### Title

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### Authors

Mokhtari, Imane

Moumou, Mohammadine

Mokhtari, Chakib

et al.



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# Nutritional Composition and Effect of Loquat Fruit (*Eriobotrya japonica* L. var. *Navela*) on Lipid Metabolism and Liver Steatosis in High-Fat High-Sucrose Diet-Fed Mice

**Imane Mokhtari[1], Mohammadine Moumou[1], Chakib Mokhtari[2], Mohamed Harnafi[1], Dragan Milenkovic[3], Souliman Amrani[1], and Hicham Harnafi[1]**

[1]Laboratory of Bioresources, Biotechnologies, Ethnopharmacology and Health, Faculty of Sciences and [2]Laboratory of Applied Chemistry and Environment, Faculty of Sciences, Mohammed First University, Oujda 60000, Morocco
[3]Department of Nutrition, College of Agricultural and Environmental Sciences, University of California, Davis, Davis, CA 95616, USA

**ABSTRACT:** Loquat (*Eriobotrya japonica* L.) is a popular fruit known for its sweet and slightly tangy flavor, which is widely consumed both fresh and in various processed forms. This study aimed to analyze the biochemical composition of loquat juice and investigate its metabolic benefits in mice fed a high-fat/high-sucrose diet (HFSD). Mice were fed either a standard diet or an HFSD and received or not the loquat juice at 4 or 8 mL/kg body weight for 8 weeks. Body weight, food efficiency ratio, plasma lipoprotein profile, plasma glucose, and lipid indices were monitored throughout the experiment. At the end of the experiment, additional assessments were performed, including lipid content measurements in liver, adipose tissue, bile, and feces; hepatic antioxidant enzyme activities (superoxide dismutase and catalase); hepatic malondialdehyde content; plasma biomarkers of liver injury; liver histology; and organ relative weight. Feeding mice with the HFSD resulted in a significant perturbation in lipid and glucose metabolism, obesity, liver steatosis, and oxidative stress-related enzymes. However, the concomitant administration of loquat juice significantly corrected this imbalance. Fresh loquat juice is low in fat and protein, moderately sugary, and energetically light; however, it is rich in minerals, vitamin C, and various phytochemicals compounds, such as phenolic acids, flavonoids, and carotenoids. The loquat juice could be considered a functional food and could be valorized through the extraction of active substances and their use as food supplements to prevent lipid metabolism disorders and the resulting health complications.

**Keywords:** *Eriobotrya japonica*, fatty liver, lipid metabolism, mice, oxidative stress

## INTRODUCTION

Loquat, scientifically known as *Eriobotrya japonica* (Thunb.) Lindl and belongs to the Rosaceae family, is a fruit tree native to southeastern China and is currently cultivated in several countries worldwide, including Mediterranean regions, Japan, California, Australia, Turkey, India, and Brazil (Surya et al., 2021). The main producer countries of this fruit are China, followed by Spain, Japan, Turkey, India and Australia (Zhang et al., 2015). Loquat cultivation is of great economic significance; however, owing to the delicate nature of the fruit's sweet flesh and thin peel, it cannot be stored for a long time. Therefore, it is best enjoyed fresh or used in various culinary creations, including jams, jellies, and pies (Dhiman et al., 2021).

In eastern Morocco, loquat is called "MZAH." This fruit was introduced to the region from Algeria during the period of French colonization in the early 20th century (Kabiri et al., 2022). The Zegzel Valley, situated in the northeast of Morocco, plays a significant role in loquat cultivation within the country. It is responsible for approximately 80% of the total land area dedicated to growing loquats. This cultivation focuses on 4 main varieties, including *Navela*, *Tanaka*, *Muscat*, and *Mkarkab* (Kodad et al., 2023). Along with its consumption as fresh pulp or juice, this fruit is known by the local population for its medicinal properties against several diseases, including metabolic disorders leading to liver diseases, oxidative stress, and related cardiometabolic complications (Ziyyat et al., 1997; Liu et al., 2016).

Among these metabolic disorders, hyperlipidemia and hyperglycemia especially with high low-density lipoprotein (LDL)-cholesterol and triglyceride (TG) levels and low high-density lipoprotein (HDL)-cholesterol levels con-

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Correspondence to Hicham Harnafi, Email: h.harnafi@ump.ac.ma

stitute the major risk factor for atherosclerosis and cardiovascular diseases (CVDs) (Alloubani et al., 2021). Furthermore, adding obesity, diabetes mellitus, and oxidative stress to these parameters generally leads to hepatic steatosis and the triggering of the atherosclerotic process (Niemann et al., 2017). LDL-cholesterol oxidation at the sub-endothelial space led to foam cell formation, inflammation, and subsequently atherogenesis (Poznyak et al., 2021). Conversely, to date, a lipid and sucrose-rich diet is the principal cause of several diseases, particularly resulting from metabolic disorders. A positive correlation has been established between western diet consumption and the occurrence of cardiovascular complications (Victorio et al., 2021). Thus, high-fat diet resulted in the elevation in blood cholesterol, TG, and glucose levels as well as obesity and hepatic steatosis development (Kobi et al., 2023). However, micronutrient-rich and low-energy foods have been recommended for patients with metabolic disorders (Annuzzi et al., 2014; Terao, 2023). The Mediterranean diet has long been proven to reduce the risk of cardiovascular accidents (Wade et al., 2018; Martínez-González et al., 2019). Consisting mainly of fruits, vegetables, whole meal bread, extra virgin olive oil, nuts, legumes, and fish, this diet is rich in micronutrients and bioactive molecules with beneficial effects on health (Wade et al., 2018).

Although fruit consumption has been shown to improve cardiovascular health (Aune et al., 2017), the chemical constituents that may be behind this effect, therapeutic doses, and presence of possible toxic effects are not well investigated, especially when there are several varieties and cultivars. The *Navela* loquat variety grown in Morocco has not yet been investigated in terms of its chemical composition and beneficial effects on the health of consumers. Therefore, in this context, this experimental study was conducted to highlight the nutritional interest and health effects of this variety and thus contribute to its valorization at national and international scales.

## MATERIALS AND METHODS

### Fruit harvest, morphologic characteristics, and juice preparation

Loquat fruits (*Eriobotrya japonica* [Thunb.] Lindl. variety *Navela*, locally called *Beldi*) were harvested at commercial maturity stage in Berkane Province (East of Morocco). The fruits were selected for integrity and uniformity in size and color.

The morphologic characteristics of loquat fruits including average fresh weight, length, width, and fruit thickness were determined by taking ten loquats as one batch and calculating their average measurements. The weight of each fruit was measured using a digital laboratory balance, whereas the length and diameter were measured using a Vernier caliper. The fruit shape index was determined for each fruit by dividing the length by the diameter.

The fresh *Navela* loquat juice (NLJ) was prepared according to the method reported by Meng et al. (2022) with slight modifications. Five kilograms of the fruits were washed with tap water before removing peels and seeds. Subsequently, the pulp was homogenized using an electric blender. The obtained slurry was filtered, and the fresh juice was used for biochemical analysis and experimental study. The juice yield was 300 g of juice per 1,000 g of fruits.

### Determination of the physicochemical parameters and nutritional composition of loquat juice

***pH, total soluble solids (TSSs), titrable acidity (TA), and TSS/TA ratio*:** The pH of the fresh juice was determined using a laboratory pH meter (HANNA HI 8424, HANNA Instruments), and the total TSS content was measured using a refractometer (ATAGO 3810-PAL-1, ATAGO) and expressed in °Brix (Association of Officical Analytical Chemists, AOAC 920.151). TA was determined by the titration of the fresh juice with sodium hydroxide solution at 0.1 N and expressed as g malic acid equivalent per 100 g of juice, and phenolphthalein (1%) was used as the indicator (AOAC 943.03). Moreover, the maturity index based on the TSS/TA ratio was calculated (Dimassi et al., 2020). All measurements were performed in triplicate.

