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Loquat fruit juice: Proximate composition and hypolipidemic effect in mice

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Abstract

Loquat fruit is consumed for its flavorful taste and a rich array of health promoting compounds like phenolic acids, flavonoids, and carotenoids. This study aimed at the biochemical characterization of fresh juice from the Moroccan Mkarkeb variety of loquat and evaluating its effects on lipid homeostasis and liver steatosis in hyperlipidemic mice. The biochemical characterization followed AOAC methods. *In vivo* study involved hyperlipidemic mice fed a high-fat, high-fructose diet for 6 weeks and treated with loquat juice at 3.5 and 7 mL/kg or fenofibrate at 4 mg/kg. The concentrations of lipids in plasma, liver, adipose tissue, feces, and bile and blood glucose levels were quantified using enzymatic kits. Liver steatosis was visually examined histologically and by measuring liver injury markers (AST, ALT, ALP, LDH, and TB). Liver oxidative stress was assessed by determining MDA content and antioxidative enzyme activities (SOD and catalase). Our findings indicate that fresh loquat juice is poor in fat and protein and contains moderate sugars amount with a low energy value (40.82±0.25 kcal/100g). It is also rich in minerals, vitamin C, phenolic acids, flavonoids, and carotenoids. The juice effectively restored lipid metabolism by enhancing reverse cholesterol transport and lowering LDL-cholesterol, triglycerides, and the atherogenic index. The studied juice decreases blood glucose and prevents weight gain and lipid accumulation in the liver and adipose tissue. The juice prevents lipotoxicity-induced liver injury, corrects toxicity markers, and improves the liver's morphological and histological structures. It also reduces oxidative stress by lowering MDA and activating SOD and catalase. The juice holds high nutritional and medicinal value and could potentially prevents lipid disorders and cardiovascular issues.

Keywords: lipid metabolism; loquat juice; hepatic steatosis; high-fat/high fructose diet;

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl), a member of the Rosaceae family, is an evergreen fruit tree that originated in China. It is now widely cultivated in temperate and subtropical regions worldwide, including the Mediterranean, Turkey, Pakistan, India, and Brazil [1]. In Morocco, loquat cultivation spans approximately 600 hectares in the Berkane province of Oriental Morocco, accounting for more than 80% of the country's loquat-growing area. This cultivation is concentrated in the communes of Zegzel, Takerboust, Tazaghine, and Ouaoullout, where four loquat varieties are produced: *Tanaka*, *Navela*, *Muscat*, and *Mkarkeb*. The Japanese variety *Mkarkeb* is known for its large size, firm yellow-orange flesh, sweet and juicy flavor, few small seeds, excellent taste quality, and late maturity. Since 2013, Zegzel loquat has been recognized with the "Protected Geographical Indication" (PGI) status. It is a key focus of the regional agricultural development plan to promote local products from the Oriental region [2].

Loquat fruit is esteemed for its health benefits, taste, and high phytochemical content, including polyphenols and carotenoids [3]. While it is usually enjoyed fresh, the mature loquat fruit has recently found favor as a key ingredient in jams and jellies, owing to its nutritional richness and appealing flavor profile [4]. Like many other fruits, the taste quality of loquat is closely linked to its sugar content and the ratio of total soluble solids to titratable acidity (TSS/TA ratio) [5]. Nutritionally, loquat is rich in sugars such as glucose, fructose, and sucrose, and it also contains dietary fibers, organic acids, vitamins, and minerals [3]. Loquat is also a significant source of various dietary bioactive compounds, including polyphenols, carotenoids, and triterpenoids, known for their pharmacological activities [3]. Numerous experimental studies have demonstrated the potential health benefits of loquat in animal and cell line models, such as its hypoglycemic, anti-inflammatory, antioxidative, and hypolipidemic properties [4]. However, further research is needed to understand better the correlation between these bioactive compounds and their biological activities.

Cardiovascular diseases (CVD) impact over 100 million people globally [6]. The primary cause of CVD is atherosclerosis, a complex and multifactorial condition characterized by hyperlipidemia, oxidative stress, and inflammation [7]. It is well established that dyslipidemia, particularly elevated levels of plasma low-density lipoproteins (LDL) and low levels of high-density lipoproteins (HDL), is a major risk factor for atherosclerosis [8]. Oxidative stress leads to the oxidation of LDL, marking the initial stage of the atherogenic process [9]. Additionally, oxidative stress associated with hypercholesterolemia increases the production of free radicals, which contribute to atherosclerosis and cause biological alterations such as lipid peroxidation,

protein oxidation, and reduced activity of antioxidant defenses [10]. Moreover, hyperlipidemia can result in excessive lipid accumulation in various tissues, including the liver and kidneys, which leads to cellular lipotoxicity [11]. To study hyperlipidemia and related metabolic disorders, researchers commonly use animal models induced by high-fat and high-fructose diets [12]. Conversely, numerous epidemiological and preclinical studies have underscored the benefits of the Mediterranean diet. This diet, rich in fruits, vegetables, whole grains, healthy fats, and lean proteins, has been linked to a lower incidence of obesity, liver disorders, and metabolic syndrome in human populations [13].

In this context, the present study aimed to analyze the biochemical composition of loquat fruit (variety *Mkarkeb*) cultivated in Eastern Morocco. This characterization is crucial for understanding the fruit's potential value for direct consumption or industrial processing. Additionally, we evaluated the fruit's positive effects on hyperlipidemia and associated cellular damage using a mouse model. Notably, to the best of our knowledge, this is the first study to explore this specific variety of loquat fruit in such detail.

MATERIALS AND METHODS

Morphologic characteristics and juice preparation

Ripe loquat fruits (*Eriobotrya japonica* (Thunb.) Lindl., Variety *Mkarkeb*) were collected from the Zegzel Valley in Berkane Province, Oriental Morocco, in May 2022. Fruits were selected for integrity and uniformity in size and color. The morphologic characteristics of loquat fruits, such as average fresh weight, length (from the tallest point), width (from the widest point), and fruit thickness, were done by taking ten loquats as one batch and calculating their average measurements. The weight of each fruit was measured using a digital weighing balance, while their length and diameter were measured using Vernier calipers. The fruit shape index, known as the length-to-width ratio, was determined for each fruit by dividing its length by diameter. The loquat juice was prepared according to the method outlined by Meng et al. [14] slightly modified. In brief, the fruits were thoroughly washed with tap water before removing peels and seeds. Subsequently, the loquat flesh was pulped using an electric blender. The slurry was filtered, and fresh juice was obtained for biochemical analysis. The rest was aliquoted and stored at - 20°C until use. Each 1 Kg of loquat flesh gave around 400 g of juice and 600 g of leftovers.

Determination of the nutritional composition of loquat juice

pH, total soluble solids (TSS), titrable acidity (TA), and TSS/TA ratio

The pH of the fresh juice was measured using a pH meter. Total soluble solids (TSS) were determined with a refractometer and reported in °Brix (AOAC 920.151). Titrable acidity (TA) was assessed by titrating the fresh juice with a 0.1 N sodium hydroxide solution, and the results were expressed as grams of malic acid equivalent per 100 grams of juice, using phenolphthalein (1%) as an indicator (AOAC 943.03). The maturity index, calculated as the TSS/TA ratio, was also determined [5]. 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 Chen [15]. 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. Total sugar content is determined using a linear regression equation derived from the calibration curve constructed with D-fructose standard solutions. The results are expressed in grams of fructose per 100 g of fresh juice. The sugar profile and content of the loquat fresh juice were determined using the HPLC method as outlined by Yu et al. [16]. In brief, the fresh juice was diluted and filtered through a 0.45 µm syringe filter to obtain a clear filtrate. Subsequently, 10 µL were injected in a Supelcosil LC column (25 cm × 0.46 cm × 5 µm). The mobile phase consisted of a mixture of acetonitrile/water (60/40, v/v), and the elution was carried out at a flow rate of 1 mL min^{-1}. The identification of sugar peaks was based on retention times compared to standards. The determination of each individual sugar content was performed based on a standard curve. The results were presented in g/100g of fresh juice.

The sweetness index of the fresh loquat juice was determined by considering the amount and sweetness characteristics of each identified sugar [17]. Thus, the sweetness index estimation considers fructose approximately 2.30 times sweeter than glucose and sucrose about 1.35 times sweeter than glucose. The sweetness index of loquat juice was calculated as follows:

SI = (1.00 [glucose g/100g]) + (2.30 [fructose g/100g]) + (1.35 [sucrose g/100g]).

