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Date (*Phoenix dactylifera* L.) Fruits as a Potential Lipid-Lowering Therapy: Effect on High-Fat Diet and Triton-WR-1339-Induced Hyperlipidemic Rats

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Abstract: The present study was designed to establish the phenolic profile and explore the potential lipid-lowering effect of two Moroccan date fruit varieties (*Majhoul* and *Bousrdoun*). HPLC-DAD has been used for phenolic profiling. Lipid peroxidation was measured in terms of thiobarbituric acid-reactive substances (TBARS) by using egg yolk homogenate as lipid-rich media. The anti-hyperlipidemic effect of the methanolic extract was examined using both models Triton-WR-1339 and chronic high-fat-diet-induced hyperlipemic rats. Further, the serum lipid profile was determined. The HPLC-DAD analysis revealed the presence of seven phenolic acids and three flavonoids, of which gallic, caffeic acids and rutin were found to be the most abundant compounds. The gathered results indicate that rats treated with both varieties showed a significant decrease in serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol levels as well as an increase in high-density lipoprotein cholesterol levels compared with Triton and high-fat diet controls. Moreover, a significant decrease in body weight was observed in the date-treated groups when compared to the hyperlipidemic control group. A thiobarbituric acid reactive substances test showed that these extracts significantly inhibited lipid peroxidation. *Bousrdoun*, which showed the highest lipid-lowering effects, is the one that displayed the greatest inhibition of lipid peroxidation and contains the largest amount of caffeic, p-coumaric, gallic, vanillic acids, rutin and luteolin. Accordingly, dates could be used as a potential functional food, which may be used to prevent lipid disorders and oxidation.

Keywords: hypolipidemic; antioxidant; polyphenols; date fruit; high-fat diet

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1. Introduction

Cardiovascular disease remains among the leading causes of death in Morocco and many other countries around the world. Oxidative stress associated with elevated cholesterol, triglyceride, and LDL-cholesterol levels, as well as decreased HDL-cholesterol levelw, are among the most critical risk factors for the development of cardiovascular diseases [1]. Therefore, the management of these parameters is necessary for cardiovascular health. Hypolipidemic treatments, such as fibrates, niacin, statins, and bile acid sequestrants, which are currently available, have been linked to numerous side effects, including severe muscle damage, diarrhea, nausea, gastric irritation, liver and kidney dysfunction, and skin dryness [2]. The fundamental mechanism in atherosclerosis physiopathology

is recognized to be hyperlipidemia and low-density lipoprotein oxidation, which can be addressed with hypolipidemic and antioxidant therapies [3]. In this sense, efforts have recently been committed to researching hypolipidemic and antioxidant nutraceuticals and functional foods.

Date palm (*Phoenix dactylifera* L.) fruits are used in traditional medicine to cure various ailments, including fever, inflammation, neurological disorders, intestinal illness, and liver problems, as well as to prevent alcohol intoxication [4]. In addition, in the Moroccan oasis, folk medicine practitioners recommended including dates in weight loss diets. It is also touted as beneficial for heart health. Moreover, earlier studies have shown that dates have potent anti-inflammatory, antioxidant, antibacterial, hepatoprotective, gastroprotective and nephroprotective properties, indicating their utility in various types of disorders [5–7].

Furthermore, dates contain niacin [6], which is known to have favorable effects on multiple lipid parameters, including raising high-density lipoprotein cholesterol (HDL-C) levels and lowering triglycerides (TGs), lipoprotein(a) and low-density lipoprotein cholesterol (LDL-C) [8]. In addition, it contains numerous chemical compounds, including anthocyanins, procyanidins, carotenoids, phenolic acid, flavonoids, and sterols [9]. These phytochemical compounds are well-known for their numerous health benefits, including obesity treatment and lipid-lowering activity [10]. It has previously been discovered that date seed extract has a significant hypolipidemic effect [11], and the purpose of this investigation is to see if this activity is preserved in the edible part of the fruit. Considering these properties, this study aims to establish the phenolic profile and to investigate the hypolipidemic activity of two Moroccan date fruit varieties (*Bousrdoun* and *Majhoul*) in Triton WR-1339 and high-fat-diet-induced hyperlipidemic rats.