***Total sugar content, sugar profile, and sweetness index*:** The total sugar content was measured using the phenol-sulfuric acid method as described by Khatun and Mollah (2024). Briefly, the fresh juice was adequately diluted and mixed with phenol (5%) and concentrated sulfuric acid. The resulting coloration was measured at 490 nm against a blank. The total sugar content was determined using a linear regression equation derived from the calibration curve constructed using D-fructose standard solutions. The results were expressed in gram fructose per 100 g of fresh juice. The sugar profile and content of the loquat fresh juice was determined using the high-performance liquid chromatography (HPLC) method as outlined by Yu et al. (2021). Briefly, the fresh juice was adequately diluted and filtered through a 0.45-μm syringe filter to obtain a clear filtrate. Subsequently, 10 μL of filtrate were injected in a Supelcosil LC column (25 cm×0.46 cm×5 μm, Merck). The mobile phase comprised a mixture of acetonitrile/water (60/40, v/v), and elution was performed at a 1-mL/min flow rate. Sugar peak identification was based on retention times compared with those of the standards. The determination of individual sugar content was performed on the basis of the standard curve. The results were presented in g/100 g of fresh juice.

The sweetness index of the fresh loquat juice was determined by considering the amount and sweetness characteristics of each identified sugar (Keutgen and Pawelzik, 2007). Thus, sweetness index estimation considers that fructose is approximately 2.30-fold sweeter than glucose, and sucrose is approximately 1.35-fold sweeter than glucose. The sweetness index of loquat juice was calculated as follows: SI=[1.00 (glucose g/100 g)]+[2.30 (fructose g/100 g)]+[1.35 (sucrose g/100 g)].

***Organic acid composition*:** The analysis of organic acids in loquat juice was preformed following the method described by Deng et al. (2023) with some modifications. The fresh juice was adequately diluted in ultrapure water and filtered through a 0.45-μm syringe filter. Ten microliters of the diluted juice were injected in a C18 column (250 mm×4.6 mm; particle size, 5 μm, Merck) at 30°C. The mobile phase composed of sodium phosphate buffer solution (25 mM) with a pH finely tuned to 2.45 through the addition of phosphoric acid. The flow rate was 1 mL/min. Organic acids were identified by comparing their chromatographic parameters with those of the standards, including malic, tartaric, succinic, and oxalic acid. Quantitative analysis was performed on the basis of the calibration curve of malic, tartaric, succinic, and oxalic acid. The results were expressed as mg equivalent malic acid per 100 g of juice.

***Fat and protein*:** The fat content was calculated according to the official method AOAC 963.15. The juice was initially dried in a ventilated oven at 40°C for 24 h. Subsequently, the dry residue was scraped and weighed. Five grams of dried juice powder were put in a cartridge and subsequently placed in the Soxhlet apparatus. The total fat content was extracted with diethyl ether at 45°C for 16 h. Next, the solvent was evaporated in a rotatory evaporator, resulting in a viscous solid residue containing the fat. The results were expressed as gram fat per 100 g of juice. The total protein content was determined according to the official method AOAC 920.152.

***Ash and mineral*:** The ash content was determined using the gravimetric method (AOAC 940.26). Briefly, 25 g of loquat juice were placed into a pre-weighed porcelain crucible. The sample was subsequently subjected to heating in a muffle furnace at 525°C for 2 h. Then, the crucible was removed from the furnace and cooled in a desiccator. Subsequently, to determine the weight of the ash, the crucible was re-weighed. The results were expressed in gram per 100 g of juice. The mineral composition was determined from the ash sample using the atomic spectrometry as described (AOAC 968.08).

***Crude fiber*:** The crude fiber content was estimated according to the Weende method (AOAC 978.10). Five grams of the previously dried juice (as outlined above) were treated with sulfuric acid 1.25% solution to extract the fibers, followed by filtration and rinsing. The extracted fibers were subsequently treated with sodium hydroxide 1.25% solution, filtered again, and rinsed. Finally, the obtained crude fibers were dried in an oven at 105°C for 1 h. After The obtained residue represented the crude fibers from which the ash content, was subtracted. The results were expressed as gram fibers per 100 g of juice.

***Total polyphenols, flavonoids, carotenoids, and vitamin C*:** The total polyphenol content was estimated using the Folin-Ciocalteu method as described by Mokhtari et al. (2023a). Next, 0.5 mL of the adequately diluted loquat juice was mixed with 0.25 mL of Folin-Ciocalteu reagent (Sigma-Aldrich) and 0.5 mL of aqueous sodium carbonate solution (20%). After incubating the mixture at 25°C in the dark for 30 min, the absorbance was measured at 725 nm. The polyphenol content was quantified using a calibration curve prepared with chlorogenic acid. The results were expressed in milligrams per gram of juice.

Flavonoids were selected for analysis because they are the most abundant class of polyphenols present in mature loquat fruits alongside phenolic acids. Their quantification was undertaken using our previous method (Mokhtari et al., 2023a). One milliliter of the aluminum chloride reagent was added to 0.5 mL of adequately diluted loquat juice, and the resulting yellow color was measured at 430 nm. The flavonoid concentration was calculated by referring to the calibration curve of rutin standard solutions and expressed as milligram per gram of juice.

The individual phenolic compounds present in loquat juice were analyzed using HPLC as previously described (Mokhtari et al., 2023b). Briefly, 10 μL of the adequately diluted juice samples were injected into a C18 column (250×4.6 mm; particle size, 5 μm, Merck). Elution was performed by a gradient of ultrapure water/acetic acid (0.5%) (A) and methanol (B) at a flow rate of 1 mL/min and a temperature of 20°C. The gradient was set as follows: 0 min: 80% A, 20% B; 20 min: 100% B; 25 min: 100% B; and 35 – 40 min: 80% A, 20% B. Chromatograms were recorded at 340 nm, and compound identification was achieved through their retention times and ultraviolet-visible spectra by referencing a database of standard phenolic compounds. Individual phenolic compounds were quantified on the basis of the calibration curve of external standards.

The carotenoid content was determined according to the method outlined by Kabiri et al. (2022). Five milliliters of loquat juice were extracted three times with 50 mL of n-hexane in a separating funnel under manual agitation. Subsequently, the two phases were allowed to separate for 30 min. The absorbance of organic phase containing carotenoids was measured at 470 nm against a blank containing n-hexane. The carotenoid content was estimated using a standard curve of β-carotene.

Vitamin C was quantified using the 2,6-dichlorophenolindophenol titrimetric method as described by Kabiri et

al. (2022) with some modifications. Ten milliliters of the diluted flesh juice were mixed with 1 mL of glacial acetic acid and titrated to a faint permanent pink color. The vitamin C content was calculated according to the standard curve of L-ascorbic acid.

***Energy value*:** The total energy value of loquat fruit was determined using standard Atwater factors: 4, 9, and 4 kcal for proteins, lipids (fats), and carbohydrates, respectively, to calculate the caloric content. The sum of the respective values multiplied by proteins, lipids, and total carbohydrate is provided below: Energy value (kcal/100 g) = (protein×4) + (total carbohydrate×4) + (lipids×9).

***Animals and treatments*:** Adult male *albino* mice weighing 20 – 25 g bred in the animal house of the Faculty of Sciences (Oujda, Morocco), were provided free access to diet and water *ad libitum* throughout the experimental duration. Their housing was maintained at 22°C with a 12-h light-dark cycle. Mice were used according to the internationally accepted standard guidelines for the use of laboratory animals. The Faculty of Medicine, Mohammed First University, Oujda, approved this study (approval number: 002016).

High-fat/high-sucrose diet (HFSD) was prepared by mixing a standard mice diet obtained from the Society Alf Sahel (Meknes, Morocco) with beef fat (16%), cholesterol (1.5%), fructose (10%), egg yolk (10%), and deoxycholic acid (0.2%).

