Research Article

Hypolipidemic Effect of Hemp Seed Oil from the Northern Morocco Endemic Beldiya Ecotype in a Mice Model: Comparison with Fenofibrate Hypolipidemic Drugs

Imane Mokhtari,1 Yassine Taaifi,2 Mohamed Harnafi,1 Kamal Belhaj ,3 Farid Mansouri ,2 Reda Melhaoui ,2 Mohamed Addi ,2 Christophe Hano ,4 Souliman Amrani,1 Hicham Harnafi,1 and Ahmed El Amrani2

1Laboratory of Bioresources, Biotechnologies, Ethnopharmacology and Health, Faculty of Sciences, University Mohammed First, Oujda 60000, Morocco
2Laboratory of Agricultural Production Improvement, Biotechnology and Environment, Faculty of Sciences, University Mohammed First, Oujda 60000, Morocco
3Higher School of Technology-Sidi Bennour, Chouaib Doukkali University, El Jadida, Morocco
4Laboratoire de Biologie des Ligneurs et des Grandes Cultures, INRAE USC1328, Campus Eure et Loir, Orleans University, Chartres 28000, France

Correspondence should be addressed to Christophe Hano; hano@univ-orleans.fr

Received 17 March 2022; Revised 16 May 2022; Accepted 21 May 2022; Published 16 June 2022

1. Introduction

Cannabis sativa L. is an annual plant belonging to the family of Cannabinaceae, widely spread in several countries of the world, especially in Asian countries, Canada, the United States (US), Europe, and Africa [1]. The ecotype “Beldiya” is an herbaceous crop endemic to the Rif region of Morocco [2]. It has been traditionally cultivated for centuries and is adapted to its natural and cultural environment. This ecotype is characterized by its high tolerance to biotic and abiotic stress and its capacity to be cultivated in a region with low and irregular rainfall [2]. It is one of the oldest herbs cultivated to provide nutritional and medicinal benefits and a versatile crop that can be cultivated for production of fiber,
seed, and oil [3–5]. Hemp seed oil is deep green oil rich in essential fatty acids, tocopherols, and other secondary metabolites [6–8]. It is characterized by a high amount of unsaturated fatty acids with respect to saturated ones and has a perfect ratio of \(\omega - 6\) linoleic fatty acid (18:2n6) to \(\omega - 3\) α-linolenic fatty acid (18:3n3) [9, 10]. This ratio of 3:1 is optimal for healthy human nutrition and confers a high nutritional value to the hemp oil. Further, the presence of micronutrients such as tocopherols particularly \(\gamma\)-tocoph-
erols, polyphenols, and carotenoids with antioxidant, anti-
flammatory, and plasma lipid-lowering activities increases the health benefits of the oil [9].

Cardiovascular diseases (CVD) represent the main cause of morbidity and mortality in many developed and developing countries [11, 12]. The most common underlying cause of onset and complication of such pathologies is hyperlipidemia [13]. Many nutritional reports have recently recommended the intake of edible vegetable oils to provide improvement of lipid metabolism and significantly reduce the risk of CVD complications [14, 15]. In fact, it is reported that numerous oils such as Moroccan argan oil decreases plasma cholesterol and triglycerides [16]. Furthermore Oladapo et al. [17] demonstrated the beneficial effect of palm, soya, and olive oils on cardiometabolic health regarding total cholesterol, HDL-cholesterol, non-HDL cho-

High cholesterol levels are a significant risk factor for 
CVD complications [14, 15]. In fact, it is reported that numerous oils such as Moroccan argan oil decreases plasma cholesterol and triglycerides [16]. Furthermore Oladapo et al. [17] demonstrated the beneficial effect of palm, soya, and olive oils on cardiometabolic health regarding total cholesterol, HDL-cholesterol, non-HDL cholesterol, and atherogenic index. On the other hand, it is showed that a blend of soy oil, sunflower oil, and non-
conventional flaxseed oil significantly decreased atherogenic circulation lipid including total cholesterol, triacylglycerol, and low-density lipoprotein in high-fat diet-fed rats [18].

