Chapter 60

Cold pressed rosemary (*Rosmarinus officinalis*) oil

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Abbreviations

CERs	cerebrosides
CK	creatine phosphokinase
CPRO	cold pressed rosemary (*Rosmarinus officinalis*) oil
DG	diacylglycerol
DGDs	digalactosyldiglycerides
DM	diabetes mellitus
DPPH$^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl
ESGs	esterified sterylglycosides
FBG	fasting blood glucose
FFA	free fatty acid
GC-FID	gas chromatography-flame ionization detector
HDL-C	high dentistry lipoprotein-cholesterol
HPLC	high-performance liquid chromatography
LDL-C	low dentistry lipoprotein-cholesterol
MG	monoacylglycerol
MGDs	monogalactosyldiglycerides
MIC	minimum inhibitory concentration
REO	rosemary essential oil
RSA	radical scavenging activity
SCF	supercritical fluid
SFA	saturated fatty acid
SQD	sulphoquinovosyldiacylglycerol
STEs	esterified sterols
STZ	streptozotocin
T1DM	DM type 1
T2DM	DM type 2
TAG	triacylglycerol
TC	total cholesterol
TGs	total triacylglycerols
TLs	total lipids

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TPCs total phenolic compounds
VLDL-C very low dentistry lipoprotein-cholesterol
WHO World Health Organization

1 Introduction

Medicinal and aromatic plants are rich in bioactive phytochemicals, which are used in nutraceuticals, functional foods, and pharmaceuticals with biological properties to promote health (Al-Kalaldeh, Abu-Dahab, & Afifi, 2010; Albano & Miguel, 2011; Elbanna et al., 2014; El-Hadary & Ramadan, 2019; Ramadan, Amer, & Awad, 2008; Ramadan & Elsanhoty, 2012; Ramadan, Sharanabasappa, Seetharam, Seshagiri, & Moersel, 2006). Rosemary (*Rosmarinus officinalis*, family Lamiaceae) is a perennial herb and native Mediterranean shrub (El-Naggara, Abdel-Farid, Germoush, Elgebaly, & Alm-Eldeen, 2016; Elbanna et al., 2018). Andrade et al. (2018) showed an interest in *R*. officinalis, with about 120 studies every year since 2010. Sadeh et al. (2019) highlighted the impact of production process and the genetic variation on *R*. *officinalis* oil composition. They reflected the importance of studying the impact of genetic and environmental factors and processing on oil composition for industrial breeding and oil production. Sarmoum et al. (2019) investigated the impact of water stresses and salinity on the constituents of *R*. *officinalis* essential oil (REO). *R*. *officinalis* plants were subjected to tap water, salt water, and without irrigation. Nonirrigated plants contained the highest oil yield. Differences in the oil constituents were highlighted in relation to water stress.

R. *officinalis* extracts and REO are in edible applications as preservatives and for treating some diseases (Elbanna et al., 2018; Olmedo, Nepote, & Grosso, 2013; Wollinger et al., 2016). *R*. *officinalis* extracts are natural sources of antioxidants (Commission Regulation (EU) no. 1130/2011, 2011; Ojeda-Sana, Baren, Elechosa, Juárez, & Moreno, 2013; Yang et al., 2016), wherein the antioxidant potential has been attributed to phenolic diterpenes (Gallego, Gordon, Segovia, Skowyra, & Almajano, 2013). Bioactive constituents of REO are 1,8-cineole, camphor, carnosic acid, and rosmarinic acid (Borges, Ortiz, Pereira, Keita, & Carvalho, 2019; Terpinc, Bezjak, & Abramovic, 2009). REO exhibited antibacterial (Ojeda-Sana et al., 2013), antifungal (Soylu, Kurt, & Soylu, 2010), and hepatoprotective (Amin & Hamza, 2005) properties. *R*. *officinalis* extracts showed hepatoprotective effects against hepatotoxic agents such as t-BHP (Joyeux, Roland, Fleurentin, Mortier, & Dorfman, 1990), CCl_4 (Fahim, Esmat, Fadel, & Hassan, 1999), and cyclophosphamide (Fahim et al., 1999). In addition, *R*. officinalis showed a protective impact against Azathioprine-induced liver damage in animals and blocked serum high levels of alanine aminotransferase and aspartate aminotransferase (Amin & Hamza, 2005). Extract from *R*. officinalis leaves mitigated cyclophosphamide-induced (El-Naggara et al., 2016) and creosote-induced (El-Demerdash, Abbady, & Baghdadi, 2016) hepatotoxicity in rats. *R*. *officinalis* oleoresin was used to develop stabile vegetable oil blends used for frying (Upadhyay, Sehwag, & Mishra, 2017; Yang et al., 2016).