Organic acids composition

The analysis of organic acids in loquat fruit juice was undertaken according to the method described by Deng et al. [18] with some modifications. The fresh juice was adequately diluted in ultrapure water and filtered through a 0.45 µm syringe filter. 10 µL of diluted juice were

injected in a C18 column (250 mm × 4.6 mm, particle size 5 μm) at 30°C. The mobile phase comprises sodium phosphate buffer solution (25 mM) with a pH finely tuned to 2.45 by adding phosphoric acid. The flow rate is 1 mL min^{-1}. The organic acids were identified by comparing their chromatographic parameters with the standards, including malic acid, tartaric acid, succinic acid and oxalic acid. The quantitative analysis used calibration curves for malic acid, tartaric, succinic, and oxalic acid. The results were then expressed in milligrams per 100 grams of juice.

Fat and protein contents

The fat content was calculated according to the official method AOAC 963.15. The juice is dried in a ventilated oven at 40°C for 24 hours. Then, the dry residue is scraped and weighed. 5g were put in a cartridge and placed in the Soxhlet apparatus. The total fat was extracted with diethyl ether at 45°C for 16 hours. After that, 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 juice. The total protein content was determined according to the official method (AOAC 920.152).

Ash and mineral contents

The ash content was determined by a gravimetric method (AOAC 940.26). Briefly, 25 grams of loquat juice were placed into a pre-weighed porcelain crucible. The sample was heated in a muffle furnace at 525°C for 2 hours. Then, the crucible was removed from the furnace and cooled in a desiccator. Subsequently, the crucible was weighed again to determine the weight of the ash. The results are represented in grams per 100g juice. The mineral composition was determined from the ash sample by using the atomic spectrometry as described (AOAC 968.08).

Crude fiber contents

The crude fiber content was estimated using the Weende method (AOAC 978.10). Five grams of previously dried juice (as outlined above) were treated with a sulfuric acid solution of 1.25% to extract the fibers, followed by filtration and rinsing. The extracted fibers are then treated with a solution of sodium hydroxide of 1.25%, filtered again, and rinsed. Finally, the obtained crude fibers are dried in an oven at 105°C for one hour. The obtained residue represents the crude fibers and the ash content that must be subtracted. The results were expressed as gram fibers per 100 g juice.

Total polyphenols, flavonoids, carotenoids and Vitamin C

The total polyphenol content was estimated using the Folin-Ciocalteu method described by Mokhtari et al. [19]. 0.5 mL of loquat juice adequately diluted was mixed with 0.25 mL of Folin-Ciocalteu reagent and 0.5 mL of aqueous sodium carbonate solution (20%). Following a 30-minute incubation at room temperature in the dark, the absorbance was measured at 725 nm. The quantification of polyphenol content was achieved through the use of a calibration curve prepared with chlorogenic acid. The obtained results are expressed in milligrams per gram of juice.

The flavonoids were quantified using our previous method [19]. The concentration of flavonoids was determined by referencing a calibration curve generated from rutin standard solutions, and the results are expressed in milligrams per gram of juice.

The individual phenolic compounds present in loquat juice were analyzed by HPLC according to our previous method [20]. Briefly, 10 µL of the juice samples adequately diluted were injected into a C18 column (250 × 4.6 mm with a particle size of 5 µm). The elution was executed using a gradient of ultrapure water/acetic acid (0.5%) (A) and acetonitril (B) with a flow rate of 1 mL min^{-1} and a temperature set at 20°C. The gradient composition was as follows: 0 min -15min: 80% A, 20% B; 15-20 min: 60% A, 40% B; 20- 35min: 40% A, 60%B; 35-40 min: 95% A, 5% B. Chromatograms were registered at 340 nm, and the identification of compounds relied on analyzing their retention times and UV-visible spectra with guidance from a database containing standard phenolic compounds.

The carotenoid content was determined according to the method outlined by Kabiri et al. [2] slightly adapted. 50 mL of loquat juice were extracted thrice 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 the organic phase containing carotenoids was measured at 470 nm against a blank. The carotenoid content was estimated using a standard curve of β-carotene.

The vitamin C was quantified using a 2,6-dichlorophenol-indophenol (DPIP) titrimetric method as described by Kabiri et al. [2] with some modifications. 10 mL of diluted flesh juice were mixed with 1 mL of glacial acetic acid and titrated to a faint permanent pink color. The amount of vitamin C was calculated according to a standard curve of L-ascorbic acid.

Energy Value

The total energy value of loquat fruit was determined using standard Atwater factors: 4 kcal for proteins, 9 kcal for lipids (fats) and 4 kcal for carbohydrates to calculate the caloric content. The sum of the respective values multiplied by proteins, lipids, and total carbohydrates is provided below:

Energy value (kcal 100 g^{-1}) = (Protein (g) × 4) + (Carbohydrate (g) × 4) + (lipid (g) × 9).

Effect of Mkarkeb loquat juice on mice fed a high-fat high-fructose Diet (HFFD)

Animals and Treatment

Male Swiss *Albino* mice (27-28 g) were bred at the Faculty of Sciences, University Mohammed I in Oujda, Morocco. The mice were housed under standard conditions at 22 °C with a 12-hour light/dark cycle and had unrestricted access to food and water. The animal experiments were conducted according to the Care and Use of Laboratory Animals Guidelines set forth by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and received approval from the local animal use committee (approval number: 002023).

Animal diet

The high-fat high fructose diet (HFFD) was prepared with minor modifications based on the method described by Yustisia et al. [21]. A standard mice diet from the Society Alf Sahel, (Morocco) was combined with specific amounts of beef fat (16%), cholesterol (1.5%), fructose (10%), egg yolk (10%), and deoxycholic acid (0.2%).

Experimental design

After a 2-week acclimatization period, the mice were randomly assigned to one of six groups, each consisting of eight animals, and housed in individual cages. The experimental groups were treated as follows: NC (Normal Control): fed a standard diet and daily gavaged with distilled water. JCG (Juice Control): fed a standard diet and daily gavaged with 0.5 mL of loquat fresh juice (7 mL kg^{-1}) for 6 weeks. HC (Hyperlipidemic Control): fed a high-fat, high-fructose diet (HFFD) and daily gavaged with distilled water for 6 weeks. JTG (3.5 mL kg^{-1}): fed the HFFD and daily gavaged with loquat juice at 3.5 mL kg^{-1}. JTG (7 mL kg^{-1}): fed the HFFD and daily gavaged with loquat juice at 7 mL kg^{-1}. FG (Fenofibrate Group): fed the HFFD and daily gavaged with fenofibrate at 4 mg kg^{-1}. Food consumption was recorded daily and body weight every fortnight.

Fecal samples, blood and organ collection

After 2, 4 and 6 weeks, fecal samples were collected and stored at -20°C. All animals were fasted overnight, lightly anesthetized with diethyl ether and then blood samples were taken under trisodium citrate. The blood was promptly centrifuged at 3000 rpm for 15 minutes, and the plasma obtained was utilized for biochemical analyses. Following the sacrifice of the animals, the hepatic and abdominal adipose tissues were meticulously excised, rinsed in a cold saline solution, and then weighed. The weights were expressed as g $100g^{-1}$ of body weight.

Plasma biochemical analysis

Plasma levels of total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and total bilirubin (TB) were assessed using biomedical kit methods. The detailed procedures for these analyses can be found in our previous studies [19-20]. Lipid indices were determined using established formulas, as outlined in prior research by Mokhtari et al. [20].

Analysis of hepatic, adipose tissue, biliary and fecal lipids

Lipid extraction from the liver, abdominal adipose tissue, and fecal matter were conducted following the method described by Mokhtari et al. [19]. Bile was obtained from the gall bladder through the use of a 30-gauge needle. The assessment of biliary cholesterol levels was undertaken using a special enzymatic kit.

Liver histology

Fresh animal tissues were cut into approximately 1 cm squares, fixed in a buffered formalin solution (10%), dehydrated, and subsequently embedded in paraffin. The tissues were then sliced into 4 to 5 µm thick sections using a microtome. These histological sections underwent cleaning with toluene hydration through decreasing alcohol baths and were ultimately stained with hematoxylin and eosin. The prepared samples were then mounted on glass slides using a light microscope for histological scrutiny.

Assessment of liver malondialdehyde content, SOD, and catalase activities

The liver lipid peroxidation was estimated by measuring the content of malondialdehydes (MDA) using our previously published method [20]. Liver SOD and catalase activites were determined following the modified method of Yaribeygi [22].

Statistical analysis

The obtained data were presented as mean ± standard error of the mean (SEM) and compared by one-way variance analysis (ANOVA). This statistical analysis was executed using GraphPad Prism 9.5.0 software, with significance at $P < 0.05$.