2. Results

2.1. Analysis of Polyphenolic Profile

The HPLC chromatogram of phenolic profiling in the *Bousrdoun* date fruit variety is shown in Figure 1. Seven phenolic acids and three flavonoids were found and varied significantly ($p < 0.01$) between the two varieties (Table 1). The greatest phenolic component in studied dates varieties was gallic acid (8.53–10.38 mg/100 g DW), followed by ferulic acid (3.17–3.86 mg/100 g DW), caffeic acid (1.82–2.72 mg/100 g DW), p-coumaric acid (1.43–1.92 mg/100 g DW), syringic acid (1.47–1.73 mg/100 g DW) and chlorogenic acid (0.83–1.32 mg/100 g DW). Vanillic acid was found in *Bousrdoun* (0.41 mg/100 g DW) but was not detected in *Majhoul*. Rutin was the main flavonoid compound in both date fruit varieties (1.84–3.11 mg/100 g DW), then quercetin (0.73–1.09 mg/100 g DW) and the luteolin (0.28–0.43 mg/100 g DW) was the least abundant. The highest amount of luteolin, rutin caffeic, p-coumaric, and gallic acids was found in *Bousrdoun*, while *Majhoul* contained the highest amount of quercetin, chlorogenic, ferulic, and syringic acid.

Table 1. Phenolic acids and flavonoid composition of *Bousrdoun* and *Majhoul* date fruit varieties (mg/100g DW).

		Bousrdoun	***Majhoul***
Phenolic acid	Caffeic acid	2.72 ± 0.13 [a]	1.82 ± 0.10 [b]
	Chlorogenic acid	0.83 ± 0.05 [b]	1.32 ±0.07 [a]
	p-Coumaric acid	1.92 ± 0.09 [a]	1.43 ± 0.08 [b]
	Ferulic acid	3.17 ± 0.12 [b]	3.86 ± 0.15 [a]
	Gallic acid	10.38± 0.21 [a]	8.53 ± 0.23 [b]
	Syringic acid	1.47± 0.10 [b]	1.73 ± 0.07 [a]
	Vanillic acid	0.41 ± 0.05	Nd

Table 1. *Cont.*

		Bousrdoun	*Majhoul*
Flavonoids	Luteolin	0.43 ± 0.03 [b]	0.28 ± 0.04 [a]
	Quercetin	0.73 ± 0.05 [b]	1.09 ± 0.06 [a]
	Rutin	3.11 ± 0.12 [a]	1.84 ± 0.13 [b]

Values are expressed as a mean ± SD (n = 3). Averages in the same line with different letters are significantly different using post hoc Bonferroni test ($p < 0.01$). Nd, not determined.

