesicular and microvesicular steatosis, sinusoidal dilation, decreased glycogen content (evidenced by reduced PAS staining), and noticeable chromatin condensation within hepatocyte nuclei, suggesting early apoptotic changes. These findings reflect hepatic cellular stress and metabolic dysfunction induced by dietary excess.

However, daily administration of *Malus domestica* juice (15 mL/kg for 30 days) markedly alleviated these alterations, as evidenced by reduced lipid accumulation, partial restoration of glycogen stores, and decreased nuclear chromatin condensation (Fig. 6D, 6G). These observations suggest a hepatoprotective effect of apple juice, potentially mediated by its antioxidant and bioactive compound content.

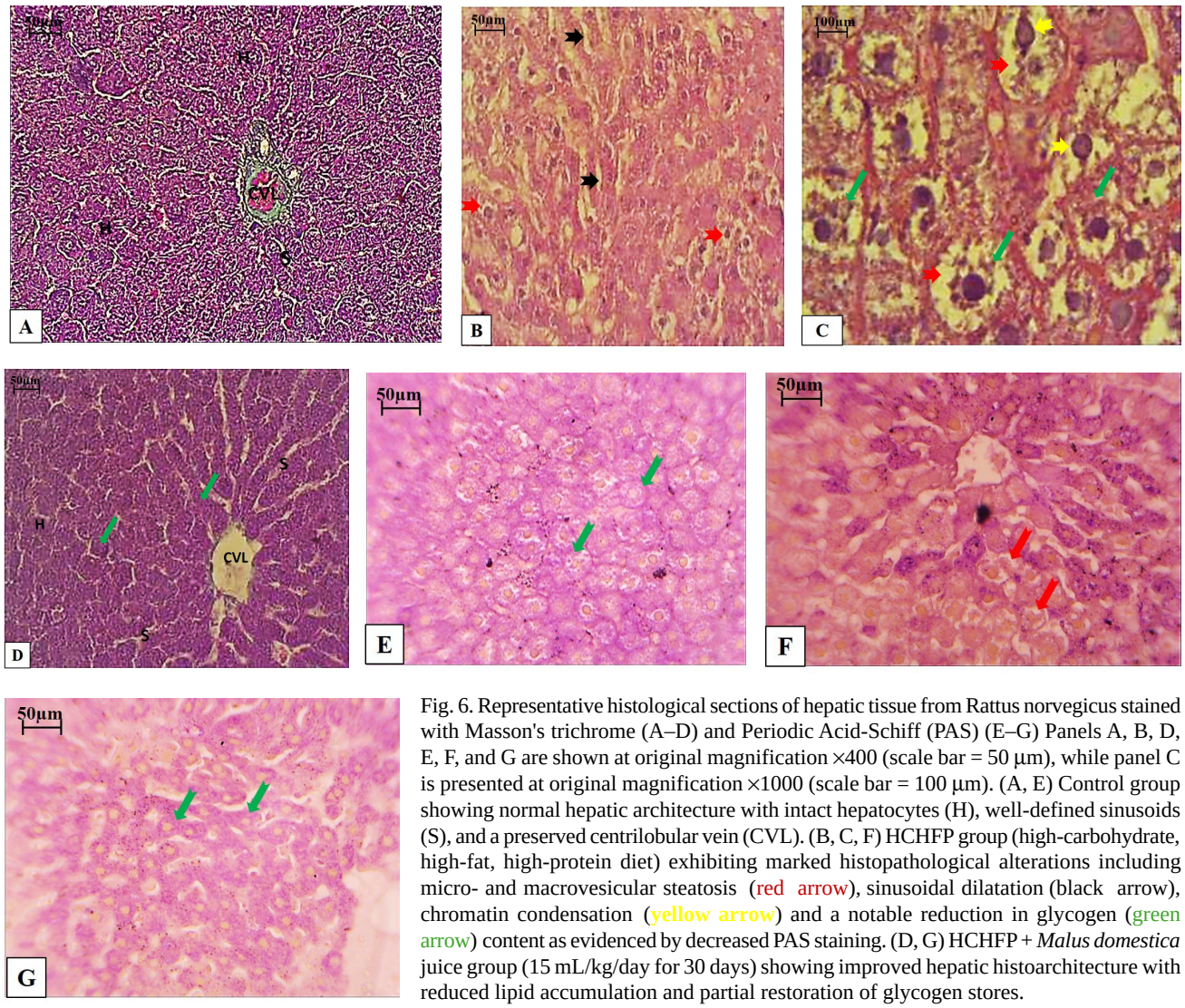

Fig. 6. Representative histological sections of hepatic tissue from Rattus norvegicus stained with Masson's trichrome (A–D) and Periodic Acid-Schiff (PAS) (E–G) Panels A, B, D, E, F, and G are shown at original magnification ×400 (scale bar = 50 µm), while panel C is presented at original magnification ×1000 (scale bar = 100 µm). (A, E) Control group showing normal hepatic architecture with intact hepatocytes (H), well-defined sinusoids (S), and a preserved centrilobular vein (CVL). (B, C, F) HCHFP group (high-carbohydrate, high-fat, high-protein diet) exhibiting marked histopathological alterations including micro- and macrovesicular steatosis (red arrow), sinusoidal dilatation (black arrow), chromatin condensation (yellow arrow) and a notable reduction in glycogen (green