RESULTS

Physical properties of loquat fruit and nutritional composition of fresh juice

Table 1 details various attributes of loquat fruits and its fresjuice, including physical dimensions and chemical properties. The average weight of the mature fruit is 68.73 ± 2.19 g, with a length of 41.27 ± 1.30 mm and a width of 56.09 ± 2.44 mm. The fruit thickness measures 24.08 ± 1.94 mm, and the fruit shape index is 0.73 ± 0.13, indicating the fruit's relative proportions. The juice has a pH of 3.71 ± 0.22 and a titrable acidity of 0.64 ± 0.03%, contributing to its overall tartness. Total soluble solids (TSS) are 12.40 ± 0.25 °Brix, and the TSS/TA ratio is 19.37 ± 0.17, suggesting a well-balanced sweetness-to-acidity ratio. The total sugar content is 9.71 ± 0.07 g $100g^{-1}$, with fructose and glucose levels of 4.72 ± 0.28 g/100g and 2.44 ± 0.34 g $100g^{-1}$, respectively. Sucrose was not detected in the juice. The sweetness index is 16.15 ± 0.31, reflecting the perceived sweetness of the juice. Nutritionally, the juice is low in fat (0.052 ± 0.0029 g $100g^{-1}$) and protein (0.38 ± 0.04 g $100g^{-1}$), with minimal crude fiber (0.11 ± 0.06 g $100g^{-1}$). The predominant organic acid is malic acid, present at 537.08 ± 11.04 mg $100g^{-1}$, followed by tartaric acid (48.02 ± 3.14 mg $100g^{-1}$), succinic acid (16.10 ± 1.84 mg $100g^{-1}$), and oxalic acid (9.10 ± 1.06 mg $100g^{-1}$). Vitamin C content is 7.10 ± 0.08 mg $100g^{-1}$, and carotenoids are 58.01 ± 4.05 µg g^{-1}. Mineral content includes potassium (259.01 ± 2.01 mg $100g^{-1}$), sodium (59.07 ± 1.40 mg $100g^{-1}$), phosphorus (23.10 ± 1.50 mg $100g^{-1}$), calcium (19.10 ± 1.01 mg $100g^{-1}$), magnesium (18.29 ± 0.99 mg $100g^{-1}$), and iron (2.91 ± 0.10 mg $100g^{-1}$). The energy value of the juice is 40.82 ± 0.25 kcal $100g^{-1}$. The total polyphenol content is 138.08 ± 0.71 mg $100g^{-1}$, and flavonoids are at 85.18 ± 4.53 mg $100g^{-1}$. The mature *Mkerkeb* loquat juice is characterized by a low fat and protein content, moderate sweetness, and notable levels of organic acids, vitamins, minerals, and bioactive compounds, contributing to its nutritional and health-promoting potential.

Table 1. Physical characteristics of loquat fruit and biochemical composition of its fresh juice

Parameters	Content
Weight (g)	68.73±2.19
Length (mm)	41.27. ±1.30
Width (mm)	56.09±2.44
Fruit thickness (mm)	24.08± 1.94
Fruit shape index	0.73 ±0.13
pH	3.71±0.22
Titrable acidity (TA) %	0.64± 0.03
Total soluble solids (TSS) (°Brix)	12.40±0.25
TSS/TA	19.37 ± 0.17
Total sugars (g/100g)	9.71± 0.07
Fructose (g/100g)	4.72± 0.28
Glucose (g/100g)	2.44 ± 0.34
Sucrose (g/100g)	Not found
Sweetness index (SI)	16.15±0.31
Fat (g/100g)	0.052±0.0029
Protein (g/100g)	0.38 ± 0.04
Crude fiber (g/100g)	0.11 ± 0.06
Malic acid (mg/100g)	537.08±11.04
Tartaric acid (mg/100g)	48.02 ±3.14
Succinic acid (mg/100g)	16.10±1.84
Oxalic acid (mg/100g)	9.10± 1.06
Vitamin C (mg/100g)	7.10±0.08
Carotenoids (µg/g)	58.01±4.05
Ash (mg/100g)	0.43±0.02
Potassium (mg/100g)	259.01±2.01
Sodium (mg/100g)	59.07±1.40
Phosphorus (mg/100g)	23.10±1.50
Calcium (mg/100g)	19.10±1.01
Magnesium (mg/100g)	18.29±0.99
Iron (mg/100g)	2.91±0.10
Energy Value (Kcal/100g)	40.82 ±0.25
Total polyphenols (mg/100g)	138.08 ± 0.71
Flavonoids (mg/100g)	65.18± 4.53

HPLC analysis of loquat juice polyphenols

The chromatogram shown in **Figure 1** offers specific insights into the phenolic composition of *Mkerkeb* loquat juice. It reveals distinct peaks representing five identifiable phenolic compounds: peak 1 (caffeic acid, 34%), peak 2 (neochlorogenic acid, 27%), Peak 3 (chlorogenic acid, 17%), Peak 4 (quercetin, 10%), peak 5 (rutin, 10%).

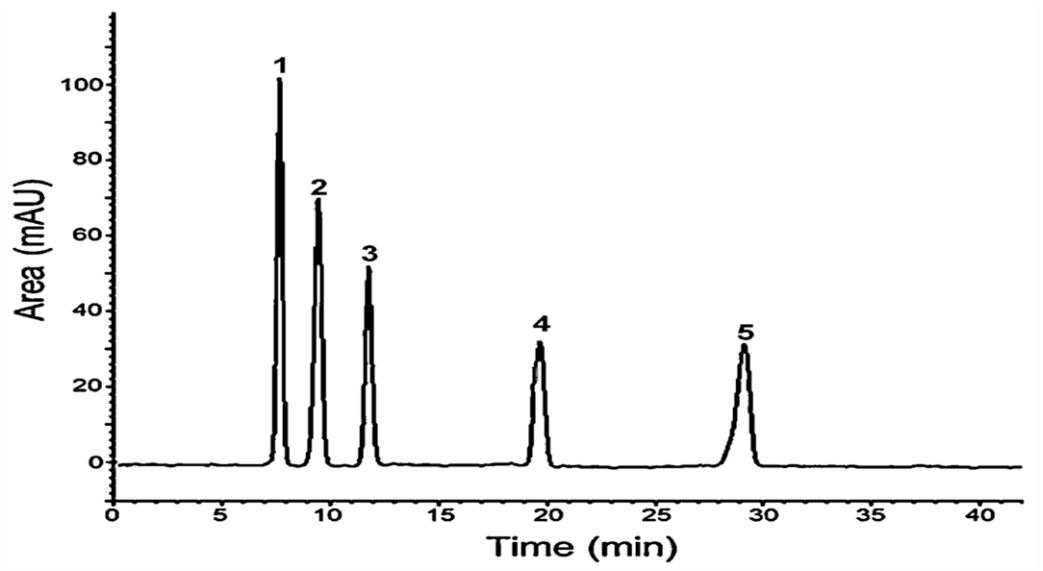

Figure 1. HPLC analysis of loquat juice polyphenols,

1: caffeic acid, 2 neochlorogenic acid, 3 chlorogenic acid, 4: quercetin, 5 rutin.

Effect of *Mkarkeb* loquat juice on plasma lipid profile in HFFD-fed mice

The impact of *Mkarkeb* loquat juice at dosages of 3.5 mL/kg and 7 mL/kg on plasma lipid parameters was evaluated across all mice groups at intervals of 2, 4, and 6 weeks (**Table 2**). In the hyperlipidemic control (HC) group, there was a notable increase in total cholesterol (TC) by 80% ($P<0.001$), triglycerides (TG) by 36% ($P<0.001$), low-density lipoprotein cholesterol (LDL-C) by 191% ($P<0.001$), atherogenic index (AI) by 21% ($P<0.001$), and LDL-C/HDL-C ratio by 582% ($P<0.001$) observed as early as the second week of the study. These levels continued to rise significantly, reaching increases of 201% ($P<0.001$), 146% ($P<0.001$), 1037% ($P<0.001$), 311% ($P<0.001$), and 1185% ($P<0.001$) respectively, by the end of six weeks of treatment. These results indicate the substantial effect of the high-fat, high-fructose diet (HFFD) on elevating plasma lipid levels. The decrease in high-density lipoprotein cholesterol (HDL-C) began subtly at week 2, and became statistically significant at week 4 with a 9% ($P<0.05$).