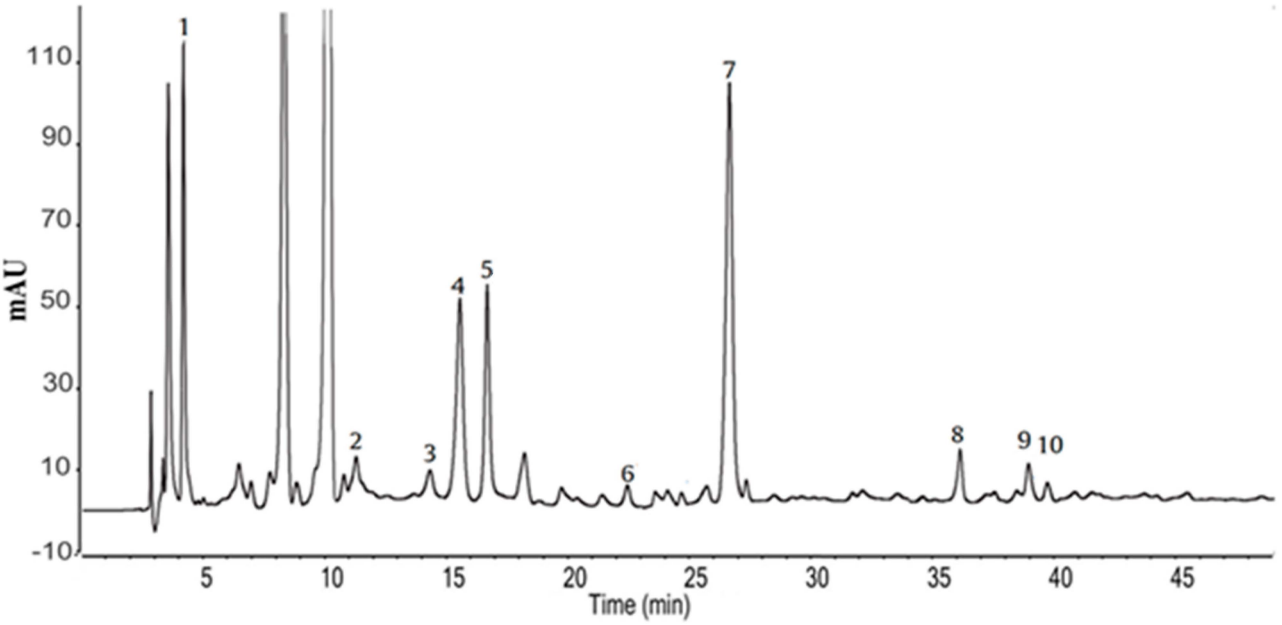

Figure 1. HPLC-DAD chromatogram of *Bousrdoun* date fruit variety. Peak numbers: (1) gallic acid; (2) chlorogenic acid; (3) vanillic acid; (4) caffeic acid; (5) syringic acid; (6) p-coumaric acid; (7) ferulic acid; (8) rutin; (9) luteolin; (10) quercetin.

2.2. Lipid Peroxidation Inhibition Effect of Dates Extracts

The TBARS method is a commonly used method to assess the lipid oxidation level through MDA, which occurs because of polyunsaturated fatty acid lipid peroxidation. As displayed in Table 2, the extracts inhibited lipid peroxidation significantly ($p < 0.01$) and in a concentration-dependent manner. *Bousrdoun* date fruit variety exhibited the highest inhibition of lipid peroxidation (IC_{50} = 371.38 µg/mL), while *Majhoul* revealed the lowest antioxidant power (IC_{50} = 492.81 µg/mL). The antioxidant power of the studied date fruit varieties is still lower compared to that of ascorbic acid (IC_{50} = 101.62 µg/mL).

Table 2. Date fruit extract inhibition of lipid peroxidation.

	Majhoul	*Bousrdoun*	Ascorbic Acid
TBARS IC_{50} (µg/mL)	492.81 ± 4.07 [a]	371.38 ± 4.82 [b]	101.62 ± 2.97 [c]

The data are represented by means ± SEM (n = 6). Averages with the same letters are not significantly different using post hoc Bonferroni test ($p < 0.01$).

2.3. Effect of Date Fruit Extracts on Serum Lipid Profile in in Triton-Induced Acute Hyperlipidemia

Rats treated with Triton WR-1339 displayed a substantial increase in triglycerides (8.35-fold), total cholesterol (4.65-fold), LDL-C (6.94-fold) along with a significant decrease in HDL-C (2.62-fold) serum levels when compared to the normal group ($p < 0.01$) (Table 3). The administration of *Majhoul* and *Bousrdoun* extracts or simvastatin to these hyperlipidemic

rats resulted in a decrease in serum concentrations of TG, TC and LDL-C and a rise in serum HDL-C levels. The administration of the *Bousrdoun* date fruit extract, which was highly potent at 1200 mg/kg b.w., induced a 4.54, 2.89, and 2.82-fold decreases in the levels of TG, TC and LDL-C, respectively, with a concomitant increase in HDL-C levels (3.06-fold) when compared to the triton group ($p < 0.01$). Likewise, treatment with the *Majhoul* date fruit extract at 1200 mg/kg b.w. 1200 mg/kg b.w reduced the levels of TG, TC, and LDL-C by 2.51, 1.91 and 1.61-fold, respectively, along with a rise in HDL-C levels (2.32-fold). The results found for the two-studied date fruit extracts are close to those reported for the standard drug simvastatin (15 mg/Kg BW), which caused 3.57, 3.54 and 3.75-fold reduction in TG, TC and LDL-C serum levels, respectively, and 3.08-fold increase in HDL-C. In addition, when compared to triton control, *Majhoul* and *Bousrdoun* fruit extracts at 1200 mg/kg BW and simvastatin at 15 mg/kg BW supplementation caused a 4.66, 10.91 and 14.20-fold reduction in AI, respectively.

Table 3. Effect of date fruit extracts on serum lipid profile in in triton-induced acute hyperlipidemia.