The hyperlipidemic control group (HFSDG) was fed with HFSD and gavaged with distilled water. The HFSD-NLJ-treated groups (HFSD-NLJG$_4$ and HFSD-NLJG$_8$) were fed with HFSD and received an oral administration of NLJ at 4 and 8 mL/kg body weight (BW), respectively. The fenofibrate-treated group was fed with HFSD and gavaged with fenofibrate at 3 mg/kg BW in the same manner. Fenofibrate (Fenogal 160 mg) was purchased from the Sothema Society. The powder was dissolved in distilled water at 180 mg/L in the presence of Tween 40 as a surfactant. Subsequently, the mixture was stirred by heating to 40°C for 10 min. The BW and food intake of each animal were recorded weekly during the treatment period. After 4 and 8 weeks, blood samples were taken from the retro-orbital sinus under sodium citrate. Samples were immediately centrifuged (419 *g*/10 min), and the plasma was used for biochemical analysis. Mice were sacrificed at the end of the experiment, and their liver and abdominal adipose tissues were removed, washed in saline, and weighed.

***Biochemical analysis of plasma*:** Total cholesterol (TC), TGs, HDL-cholesterol, LDL-cholesterol, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were analyzed in plasma using specific biomedical kits as described in our previous study (Mokhtari et al., 2023a).

Lipid indices were calculated using the following formulas (Oršolić et al., 2019):

Atherogenic index of plasma (AIP) = log (TG/HDL-C)
Cardiac risk ratio (CRR) = TC/HDL-C
Atherogenic coefficient (AC) = (TC − HDL-C)/HDL-C
Cardiovascular protective index (CPI) = HDL-C/LDL-C

### Determination of liver, adipose tissue, and fecal total lipids

Total lipids were extracted from the liver, adipose tissues, and feces according to our previous method (Mokhtari et al., 2023b). Briefly, 1 g of adipose tissues or fecal matter was extracted in cold isopropanol and incubated overnight at 4°C. After centrifugation at 419 *g* for 15 min, the collected supernatants were used for TC and TG measurements using enzymatic kits.

### Liver lipid peroxidation measurement: malondialdehyde (MDA), superoxide dismutase (SOD), and catalase enzymes

Liver lipid peroxidation was determined by measuring the amount of MDA. Briefly, liver tissue samples were homogenized in potassium phosphate buffer. After centrifugation at 1,073 *g* for 10 min, supernatants were recovered to analyze the MDA by adding trichloroacetic and thiobarbituric acid. The absorbance of the resulted red chromophore was recorded at 532 nm. The results were expressed in nmol MDA/mg of liver tissues. Hepatic SOD and catalase activities were measured according to methods outlined in our previous studies (Mokhtari et al., 2023a).

### Liver histological study

Fresh liver tissues were cut into 1-cm pieces, fixed in 10% formalin solution, and embedded in paraffin as described in our previous study (Mokhtari et al., 2023a). The fixed tissues were subsequently sliced into thin sections using a microtome. Then, the obtained sections were deparaffinized in toluene and rehydrated in dilutions of ethanol. The samples were stained with hematoxylin and eosin and examined under optic microscopy.

### Statistical analysis

Data were analyzed using student's *t*-test. Differences with *P*-values of $<0.05$ were considered statistically significant. The results were expressed as means±standard error of the mean.

## RESULTS

### Biochemical composition of NLJ

NLJ was analyzed for its chemical and nutritional composition (Table 1). The results indicate that the TSS content was 13.65±0.88°Brix, indicating that the loquat

**Table 1.** Physicochemical parameters and biochemical composition of *Navela* loquat juice

| Parameters | Values |
|---|---|
| Weight (g) | 71.52±4.23 |
| Length (mm) | 56.88±1.53 |
| Width (mm) | 47.17±2.22 |
| Fruit thickness (mm) | 18.43±1.85 |
| Fruit shape index | 1.18±0.11 |
| pH | 4.00±0.23 |
| TA (%) | 0.73±0.03 |
| TSSs (°Brix) | 13.65±0.88 |
| TSS/TA | 18.67±0.33 |
| Total sugars (g/100 g) | 10.22±1.01 |
| Sugars (g/100 g) | |
| Fructose | 4.01±0.21 |
| Glucose | 2.52±0.15 |
| Sucrose | 1.49±0.11 |
| Sweetness index | 13.70±0.38 |
| Fat (g/100 g) | 0.08±0.003 |
| Protein (g/100 g) | 0.41±0.02 |
| Crude fiber (g/100 g) | 0.12±0.04 |
| Organic acids (mg/100 g) | |
| Malic acid | 610.08±19.55 |
| Tartaric acid | 73.17±5.22 |
| Succinic acid | 26.11±1.83 |
| Oxalic acid | 15.93±1.13 |
| Vitamin C (mg/100 g) | 10.52±0.29 |
| Carotenoids (μg/g) | 53.18±4.09 |
| Polyphenols (mg/g) | 153.67±8.33 |
| Flavonoids (mg/g) | 56.00±1.22 |
| Ash (g/100 g) | 0.41±0.02 |
| Potassium (mg/100 g) | 261.58±10.23 |
| Sodium (mg/100 g) | 36.20±1.93 |
| Phosphorus (mg/100 g) | 23.66±1.41 |
| Calcium (mg/100 g) | 17.62±3.01 |
| Magnesium (mg/100 g) | 18.02±2.11 |
| Iron (mg/100 g) | 3.99±0.16 |
| Energy value (kcal/100 g) | 43.12±0.28 |

TA, titratable acidity; TSSs, total soluble solids.

juice is rich in nutrients, and the fruit has reached the stage of maturity since a minimum soluble solid content of 10°Brix is frequently required for commercialization. Moreover, the TA of the juice is well within the standards, with an average value of 0.73%±0.03%, justifying the non-acid taste of this variety of loquat as known locally among farmers. The balance between TSS and acidity, generally measured as the TSS/TA ratio, is highly significant for judging the fruit taste and its post-harvest quality. Therefore, the high obtained value of the ratio (TSS/TA=18.67±0.33) indicates richness in sugar, making the fruit sweet, mature, and more suitable for consumption or industrial processing. We concluded that the total sugar content of loquat juice was 10.22%±1.01%. The sugar amount is essentially represented by fructose (4.01%±0.21%), glucose (2.52%±0.15%), and sucrose (1.49%±0.11%), and the rest represent other sugars that responded less and were not analyzed in this study, including maltose and sorbitol. The organic acids responsible for the TA of the juice were mainly represented by malic acid, which is the majority constituent (610.08± 19.55 mg/100g), followed by tartaric acid (73.17±5.22 mg/100g), succinic acid (26.11±1.83 mg/100 g), and oxalic acid (15.93±1.13 mg/100 g). Furthermore, we noted that the juice was very low in liqids (0.08%±0.003%) and proteins (0.41%±0.02%). However, it contained 0.41%± 0.02% ash, which was essentially represented by potassium (261.58±10.23 mg/100 g), sodium (36.20±1.93 mg/100 g), phosphorus (23.66±1.41 mg/100 g), calcium (17.62±3.01 mg/100 g), magnesium (18.02±2.11 mg/100 g), and iron (3.99±0.16 mg/100 g). The juice was a good source of vitamin C (10.52±0.29 mg/100 g), carotenoids (53.18±4.09 μg/g), and polyphenols (153.67 ±8.33 mg/g), of which flavonoids represent 56±1.22 mg/g, making the juice a good functional food with a low calorie content (43.12±0.28 kcal/100 g).