Concerning loquat treated group, we observed that the juice at a 3.5 mL/kg dose on plasma lipids were not significant throughout the treatment period. However, when the loquat juice dosage was increased to 7 mL/kg, a substantial and pronounced effect on the plasma lipid

profile was observed as early as 2 weeks into the treatment, with the impact being time-dependent. After 2 weeks, there were notable reductions in TC by 4.25% ($P<0.05$), TG by 5.97% ($P<0.01$), and LDL-C by 11.24 % ($P<0.05$) compared to the HC group. AI showed a significant decrease of 10% ($P<0.001$), and the LDL-C/HDL-C ratio was reduced by 16% ($P<0.001$). These positive changes persisted and even improved by week 4, with TC, TG, LDL-C, AI, and LDL-C/HDL-C ratios showing respective decreases of 7% ($P<0.001$), 16% ($P<0.001$), 12% ($P<0.05$), 28% ($P<0.001$), and 29% ($P<0.001$). HDL-C levels rose by 9% ($P<0.001$). By the end of the 6 weeks, the group receiving 7 mL/kg of loquat juice exhibited even more significant reductions: TC and TG levels decreased by 20% ($P<0.001$) and 21% ($P<0.001$), respectively, and LDL-C, AI, and LDL-C/HDL-C ratio were reduced by 22% ($P<0.001$), 44% ($P<0.001$), and 46% ($P<0.001$) compared to the HC group. Furthermore, HDL-C levels saw a substantial increase of 45% ($P<0.001$) compared to the HC group at 6 weeks.

For comparison, the standard hypolipidemic drug, fenofibrate, administered at a dose of 4 mg/kg, led to statistically significant reductions in plasma TC (14%, $P<0.01$), TG (18%, $P<0.01$), LDL-C (13%, $P<0.01$), AI (30%, $P<0.001$), and LDL-C/HDL-C ratio (35%, $P<0.001$) after 4 weeks, along with a 33% ($P<0.001$) increase in HDL-C levels. As the study progressed to 6 weeks, fenofibrate continued to show impressive effects, with TC levels decreasing by 34% ($P<0.001$) and TG levels by 37% ($P<0.001$). LDL-C, AI, and LDL-C/HDL-C ratios experienced further reductions of 56% ($P<0.001$), 54% ($P<0.001$), and 68% ($P<0.001$), respectively. HDL-C levels maintained their upward trend, increasing by 34% ($P<0.01$). Notably, at a dose of 7 mL/kg, the effect of the loquat juice on the lipid profile was comparable to that of fenofibrate. It is worth mentioning that loquat juice administered to mice on a standard diet did not result in significant changes in lipid parameters compared to the NC group, emphasizing the specific impact of loquat juice on lipid metabolism disorders induced by the HFFD.

Effect of *Mkarkeb* loquat juice on the plasma glucose level

The data presented in **Table 2** shows a significant increase in plasma glucose levels in the hyperlipidemic group, with a 57% rise ($P<0.001$) compared to the normolipidemic group. Conversely, administering *Mkarkeb* loquat fruit juice to normolipidemic mice did not significantly change the glucose levels compared to the normolipidemic group ($P>0.05$). However, in hyperlipidemic mice, loquat juice supplementation led to a dose-dependent decrease in plasma glucose. The JTG 3.5 mL/kg group showed no significant effect, while the JTG 7 mL/kg group experienced a substantial reduction of 12% ($P<0.01$). Fenofibrate resulted in a greater reduction in plasma glucose levels, with a decrease of 33% ($P<0.01$).

Table 2. Effects of *Mkarkeb* loquat juice on plasma lipid and glucose over time in mice

Treatments	Parameters	0 weeks	2 weeks	4 weeks	6 weeks
NC	TC (mg/dL)	82.10±1.04	82.46±1.50	83.06±1.12	84.04±2.09
	TG (mg/dL)	45.12±1.07	46.18±1.05	49.27±1.08	50.01±1.01
	LDL-C (mg/dL)	8.57±1.28	8.88±2.51	8.21±2.06	8.10±1.43
	HDL-C (mg/dL)	18.17±0.99	18.10±0.45	19.08±0.49	19.09±0.52
	AI	3.51±0.79	3.55±0.60	3.53±0.14	3.40±0.09
	LDL-C/HDL-C	0.47±0.15	0.49±0.17	0.43±0.05	0.42±0.05
	Glucose (mg/dL)	96.55±3.19	98.18±6.50	99.50±3.09	100.1±1.12
JCG	TC (mg/dL)	82.7±1.21	79.18±2.44	80.20±3.87	83.10±4.77
	TG (mg/dL)	49.1±3.17	49.10±1.70	50.07±1.11	51.90±1.21
	LDL-C (mg/dL)	8.37±1.24	8.78±1.31	7.95±2.61	7.99±2.44
	HDL-C (mg/dL)	19.05±0.36	19.08±0.77	20.40±1.88	20.10±3.40
	AI	2.98±0.71	3.14±0.33	2.93±0.73	3.14±0.61
	LDL-C/HDL-C	0.40±0.10	0.46±0.29	0.38±0.31	0.39±0.81
	Glucose (mg/dL)	95.34±5.10	99.03±3.93	100.09±4.88	101.1±12
HC	TC (mg/dL)	81.10±2.09	145.19±2.17***	188.3±1.12**	251.70±3.84**
	TG (mg/dL)	51.29±1.32	68.08±1.14***	99.8±1.70**	129.18±1.61**
	LDL-C (mg/dL)	8.09±3.17	58.70±1.70***	57.18±2.11**	92.81±1.51**
	HDL-C (mg/dL)	20.02±1.90	19.12±0.82	18.18±0.79*	18.03±0.19*
	AI	3.19±0.39	6.59±0.17***	9.35±0.28**	12.96±1.09**
	LDL-C/HDL-C	0.30±0.17	3.07±0.09***	3.14±0.05**	5.14±0.08**
	Glucose (mg/dL)	100.09±4.09	103.77±5.19	106±3.62	171.09±6.10***
JTG 3.5mL/kg	TC (mg/dL)	84.12±3.11	143.99±1.41	186.22±3.10	249.11±3.81
	TG (mg/dL)	52.22±1.19	67.26± 1.54	102.10±2.71	127.12±2.03
	LDL-C (mg/dL)	8.16±1.05	58.91±0.63	59.11±1.11	89.99±2.53
	HDL-C (mg/dL)	20.10±1.32	20.09±1.21	20.03±2.07	20.98±2.94
	AI	3.18±0.11	6.16±1.95	8.29±1.12	10.87±1.33
	LDL-C/HDL-C	0.40±0.19	2.94±0.08	2.95±0.19	4.28±0.53
	Glucose (mg/dL)	101.18±4.11	99.81±7.44	101.01±7.90	169.01±3.01
JTG 7 mL/kg	TC (mg/dL)	84.77±1.31	139.01±1.15[a]	176.12±1.46[c]	199.01±2.10[c]
	TG (mg/dL)	52.92±1.87	64.01±0.23[b]	87.10±1.04[c]	101.72±2.01[c]
	LDL-C (mg/dL)	8.29±1.84	52.10±0.05[a]	52.08±0.46 [a]	72.41±1.06[c]
	HDL-C (mg/dL)	20.17±1.39	20.21±0.21	23.04±0.22[c]	26.17±1.18[c]
	AI	3.20±0.18	5.87±0.10[c]	6.64±0.06[c]	7.15±0.04[c]
	LDL-C/HDL-C	0.40±0.04	2.57±0.07[c]	2.26±0.08[c]	2.76±0.09[c]
	Glucose (mg/dL)	102.12±4.10	101.81±4.44	102.07±6.06	149.1±4.2[b]

	TC (mg/dL)	83.19±2.66	140.01±1.71	181.5±1.37[b]	164.5±1.51[c]
	TG (mg/dL)	50.74±2.23	64.1±2.07	91.70±1.90[b]	76.02±1.11[c]
	LDL-C (mg/dL)	8.19±1.47	55.92±1.60	49.60±1.27[b]	39.19±1.46[c]
FG	HDL-C (mg/dL)	21.91±1.71	21.23±1.09	24.27±1.71[c]	24.01±1.64[b]
	AI	2.79±0.43	5.59±1.45	6.47±0.09[c]	5.85±0.07[c]
	LDL-C/HDL-C	0.37±0.11	2.63±0.30	2.04±0.28[c]	1.63±0.05[c]
	Glucose (mg/dL)	104.16±4.19	107.16±4.08	108.15±5.01	121.06±11[b]

HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; AI, atherogenic index; LDL-C/HDL-C, ratio of low-density lipoprotein-cholesterol to high-density lipoprotein-cholesterol. NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. The results are expressed as mean ± SEM (n=8). *P<0.05,**P<0.01 and ***P<0.001 vs. NC, [a]P<0.05, [b]P<0.01, [c]P<0.001 against HC.