	TC (mg/dL)	TG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	AI
Normal control	77.90 ± 5.94^{f}	92.85 ± 7.37^{f}	33.68 ± 4.79^{f}	$26.43 \pm 2.77^{b,c}$	1.87 ± 0.53^{e}
Triton control	362.34 ± 18.39^{a}	775.03 ± 29.20^{a}	233.59 ± 23.29^{a}	10.09 ± 0.61^{e}	34.37 ± 3.81^{a}
Triton + BDFE (600 mg/Kg BW)	165.15 ± 11.25^{c}	232.19 ± 17.94^{d}	114.21 ± 9.72^{c}	$28.07 \pm 2.27^{a,b}$	$4.93 \pm 1.17^{c,d}$
Triton + BDFE (1200 mg/Kg BW)	125.31 ± 10.91^{d}	170.80 ± 14.12^{e}	82.71 ± 9.45^{d}	$30.85 \pm 2.16^{a,b}$	$3.15 \pm 0.92^{d,e}$
Triton + MDFE (600 mg/Kg BW)	228.75 ± 15.63^{b}	354.51 ± 17.08^{b}	168.22 ± 10.73^{b}	18.83 ± 2.16^{d}	12.21 ± 1.09^{b}
Triton + MDFE (1200 mg/Kg BW)	187.85 ± 12.27^{c}	308.91 ± 17.11^{c}	145.01 ± 10.28^{b}	23.45 ± 2.32^{c}	7.37 ± 1.32^{c}
Triton + Sim (15 mg/Kg BW)	102.28 ± 9.79^{e}	217.26 ± 19.94^{d}	62.27 ± 6.79^{e}	31.10 ± 2.18^{a}	2.42 ± 0.42^{e}

Values are expressed as means ± SEM for six animals. Statistical analysis was carried out using one-way analysis of variances followed by Bonferroni post hoc test. Averages, in the same column values followed by the same letters are not significantly different ($p < 0.01$). TC: total cholesterol; TG: triglycerides; LDL-C: LDL-cholesterol; HDL-C: HDL-cholesterol; AI: atherogenic index; MDFE: *Majhoul* date fruit extract; BDFE: *Bousrdoun* date fruit extract; Sim: simvastatin.

2.4. Effect of Date Fruit Extracts on Serum Lipid Profile in High-Fat Diet-Induced Hyperlipidemia

Feeding HFD to rats leads to hyperlipidemia as evidenced by a significant increase in serum levels of TC, LDL-C and TG by 3.45, 4.71 and 4.21-fold and a marked diminution in serum HDL-C levels by 1.93-fold in comparison with the normal group (Table 4). The feeding of simvastatin or date fruit extract of both varieties at different doses to rats given the HFD led to a significant ($p < 0.01$) decrease in the serum levels of TC, TG, and LDL-C serum levels, as well as a remarkable ($p < 0.01$) rise in HDL-C levels when compared to HFD group. Indeed, the hyperlipidaemic rats that received *Bousrdoun* at 1200 mg/Kg showed a 3.24-, 3.00- and 2.95-fold reduction in TG, TC and LDL-C and a 2.13-fold increase in HDL-C serum level. The oral administration of *Majhoul* extract showed a lower effect compared to the *Bousrdoun* extract. In fact, giving *Majhoul* extract at 1200 mg/Kg to hyperlipidaemic rats caused 1.66-, 2.16- and 1.64-fold decreases in TG, TC and LDL-C as well as 1.68-fold increases in HDL-C as compared to rats of hyperlipidaemic control. Significant differences ($p < 0.01$) in lipid-lowering effect were observed between studied varieties. Similarly, the standard drug simvastatin (15 mg/Kg b.w.) showed 2.16-, 3.20-, and 3.74-fold decreases for TG, TC, and LDL-C levels, respectively, while it caused a significant increase in serum HDL-C by 1.61-fold compared to HFD control. Compared to the HFD group, the AI was reduced significantly ($p < 0.01$) by about 4.26-, 10.13- and 6.92-fold in rats receiving the

Majhoul extract (1200 mg/kg BW), *Bousrdoun* extract (1200 mg/kg BW), and simvastatin (15 mg/kg BW), respectively.

Table 4. Effect of date fruit extracts on serum lipid profile in high-fat diet-induced hyperlipidemia.