### Phenolic profile of NLJ

The HPLC chromatogram (Fig. 1) shows the presence of 5 phenolic compounds in NLJ. The first peak was identified as 5-caffeoylquinic acid, representing 18.70±1.12 mg/g dry juice. The second and third peaks corresponded to 3-*p*-coumaroylquinic acid (0.46±0.02 mg/g dry juice) and 3-caffeoylquinic acid (42.09±3.32 mg/g dry juice), respectively. The fourth and fifth peaks were identified

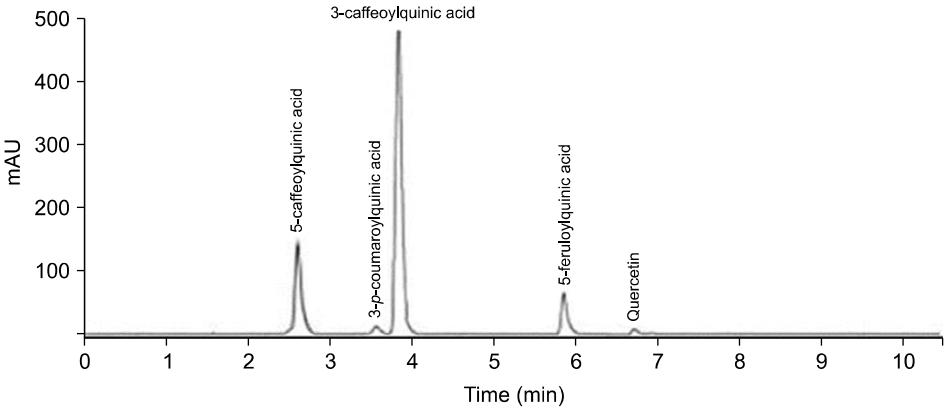


**Fig. 1.** Polyphenol high-performance liquid chromatography analysis of *Navela* loquat juice.

as 5-feruloylquinic acid (6.67±0.73 mg/g dry juice) and quercetin (0.28±0.04 mg/g dry juice), respectively.

**Effect of the HFSD on the metabolic parameters in mice**

The results of this study suggested that HFSD, compared with the standard diet, caused several disorders at both metabolic and tissue levels. Metabolically, the high-calorie diet caused a real disturbance in lipid and carbohydrate homeostasis.

This disruption was manifested by an increase in plasma TC ranging from 90% ($P$<0.001) after 4 weeks to 94% ($P$<0.001) after 8 weeks. The trend was mirrored in other plasma lipid parameters: TGs showed an increase of 107% and 108% ($P$<0.001), very low density lipoprotein (VLDL) increased by 107% and 109% ($P$<0.01), and LDL-cholesterol surged by 420% and 442% ($P$<0.001) after 4 and 8 weeks, respectively. Glucose, for its part, showed an increase of 30% and 40% ($P$<0.01) after 4 and 8 weeks, respectively (Table 2).

After 4 weeks, these changes collectively increased the atherogenic and cardiac risk indices, including the AIP (+164%, $P$<0.001), CRR (+301%, $P$<0.001), and AC (+715%, $P$<0.001). After 8 weeks, the increase persisted, with the AIP, CRR, and AC increasing by approximately +165% ($P$<0.001), +323% ($P$<0.001), and +782% ($P$<0.001), respectively, indicating a heightened susceptibility to CVDs owing to hyperlipidemia. Consequently, the CPI (−91%, $P$<0.001) showed a reduced defense against such diseases (Table 3).

In the liver, adipose tissue, feces, and bile, hyperlipidemia was translated into a significant increase in cholesterol and TG contents (Table 4).

Furthermore, following sacrifice of the mice, a clear difference was observed in the morphology of the liver and adipose tissue compared with normal mice. The liver appeared enlarged, fatter, and whitish in color. Adipose tissues were highly abundant, particularly in the abdominal area (Fig. 2A). This observation was supported, on

**Table 2.** Effect of *Navela* loquat juice on plasma lipid parameters and glucose in mice

| Groups | Lipid parameters (mg/dL) | 2 weeks | 4 weeks | 8 weeks |
|---|---|---|---|---|
| NC | TC | 118.22±11.13 | 119.10±10.60 | 119.51±12.18 |
| | TG | 65.17±9.95 | 65.93±8.43 | 67.07±11.22 |
| | VLDL | 13.10±2.11 | 13.21±1.18 | 13.40±2.02 |
| | LDL-C | 27.89±5.53 | 28.45±3.30 | 28.79±4.63 |
| | HDL-C | 68.32±8.76 | 69.02±9.21 | 71.22±10.50 |
| | Glucose | 90.12±5.77 | 92.53±4.73 | 92.20±4.82 |
| HFSDG | TC | 117.45±11.04 | 227.26±10.24** | 232.56±11.18** |
| | TG | 64.58±7.17 | 136.23±6.13** | 140.45±9.62** |
| | VLDL | 12.91±3.12 | 27.24±4.10* | 28.06±4.23* |
| | LDL-C | 23.93±10.02 | 148.22±9.78** | 152.23±10.19** |
| | HDL-C | 73.11±5.30 | 33.78±4.10* | 33.92±4.52* |
| | Glucose | 93.18±5.92 | 120.95±7.62* | 129.44±7.06* |
| HFSD-NLJG$_4$ | TC | 116.12±10.22 | 204.14±9.32 | 184.25±6.24$^b$ |
| | TG | 61.83±6.03 | 122.70±7.36 | 115.53±4.10$^a$ |
| | VLDL | 12.36±2.98 | 24.54±3.11 | 21.09±2.33 |
| | LDL-C | 24.15±4.33 | 133.27±6.78 | 120.33±5.16$^a$ |
| | HDL-C | 69.69±5.44 | 40.33±4.5 | 50.09±3.60$^a$ |
| | Glucose | 90.16±4.03 | 109.21±6.23 | 108.65±5.98$^a$ |
| HFSD-NLJG$_8$ | TC | 118.25±10.22 | 196.56±9.32$^a$ | 173.21±6.15$^c$ |
| | TG | 53.43±4.03 | 120.02±3.72$^a$ | 105.56±3.43$^b$ |
| | VLDL | 10.65±2.04 | 21.18±1.66 | 14.50±1.15$^b$ |
| | LDL-C | 25.11±3.37 | 118.98±6.88$^a$ | 77.63±4.12$^c$ |
| | HDL-C | 70.69±5.25 | 52.55±4.32$^b$ | 66.56±3.93$^c$ |
| | Glucose | 91.05±5.21 | 96.01±3.11$^b$ | 101.21±3.05$^b$ |
| HFSD-FFG | TC | 119.13±9.80 | 195.33±8.17$^a$ | 167.05±4.73$^c$ |
| | TG | 56.21±3.47 | 118.20±4.96$^a$ | 103.55±3.88$^b$ |
| | VLDL | 11.24±1.18 | 20.63±1.56 | 13.90±1.27$^b$ |
| | LDL-C | 21.89±3.66 | 113.74±7.02$^a$ | 73.13±3.48$^c$ |
| | HDL-C | 72.17±5.96 | 58.93±4.98$^b$ | 70.88±4.04$^c$ |
| | Glucose | 93.11±6.66 | 95.78±2.99$^b$ | 99.92±3.14$^b$ |

*$P$<0.05 and **$P$<0.001 against NC.
$^a$$P$<0.05, $^b$$P$<0.01, and $^c$$P$<0.001 against HFSDG.
NC, normal control; HFSD, high-fat/high-sucrose diet; HFSDG, hyperlipidemic control group; HFSD-NLJG$_4$, HFSD-*Navela* loquat juice-treated group at 4 mL/kg; HFSD-NLJG$_8$, HFSD-*Navela* loquat juice-treated group at 8 mL/kg; HFSD-FFG, HFSD-fenofibrate-treated group; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