Effect of *Mkarkeb* loquat juice on liver and adipose tissue lipids

Table 4 illustrates the impact of loquat juice on liver and adipose tissue lipid levels in mice subjected to an HFFD for 6 weeks. In the group of mice that received a standard diet and were administered loquat juice at a dosage of 7 mL/kg, there was no significant change in liver and adipose tissue lipids compared to the normolipidemic group. However, the HFFD led to a significant increase in total cholesterol (TC) by 101% (P<0.001) and triglycerides (TG) by 398% (P<0.001) in the liver tissue of hyperlipidemic mice, compared to normolipidemic controls. Interestingly, loquat juice at 3.5 mL/kg alongside the HFFD showed no significant reduction in liver or adipose tissue lipids. However, at a higher dose of 7 mL/kg, loquat juice demonstrated greater efficacy, leading to a significant decrease in hepatic TC by 41% (P<0.001) and TG by 37% (P<0.001). Additionally, it resulted in a reduction of adipose tissue TC by 41% (P<0.001) and TG by 29% (P<0.001). Similarly, the administration of fenofibrate significantly reduced hepatic TC by 40% (P<0.001) and TG by 26% (P<0.01), along with a significant decrease in adipose tissue TC by 38% (P<0.001) and TG by 28% (P<0.001).

Table 3. Liver and adipose tissue lipid profile modulation by *Mkarkeb* loquat juice in HFFD-induced hyperlipidemic mice.

	Parameters (mg/g)	NC	JCG	HC	JTG 3.5 mL/kg	JTG 7 mL/kg	FG
Liver	TC	10.26±0.61	10.77±0.76	20.64±1.51*	18.15±2.71	12.10±0.40^{b}	12.27±0.24^{b}
	TG	4.56±0.67	4.53±0.98	22.71±1.76*	23.15±0.75	14.17±0.27^{b}	16.76±0.41^{a}
Adipose tissue	TC	1.89±0.12	1.91±0.15	3.19±0.17*	2.41±0.20	1.86±0.21^{b}	1.97±0.10^{b}
	TG	7.56±0.68	7.77±0.81	27.71±0.29*	25.14±1.21	19.61±1.21^{b}	20.12±0.54^{b}

NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. Data are mean ± SEM (n=8). *P <0.001 against NC. ^{a}P<0.05 ^{b}P<0.01 and ***P<0.001 against HC.

Effect of *Mkarkeb* loquat juice on biliary cholesterol excretion

Figure 2 illustrates the variations in biliary cholesterol levels across different treatment groups. Mice on a HFFD for six weeks showed a significant increase in bile cholesterol excretion, rising by 198% (P<0.001) compared to the control group. However, administration of loquat juice at a dose of 7 mL/kg further increased biliary cholesterol excretion, with rises of 25% (P<0.001) compared to the hyperlipidemic group. In contrast, the 3.5 mL/kg dose did not result in a significant increase compared to the hyperlipidemic group. Fenofibrate also produced a similar effect, increasing biliary cholesterol excretion by 32% (P<0.001). However, Loquat juice administered to normolipidemic mice maintained biliary cholesterol levels identical to those observed in the normolipidemic control group (P>0.05).

Effect of *Mkarkeb* loquat juice on fecal cholesterol and triglyceride excretion

Figure 3 displays the impact of loquat juice (3.5 mL/kg and 7 mL/kg) on fecal cholesterol and triglyceride excretion. Mice on an HFFD exhibited a 31% (P<0.001) increase in total cholesterol (TC) and a 22% (P<0.001) increase in triglycerides (TG) in their feces after two weeks. These levels progressively increased throughout the 6-week treatment period compared to mice on a standard diet (NC).

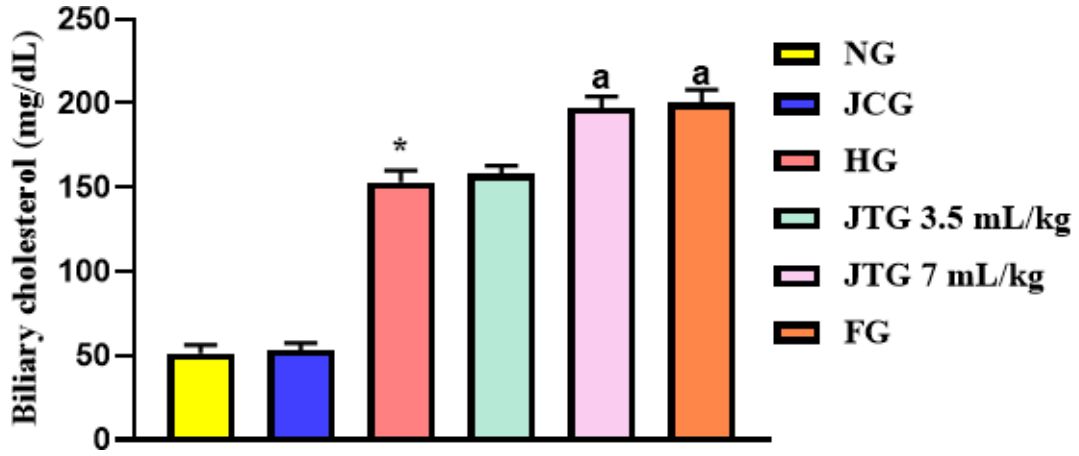

Figure 2. Effect of *Mkarkeb* loquat juice on biliary cholesterol in HFFD-induced hyperlipidemic mice. NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. Each value is a mean ±SEM (n=8). $^{*}P<0.001$ against NC. $^{a}P<0.001$ against HC.

Administering loquat juice at 7 mL/kg resulted in a notable rise in fecal TC and TG levels after two weeks, with increases of 14% ($P<0.05$) and 11% ($P<0.01$), respectively. The same dosage led to a significant increase in fecal TC by 26% ($P<0.001$) after four weeks and 46% ($P<0.001$) after six weeks. TG excretion also significantly rose by 20% ($P<0.001$) after four weeks, with a further increase to 48% ($P<0.001$) after six weeks. Conversely, loquat juice at 3.5 mL/kg did not significantly affect TC and TG excretion alongside the period treatment. Fenofibrate enhanced TC and TG fecal excretion, showing comparable results to loquat juice. Specifically, it increased TC excretion by 25% ($P<0.001$) and TG by 26% ($P<0.001$) after four weeks. This effect became more pronounced after six weeks, with TC excretion rising by 61% ($P<0.01$) and TG by 48% ($P<0.001$). In contrast, the 3.5 mL/kg dose of loquat juice had no significant effect on fecal cholesterol and triglyceride levels.

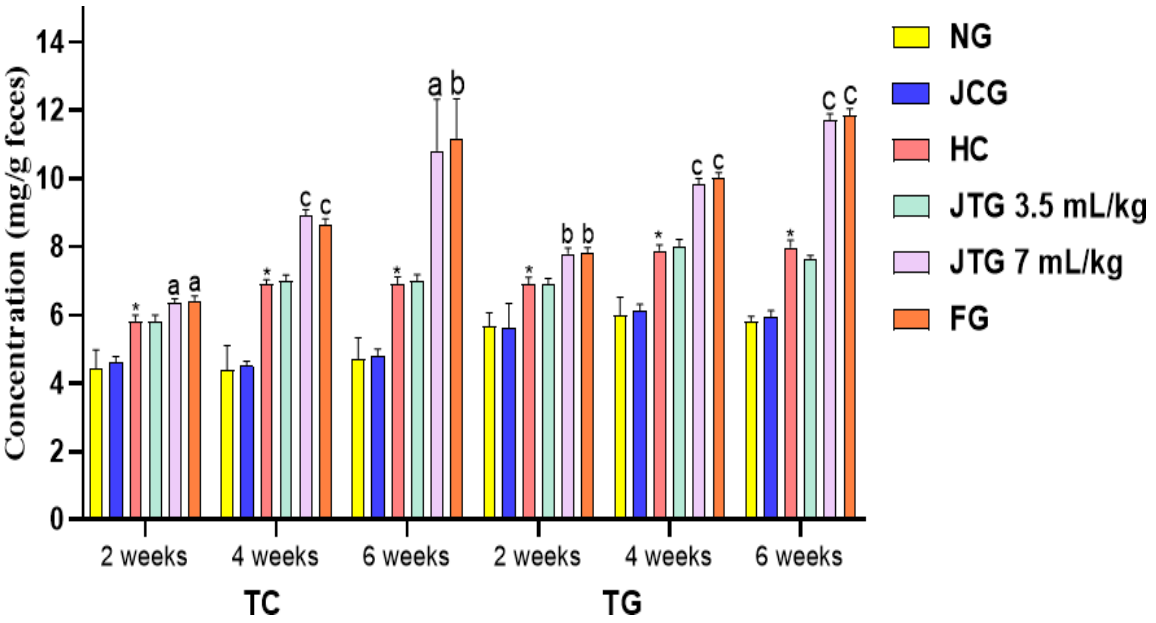

Figure 3. Effect of *Mkarkeb* loquat juice on excretion of fecal cholesterol and triglycerides. NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group. The values are means± SEM (n=8). $^{*}P<0.001$ against NC. $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$ against HC.