So, to demonstrate the beneficial impact of these oils on cardiometabolic health, many studies were conducted to show their hypolipidemic and antiatherogenic properties in different experimental models such as Wistar and Meriones shawi rats [14, 16, 19]. However, no experimental study has been undertaken in Morocco to demonstrate the possible beneficial effect of hemp oil in this field. The TritonWR-1339 mice model is widely used to screen hypocholesterolemic compounds were quantified by external calibration using standard tocopherol compounds obtained from Sigma-Aldrich (Stein-
heim, Germany).

2.4. Total Phenols and \(\beta\)-Carotene Quantification. The amount of total phenols was determined by the Folin–Ciocalteu spectrophotometric method (spectrophotometer, RAYLEIGH UV1800, UV-Visible) according to the procedure described by Moumen et al. [22]. The \(\beta\)-carotene content was determined spectrophotometri-
cally, and the results were expressed with reference to a standard curve established from a range of \(\beta\)-carotene concentrations (Sigma-Aldrich, St-Louis, MO, USA) as previously described [23].

2.5. Experimental Animal Design. Forty albinos’ mice bred in the animal house of the Faculty of Sciences (Oujda, Mor-
rocco) and weighing 30 g were fasted overnight and divided randomly into five groups of eight animals each; the first served as the control group (CG) which received 0.5 ml of 9% NaCl by intraperitoneal injection and gavaged with

July 2020. The collected samples were cleaned by hexane to remove \(\Delta 9\)-THC [15], which could have contaminated them during the harvest, and stored at \(\pm 2^\circ C\) in plastic containers sealed until use. The oil was prepared by triturating in an oil screw press (KOMET Model DDB85G); the speed was 70 RPM with a temperature of 100°C. The fine particles and debris were removed by centrifugation at 3000 RPM for 15 min. The obtained hemp oil samples were stored at 4°C until use.

2.2. Fatty Acid Analysis of “Beldiya” Hemp Seed Oil. Fatty acids were converted to fatty acid methyl esters (FAMEs) before analysis by gas chromatography coupled with a flame ionization detector (GC Agilent 6890, Agilent Technologies) as previously described [21]. The samples (1 \(\mu L\) in spirtless mode) were separated on a BPX70 capillary column (60 m length, 0.32 mm internal diameter, and 0.25 \(\mu m\) film thickness; SGE Europe). Helium was used as the carrier gas at a flow rate of 1 ml·min\(^{-1}\). The initial oven temperature was 50°C and then it was increased at a rate of 30°C·min\(^{-1}\) to 170°C, followed by an increase of 4°C·min\(^{-1}\) to 220°C. This temperature was maintained for 10 min.

The results were expressed in percentage, after the identification of fatty acids by comparison with those of standards from Sigma-Aldrich containing 37 FAMEs (Supelco, Bellefonte PA, USA).

2.3. Tocopherols Analysis. The tocopherol compounds were analyzed by HPLC-DAD (Agilent Technologies Series 1200 System) according to the AOCS Method Ce 8–89 (AOCS, 1989) [22]. The samples were dissolved in hexane and separated in an Uptisphere NH2 column (150 mm × 3 mm, 3 \(\mu m\) using hexane/2-propanol (99:1, v/v) as an isocratic mobile phase. The flow rate was 1 ml/min and temperature was set at 30°C. The detection of separated compounds was carried out at 292 nm using an ultraviolet detector. The compounds were quantified by external calibration using standard tocopherol compounds obtained from Sigma-Aldrich (Stein-

2.1. Cold Extraction of Hemp Seed Oil. Hemp seeds of Cannabis sativa L. “Beldiya” ecotype were collected in the region of Tamrot (Rif, Northern Morocco) at the maturity in

2. Material and Methods

2.1. Cold Extraction of Hemp Seed Oil. Hemp seeds of Cannabis sativa L. “Beldiya” ecotype were collected in the region of Tamrot (Rif, Northern Morocco) at the maturity in
3. Results and Discussion