Crude extracted oils are rich sources of bioactive compounds, such as phenolics, tocols, phytosterols, and fatty acids with health-promoting and functional properties (Assiri, Elbanna, Abulreesh, & Ramadan, 2016; Kiralan et al., 2017; Ramadan, Asker, & Tadros, 2012). Current interest in environmentally friendly technologies has resulted in a huge international market of natural products (Ibrahim, Attia, Maklad, Ahmed, & Ramadan, 2017). Cold pressing is popular due to the high levels of bioactives in the recovered oil. Cold pressing is an environmentally safe and simple technique that requires no chemical or thermal treatments (Assiri, Elbanna, Al-Thubiani, & Ramadan, 2016; El-Hadary & Ramadan, 2016a; Ramadan, 2013). Some cold pressed oils showed a protective impact against CCl_4-induced hepatotoxicity in rats (El-Hadary & Ramadan, 2016a, 2016b).

This chapter reviews the lipids profile, phenolics content, and antioxidant, antimicrobial, antidiabetic, and hepatoprotective properties of cold pressed rosemary (*R*. *officinalis*) oil (CPRO).

2 Extraction and processing of cold pressed *R. officinalis* oil

Ali, Chua, and Chow (2019) reviewed the history, chemical profile, and analysis of *R*. *officinalis* extraction technologies. The execution of extraction methods is endless because they stretch from conventional (maceration, Soxhlet, microwave distillation) to developed technologies (supercritical fluid extraction, ultrasound-assisted, pressurized liquid). Carnosol, carnosic acid, and rosmarinic acid, the major markers of bioassays with the highest activities in drugs, were the main compounds investigated. To produce fractions rich in those bioactive compounds, pressurized liquid and SCF extraction followed by supercritical antisolvent fractionation are among the most tested methods. With the development of novel techniques, extracting plant bioactive phytochemicals according to the desired applications is possible. The major bioactive

compounds in the *R. officinalis* volatiles are 1α-pinene, 8-cineole, and camphor, while bioactive compounds in the non-volatile extract are carnosol, carnosic acid, and rosmarinic acid.

3 Acyl lipids and fatty acid profile of cold pressed *R. officinalis* oil

3.1 Lipid classes

Neutral lipids (NL), phospholipids (PL), and glycolipids (GL) represent the main lipid classes in the most of crude vegetable oils. The proportions of lipid classes in CPRO are shown in Table 1 (Elbanna et al., 2018). In the CPRO, NL fraction was the highest, followed by PL and GL. Triacylglycerol (TAG), diacylglycerol (DG), free fatty acids (FFAs), monoacylglycerol (MG), and esterified sterols (STEs) were the main NL classes. Classes of GL were sterylglycosides (SGs), sulphoquinovosyldiacylglycerol (SQD), digalactosyldiglycerides (DGDs), monogalactosyldiglycerides (MGDs), cerebrosides (CERs), and esterified sterylglycosides (ESGs). The main PL subclasses were phosphatidylcholine, followed by phosphatidylethanolamine. Polar lipids (GL and PL) in crude vegetable oils exhibited antiradical and antioxidant traits (Ramadan, 2008, 2012; Ramadan and Asker, 2009).