Effect of *Mkarkeb* loquat juice on food intake and body weight

None of the experimental animals experienced mortality or exhibited any behavioral abnormalities throughout the study. Additionally, there were no significant differences in food intake among the various treatment groups, suggesting that neither loquat juice nor fenofibrate affected mice's appetite on an HFFD, as detailed in **Table 4**. Compared to the normal group, mice treated with loquat juice showed no significant change in body weight, maintaining a weight level similar to that of healthy mice. In contrast, the hyperlipidemic group exhibited a gradual increase in body weight, with 8% increase ($P<0.001$) after 2 weeks, 30% increase ($P<0.001$) after 4 weeks, and 33% increase ($P<0.001$) after 6 weeks compared to the normal group. However, mice treated with loquat juice at a higher dose of 7 mL/kg experienced a reduction in body weight, with a 15% decrease ($P<0.001$) after 4 weeks, and this reduction persisted after 6 weeks of treatment ($P<0.001$), although no significant change was observed after the first 2 weeks. Similarly, the group treated with fenofibrate showed a 17% reduction ($P<0.001$) in body weight after 4 weeks and an 18% reduction ($P<0.001$) after 6 weeks compared to the hyperlipidemic group, as shown in **Table 4**. These findings demonstrate that the higher dose of loquat juice, like fenofibrate, effectively counteracted the weight gain typically associated with HFFD, contributing to a healthier body weight.

Table 4. Effect of *Mkarkeb* loquat juice on food intake and body weight in HFFD induced hyperlipidemic mice.

	2 weeks		**4 weeks**		**6 weeks**	
	BW (g)	**FI (g /day)**	**BW (g)**	**FI (g/day)**	**BW (g)**	**FI (g/day)**
NC	28.03±0.12	2.19±0.06	28.46±0.51	2.05±0.04	28.46±0.51	2.02±0.12
JCG	28.17±0.23	2.27±0.18	28.81±0.37	2.12±0.10	28.81±0.37	2.20±0.09
HC	29.95±0.24*	2.05±0.32	35.41±1.18*	2.15±0.19	35.41±1.18*	2.35±0.08
JTG 3.5 mL/ kg	29.51±0.97	2.11±0.81	35.17±1.27	2.05±0.72	35.17±1.27	2.02±0.51
JTG 7 mL/kg	29.01±0.41	2.13±0.42	30.11±0.40[a]	2.03±0.14	30.11±0.40[a]	2.03±0.22
FG	28.30±1.21	2.16±0.12	29.07±1.09[a]	2.07±0.21	29.07±1.09[a]	2.22±0.31

BW: Body weight (g/animal), FI: Food intake (g/animal/day), NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. All values represent the mean ±SEM. *P <0.001 against NC. [a]P<0.001 against HC.

Effect of *Mkarkeb* loquat juice on hepatic injury biomarkers in mice

This study examined the impact of hyperlipidemia on liver function, a crucial organ involved in many vital physiological processes. **Table 5** shows that mice fed a HFFD exhibited significant increases in several hepatic injury biomarkers: AST levels rose by 78% (P<0.001), ALT by 127% (P<0.001), ALP by 67% (P<0.001), TB by 86% (P<0.05), and LDH by 121% (P<0.001) compared to the normal control group. Administering loquat juice at dose of 3.5 mL/kg resulted in a slight, non-significant decrease in these biomarkers. However, a higher dose of 7 mL/kg effectively mitigated these elevations, reducing plasma AST levels by 10% (P<0.05), ALT by 21% (P<0.001), ALP by 22% (P<0.001), TB by 41% (P<0.001), and LDH by 9% (P<0.001). Fenofibrate also showed significant reductions in these biomarkers: AST levels decreased by 14% (P<0.01), ALT by 11% (P<0.001), ALP by 30% (P<0.001), TB by 40% (P<0.05), and LDH by 35% (P<0.001). Notably, the protective effects of *Mkarkeb* loquat juice at a dose of 7 mL/kg were comparable to those of fenofibrate, highlighting its potential efficacy in preventing hepatic injury associated with hyperlipidemia.

Table 5. Effect of *Mkarkeb* loquat juice on various biomarkers of hepatic injury in HFFD-induced hyperlipidemic mice.

	AST (U/L)	**ALT (U/L)**	**ALP (U/L)**	**TB (mg/dL)**	**LDH (U/L)**
NC	53.79±1.94	39.18±2.73	59.71±0.96	1.87±0.74	61.74±1.91
JCG	52.1±2.14	38.21±2.17	58.19±1.91	1.53±0.70	60.71±1.41
HC	96.07±3.19**	89.14±3.19**	99.73±1.71**	3.49±0.09*	137.17±2.40**
JTG 3.5 mL/kg	95.84±2.08	86.77±2.17	99.07±2.09	2.51±0.78	132.11±2.09
JTG 7 mL/kg	85.71±3.01^{a}	70.15±2.44^{c}	78.01±1.79^{c}	2.05±0.14^{c}	126.00±1.01^{c}
FG	82.42±1.19^{b}	73.25±2.09^{c}	86.19±1.67^{c}	2.22±0.33^{b}	111.89±2.10^{c}

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; LDH, lactate dehydrogenase; NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. The values are 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 HC.

Effect of *Mkarkeb* loquat juice on liver lipid peroxidation

Malondialdehyde (MDA) formation, an indicator of lipid peroxidation resulting from the breakdown of polyunsaturated fatty acids, was utilized to assess oxidative stress in the livers of mice fed a HFFD. As shown in **Figure 4**, hyperlipidemic mice exhibited a dramatic increase in liver MDA levels, rising by over 318% (P<0.001). A dose of 3.5 mL/kg of *Mkarkeb* loquat juice led to a significant reduction in MDA levels by 27% (P<0.001), while a higher dose of 7 mL/ kg resulted in a more substantial decrease of 39% (P<0.001). In comparison, fenofibrate treatment resulted in a 26% reduction in lipid peroxidation (P<0.01), although its effectiveness was still slightly lower than that of the higher dose of loquat juice.

On the other hand, when *Mkarkeb* loquat juice was administered to mice on a standard diet, it did not significantly affect liver lipid peroxidation compared to normolipidemic controls.

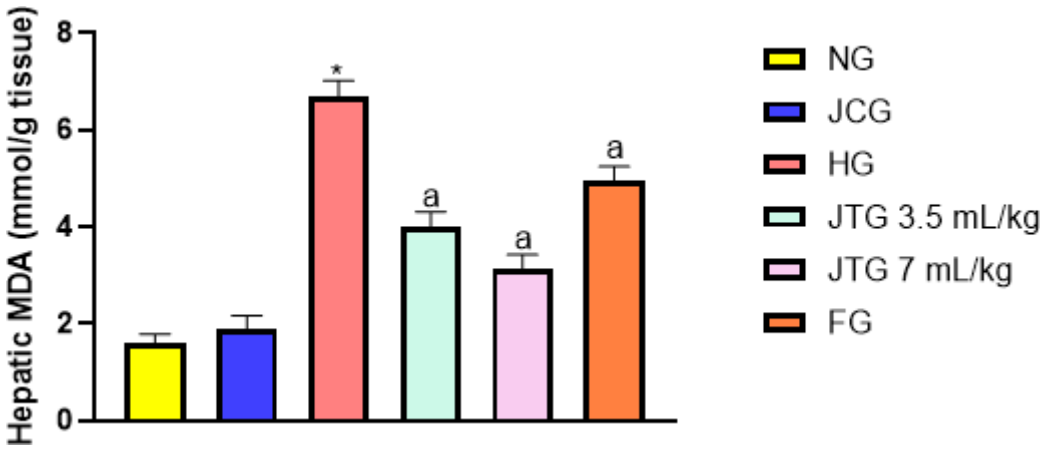

Figure 4. Effect of *Mkarkeb* loquat juice on liver lipid peroxidation in HFFD fed mice. NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. Data are mean± SEM (n=8). *P<0.001 against NC. ^{a}P<0.001 against HC.

Effect of *Mkarkeb* loquat juice on liver antioxidant enzymes activities

Based on **Figure 5**, HFFD feeding led to a significant fall in the liver SOD and catalase (CAT) activities in the hyperlipidemic group compared to the normolipidemic one, with reductions of 18% (P<0.001) and 42% (P<0.001), respectively. However, in treated groups, there was a significant rise in enzyme activities in a dose-dependent manner. *Mkarkeb* loquat juice, in particular, restored SOD and CAT activities by 12% (P<0.01) and 22% (P<0.05), respectively, at a dose of 3.5 mL/kg, and by 25% (P<0.001) and 36% (P<0.05), respectively, at a dose of 7 mL/kg. Furthermore, fenofibrate treatment increased SOD and CAT activity by approximately 21% (P<0.001) and 36% (P<0.05), respectively.