3.1. Fatty Acid, Tocopherol, β-Carotene, and Total Phenol Content of “Beldiya” Hemp Seed Oil. The analysis of the hemp oil fatty acids (Table 1) shows that linoleic acid (48.85%) is the main fatty acid followed by oleic acid (21.82%), α-linolenic acid (14.17%), and palmitic acid (8.03%). It is also noted from several studies that the presence of γ-linolenic acid (0.55%) has beneficial effects on human health [23]. These results are widely comparable to that of other hemp seed oils previously reported in the literature [4, 7, 25]. In addition, the balance of saturated, monounsaturated, and polyunsaturated fatty acids brings out values of 12.46%, 23.71%, and 63.83%, respectively. In comparison with cannabis seed oils from Morocco or other origins described in the literature, no discrepancies were found [15].

On the other hand, the polyunsaturated fatty acid/saturated fatty acid ratio of this oil was evaluated at 5/1; this fairly high ratio could be favorable for reducing hyperlipidemia and preventing atherosclerosis [14, 26]. Finally, the ω6/ω3 ratio of our oil was estimated at 3.48 which is very close to those reported by previous works studying cannabis oil from different regions [10, 15, 25, 27, 28]. According to the nutritional recommendations, this ratio lower than 5 could be ideal and nutritionally beneficial compared to those given by the current alimentation ranged between 10 and 30 [23, 29].

Minor oil fractions analysis (Table 2) showed a high content of total tocopherol (498.64 mg·kg⁻¹ oil) with a dominance of α-tocopherol (456 mg·kg⁻¹ oil). The hemp seed oil is rich in β-carotene (17.73 mg·kg⁻¹ oil) and total phenols (39.02 mg·kg⁻¹ oil).

The amounts obtained are within the range of values usually found in the literature [15]. This richness in antioxidants, especially, γ-tocopherol, could constitute a considerable asset in the use of Moroccan hemp oil as an important source of natural tocopherols as well as a protective agent against oxidation [30, 31]. Also, during the last 25 years, research has revealed that γ-tocopherol has antioxidant and anti-inflammatory activities relevant to disease prevention compared to α-tocopherol [32].

3.2. Hypolipidemic Effect of Hemp Seed Oil. The induction of hyperlipidemia in animal models by using the nonionic surfactant Triton WR-1339 is a strategy widely used in experimental studies in order to search for novel hypo-lipidemic substances [24]. This surfactant can inhibit the enzymatic activity of lipoprotein lipase (LPL) and lecithin cholesterol acyl transferase (LCAT), resulting in the accumulation of plasma total cholesterol, triglycerides, and very low-density lipoprotein [24]. The concentrations of plasma total cholesterol and triglyceride in Triton-treated mice and controls are summarized in Figure 1. As can be seen, in comparison with the normal control group, Triton WR-1339 caused a marked increase in cholesterol and triglyceride levels measured 24 h after injection. The plasma total cholesterol was increased by 234% (P < 0.001) and triglycerides by more than 660% (P < 0.001). After 24 h, the
administration of hemp seed oil in Triton-injected mice at a dose of 3.5 mg/kg, respectively, decreases both plasma total cholesterol and triglycerides by 45.7% and 82% \((P < 0.001)\), with respect to the hyperlipidemic group. At 7 mg/kg, the hemp seed oil reduced plasma total cholesterol by 57% and triglycerides by 87% \((P < 0.001)\) (Figure 1).

The LDL and HDL-cholesterol levels are reported in Figure 2. When the Triton-injected group was compared with control, we observed that the LDL cholesterol was increased by 207% \((P < 0.001)\). While the HDL cholesterol was not significantly hindered, LDL cholesterol was reduced by 61.7% \((P < 0.001)\) and HDL cholesterol increased by 324% \((P < 0.001)\) after 24 h of the administration of hemp seed oil (Figure 2).