	TC (mg/dL)	TG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	AI
Normal control	80.20 ± 5.74 ^{d}	84.51 ± 5.97 ^{f}	32.38 ± 3.69 ^{e}	32.84 ± 3.25 ^{a,b}	1.48 ± 0.59 ^{d}
HFD control	276.50 ± 17.90 ^{a}	355.78 ± 16.79 ^{a}	152.45 ± 11.92 ^{a}	16.97 ±1.90 ^{c}	15.30 ± 1.01 ^{a}
HFD + BDFE (600 mg/Kg BW)	115.16 ± 11.63 ^{c}	106.71 ± 12.63 ^{e}	75.38 ± 7.89 ^{c}	30.89 ± 3.34 ^{a,b}	2.59 ± 0.81 ^{c}
HFD + BDFE (1200 mg/Kg BW)	92.23 ± 7.95 ^{d}	109.92 ± 13.87 ^{e}	51.67 ± 5.19 ^{d}	36.19 ± 2.48 ^{a}	1.51 ± 0.72 ^{d}
HFD + MDFE (600 mg/Kg BW)	141.19 ± 11.39 ^{b}	259.01 ± 16.07 ^{b}	108.45 ± 10.11 ^{b}	21.95 ± 1.94 ^{c}	5.58 ± 0.67 ^{b}
HFD + MDFE (1200 mg/Kg BW)	127.53 ± 12.19 ^{b,c}	214.93 ± 17.85 ^{c}	92.77 ± 10.48 ^{b,c}	28.57 ± 2.31 ^{b}	3.59 ± 0.82 ^{c}
HFD + Sim (15 mg/Kg BW)	86.40 ± 8.54 ^{d}	164.37 ± 14.09 ^{d}	40.73 ± 4.71 ^{e}	27.31 ± 2.24 ^{b}	2.21 ± 0.78 ^{c}

Values are expressed as means ± SEM for six animals. Statistical analysis was carried out using one-way analysis of variances followed by Bonferroni post hoc test. Averages, in the same column values followed by the same letters are not significantly different ($p < 0.01$).; TC: total cholesterol; TG: triglycerides; LDL-C: LDL-cholesterol; HDL-C: HDL-cholesterol; AI: atherogenic index; MDFE: *Majhoul* date fruit extract; BDFE: *Bousrdoun* date fruit extract; Sim: simvastatin.

2.5. Effect of Date Fruit Extracts on Body Weight Gain of Rats under High-Fat Diet

As shown in Table 5, body weight gain in hyperlipidemic rats was significantly ($p < 0.01$) higher, which was found to be 2.74 times higher in this group of rats when compared to the control group. The treatment with different date fruit varieties at different doses significantly reduced body weight gain when compared to the hyperlipidemic group that exclusively received the HFD. *Bousrdoun* and *Majhoul* date fruit extracts at 1200 mg/kg b.w caused a 2.70- and 2.09-fold reduction in body weight gain, respectively, as compared to the hyperlipidemic group. Similar results were observed in the group receiving simvastatin (15 mg/Kg BW), which reduced the body weight gain by 2.14-fold when compared to the same control.

Table 5. Effect of date fruit extracts on date fruit extracts effect on body weight gain of rats under high-fat diet.

	Body Weight Gain (g)
Normal control	26.13 ± 2.40 ^{d}
HFD control	71.57 ± 2.38 ^{a}
HFD + BDFE (600 mg/Kg BW)	39.36 ± 3.41 ^{b,c}
HFD + BDFE (1200 mg/Kg BW)	26.43 ± 2.47 ^{d}
HFD + MDFE (600 mg/Kg BW)	46.67 ± 4.22 ^{b}
HFD + MDFE (1200 mg/Kg BW)	34.25 ± 4.64 ^{c}
HFD + Sim (15 mg/Kg BW)	33.43 ± 3.54 ^{c}

Values are expressed as means ± SEM for six animals. Statistical analysis was carried out using one-way analysis of variances followed by Bonferroni post hoc test. Averages, in the same column, values followed by the same letters are not significantly different ($p < 0.01$); HFD: high-fat diet; BDFE: *Bousrdoun* date fruit extract; MDFE: *Majhoul* date fruit extract; Sim: simvastatin.