3.2 Fatty acids

Fatty acid profiles of the R. officinalis total lipids (TL) and lipid classes in CPRO are given in Table 2 (Elbanna et al., 2018). Lipid classes have a similar fatty acid composition, whereas linoleic (C18:2) and oleic (C18:1) acids were the major acids. Palmitic (C16:0) and stearic (C18:0) acids were the major identified saturated fatty acids (SFAs). The amounts of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), and SFAs were 42.30%, 41.70%, and 15.80%, respectively. The U/S ratio of CPRO was 5.3, and CPRO might be included into oleic/linoleic oils group. The CPRO fatty acids profile was similar to that of pumpkin, sunflower, and maize oils (Tuberoso, Kowalczyk, Sarritzu, & Cabras, 2007). MUFA levels were comparable to cranberry, blueberry, hemp, and onion cold pressed oils (Parker, Adams, Zhou, Harris, & Yu, 2003; Parry et al., 2006). High levels of PUFAs and MUFAs make CPRO a valuable oil in human nutrition (Elbanna et al., 2018).

4 Minor bioactive lipids in cold pressed *R. officinalis* oil

Tocols are inhibitors of free radical chain reactions and delay lipids oxidation (Hassanien et al., 2014Parry et al., 2006). CPRO contained high amounts (25 g/kg oil) of unsaponifiables. α-, β-, γ-, and δ-tocopherols in CPRO accounted for 291, 22, 1145, and 41 mg/100 g CPRO, respectively. Levels of α-, β-, γ-, and δ-tocotrienols accounted for 18, 12, 29, and 158 mg/100 g CPRO, respectively (Elbanna et al., 2018). The major tocopherol homologue was γ-tocopherol (more than 66% of the tocols), followed by δ-tocopherol. According to the levels of tocochromanols (Hassanien et al., 2014), oils could be divided to oils with high α-tocopherol amounts (sunflower, almond, olive oil, hazelnut, and wheat germ) and oils with high γ-tocopherol amounts (pumpkin, black cumin, flaxseed, poppy, apricot kernel, and sesame). The most efficient antioxidant

TABLE 1 Neutral lipids (NL), glycolipids (GL), and phospholipids (PL) classes (g/kg total lipids) of CPRO.

NL class	Total lipids (g/kg)	GL class	Total lipids (g/kg)	PL class	Total lipids (g/kg)
MG	2.55	SQD	0.70	PS	1.20
DG	7.59	DGD	0.55	PI	2.10
FFAs	10.9	CER	3.26	PC	3.44
TG	833	SG	2.45	PE	2.50
STEs	6.88	MGD	0.33		
		ESG	1.55		

MAGs, monoacylglycerols; *DAGs*, diacylglycerols; *TAGs*, triacylglycerols; *FFAs*, free fatty acids; *STEs*, sterol esters; *SQD*, sulphoquinovosyldiacylglycerol; *DGD*, digalactosyldiacylglycerol; *CERs*, cerebrosides; *SG*, steryl glucoside; *MGD*, monogalactosyldiacylglycerol; *ESG*, esterified steryl glucoside; *PS*, phosphatidylserine; *PI*, phosphatidylinositol; *PC*, phosphatidylcholine; *PE*, phosphatidylethanolamine.
(Adapted from Elbanna, K., Assiri, A. M. A., Tadros, M., Khider, M., Assaeedi, A., Mohdaly, A. A. A., & Ramadan, M. F. (2018). Rosemary (*Rosmarinus officinalis*) oil: Composition and functionality of the cold-pressed extract. *Journal of Food Measurement and Characterization, 12*, 1601–1609.)

TABLE 2 Fatty acid profile of CPRO and lipid classes.

	CPRO	NL	GL	PL
C 10:0	0.07	0.07	0.06	0.07
C 12:0	0.04	0.03	0.02	0.02
C 14:0	0.09	0.05	0.03	0.03
C 16:0	9.10	8.95	8.40	8.90
C 16:1	0.54	0.53	0.55	0.54
C 18:0	6.55	6.41	5.50	5.96
C 18:1*n*-9	41.0	41.1	41.6	41.2
C 18:2*n*-6	41.1	41.4	41.9	41.7
C 20:1	0.25	0.26	0.38	0.28
C 18:3	1.21	1.20	1.50	1.30
ΣSFA	15.85	15.51	14.01	14.98
ΣMUFA	41.79	41.89	42.59	42.02
ΣPUFA	42.36	42.60	43.40	43.00
U/S	5.30	5.44	6.09	5.67
n-6/*n*-3	34.01	34.50	27.93	32.08

(Adapted from Elbanna, K., Assiri, A. M. A., Tadros, M., Khider, M., Assaeedi, A., Mohdaly, A. A. A., & Ramadan, M. F. (2018). Rosemary (*Rosmarinus officinalis*) oil: Composition and functionality of the cold-pressed extract. *Journal of Food Measurement and Characterization, 12,* 1601–1609.)

of tocols is α-tocopherol, while γ-tocopherol has 35% of the antioxidant impact of γ-tocopherol (Ramadan, 2013). The amounts of tocochromanols in CPRO suggest that it might effectively resist oxidation in vivo and in vitro.