After 24 h, a significant increase was noticed in the atherogenic index (117%, \(P < 0.001\)) and LDL/HDL ratio (111%, \(P < 0.001\)) (Figure 3). In addition, the hypolipidemic effect of the studied oil resulted in a significant decrease of the atherogenic index (−50.2%) and LDL/HDL ratio (−90%) compared to the hyperlipidemic control (Figure 3). Furthermore, the LDL cholesterol was 80% reduced and HDL cholesterol 380% increased comparatively to mice received Triton alone \((P < 0.001)\). As a consequence, the atherogenic index was reduced by 66% and LDL/HDL ratio by 95%. Thus, from these results, it appears clearly that the hypolipidemic effect of the hemp oil is dose-dependent and affects all lipid parameters which represent a high-risk factor for atherosclerosis and related cardiovascular diseases.

In fenofibrate-treated mice at a dose of 3.5 mg/kg, similar patterns of change were observed. Indeed, total cholesterol was lowered by 61.7% and triglycerides by 87% (Figure 1). Furthermore, the LDL cholesterol was significantly reduced (−87.4%) and HDL cholesterol raised (+408%) in comparison to the hyperlipidemic group (Figure 2). This results in a significant decrease in the atherogenic index (−84.6%, \(P < 0.001\)) and LDL/HDL ratio (−97%, \(P < 0.001\)) (Figure 3). It is noted that the hypolipidemic effect of fenofibrate is relatively more marked compared to that of hemp seed oil especially concerning total cholesterol and its LDL fraction. However, the oil can be included in diet as a functional food unlike drugs which can only be taken for therapeutic purposes. Furthermore, the oil is a natural product with ordinary fatty acids, tocopherols, and polyphenols which could be safer compared to synthetic drug fenofibrate.

The hypocholesterolemic effect of the studied hemp seed oil was associated with a significant drop in the atherogenic LDL fraction considered for a long time as the main

### Table 1: Fatty acids’ composition of hemp seed oil (Beldiya ecotype) cultivated in the Tamrote region (north of Morocco).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>(g/100 g fatty acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>C16:0</td>
<td>8.03 ± 0.04</td>
</tr>
<tr>
<td>C16:1n7</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>C16:1n9</td>
<td>0.13 ± 0.00</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>C17:1</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.13 ± 0.02</td>
</tr>
<tr>
<td>C18:1n9</td>
<td>21.82 ± 0.12</td>
</tr>
<tr>
<td>C18:1n7</td>
<td>1.24 ± 0.04</td>
</tr>
<tr>
<td>C18:2n6</td>
<td>48.85 ± 0.08</td>
</tr>
<tr>
<td>C18:3n6</td>
<td>0.55 ± 0.01</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>14.17 ± 0.08</td>
</tr>
<tr>
<td>C18:4n3</td>
<td>0.07 ± 0.00</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>C20:2n6</td>
<td>0.19 ± 0.00</td>
</tr>
<tr>
<td>C20:1n9</td>
<td>0.45 ± 0.00</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>SFA</td>
<td>12.46 ± 0.05</td>
</tr>
<tr>
<td>UFA</td>
<td>87.54 ± 0.05</td>
</tr>
<tr>
<td>MUFA</td>
<td>23.71 ± 0.14</td>
</tr>
<tr>
<td>PPUA</td>
<td>63.83 ± 0.14</td>
</tr>
<tr>
<td>n−6</td>
<td>49.60 ± 0.08</td>
</tr>
<tr>
<td>n−3</td>
<td>14.24 ± 0.08</td>
</tr>
<tr>
<td>n−6/n−3</td>
<td>3.48 ± 0.02</td>
</tr>
</tbody>
</table>


### Table 2: Tocopherol, total phenol, and carotene contents of hemp seed oil (Beldiya ecotype) cultivated in the Tamrote region (north of Morocco).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>23.31 ± 2.22(^a)</td>
</tr>
<tr>
<td>β-tocopherol</td>
<td>0.20 ± 0.01(^a)</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>456.24 ± 18.80(^a)</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>18.89 ± 0.85(^a)</td>
</tr>
<tr>
<td>Total tocopherols</td>
<td>498.64 ± 21.07(^a)</td>
</tr>
<tr>
<td>Seed total phenols</td>
<td>191.20 ± 1.94(^b)</td>
</tr>
<tr>
<td>Oil total phenols</td>
<td>39.92 ± 4.08(^b)</td>
</tr>
<tr>
<td>β-carotene</td>
<td>17.73 ± 0.38(^b)</td>
</tr>
</tbody>
</table>

\(^a\)mg/kg oil, \(^b\)mg gallic acid equivalent/100 g seeds, \(^c\)mg gallic acid equivalent/kg oil, \(^d\)mg/kg oil.