3. Discussion

In the current research, triton WR-1339 and HFD-induced hyperlipidemia were selected to investigate the lipid-lowering effect of two date fruit varieties named *Bousrdoun* and *Majhoul*. Triton WR-1339 (also known as Tyloxapol), a non-ionic detergent, provokes a marked elevation in serum cholesterol and triglycerides levels because it increases the HMG Co-A (3-hydroxy-3-methyl-glutaryl Co-A) reductase activity involved in hepatic cholesterol synthesis and suppresses triglycerides hydrolysis by lipoprotein lipase (LPL) and thereby blocks the uptake of triacylglycerol-rich lipoproteins from circulation by extrahepatic tissues [12]. Moreover, the dissociation of apolipoprotein A-I (apo A-I) and apo C-II from high-density lipoproteins was demonstrated by triton [13]. The oral administration of date fruit extracts *Majhoul* and *Bousrdoun* to rats treated with Triton WR 1339 was able to avoid the increase in TG, TC and LDL-cholesterol levels and the decline in HDL-cholesterol levels. Since triton-induced hyperlipidemia occurs under fasting conditions, endogenous lipid synthesis is the only source of serum lipid. Hence, this lipid-lowering effect of date fruit extract indicates that it acts in lipid metabolism. In addition, since lipoprotein lipase and hepatic lipase are believed to play an important role in the catabolism of triacylglycerol and HDL, it can be deduced that the antihyperlipidemic effect observed because of the administration of the two extracts of date varieties is probably due to the reactivation of these enzymes [14]. The ability of date fruit extract to lower cholesterol might be due to their ability to regulate the enzymes such as lecithin cholesteryl acyltransferase (LCAT), HMG-CoA reductase and acyl CoA cholesterol acyltransferase (ACAT), which are implicated in cholesterol metabolism [15]. The low amount of hepatocyte cholesterol increases their ability to remove LDL-C from the blood by increasing the LDL receptor densities on liver cell membranes [16]. The bile acid excretion process may be enhanced by date fruit extracts, which may lead to a reduction in blood cholesterol levels, as found for some plant extracts [17]. HFD-induced hyperlipidemia is a suitable model since it imitates pathological conditions, such as in humans in rats. Feeding with an HFD for twenty-one days caused a significant elevation of serum TG, TC, and LDL-C levels and a marked decrease in HDL-C levels in treated rats compared to rats fed a normal diet. The administration of extracts of both date fruit varieties *Majhoul* and *Bousrdoun* at 600 and 1200 mg/kg BW for three weeks along with HFD significantly decreased the serum concentration of TC, TG, and LDL-C and increased the concentration of HDL-C when compared with the HFD group. As found in our previous study, date fruit contains a high amount of niacin, and *Majhoul* (2318.94 µg/100 g DW) and *Bousrdoun* (2061.72 µg/100 g DW) [6], which hasten the ApoB degradation within cells by inhibiting triacylglycerol synthesis and, in turn, reduce VLDL and LDL particle release [18]. It also reduces free fatty acid release and mobilization from adipose tissue and, consequently, decreases TGs blood levels [18]. Moreover, niacin reduces HDL-apo A-I catabolism and increases the half-life of the HDL, which in turn promotes reverse cholesterol transport [18]. The hypolipidemic action of these date fruits might be due to the synergetic effect of their main bioactive compounds. As compared to the high-fat group, Alam et al. [19] have reported that the administration of chlorogenic, caffeic and ferulic acids considerably decreased the ACAT and HMG-CoA reductase activities, suppressed fatty acid synthase and increased fatty acid β-oxidation and PPAR α expression in the liver. In addition, Yeh et al. [20] have found that the administration of ferulic, coumaric and caffeic acids to rats enhances the excretion of neutral and acidic sterols, thus lowering dietary cholesterol absorption and plasma hepatic cholesterol. In the same study, these phenolic acids showed a potent inhibition of HMG-CoA reductase and hepatic ACAT activities. In addition, Martinez-Gonzalez et al. [21] established that quercetin, caffeic acid and p-coumaric acid inhibit pancreatic lipase activity. Ferulic acid, gallic acid, caffeic acid, syringic acid and vanillic have a strong capacity to inhibit pancreatic phospholipase A2 activity [22], which might be the primary reason for decreased lipid absorption since luminal phospholipids hydrolysis is essential for intestinal lipid digestion and absorption [23]. Prince and Kannan [24] studied the effect of rutin on enzymes of lipid metabolism and found that rutin reduces the activity of HMG-CoA reductase while increasing plasma LPL

and LCAT activities. Gnoni et al. [25] have found that quercetin strongly reduced the activity of acetyl-CoA carboxylase (ACC), the initial crucial step in the biosynthesis of fatty acids, fatty acid synthase (FAS), and HMG-CoA reductase in rat liver cells. Luteolin and quercetin have been found to block NPC1L1-mediated intestinal cholesterol absorption to block NPC1L1-mediated intestinal cholesterol absorption, lowering elevated cholesterol levels in the blood [26]. This hypolipidemic action might be attributed to a synergistic impact of bioactive molecules found in dates, which can act on lipid metabolism in different pathways. In addition, some of these substances restrict the intestinal assimilation of fats and biliary salts, others increase the hepatic uptake of cholesterol, and others inhibit the hepatic synthesis of cholesterol and fatty acids. Feeding with an HFD causes the overproduction of reactive oxygen species, which promotes lipid peroxidation, leading to MDA production and hastening the establishment and development of atherogenesis [27]. These date fruit cultivars have been shown to have a significant radical scavenging power against DPPH and ABTS radicals, a higher ferrous ion chelating activity [9], as well as a strong nitric oxide scavenging ability [7]. The ability of date fruit extracts to decrease lipid peroxidation, as well as to lower blood LDL-cholesterol levels, suggests their ability to act as an anti-atherogenic agent.