arrow) content as evidenced by decreased PAS staining. (D, G) HCHFP + *Malus domestica* juice group (15 mL/kg/day for 30 days) showing improved hepatic histoarchitecture with reduced lipid accumulation and partial restoration of glycogen stores.

DISCUSSION

The juice of *Malus domestica* exhibited a high total phenolic content (114.89 µg GAE/mg), indicative of a rich composition in polyphenolic compounds known for their antioxidant potential (Scalbert *et al*., 2005). However, its DPPH radical scavenging activity (IC50 = 37.83 ± 1.41 µg/mL) was lower than that of synthetic antioxidants such as BHA and BHT, suggesting moderate in vitro antioxidant efficacy. This observation is consistent with previous findings indicating that antioxidant activity depends not only on the total phenolic content but also on the specific composition and structure of the phenolic constituents (Scalbert *et al*., 2005). Phytochemical screening revealed the presence of major classes of phenolic secondary metabolites, including tannins, flavonoids (flavones, flavonols, leucoanthocyanins, and anthocyanins), anthracene derivatives, and reducing compounds. The absence of alkaloids, confirmed by classical reagents (Bouchardat, Dragendorff, Mayer), suggests the lack of nitrogen-containing alkaloids. This polyphenolic richness, known for its antioxidant, anti-inflammatory, and metabolic regulatory properties (Scalbert *et al*., 2005), may underlie the observed *in vivo* antioxidant and cardiometabolic effects. Rats subjected to the high-carbohydrate, high-fat,