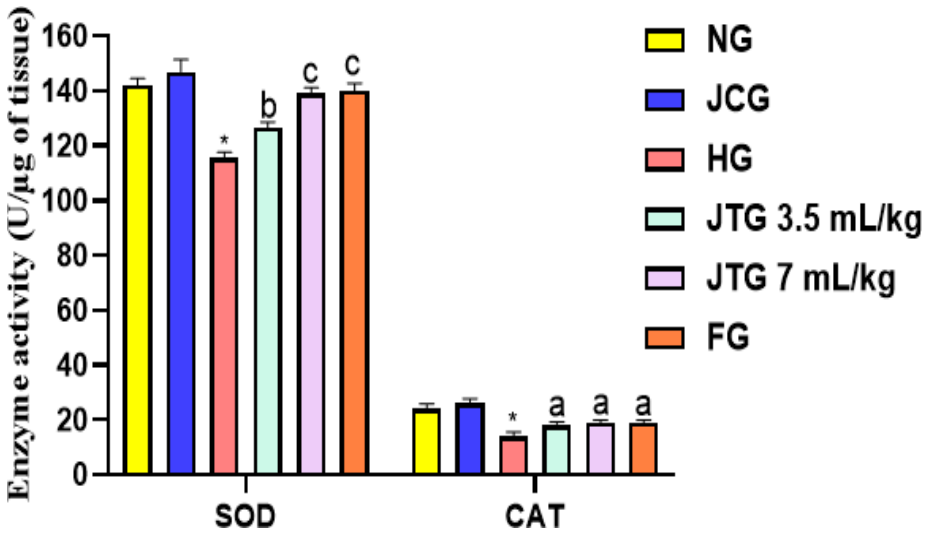

Figure 5. Effect of *Mkarkeb* loquat juice on superoxide dismutase (SOD) and catalase (CAT) activities in mice. NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. Data are mean± SEM (n=8). $^{*}P<0.001$ against NC. $^{a}P<0.01$; $^{b}P<0.01$ and $^{c}P<0.001$ against HC.

Effect of *Mkarkeb* loquat juice on liver morphology and histology and abdominal adipose tissue accumulation

In **Figure 6.A**, we visually compared the liver morphologies among different groups. The livers of the healthy control group displayed a glossy, sleek appearance with a dark red color, indicative of their normalcy. In contrast, the livers of hyperlipidemic mice appeared enlarged, with a yellowish tint and an irregular surface, suggesting the presence of a fatty liver condition. Following treatment with both loquat juice and fenofibrate, livers exhibited a reddish-brown appearance with smooth surfaces, resembling the livers in the control group. To provide detailed insights into the cellular changes underlying macroscopic observations, we present histological cross-sections of liver tissues observed under an optical microscope in **Figure 6.B**. As can be observed, the livers from normolipidemic control mice showcased a regular appearance with well-organized hepatocytes, clear cell borders, violet nuclei, and ample cytoplasm. The picture is strikingly different in hyperlipidemic mice; their hepatocytes exhibited round and excessively swollen characteristics, signifying hepatocyte hyperplasia compared to normolipidemic mice.

Additionally, an abundance of empty cytoplasmic vacuoles of varying sizes was observed within the hepatocytes, previously identified as granular degeneration or lipid droplets. This suggests that HFFD induces a substantial influx of lipids into hepatocytes, accumulating lipid vacuoles. These observations are consistent with our results regarding elevated TC and TG

levels in hyperlipidemic mice livers. In contrast to the hyperlipidemic group, mice treated with *Mkarkeb* loquat juice and fenofibrate exhibit dose-dependent mitigation of these histopathological modifications (quantity and dimensions of vacuoles and granular degeneration). These conclusions find further support in the corresponding liver lipid data, indicating lower TC and TG levels in the livers of *Mkarkeb* loquat juice-treated mice relative to hyperlipidemic ones. Additionally, as depicted in **Figure 6.C**, the relative liver weight of HFFD-fed mice was significantly higher than that of NC by 70% ($P<0.001$). However, concurrent treatment with *Mkarkeb* loquat juice led to a reduction of this parameter by 15% ($P<0.01$) and 31% ($P<0.001$) at doses of 3.5 mL/kg and 7 mL/kg, respectively. Notably, Fenofibrate exhibited a comparable effect, reducing relative liver weight by 46% ($P<0.001$).

Besides, a notable three-fold elevation ($P<0.001$) in the relative weight of abdominal adipose tissue was observed in hyperlipidemic mice (**Figure 6.C).** In contrast, a significant decrease in adipose tissue relative mass was observed in the group that received *Mkarkeb* loquat juice at doses of 7 mL/kg (49%, $P<0.001$).

It is important to note also that the fenofibrate's effect was comparable to that of *Mkarkeb* loquat juice (58%, $P<0.001$) concerning decreasing the mass of adipose tissues.The group treated with *Mkarkeb* loquat juice alone displayed no noteworthy disparities in livers' macroscopic or microscopic characteristics. Furthermore, there were no discernible differences in the relative weights of the hepatic and adipose tissues. This suggests that the administration of *Mkarkeb* loquat juice did not significantly change the appearance or weight of these organs compared to the normolipidemic group.

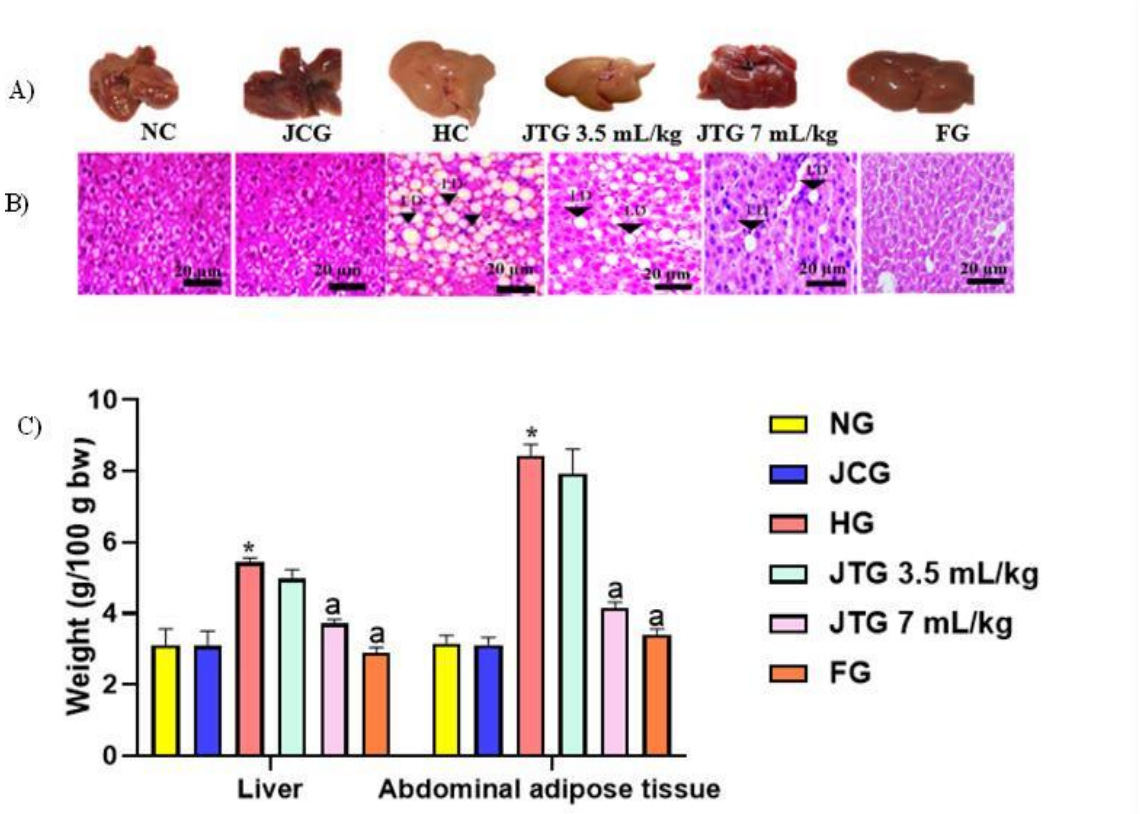

Figure 6. Effect of *Mkarkeb* loquat juice on liver and abdominal adipose tissue in HFFD fed mice. NC, normolipidemic control; HC, hyperlipidemic control; JCG, *Mkarkeb* loquat juice control group; JTG, *Mkarkeb* loquat juice-treated groups; FG, fenofibrate group; HFFD, high fat-high fructose diet. Data are mean± SEM (n=8). $^{*}P<0.001$ against NC. $^{a}P<0.001$ against HC.