**Table 3.** Effect of *Navela* loquat juice on mice lipid indices

| Groups | Lipid indices | 2 weeks | 4 weeks | 8 weeks |
|---|---|---|---|---|
| NC | AIP | −0.38±0.02 | −0.38±0.01 | −0.39±0.03 |
| | CRR | 1.72±0.12 | 1.71±0.13 | 1.66±0.10 |
| | AC | 0.73±0.02 | 0.72±0.011 | 0.68±0.013 |
| | CPI | 2.51±0.12 | 2.46±0.11 | 2.53±0.13 |
| HFSDG | AIP | −0.41±0.04 | 0.24±0.06* | 0.25±0.06* |
| | CRR | 1.60±0.13 | 6.87±1.25* | 7.03±1.34* |
| | AC | 0.60±0.02 | 5.87±0.88* | 6.00±1.10* |
| | CPI | 3.17±0.17 | 0.22±0.01* | 0.21±0.009* |
| HFSD-NLJG$_4$ | AIP | −0.40±0.02 | 0.12±0.05 | 0.002±0.001[c] |
| | CRR | 1.68±0.15 | 5.11±1.10 | 3.06±1.02[a] |
| | AC | 0.68±0.013 | 4.10±0.99 | 2.06±0.87[a] |
| | CPI | 2.87±0.17 | 0.30±0.04 | 0.53±0.14[a] |
| HFSD-NLJG$_8$ | AIP | −0.48±0.05 | 0.003±0.001[b] | −0.15±0.02[c] |
| | CRR | 1.68±0.16 | 3.76±1.24 | 2.62±0.99[a] |
| | AC | 0.67±0.016 | 2.76±0.17[b] | 1.24±0.13[c] |
| | CPI | 2.80±0.12 | 0.44±0.02[c] | 0.85±0.017[c] |
| HFSD-FFG | AIP | −0.46±0.04 | −0.052±0.01[c] | −0.19±0.03[c] |
| | CRR | 1.65±0.10 | 3.36±1.01[a] | 2.38±0.93[a] |
| | AC | 0.65±0.02 | 2.34±0.13[b] | 1.38±0.11[c] |
| | CPI | 3.42±0.17 | 0.51±0.012[c] | 0.95±0.07[c] |

*$P$<0.001 against NC.
[a]$P$<0.05, [b]$P$<0.01, and [c]$P$<0.001 against HFSDG.
NC, normal control; HFSD, high-fat/high-sucrose diet; HFSDG, hyperlipidemic control group; HFSD-NLJG$_4$, HFSD-*Navela* loquat juice-treated group at 4 mL/kg; HFSD-NLJG$_8$, HFSD-*Navela* loquat juice-treated group at 8 mL/kg; HFSD-FFG, HFSD-fenofibrate-treated group; AIP, atherogenic index of plasma; CRR, cardiac risk ratio; AC, atherogenic coefficient; CPI, cardiovascular protective index.

the one hand, by a comparison of relative organ weights, showing an increase of 103% ($P$<0.05) in liver mass and 186% ($P$<0.05) in adipose tissue mass (Fig. 2C). On the other hand, microscopic examination of the liver tissues highlighted the difference in histology and, above all, the massive presence of lipid deposits in animals on the HFSD diet compared with controls (Fig. 2B).

In conjunction with this histological observation, the results of this study unveiled a cascade of HFSD-induced metabolic and tissue changes. These changes culminated in a significant increase in BW. Specifically, after the 4-week HFSD treatment, the BW increased by 136% ($P$<0.001); after 8 weeks, it further increased by 138% ($P$<0.001). Notably, these changes were not accompanied by any significant increase in food intake, indicating that it was not the quantity of food ingested that caused the weight gain, but rather its high calorie content (Fig. 3).

Moreover, these alterations were accompanied by a marked change in liver tissue integrity, which was manifested by an increase in enzymatic and non-enzymatic markers of cytotoxicity, including AST (+50%, $P$<0.001), ALT (+46%, $P$<0.01), and ALP (+26%, $P$<0.05). Moreover, this hypercaloric diet was responsible for the remarkable diminution in antioxidant status, as reflected in the increased hepatic MDA levels (+157%, $P$<0.001) and the remarkable decrease in the activity of the following antioxidant enzymes: SOD (−15%, $P$<0.05) and catalase (−21%, $P$<0.05) (Fig. 4 and 5).

**Metabolic effects of NLJ in HFSD-fed mice**

General analysis of the results of this study suggested that NLJ exerted significant effects on several levels.

First, we observed that the juice significantly prevented lipid metabolism disorders at doses of either 4 or 8 mL/kg/d, except that the first dose only showed significant effects after 8 weeks, unlike the second, which was active from the 4th week. At the end of this study, the 8-mL/d juice was the most active, decreasing TC, TG, VLDL, LDL-cholesterol, and HDL-cholesterol levels by 25% ($P$<0.001), 25% ($P$<0.01), 50% ($P$<0.01), 49% ($P$<0.001), and 100% ($P$<0.001), respectively. Of note, this impact was dependent on treatment duration, as the effect was largely moderate after 4 weeks. The juice reduced TC, TG, and LDL-cholesterol levels by only 13% ($P$<0.05), 11% ($P$<0.05), and 20% ($P$<0.05), respectively, and increased HDL-cholesterol levels by 57% ($P$<0.01); however, the effect on VLDL levels was insignificant (Table 2).

These changes in plasma lipid profile positively affected the indices of atherogenicity and cardiovascular prevention. Thus, at the end of the experiment, all risk indices were significantly lowered, with an increase in the cardiovascular protection index. Taking the case of the higher dose (8 mL/kg/d dose for 8 weeks), the AIP, CRR, and AC were reduced by 40% ($P$<0.001), 62% ($P$<0.05), and 79% ($P$<0.001), respectively, whereas the CPI increased by 304% ($P$<0.001) (Table 3).

**Table 4.** Effect of *Navela* loquat juice on mice hepatic, adipose tissue, biliary, and fecal lipids

| Groups | | Lipid parameters | Levels |
|---|---|---|---|
| NC | Liver | TC (mg/g) | 9.93±1.86 |
| | | TG (mg/g) | 5.24±1.12 |
| | Adipose tissues | TC (mg/g) | 3.44±0.24 |
| | | TG (mg/g) | 16.28±1.12 |
| | Bile | TC (mg/dL) | 80.22±6.41 |
| | Feces | TC (mg/g) | 4.05±0.13 |
| | | TG (mg/g) | 6.02±1.23 |
| HFSDG | Liver | TC (mg/g) | 17.53±2.43* |
| | | TG (mg/g) | 19.28±3.01*** |
| | Adipose tissues | TC (mg/g) | 5.52±0.63** |
| | | TG (mg/g) | 28.66±1.12*** |
| | Bile | TC (mg/dL) | 96.03±5.25*** |
| | Feces | TC (mg/g) | 8.73±1.88* |
| | | TG (mg/g) | 9.74±1.02* |
| HFSD-NLJG$_4$ | Liver | TC (mg/g) | 13.66±1.21 |
| | | TG (mg/g) | 15.14±1.36 |
| | Adipose tissues | TC (mg/g) | 3.87±0.51 |
| | | TG (mg/g) | 24.22±1.16 |
| | Bile | TC (mg/dL) | 120.79±6.11$^b$ |
| | Feces | TC (mg/g) | 10.89±0.93 |
| | | TG (mg/g) | 11.93±1.00 |
| HFSD-NLJG$_8$ | Liver | TC (mg/dL) | 10.25±1.35$^a$ |
| | | TG (mg/g) | 9.78±2.72$^a$ |
| | Adipose tissues | TC (mg/g) | 3.23±0.47$^a$ |
| | | TG (mg/g) | 20.03±2.43$^b$ |
| | Bile | TC (mg/dL) | 140.22±6.78$^c$ |
| | Feces | TC (mg/g) | 13.96±1.32$^a$ |
| | | TG (mg/g) | 13.98±1.44$^a$ |
| HFSD-FFG | Liver | TC (mg/g) | 9.73±1.17$^a$ |
| | | TG (mg/g) | 8.22±1.60$^b$ |
| | Adipose tissues | TC (mg/g) | 3.17±0.51$^a$ |
| | | TG (mg/g) | 19.63±2.61$^b$ |
| | Bile | TC (mg/dL) | 145.86±7.05$^c$ |
| | Feces | TC (mg/g) | 13.52±1.01$^a$ |
| | | TC (mg/g) | 14.08±1.55$^a$ |

*$P$<0.05, **$P$<0.01, and ***$P$<0.001 against NC. $^a$$P$<0.05, $^b$$P$<0.01, and $^c$$P$<0.001 against HFSDG.
NC, normal control; HFSD, high-fat/high-sucrose diet; HFSDG, hyperlipidemic control group; HFSD-NLJG$_4$, HFSD-*Navela* loquat juice-treated group at 4 mL/kg; HFSD-NLJG$_8$, HFSD-*Navela* loquat juice-treated group at 8 mL/kg; HFSD-FFG, HFSD-fenofibrate-treated group; TC, total cholesterol; TG, triglyceride.