high-protein (HCHFP) diet exhibited profound disturbances in glucose and lipid homeostasis, as evidenced by significantly increased glycemia, insulinemia, total cholesterol, and plasma protein levels at T1 and T2. These alterations are indicative of insulin resistance, hyperglycemia, and dyslipidemia. Elevated insulin levels further suggest a compensatory pancreatic response to peripheral insulin resistance, a hallmark of emerging metabolic syndrome. These metabolic derangements are in line with previous reports linking hypercaloric diets to metabolic disorders associated with obesity and chronic systemic inflammation (de Souza *et al*., 2015). Treatment with *Malus domestica* juice significantly improved these parameters at T2. The observed reductions in glucose and insulin levels suggest enhanced insulin sensitivity or increased glucose uptake, potentially mediated by polyphenols, which are known to modulate carbohydrate metabolism and insulin signaling pathways (Hyson, 2011). Similarly, the reduction in total cholesterol may be attributed to the hypolipidemic effects of apple-derived flavonoids, which regulate lipid metabolism and inhibit hepatic lipogenesis (Liu, 2013). Moreover, elevated CK-MB levels in the HCHFP group, a biomarker of myocardial injury, point to cardiac damage likely driven by lipid accumulation, oxidative stress, and low-grade chronic inflammation. The significant reduction in CK-MB levels following *Malus domestica* juice administration suggests a cardioprotective effect, likely mediated by antioxidant and anti-inflammatory constituents such as quercetin, catechins, and anthocyanins (Liu, 2013), as further supported by histological improvements in myocardial tissue. Cardiac oxidative stress was marked by increased malondialdehyde (MDA) and advanced oxidation protein products (AOPP), along with decreased superoxide dismutase (SOD) activity, indicating a redox imbalance characteristic of metabolic stress and cardiovascular dysfunction (Montezano *et al*., 2015). These findings reflect mitochondrial dysfunction and excessive reactive oxygen species (ROS) production, commonly associated with diets high in fat and carbohydrates (Montezano *et al*., 2015). *Malus domestica* juice mitigated these oxidative changes, suggesting a potent antioxidant effect. The partial restoration of SOD activity and reductions in MDA and AOPP levels may be attributed to polyphenolic compounds such as flavonoids and phenolic acids that scavenge ROS and enhance endogenous antioxidant defenses (Hyson, 2011). In addition, the HCHFP diet promoted a pro-inflammatory cardiac milieu, as indicated by elevated levels of NO, TNF-a, and MCP-1 key mediators of inflammation and myocardial remodeling. These biomarkers are often upregulated in obesity-related cardiometabolic disorders and contribute to endothelial dysfunction and myocardial injury. *Malus domestica* juice supplementation significantly lowered these pro-inflammatory markers, reinforcing its anti-inflammatory potential, likely via polyphenol-mediated modulation of oxidative stress-sensitive transcription factors such as NF-kB (Grand *et al*., 2001). Furthermore, the upregulation of AMPK and p38 MAPK in the HCHFP group reflects a cellular response to metabolic stress. Although AMPK activation is generally protective, excessive activation in inflammatory conditions may exacerbate stress signaling (Zhao *et al*., 2017). The normalization of AMPK and p38 MAPK expression following juice treatment suggests restored signaling balance and supports the cardioprotective role of *Malus domestica* (Zhao *et al*., 2017). Masson's trichrome staining revealed that the HCHFP diet induced major structural disruptions in cardiac tissue, including cardiomyocyte disorganization, fiber fragmentation, trabecular splitting, and inflammatory infiltration hallmarks of pathological cardiac remodeling seen in obesity-related cardiometabolic diseases (Sahraoui *et al*., 2020). Juice-treated rats showed partial restoration of myocardial architecture, reduced inflammation, and improved fiber alignment. These histological improvements corroborate the biochemical findings and further highlight the cardioprotective properties of apple juice, likely mediated by its polyphenolic content known to exert antioxidant, anti-inflammatory, and anti-fibrotic effects in cardiac models (Zou *et al.*, 2020). The HCHFP di*et al*so triggered hepatic disturbances, as evidenced by increased lipid content and depleted glycogen stores hallmarks of hepatic steatosis and impaired energy metabolism characteristic of non-alcoholic fatty liver disease (NAFLD) (Milton-Laskibar *et al*., 2020). Juice administration significantly reduced hepatic lipid accumulation and partially restored glycogen content, effects likely mediated by polyphenols such as quercetin and chlorogenic acid, which modulate lipid metabolism and improve hepatic insulin sensitivity (Milton-Laskibar *et al*., 2020). Polyphenols activate AMPK pathways, inhibit lipogenesis, and promote glycogen synthesis, countering steatosis and glucose dysregulation (Zou *et al*.,2020). Histopathological examination confirmed hepatic damage in HCHFP-fed rats, including steatosis, sinusoidal dilation, glycogen depletion, and nuclear condensation suggestive of apoptosis. These changes are characteristic of early-stage non-alcoholic steatohepatitis (NASH), a progressive form of NAFLD driven by lipotoxicity, oxidative stress, and mitochondrial dysfunction. Macro- and microvesicular steatosis reflects disrupted lipid metabolism, while reduced PAS staining indicates compromised glycogen reserves (Marcolin *et al*., 2012). Juice treatment significantly ameliorated these histological alterations, suggesting improved hepatic metabolism and reduced oxidative damage. These protective effects are attributed to polyphenols such as phloretin and quercetin, which possess antioxidant, anti-inflammatory, and anti-apoptotic properties (Milton-Laskibar *et al*., 2020).