5 Health-promoting properties of cold pressed *R. officinalis* oil and oil constituents

5.1 Antioxidant activity

Radical scavenging activity (RSA) of CPRO and extra virgin olive oil (EVOO) was evaluated against galvinoxyl and DPPH• free radicals (Elbanna et al., 2018). CPRO exhibited more RSA than EVOO (Fig. 1). After 60 min of incubation with DPPH• free radicals, 67% of free radicals were quenched by CPRO. In the course of 60 min, CPRO quenched 55% of galvinoxyl free radicals. CPRO contained higher total phenolics (7.2 mg GAE/g) than EVOO (3.6 mg GAE/g). The same study (Elbanna et al., 2018) reported that the induction period (IP) of CPRO and sunflower oil blend (1:9, *v*/*v*) was 390 min, while the IP of CPRO blended with sunflower oil (2:8, *v*/*v*) was 540 min. The antioxidant effect of CPRO was likely to be due to the high amounts of tocochromanols and phenolic compounds in the cold pressed oil.

5.2 Antimicrobial activity

Nowadays, there is an interest in use of natural preservatives in the production of foodstuffs. Rosemary extracts showed biological activities such as insecticide, antioxidant, hepatoprotective, antifungal, and antibacterial. The antimicrobial activity (AA) of CPRO was tested against dermatophyte fungi (*Trichophyton mentagrophytes*, and T*richophyton* rubrum), and food pathogens (*Salmonella enteritidis*, L*isteria* monocytogenes, and E*scherichia* coli). CPRO exhibited a broad AA spectrum (Table 3) against food-borne pathogens and dermatophyte fungi (Elbanna et al., 2018). The inhibition effect of CPRO against selected microorganisms is shown in Fig. 2. AA, measured as a clear zone diameter (CZD), was 29 mm, 17 mm, and 14 mm for E. coli, *S. enteritidis*, and L. monocytogenes, respectively. CPRO had activity against the pathogenic microorganisms with minimum inhibitory concentration (MIC) ranging from 160 to 320 μg/mL. CPRO exhibited a high AA compared to different antibiotics (Chloramphenicol, Flucoral, Augmentin, and Mycosat) as given in Table 3. On the other hand, CPRO did not inhibit *Staphylococcus* aureus growth. Dermatophytic fungi such as *T. rubrum* are anthropophilic

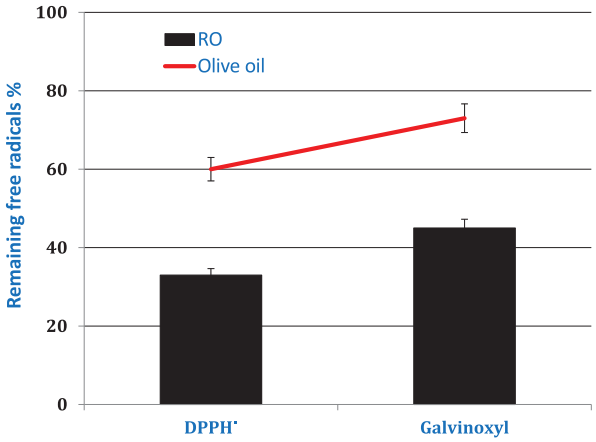

FIG. 1 Antiradical impact after 60 min of incubation for CPRO and EVOO on DPPH· and galvinoxyl free radicals. *(Adapted from Elbanna, K., Assiri, A. M. A., Tadros, M., Khider, M., Assaeedi, A., Mohdaly, A. A. A., & Ramadan, M. F. (2018). Rosemary (*Rosmarinus officinalis*) oil: Composition and functionality of the cold-pressed extract.* Journal of Food Measurement and Characterization, 12, *1601–1609.)*