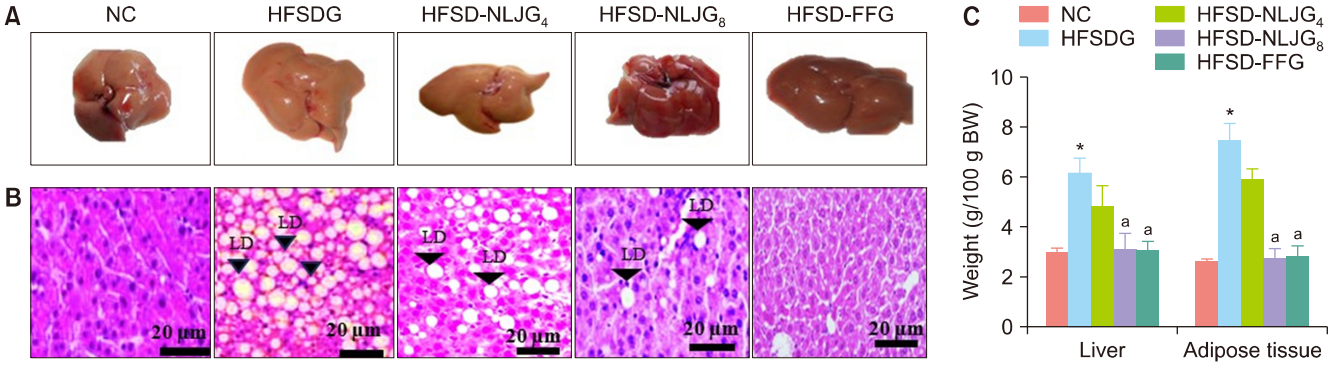


**Fig. 2.** Effect of *Navela* loquat juice on liver health in hyperlipidemic mice. (A) Macroscopic aspect. (B) Microscopic liver aspect using H&E stain. Magnification: 20x. (C) Relative liver and abdominal adipose tissue weights. Data are presented as means±SEM (n=8). *$P$<0.001 against NC. $^a$$P$<0.001 against HFSDG. SEM, standard error of the mean; BW, body weight; NC, normolipidemic control; HFSD, high-fat/high-sucrose diet; HFSDG, hyperlipidemic control; HFSD-NLJG, HFSD-*Navela* loquat juice-treated group; HFSD-FFG, HFSD-fenofibrate group; LD, lipid droplet.

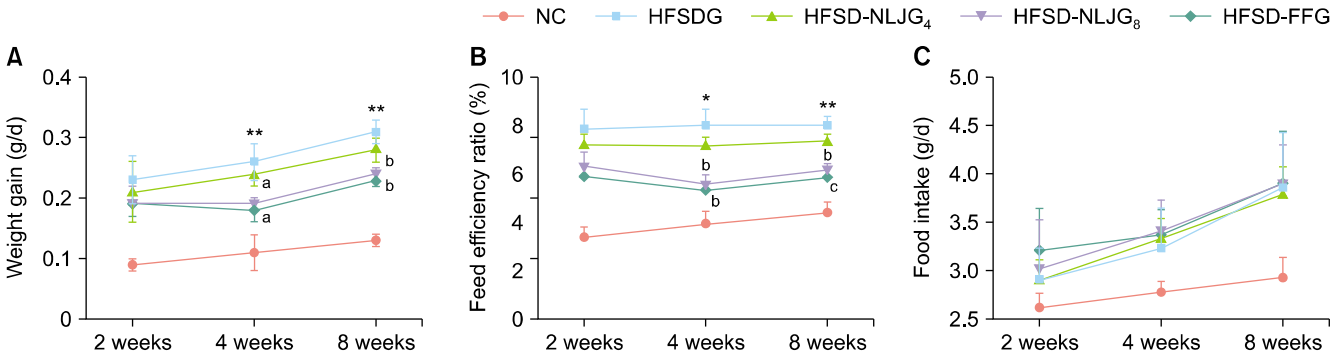


**Fig. 3.** Beneficial effects *Navela* loquat juice on body weight, feed efficiency, and food intake. (A) Weight gain, (B) feed efficiency, and (C) food intake. Data are presented as means±SEM (n=8). *$P$<0.05 and **$P$<0.001 against NC. [a]$P$<0.05, [b]$P$<0.01, and [c]$P$<0.001 against HFSDG. SEM, standard error of the mean; NC, normolipidemic control; HFSD, high-fat/high-sucrose diet; HFSDG, hyperlipidemic control; HFSD-NLJG, HFSD-*Navela* loquat juice-treated group; HFSD-FFG, HFSD-fenofibrate group.

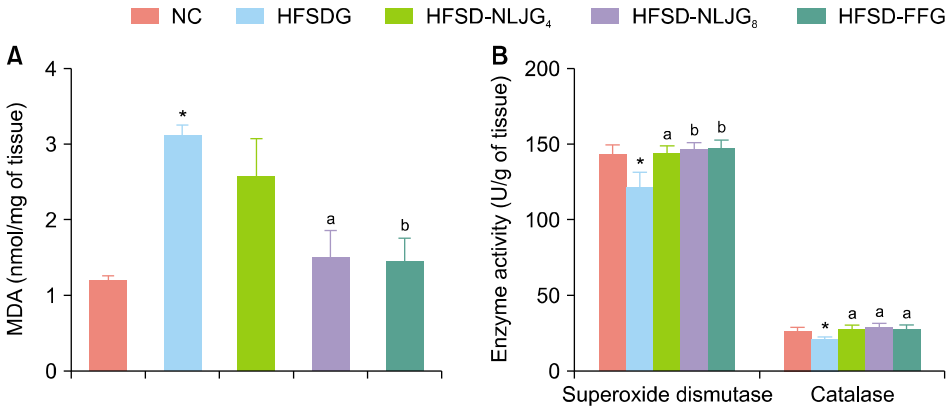


**Fig. 4.** Effect of *Navela* loquat juice on oxidative status in HFSD-fed mice. (A) Liver lipid peroxidation, (B) superoxide dismutase and catalase activities. Data are presented as means±SEM (n=8). *$P$<0.001 against NC. [a]$P$<0.05, and [b]$P$<0.01 against HFSDG. SEM, standard error of the mean; MDA, malondialdehyde; NC, normolipidemic control; HFSD, high-fat/high-sucrose diet; HFSDG, hyperlipidemic control; HFSD-NLJG, HFSD-*Navela* loquat juice-treated group; HFSD-FFG, HFSD-fenofibrate group.