CONCLUSION

In summary, this study demonstrates that *Malus domestica* juice exerts significant metabolic benefits in rats subjected to a high-fat, high-carbohydrate, high-protein (HCHFP) diet. The juice effectively reduced hyperglycemia, hyperinsulinemia, and hypercholesterolemia, while improving both hepatic and cardiac metabolic profiles. Furthermore, it restored redox homeostasis and attenuated inflammation by downregulating key pro-inflammatory mediators, including TNF-α, NO, and MCP-1. It also suppressed the diet-induced activation of AMPK and p38 MAPK signaling pathways in cardiac tissue. These findings suggest that the naturally occurring bioactive compounds in *Malus domestica* juice may offer promising potential for the prevention and early management of metabolic syndrome and its associated hepatic and cardiovascular complications.

REZKALLAH, N.; SMAIL, L.; BOUMAZA, S.; MAAMRI, S.; HEMILA, K.; AMALOU, A.; KACIMI, G.; HAMLAT, N. & BOUGUERRA, S. A. Evaluación histomorfológica de los efectos protectores hepatocardíacos del jugo de *Malus domestica* en ratas expuestas a una dieta obesogénica. *Int. J. Morphol., 43(6)*:2093-2103, 2025.

RESUMEN: Los trastornos cardiometabólicos, como la esteatosis hepática, la resistencia a la insulina y las enfermedades cardiovasculares, representan importantes desafíos para la salud pública y frecuentemente son asociados con las dietas occidentales ricas en grasas, carbohidratos y proteínas. Los polifenoles dietéticos, en particular los presentes en las manzanas, han mostrado prometedores efectos protectores contra disfunciones metabólicas y cardiovasculares. Este estudio investigó el potencial terapéutico del jugo de manzana en ratas Wistar hembra sometidas a un modelo de síndrome metabólico inducido por la dieta. Los animales se dividieron aleatoriamente en tres grupos: un grupo control (C) alimentado con una dieta estándar; un grupo alimentado con una dieta alta en carbohidratos, grasas y proteínas (HCHFP; 60 % grasa, 30 % carbohidratos y 9 % proteína) durante ocho semanas; y un tercer grupo (HCHFP + A) que recibió la misma dieta, suplementada por vía oral con jugo de manzana fresco (*Malus domestica*) (15 mL/kg/día) durante los últimos 30 días del período experimental. Se evaluaron los parámetros bioquímicos en plasma y tejidos para evaluar el metabolismo de lípidos y glucosa, estrés oxidativo (MDA, AOPP, SOD, CAT), marcadores inflamatorios (NO, MCP-1, TNF-a) y reguladores metabólicos (AMPK). También se realizaron exámenes histológicos de tejidos hepáticos y cardíacos. La suplementación con jugo de manzana mejoró significativamente los perfiles lipídicos y glucémicos, mejoró las actividades de las enzimas antioxidantes, redujo la inflamación y alivió la esteatosis hepática y el daño miocárdico. Estos hallazgos resaltan el potencial del jugo de manzana como una intervención nutricional funcional con propiedades antioxidantes, antiinflamatorias y reguladoras metabólicas contra los trastornos cardiometabólicos inducidos por la dieta.

PALABRAS CLAVE: Jugo de manzana; *Malus domestica*; Estrés oxidativo; Inflamación; Esteatosis hepática; Cardiovascular.

REFERENCES

Beauchamp, C. & Fridovich, I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem., 44(1)*:276-87, 1971.

Boden, G. Obesity, insulin resistance and free fatty acids. *Curr. Opin. Endocrinol. Diabetes Obes., 18(2)*:139-43, 2011.

Brand-Williams, W.; Cuvelier, M. E. & Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Sci. Technol., 28(1)*:25-30, 1995.

de Souza, R. J.; Mente, A.; Maroleanu, A.; Cozma, A. I.; Ha, V.; Kishibe, T.; Uleryk, E.; Budylowski, P.; Schünemann, H.; Beyene, J.; *et al*. Intake of saturated and trans unsaturated fatty acids and risk of all-cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ, 351*:h3978, 2015.

Folch, J.; Lees, M. & Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem., 226(1)*:497-509, 1957.

Giacco, F. & Brownlee, M. Oxidative stress and diabetic complications. *Circ. Res., 107(9)*:1058-70, 2010.