and cause acute inflammatory tinea corporis, which is dermatophytosis of the arms and legs (Burmester et al., 2011). CPRO inhibited *T. mentagrophytes* and T. rubrum with CZDs of 25 mm and 23 mm, respectively, while the MLC for both fungi was 160 µg/mL. It was worth noting that the AA of CPRO against tested microorganisms kept the same CZD for long time (more than 10 days). The AA of CPRO might be due to the presence of tococls and phenolics in CPRO (Elbanna et al., 2018). The proposed mode of action of CPRO is that the cell walls and membranes of the pathogen microorganisms were damaged with the loss of cytoplasmic materials. A similar observation was reported by Carson, Mee, & Riley (2002), who showed the electron micrographs captured for the cell wall and membrane of *S. aureus* MRSA by the cold Valencia orange. Cell walls were damaged with the loss of cytoplasmic materials.

Burt (2004) and Sirocchi et al. (2013) found that the rosemary oil exhibited potential AA against both Gram-negative and Gram-positive bacteria including *Bacillus cereus*, *S. aureus*, *Clostridium perfringens*, *E. coli*, *Aeromonas hydrophila*, and *Salmonella choleraesuis*. Fung, Taylor, and Kahan (1977) mentioned that the inhibitory effect of the rosemary essential oil refers to the action of carnosic acid, rosmanol, carnosol, epirosmanol, rosmarinic acid, rosmaridiphenol, and isorosmanol on the cell membrane, causing changes in nutrients and genetic material and nutrients, leakage of cellular components, altering the transport of electrons, and changing the fatty acids. Furthermore, it produces an interaction with the membrane of proteins that causes the loss of membrane structure and functionality. Khezri, Farahpour, and Mounesi Rad (2019) evaluated the efficiency of rosemary essential oil loaded in the nanostructured lipid carrier (REO-NLC) on in vitro AA and in vivo infected wound healing in experimental animals. REO-NLC showed AA against *E. coli*, *Staphylococcus epidermidis*, *S. aureus*, *L. monocytogenes*, and *Psudomonas aeruginosa*. REO-NLCs reduced the rate of tissue bacterial colonization and wound size. In a recent study, Risaliti et al. (2019) reported that *R. officinalis* oil-loaded liposomes and exhibited significant AA, antiinflammatory, and antioxidant activities. These findings might lead to effective applications of CPRO as a natural antimicrobial agent to control food-borne and food spoilage pathogens.

5.3 Antidiabetic activity

Diabetes mellitus (DM) is the fast-growing chronic disease and one of the main disorders threatening human health (Assiri, El-Beeh, Amin, & Ramadan et al., 2017; Esteves et al., 2008; Hebi, Farid, Ajebli, & Eddouks, 2017; Wang et al., 2018). The WHO reported that DM patients by 2025 will reach 300 million (Jeszka-Skowron et al., 2014; Rahimi-Madiseh, Heidarian, Kheiri, & Rafieian-Kopaei, 2017; Sarfraz, Khaliq, Khan, & Aslam, 2017). DM is classified as type 1 (T1DM) and type 2 (T2DM), with T2DM accounting for about 95% of cases (Taslimi et al., 2018). Pathogenesis of T2DM involved resistance to insulin activities, an abnormality in glucose, and inadequate insulin secretion from β-cells (Achenbach, Bonifacio, Koczwara, & Ziegler, 2005; Goldstein, 2007).

El-Beeh et al. (2019) studied the ameliorative effect of CPRO against liver injury and genotoxic impacts in streptozotocin (STZ)-induced diabetic rats and offspring. Treatment with CPRO reduced the harmful impact of diabetes on the weight loss and caused an increase in the animal's body weight except of the animals in the control group. According to Wang et al. (2018), body weight reduction is one of the major markers of diabetic. On the other hand, CPRO supplementation decreased the impact of diabetes on the liver weight.