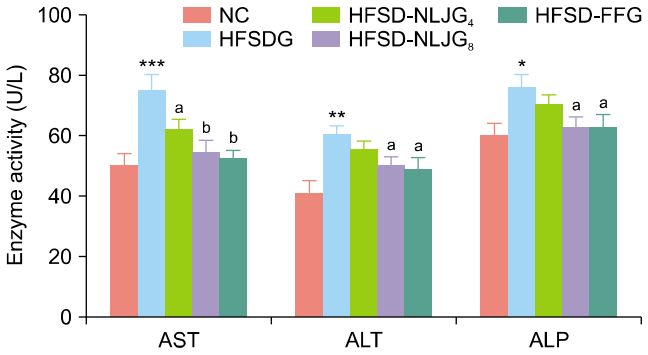


**Fig. 5.** Effect of *Navela* loquat juice on enzyme markers of liver injury in HFSD-fed mice. Data are presented as means±SEM (n=8). *$P$<0.05, **$P$<0.01, and ***$P$<0.001 against NC. [a]$P$<0.05 and [b]$P$<0.01 against HFSDG. SEM, standard error of the mean; NC, normolipidemic control; HFSD, high-fat/high-sucrose diet; HFSDG, hyperlipidemic control; HFSD-NLJG, HFSD-*Navela* loquat juice-treated group; HFSD-FFG, HFSD-fenofibrate group; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

In the liver and adipose tissues, the same pattern of changes affected TC and TGs. TC levels in the liver and adipose tissues decreased by 41% ($P$<0.05) and 40% ($P$<0.05), respectively. TG levels, in turn, decreased by 49% ($P$<0.05) and 28% ($P$<0.01) in the liver and adipose tissues, respectively. These downward changes appeared as increased cholesterol levels in the bile (+45%, $P$<0.001) and feces (+59%, $P$<0.05) as well as sequestration of TGs in the fecal matter (+43%, $P$<0.05) (Table 4), suggesting lipid reverse transport pathway activation. Furthermore, the disturbance in fasting blood glucose levels following the HFSD was significantly corrected by loquat juice at scores ranging from 16% ($P$<0.05) at the 4-mL/kg dose to 21% ($P$<0.01) at the 8-mL/kg dose.

The protective effect of loquat juice can also be observed in the color and histology of the liver, which tends to return to normal (Fig. 2A and 2B). Undoubtedly, the whitish color caused by lipid accumulation was visibly improved by the juice. This finding is supported by the results of histological examination, which showed a marked reduction in lipid droplets and an improvement in cellular integrity in treated mice compared to the control on high-fat diet alone. This finding is also evidenced by a decrease in the relative mass of the liver (−49%, $P$<0.05) and adipose tissues (−63%, $P$<0.05) (Fig. 2C). Finally, all these metabolic changes were reflected in the modulation of BW (−22%, $P$<0.01) and food efficiency ratio (−23%, $P$<0.01) without modifying food intake, thereby eliminating the likelihood of juice-related anorexic effects (Fig. 3).

Regarding the comparison, the results obtained suggest that, especially at an 8-mL/kg dose, were mostly very similar to those obtained with the fenofibrate used in

this study as the standard lipid-lowering drug.

### NLJ improved liver oxidative status and prevented liver injury

The loquat juice administered simultaneously with the high-calorie diet significantly improved the overall oxidative status in the liver (Fig. 4). As can be observed, the 8-mL/kg/d juice reduced MDA levels by 51% ($P<0.05$), increased SOD enzyme activity by 20% ($P<0.01$), and catalase activity by 37% ($P<0.05$). The effect was dose dependent. Therefore, at half dose (4 mL/kg/d), the effect on oxidative status, being moderate, was focused on the activation of SOD (+19%, $P<0.05$) and catalase (+30%, $P<0.05$) without significantly affecting MDA levels. Additionally, mice liver enzyme levels were significantly disrupted by the chronic intake of fat and sucrose-rich diets. Hyperlipidemia and oxidative stress were undoubtedly at the origin of hepatic steatosis, leading to liver injury characterized by an increase in the levels of liver enzymes, such as AST, ALT, and ALP, in the plasma. However, loquat juice especially at an 8-mL/kg/d dose almost decreased the increased levels of these three enzymes to baseline values. Therefore, AST, ALT, and ALP levels decreased by 28% ($P<0.01$), 17% ($P<0.05$), and 17% ($P<0.05$) (Fig. 5), respectively, which is a good indication of the juice's beneficial effects on liver integrity. Finally, concerning oxidative status and liver enzymes, the effect of loquat juice was broadly comparable to that of fenofibrate.

## DISCUSSION

The sugar and organic acid levels are critical indicators of loquat fruit maturity. The harmony between these components directly affects the taste and flavor of fruit that are meant for consumption either fresh or following industrial processing (Pinillos et al., 2011). In this study, mature *Navela* loquat fruits exhibited a total soluble sugar content of 12.22%±1.02%. This finding aligns with earlier reports on the sugar content of loquats from Morocco and other regions (Kabiri et al., 2022). Additionally, loquat fruits contain sucrose, glucose, and fructose as primary sugars, which corresponds with the outcomes of prior studies on the biochemical composition of loquat (Sortino et al., 2022; Ali et al., 2023). Regarding its sugar composition, the studied *Navela* variety exhibited a marked concentration of fructose, followed by glucose and sucrose. This sugar profile aligns with previous findings in various loquat varieties (Kabiri et al., 2022; Ali et al., 2023). Interestingly, although sucrose is the primary sugar in some varieties (Sortino et al., 2022), others such as the *Tanaka* variety are characterized by fructose predominance (Ali et al., 2023). The elevated fructose content likely contributes to the *Navela* variety's renowned sweetness in the local market compared with other varieties. Finally, the sugar content significantly varied depending on the harvest date, ecological conditions, and growing method (Amorós et al., 2003). The equilibrium between sweetness and acidity is another crucial factor affecting the flavor of loquats, as described by Dimassi et al. (2020) and Hasegawa et al. (2010). Thus, other varieties previously showed that the fruit reaches this sensory balance when its TA is near to 1 g of acid per 100 mL of juice and its TSS content is between 10 and 12°Brix (Pinillos et al., 2011). Therefore, to ensure high-quality fruits, a minimum TSS level of 10°Brix during the harvest stage was necessary. We discovered that the harvested fruit of the local variety (*Navela*) under investigation had a TSS content of 13.65±0.88°Brix, a TA of 0.73%±0.03%, and a TSS/TA ratio of 18.67±0.33. These values illustrate the excellent taste of the *Navela* variety, which is grown and marketed on a large scale in the Berkane region. Furthermore, this variety includes proteins, lipids, and mineral matter with levels comparable to those reported in the literature for other varieties (Li et al., 2016). Conversely, its abundance in minor elements including carotenoids, ascorbic acid, and polyphenols may position it as a fruit with high nutritional quality and functional food potential. Considering the evidence that a balanced diet of fruits and vegetables helps prevent several diseases, consumers today place a high value on the functional qualities of fruits in addition to their quality and price (Martínez-González et al., 2019). It has been demonstrated that a number of fruits, owing to their abundance in micronutrients, offer protection against hyperlipidemia and cardiovascular disorders (Yang et al., 2010; Zeng et al., 2015). During the 6-week study period, loquat juice administration significantly improved lipid metabolism among hyperlipidemic mice. Thus, the hyperlipidemic mice exhibited a plasma lipid profile marked by a significant increase in TC and LDL-cholesterol levels, accompanied by a decrease in HDL-cholesterol levels. These changes had a detrimental effect on the atherogenic and cardiovascular indices. However, loquat juice treatment reversed the observed disorders. Significant reductions in TC levels and atherogenic LDL-C fraction were observed, suggesting that loquat juice can enhance the uptake of LDL-C by the liver and peripheral tissues through LDL receptors, as previously proposed (Mokhtari et al., 2023a). Furthermore, the juice's effect on cholesterol metabolism was demonstrated by the increase in the anti-atherogenic HDL-C fraction. This particular fraction is involved in reverse cholesterol transport (RCT), a mechanism responsible for transporting cholesterol from peripheral tissues to the liver, eventually facilitating its excretion in the bile. The RCT pathway activation by loquat juice was confirmed by evaluating