Grand, F.; Guitton, J. & Goudable, J. Optimisation des paramètres du dosage des nitrites et nitrates sériques par la technique de Griess. Ann. Biol. Clin., 59:559-65, 2001.

Hardie, D. G.; Ross, F. A. & Hawley, S. A. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol., 13(4)*:251-62, 2012.

Harborne, J. B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London, Chapman and Hall, 1998.

Hyson, D. A. A comprehensive review of apples and apple components and their relationship to human health. *Adv. Nutr., 2(5)*:408-20, 2011.

Liu, R. H. Health-promoting components of fruits and vegetables in the diet. *Adv. Nutr., 4(3)*:384S-92S, 2013.

Marcolin, E.; San-Miguel, B.; Vallejo, D.; Tieppo, J.; Marroni, N.; González-Gallego, J. & Tuñón, M. J. Quercetin treatment ameliorates inflammation and fibrosis in mice with nonalcoholic steatohepatitis. *J. Nutr., 142(10)*:1821-8, 2012.

Milton-Laskibar, I.; Aguirre, L.; Gómez-Zorita, S.; Rolo, A. P. & Portillo, M. P. The influence of dietary conditions in the effects of resveratrol on hepatic steatosis. *Food Funct., 11(11)*:9432-44, 2020.

Montezano, A. C.; Dulak-Lis, M.; Tsiropoulou, S.; Harvey, A. P.; Briones, A. M. & Touyz, R. M. Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. *Can. J. Cardiol., 31(5)*:631-41, 2015.

Ohkawa, H.; Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem., 95(2)*:351-8, 1979.

Sahraoui, A.; Dewachter, C.; Vegh, G.; McEntee, K.; Naeije, R.; Dewachter, L. & Bouguerra, S. A. High-fat di*et al*tered cardiac metabolic gene profile in Psammomys obesus gerbils. *Lipids Health Dis., 19(1)*:123, 2020.

Scalbert, A.; Johnson, I. T. & Saltmarsh, M. Polyphenols: antioxidants and beyond. *Am. J. Clin. Nutr., 81(1 Suppl)*:215S-217S, 2005.

Singleton, V. L. & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic., 16(3)*:144-58, 1965.

Van der Sluis, A. A.; Dekker, M. & van Boekel, M. A. J. S. Activity and concentration of polyphenolic antioxidants in apple juice. 3. Stability during storage. *J. Agric. Food Chem., 53(4)*:1073-80, 2005.

Van Handel, E. Estimation of glycogen in small amounts of tissue. *Anal. Biochem., 11(2)*:256-65, 1965.

Viollet, B.; Horman, S.; Leclerc, J.; Lantier, L.; Foretz, M.; Billaud, M. & Andreelli, F. AMPK inhibition in health and disease. *Crit. Rev. Biochem. Mol. Biol., 45(4)*:276-95, 2010.

Witko-Sarsat, V.; Friedlander, M.; Capeillère-Blandin, C.; Nguyen Khoa, T.; Nguyen, A. T.; Zingraff, J.; Jungers, P. & Descamps-Latscha, B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int., 49(5)*:1304-13, 1996.

Zaha, V. G. & Young, L. H. AMP-activated protein kinase regulation and biological actions in the heart. *Circ. Res., 111(6)*:800-14, 2012.

Zhao, L.; Cen, F.; Tian, F.; Li, M.; Zhang, Q.; Shen, H.; Shen, X.; Zhou, M. & Du, J. Combination treatment with quercetin and resveratrol attenuates high-fat diet-induced obesity and associated inflammation in rats via the AMPKa1/SIRT1 signaling pathway. *Exp. Ther. Med., 14(6)*:5942-8, 2017.

Zou, T.; Wang, B.; Li, S.; Liu, Y. & You, J. Dietary apple polyphenols promote fat browning in high-fat diet-induced obese mice through activation of adenosine monophosphate-activated protein kinase α. *J. Sci. Food Agric., 100(6)*:2389-98, 2020.

Corresponding author:
Dr Nabila Rezkallah
Laboratory of Physiology of Organisms
Team of Cellular and Molecular Physiopathology
Faculty of Biological Sciences
University of Sciences and Technology
Houari Boumediene (USTHB)
BP 32 El Alia
16011 Algiers
ALGERIA

Email: nabila.r@gmx.fr