*Figure 1: Effect of hemp seed oil on plasma cholesterol and triglycerides in Triton WR-1339-induced hyperlipidemic mice. With (CG: control group; HG: hyperlipidemic group; HOG: hemp oil-treated group; FTG: fenofibrate-treated group; TC: total cholesterol, TG: triglycerides. \(* P < 0.001\) (HG vs CG and treated groups vs HG)).
the liver via HDL and its elimination in the form of bile returning the excess cholesterol from peripheral tissues to prevents fat deposition in the vessel endothelium by suggesting that the hypocholesterolemic effect of hemp seed oil signifi cantly reduced plasma triglycerides levels. This suggests that the oil could restore metabolism of triglycerides via the activation of endothelial lipoprotein lipase involved in the hydrolysis of VLDL at a tissue level. This mechanism was previously proposed by others when studying the hypolipidemic effect of natural products [24, 35]. Furthermore, the administration of hemp seed oil to the hyperlipidemic mice results in a signifi cant decrease of the atherogenic index and LDL/HDL ratio which are closely related to the hypocholesterolemic effect. Our fi ndings concord with several studies conducted with vegetable oils and natural substances [17, 37, 38]. This could confi rm the benefi cial effect of the studied oil on lipid metabolism and related atheromatous diseases.

So, it is recently reported that intake of polyunsaturated fatty acids (PUFAs) decreased total cholesterol, triglycerides, LDL-cholesterol, VLDL, apoprotein B, and increased HDL-cholesterol by increasing liver X receptor alpha (LXRα) gene transcription via peroxisome proliferator-activated receptors (PPARs) [39, 40]. LXRα increases the expression of cholesterol 7α-hydroxylase (CYP7), which converts cholesterol to bile acids; therefore, by stimulating CYP7 activity, PUFAs help to catabolize cholesterol [39]. Furthermore, dietary PUFAs may exert their LDL-C-lowering properties by increasing membrane fi uidity, which increases LDL receptor activity and increases LDL catabolism [26, 41]. Concerning triglyceride metabolism, it is suggested that such fatty acids act by decreasing VLDL secretion from the liver and by stimulating lipoprotein lipase, which hydrolyzes triglycerides from chylomicrons and VLDLs [39, 42, 43]. In addition, the $\omega-6/\omega-3$ ratio of the studied hemp oil was estimated at 3.48 lower than 4, which corresponds to the nutritional recommendations [43]. Minor oil fractions analysis showed high content of total tocopherol with a dominance of $\beta$-tocopherol. The oil also contains $\beta$-carotene and phenols. These compounds could prevent lipoprotein structural alteration, contributing to their normal metabolism [30].

4. Conclusion

It was concluded that the “Beldiya” hemp seed oil, native ecotype of cultivated in Morocco, could be considered as a prominent source of polyunsaturated fatty acids characterized by high nutritional and functional values. Besides, its richness in antioxidants, especially $\gamma$-tocopherol, could constitute a considerable asset in use of the oil as an important source of natural tocopherols as well as a protective agent against lipoprotein oxidation and prevention of atherosclerosis. This work could contribute as an idea to the
reorientation of exploitation of cannabis in Morocco for the production of commercial value-added oil instead of drugs.

Data Availability
All the data supporting the findings of this study are included in this article.

Ethical Approval
All applicable international and national guidelines for the care and use of animals were followed.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Acknowledgments
This work was a part of a project funded by the CNRST (Centre National pour la Recherche Scientifique et Technique, Maroc), the UMP (Université Mohamed Premier, Oujda, Maroc), the ANPMA (Agence Nationale des Plantes Medicinales et Aromatiques, Maroc), Conseil Départemental d’Eure et Loir, and the Région Centre-Val de Loire

References