cholesterol levels in the livers and bile of treated mice. Consequently, mice treated with the juice showed a decrease in cholesterol levels in the liver and an increase in cholesterol levels in the bile compared with the control group. This finding indicates that the excess peripheral cholesterol was efficiently transported back to the liver via the RCT pathway and ultimately excreted into the bile. These findings align with and complement previous studies highlighting the positive effects of loquat on lipid metabolism (Abdelrahman et al., 2023; Mokhtari et al., 2023b). Conversely, the atherogenic indices are currently regarded as more meaningful indicators for atherosclerotic risk evaluation. Therefore, maintaining these parameters at lower levels is recommended (Bo et al., 2018). In this study, loquat juice substantially rectified the atherogenic and cardiac indices in hyperlipidemic mice. This finding emphasizes the nutritional significance of the juice in preventing atherosclerosis and other lipid metabolism disorder-associated diseases. Consistent results were documented in our previous study, where we explored the effect of loquat peel extract on lipid metabolism in mice (Mokhtari et al., 2023b). Moreover, hypertriglyceridemia contributes to abdominal obesity, overweight, and liver fatty diseases, including steatosis (Netto et al., 2023). Insulin resistance may result from this condition, which may subsequently progress to hyperglycemia, diabetes, and CVD (Suren Garg et al., 2023). In this context, we showed that long-term exposure to HFSD led to a significant increase in blood TG and glucose levels as well as TG levels in the liver and adipose tissues. Additionally, HFSD increased BW and visceral adipose tissue mass. The obtained results are consistent with those of several earlier studies using the same mouse model (Mokhtari et al., 2023a, 2023b). However, when loquat juice was concomitantly administered, TG levels in the plasma, liver, and adipose tissues were significantly reduced. Simultaneously, their levels in the bile and feces were increased. Consequently, we suggest that the administered juice can enhance the uptake and catabolism of TG-rich lipoproteins through lipoprotein lipase activation, as previously hypothesized (Mokhtari et al., 2023a, 2023b). Furthermore, the juice-treated mice exhibited a significant loss in BW, as well as reduced liver and adipose mass. This finding suggests that the juice can inhibit lipogenic enzymes in adipose tissues, particularly fatty acid synthase (Bin-Jumah, 2018) and triacylglycerol acyltransferase 2 (Neuschwander-Tetri, 2010), while activating fatty acid oxidation enzymes, such as carnitine palmitoyltransferase 1 (Tacherfiout et al., 2018). Conversely, the abnormal lipid accumulation in liver cells due to hyperlipidemia leads to cellular damage and triggers inflammatory responses. This condition is frequently referred to as "lipotoxicity" (Van Herpen and Schrauwen-Hinderling, 2008). In this study, we discovered that the HFSD-induced chronic hyperlipidemia changed the color, shape, and histology of the liver, suggesting the apparition of steatosis and lipotoxicity. This finding was supported by the increase in hepatic lipid content and higher plasma enzymatic indicators of liver injury in hyperlipidemic mice (ALT, AST, and ALP). However, loquat juice treatment significantly protected hyperlipidemic mice against liver damage, as evidenced by the integrity of the hepatic histological structures and the correction of plasma biochemical indicators. This finding affirms our earlier observation regarding the protective effect of loquat peel extract against lipotoxicity (Mokhtari et al., 2023a) and seamlessly aligns with the findings of Shahat et al. (2018) who successfully demonstrated the hepatoprotective effects associated with loquat leaves. Specifically, the observed palliative effect of loquat juice on chronic hyperlipidemia-induced liver tissue damage may be attributed to its hypolipidemic effect. In this manner, the loquat juice allows the excretion of excess lipids in the bile and fecal matter, thereby preventing their harmful accumulation in liver tissues. The fat that builds up in the liver can be peroxided and subsequently produce several toxic molecules, including oxidized LDL, as well as lipid free radicals that can easily attack cell membranes, thereby causing tissue damage (Ayala et al., 2014). In response to these oxidative challenges, the body relies on crucial antioxidant mechanisms. These defense mechanisms play a vital role in neutralizing the harmful effects of oxidized molecules, underscoring the significance of maintaining a delicate balance between oxidative stress and antioxidant defenses for overall liver health (Halliwell, 2024). In a similar context, the present study reveals that the positive effect of loquat juice extends to the tissue level, specifically in safeguarding the liver against oxidative stress. This finding is achieved through MDA content reduction and the activation of key antioxidant enzymes, including SOD and catalase. These findings highlight the potential of loquat juice not only in mitigating oxidative damage but also in promoting a balance between reactive oxygen species and protective antioxidant defenses within the liver. This finding is consistent with those of previous studies suggesting the beneficial effects of loquat seeds, leaves, and peels through antioxidant activity (Hamada et al., 2004; Koba et al., 2007; Mokhtari et al., 2023a). Moreover, the observed protective effect of loquat juice may be closely linked to the presence of bioactive minor compounds, notably phenolics, fibers, and carotenoids. These compounds, recognized for their diverse pharmacological activities, have been extensively investigated for their health-promoting properties (Sagar et al., 2020). Loquat juice is abundant in phenolics, particularly phenolic acids and flavonoids, which have been identified as potential contributors to hypolipidemic activity according to studies by Kobayashi

and Ikeda (2014) and Mokhtari et al. (2023b). Further, fibers could improve lipid homeostasis by inhibiting the intestinal absorption of cholesterol and facilitating its fecal excretion as demonstrated by Bakr and Farag (2023). Conversely, these compounds, along with carotenoids and ascorbic acid, may play a crucial role in combating oxidative stress at the tissue level, as documented in previous studies (González-Peña et al., 2021; Hamdan et al., 2022). Therefore, polyphenol analysis reveals the presence of the following five major phenolic compounds in loquat juice: 5-caffeoylquinic acid, 3-*p*-coumaroylquinic acid, 3-caffeoylquinic acid, 5-feruloylquinic acid, and quercetin. Notably, 5-caffeoylquinic acid is recognized for its diverse pharmacological properties (Liu et al., 2013; Park et al., 2018). Our results are consistent with those of Zhang et al. (2015) who reported that 3-caffeoylquinic acid is the predominant phenolic acid in six loquat fruit cultivars from China. However, Xu et al. (2014) and Ding et al. (2001) indicated that 5-caffeoylquinic acid is the predominant phenolic acid in loquat fruits from China and Japan. These compounds, along with their circulating metabolites, can restore lipid metabolism, thereby preventing fat accumulation in tissues and its subsequent oxidation, which can lead to cellular damage.

To compare the overall effect of loquat juice with a reference drug, fenofibrate was used as a standard hypolipidemic drug in this study. It is therefore clear that the effect of loquat juice is comparable to a large extent to the majority of the parameters monitored in this study. This finding can only further support our hypothesis that NLJ could be of significant nutritional significance in hyperlipidemia treatment and CVD prevention.

In conclusion, the widespread cultivation of the *Navela* variety of loquat in the Zegzel Valley, east of Morocco, presents a fruit of commendable organoleptic and nutritional quality. The favorable TSS/TA ratio, coupled with its low-calorie content and richness in minerals, carotenoids, ascorbate, and polyphenols, underscores its nutritional value. The extracted juice demonstrates substantial health potential, exhibiting antihyperlipidemic, anti-hepatic steatosis, and anti-oxidative stress properties. These promising findings suggest that the regular consumption of loquat fruits can prevent hyperlipidemia and mitigate associated cardiovascular complications. Furthermore, the loquat juice's composition positions it as a natural substrate, offering opportunities for the development of functional foods or dietary supplements with potential health benefits.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Concept and design: HH. Analysis and interpretation: IM. Data collection: MM, CM. Writing the article: IM, HH. Critical revision of the article: DM, SA. Final approval of the article: all authors. Statistical analysis: MH. Obtained funding: HH. Overall responsibility: HH.

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