DISCUSSION

Lipid metabolism is crucial for maintaining the body's structural integrity and signaling processes and preserving energetic balance [23]. However, this balance can be disrupted by diets high in fat and sugar, leading to fat accumulation in the liver and adipose tissues. Such dietary habits contribute to hyperlipidemia, oxidative stress, steatosis, weight gain and an increased risk of cardiovascular diseases [24].

In contrast, diets rich in fruits and vegetables, like the Mediterranean diet, have been shown to confer health benefits primarily due to the synergistic properties of micronutrients and antioxidants [25]. These components are pivotal in reducing lipid levels, scavenging free radicals, and preventing lipid peroxidation [26].

Previously, our research demonstrated that loquat peel extract significantly reduced plasma TC and TG levels in hyperlipidemic mice, lowering their concentrations in bile and fecal matter

[17]. In this study, we further investigated the nutritional composition of *Mkarkeb* loquat juice, focusing on its potential impact on lipid disorders in mice.

At its ripe stage, the *Mkarkeb* variety of loquat fruit contains a total soluble sugar content of 9.71 ± 0.07 g $100g^{-1}$. This is consistent with findings from other studies on loquats from Morocco and elsewhere [2, 27]. The main sugars found in many loquat varieties are sucrose, glucose, and fructose [28-29]. In our studied variety, fructose was the main sugar, followed by glucose, while sucrose was not detected. The high fructose content likely contributes to the *Mkarkeb* variety's characteristic sweetness, which is highly favored in local markets compared to other varieties.

The flavor of loquat is also influenced by the balance between sweetness and acidity [5,30]. Optimal flavor in loquat varieties is typically achieved when the fruit has a total soluble solids (TSS) content between 10 and 12 °Brix and a titratable acidity of around 1 g of acid per 100 mL juice [31]. For the local *Mkarkeb* variety, we measured a TSS content of 12.40 ± 0.25° Brix and a titratable acidity of 0.64 ± 0.03%, with a TSS/TA ratio of 19.37 ± 0.17. These metrics explain why the *Mkarkeb* variety is so popular in the Berkane region. Additionally, *Mkarkeb* loquat contains proteins, lipids, and minerals in quantities similar to those found in other loquat varieties reported in the literature [3].

Beyond its macronutrient profile, *Mkarkeb* loquat's richness in minor elements like carotenoids, ascorbic acid, and polyphenols potentially positions it as a fruit of exceptional nutritional quality and a functional food. In modern diets, the functional qualities of fruits are increasingly important to consumers, especially given the evidence supporting the role of balanced fruit and vegetable intake in disease prevention [13]. Indeed, numerous studies have demonstrated the protective effects of micronutrient-rich fruits against hyperlipidemia and cardiovascular diseases [32,33].

In the present study, we reliazed that feeding mice with HFFD caused an increase in TC and LDL-C levels and a decrease in HDL-C levels in treated mice compared to normolipidemic group. This imbalance negatively impacts the atherogenic index (AI) and the LDL-C/HDL-C ratio. However, treatment with *Mkarkeb* loquat juice for 6 weeks effectively reduced TC and particularly lowering the atherogenic LDL-C fraction. This suggests that *Mkarkeb* loquat juice may enhance LDL-C uptake by the liver and peripheral tissues through LDL-receptors, as previously proposed [20].

The juice also positively impacted cholesterol metabolism by elevating the anti-atherogenic HDL-C fraction, which is crucial for reverse cholesterol transport (RCT) from peripheral tissues to the liver, where cholesterol is excreted into bile. The juice's effect on the RCT pathway was

evidenced by significant changes in cholesterol levels within the liver and bile of treated mice. Notably, mice receiving the juice had lower hepatic cholesterol and higher biliary cholesterol levels than controls, indicating effective peripheral cholesterol clearance through the RCT pathway. These findings align with and extend previous research on loquat's benefits against lipid metabolism disorders [19,34].

Furthermore, the AI and LDL-C/HDL-C ratio are considered reliable predictors of atherosclerotic risk, underscoring the importance of maintaining these parameters at low levels [35]. Our study demonstrated that loquat juice significantly improved these atherogenic parameters in hyperlipidemic mice, underscoring its potential nutritional value in atherosclerosis prevention and other lipid metabolism disorders. This supports our earlier work on loquat peels' effects on lipid metabolism [19].

Given that dyslipidemia often coexists with hypertriglyceridemia, which is closely associated with abdominal obesity, overweight, and liver diseases like steatosis, these conditions can lead to insulin resistance, hyperglycemia, diabetes mellitus, and cardiovascular disease (CVD) [36]. This study's prolonged HFFD exposure led to an increases in triglyceridemia, glycemia, hepatic and adipose tissue TG content, body weight, and visceral adipose tissue mass. These findings corroborate numerous earlier studies utilizing this mouse model [37,38,39].

Conversely, concurrent administration of *Mkarkeb* loquat juice markedly reduced TG levels in plasma, liver, and adipose tissue while enhancing their excretion in feces. This suggests that the juice may promote the uptake and catabolism of TG-rich lipoproteins via lipoprotein lipase (LPL) activation, as previously hypothesized [40].

Moreover, mice treated with the juice experienced significant reductions in body weight, liver, and adipose mass, implying the juice's potential to inhibit lipogenic enzymes in adipose tissue, such as fatty acid synthase (FAS) [41] and diacylglycerol acyltransferase 2 (DGAT2) [42], while simultaneously activating fatty acid oxidation enzymes like carnitine palmitoyltransferase 1 (CPT1) [43].

Hyperlipidemia often leads to excessive lipid deposition in the liver, causing cellular damage and inflammatory injuries, a phenomenon known as "lipotoxicity" [44]. In our study, chronic hyperlipidemia induced by HFFD altered liver color, morphology, and histology, indicating steatosis and lipotoxicity. This was corroborated by increased hepatic lipid content and elevated plasma markers of liver injury in hyperlipidemic mice (ALT, AST, ALP, LDH, and TB). However, after treatment with loquat fruit juice, we concluded that it provided significant protection against liver damage induced by lipotoxicity, as shown by the correction of plasma biochemical markers and the integrity of hepatic histological structures. This finding confirms

our previous result on the protective effect of loquat peels against lipotoxicity [19] and aligns with those reported by Shahat et al. [45], demonstrating the hepatoprotective effect of loquat leaves.

The observed protective effect of *Mkarkeb* loquat juice against chronic hyperlipidemia-induced hepatic tissue damage could be attributed to its potent hypolipidemic activity. The juice eliminates excess lipids in bile and fecal matter, preventing their harmful accumulation in liver tissue. Without this removal, accumulated lipids in the liver can undergo peroxidation, leading to the production of toxic molecules such as oxidized LDL and lipid free radicals, which can damage cell membranes and cause tissue injury [46]. In this regard, we demonstrated that *Mkarkeb* loquat juice mitigates liver oxidative stress by reducing MDA content and activating antioxidant enzymes, such as SOD and catalase. This aligns with previous studies highlighting the antioxidant properties of loquat seeds, leaves, and peels [19,47,48].

The protective effect of *Mkarkeb* loquat juice is likely due to the presence of beneficial minor compounds, such as phenolics and carotenoids, known for their pharmacological activities [49]. The fruit juice is rich in phenolics, particularly phenolic acids and flavonoids, which have been identified as natural alternative for treating and preventing against several ailments without any side effect [20,50]. Additionally, these compounds, along with carotenoids and ascorbic acid, play crucial roles in combating oxidative stress at the tissue level [13,51]. Polyphenol analysis revealed that the juice contains five major phenolic compounds: neochlorogenic acid, chlorogenic acid, quercetin, and rutin, with caffeic acid being the predominant compound. All these phenolic compounds are known for their various pharmacological properties [52,53]. Our results disagree with those reported by Xu et al. [54] and Ding et al. [55], who identified chlorogenic acid as the main phenolic acid in Chinese and Japanese loquat fruits, respectively. These compounds can restore lipid metabolism, prevent fat accumulation in tissues and subsequent oxidation, which leads to cellular damages.

In this study, fenofibrate was used to compare the effects of *Mkarkeb* loquat juice, which were found to be largely similar to those of fenofibrate for most parameters.

CONCLUSION

The *Mkarkeb* variety of loquat fruit grown in eastern Morocco stands out as a nutritionally rich and flavorful option, making it a valuable addition to a balanced diet. Its optimal TSS/TA ratio, low-calorie profile, and abundance of essential minerals, carotenoids, ascorbate, and polyphenols highlight its beneficial effects on hyperlipidemia, hepatic steatosis, and oxidative

stress. These findings indicate that incorporating *Mkarkeb* loquat fruit into the diet may be crucial in preventing hyperlipidemia and related cardiovascular diseases.

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