4. Materials and Methods

4.1. Polyphenol Extraction Method

The polyphenol extraction was carried out using the protocol previously reported by Bouhlali et al. [11]. In total, 30 g of date fruit were blended and homogenized in 150 mL of methanol–water (4:1, *v*/*v*) using a shaker for 12 h at 35 °C. After filtration through a Whatman filter paper (grade 1), the supernatants were collected and concentrated using a rotary evaporator under reduced pressure at 40 °C until complete solvent evaporation. The residues were stored in dark glass vials at—20 °C until use. The extracts were reconstituted in distilled water to obtain stock solutions of known dilution and used to assess their lipid-lowering effects and the inhibition of lipid peroxidation.

4.2. Chromatographic Equipment and Analysis of Polyphenolic Profile

Date fruit phenolic acid and flavonoid profile analysis was performed as described by Bouhlali et al. [11]. The extraction of phenolic compounds was carried out through ultrasound homogenization of two grams of dates in 25 mL of acidified methanol (methanol/HCl/water; 80/1/19) for 30 min. The mixture was centrifuged at 1000× *g* for 15 min, and then 2 mL of the supernatant was filtered through a 0.45 µm filter. Chromatographic separation was carried out using a Shimadzu HPLC-DAD system (Kyoto, Japan) equipped with an SPD-M10A diode array detector, LC-20AB binary solvent delivery module, DGU-14A solvent degasser, a SIL-20A automatic sampler, a CTO-10AC column oven and an SCL-10A System Controller. The column was Restek C18 (4.6 × 150 mm, 5 µm) (Bellefonte, PA, USA). The temperature of the column was 40 °C, the injection volume was 10 µL, and the detection wavelength was maintained at 280, 320 and 350 nm. At a flow rate of 1 mL/min, a gradient solvent system comprised of solvent A (water–acetic acid (97:3, *v*/*v*)) and solvent B (acetonitrile) was employed following the elution gradient procedure: 0–5 min, 0–8% mobile phase B; 5–25 min, 8–25% mobile phase B; 25–30 min, 25% mobile phase B; 30–50 min, 25–90% mobile phase B. The compounds were identified by comparing the RTs of the extract peaks to those of the standards, and their concentrations were calculated using a calibration curve prepared from a stock solution of standards (100 µg/mL) that included three flavonoids: luteolin, quercetin, and rutin, as well as seven phenolic acids: caffeic, p-coumaric, chlorogenic, ferulic, gallic, vanillic, and syringic acids.

4.3. Lipid Peroxidation Inhibition Effect of Dates Extracts

The ability of extracts to inhibit lipid peroxidation was measured using a modified TBARS assay described by Upadhyay et al. [28]. Briefly, 500 µL of egg yolk homogenate (10% *w*/*v* in phosphate-buffered saline (pH 7.4)) was added to 100 µL of the extract, and

the volume was made up to 1000 µL with distilled water. Then, 50 µL of ferrous sulfate ($FeSO_4$ (0.07 M)) was added to the above mixture and incubated for 30 min at 37 °C, to induce lipid peroxidation and colour reaction was carried out by adding 1.5 mL acetic acid (20%, pH 3.5), 1.5 mL of thiobarbituric acid (TBA) (0.8% *w*/*v* prepared in 1.1% sodium dodecyl sulfate) and 50 µL 20% trichloroacetic acid (TCA). The mixture was vortexed and heated in a boiling water bath for 1 h. The mixtures were cooled, and 6 mL of n-butanol was added and centrifuged. The absorbance was read using a spectrophotometer (RAY-LEIGH, VIS-723G, Beijing, China) at 532 nm against n-butanol. For the control, 100 µL of distilled water was used in place of extract.

The inhibition of lipid peroxidation (ILP) was calculated using the following formula:

$$\text{ILP (\%)} = 100 \times (\text{Abs Control} - \text{Abs Sample})/\text{Abs Control}$$

The ILP (%) was plotted against the concentrations of samples or standards to determine and IC_{50} values (concentration of extract or standard to prevent 50% of lipid oxidation).

4.4. Animals

Male albino rats (Wistar strain) weighing between (150 and 190 g) were taken from the Biology Department's animal facility (Faculty of Sciences and Techniques, Errachidia, Morocco). Then, they were divided into groups, housed in polypropylene cages and kept under hygienic and favorable conditions, including a 12 h light/dark cycle, 30–70% relative humidity, 22 ± 2 °C controlled temperature, proper ventilation and free access to water provided ad libitum and pelletized rat feeds. The procedures for the current study were authorized by the Animal Research Ethics Committee of the Faculty of Sciences and Techniques (AREC) (AREC-FSTE-12/2020), which was carried out following the National Institute of Health Guide for the Care and Use of Laboratory Animals.

4.5. Triton WR-1339 Induced Acute Hyperlipidemia Assay

As described by Bouhlali et al. [11], forty-two overnight-starved rats were randomly assigned to seven experimental groups, each with six members. Rats in the normo-lipidemic group were gavaged with distilled water after receiving normal saline injections (pH 7.4). Triton WR-1339 was injected intraperitoneally into the other groups (II–VII) at a dose of 200 mg/kg BW to cause hyperlipidemia. The hyperlipidemic control group's rats were received distilled water. *Bousrdoun* and *Majhoul* date fruit extracts were administered to the third, fourth, fifth and sixth groups at 600 and 1200 mg/Kg BW doses, respectively. Simvastatin (15 mg/kg BW) was given to the rats of the seventh (standard) group. Blood was taken from the retro-orbital sinus of rats after 24 h of treatment while they were given diethyl-ether anesthesia, centrifuged at 2500 rpm for 10 min, and the serum was used for biochemical analysis.

4.6. High-Fat-Diet-Induced Hyperlipidemia in Rats

As described by Harnafi et al. [29], high-fat diet-induced hyperlipidemic rats were used to study the hypolipidemic effect of date fruit extract. The high-fat diet (HFD) contained 10% egg yolk powder, 16% lard, 71% rat chow, 2% cholesterol and 1% cholic acid. Forty-two male rats were fed various diets for three weeks after being randomly assigned to seven experimental groups of six rats.

Group I (normo-lipidemic control): received a standard diet and daily gavaged with distilled water.

Group II (hyper-lipidemic control): fed HFD and gavaged with distilled water.

Group III (*Majhoul* group): received an HFD and *Majhoul* date fruit extract at a 600 mg/Kg BW dose.

Group IV (*Majhoul* group): received an HFD and *Majhoul* date fruit extract at a 1200 mg/Kg BW dose.

Group V (*Bousrdoun* group): fed HFD and *Bousrdoun* date fruit extract at a 600 mg/Kg BW dose.

Group VI (*Bousrdoun* group): fed HFD and *Bousrdoun* date fruit extract at a 1200 mg/Kg BW dose.

Group VII (Standard group): fed HFD and simvastatin at a 15 mg/Kg BW dose.

For three weeks, simvastatin and the extracts were dissolved in distilled water before being taken orally once a day at 9 a.m. At the end of the experiment, rats were anesthetized with light diethyl-ether. Blood samples were taken from the retro-orbital sinus using glass capillaries, centrifuged for 10 min at 2500 rpm to separate the serum, and then used for biochemical analysis.

4.7. Determination of Serum Lipids Levels

The levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were measured using enzymatic assay kits from DiaSys (Germany) and Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

The atherosclerosis index (AI) was calculated as follows: AI = (total cholesterol − HDL cholesterol)/HDL cholesterol [11].

4.8. Statistical Analysis

The data were analyzed using StatView 5.0 software (SAS Institute Inc., Cary, NC, USA). One-way ANOVA followed by a post hoc Bonferroni test was performed to determine significant group differences. Differences at $p < 0.01$ are considered significant. Data were represented as the average of six repetitions for all the experiments ± standard error of the means (SEM).

5. Conclusions

The current research demonstrates the lipid-lowering effect of *Majhoul* and *Bousrdoun* date varieties, as they prevent the elevation of serum TG, TC and LDL-C levels in rats fed a high-fat diet and in rats treated for hyperlipidemia caused by triton WR-1339. These date extracts have shown a powerful ability to suppress lipid peroxidation. The lipid-lowering effects could be due to their high content of phenols, mainly gallic, ferulic, caffeic and p-coumaric acids, as well as rutin. Therefore, our results suggest that the consumption of *Majhoul* and *Bousrdoun* date fruits may contribute to reducing dyslipidemic disorders. Further research is needed to better explain the possible mechanism of action of this lipid-lowering effect at the molecular and cellular levels.

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