TABLE 3 Antimicrobial effects of CPRO recorded by clear zone diameter (CZD, mm) and minimal lethal concentration (MLC, μg/mL).[a]

	Food-borne pathogen bacteria								Dermatophytic fungi			
	S. enteritidis		*L. monocytogenes*		*E. coli*		*S. aureus*		*T. mentagrophytes*		*T. rubrum*	
	CZD	MLC	CZD	MLC	CZD	MLC	CZD	MLC	CZD	MLC	CZD	MLC
CPRO (100 μL)	17	320	14	160	29	320	nd	nd	25	160	23	160
Augmentin (30 μg)	28	Nd[b]	28	nd	30	nd	40	nd	nd	nd	nd	nd
Chloramphenicol (30 μg)	20	nd	22	nd	27	nd	25	nd	nd	nd	nd	nd
Flucoral (100 μg/mL)	nd	nd	nd	nd	nd	nd	nd	nd	35	nd	34	nd
Mycosat (100 μg/mL)	nd	nd	nd	nd	nd	nd	nd	nd	40	nd	38	nd

[a]*The diameter of the inhibition zone was measured as the clear area centered on the agar well containing the sample.*
[b]*nd: not determined.*
(Adapted from Elbanna, K., Assiri, A. M. A., Tadros, M., Khider, M., Assaeedi, A., Mohdaly, A. A. A., & Ramadan, M. F. (2018). Rosemary (*Rosmarinus officinalis*) oil: Composition and functionality of the cold-pressed extract. *Journal of Food Measurement and Characterization, 12*, 1601–1609.)

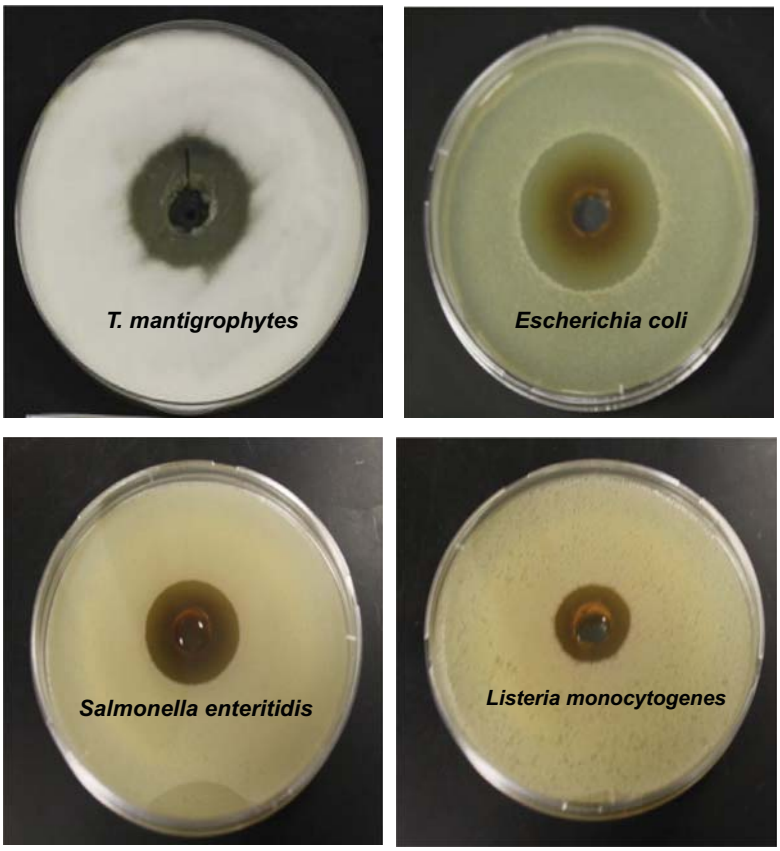

FIG. 2 Antimicrobial effect of CPRO against food-borne pathogens and dermatophyte fungi (Elbanna et al., 2018).

Liver histological graphs of maternal and offspring are shown in Fig. 3. DM rats showed degeneration of hepatic cells, congestion of blood vessels with an edematous central vein, as well as fatty degeneration. In addition, livers of offspring from DM animals showed degeneration of hepatic cords and vascularization of blood vessels, while DM animals treated with CPRO showed good recovery of histological characteristics (El-Beeh et al., 2019).

The serum biochemical parameters are given in Table 4. Group D contained high TC, TG, LDL-C, FBG, and CK as well as low HDL-C levels. Supplementation with CPRO reduced TC, TG, LDL-C, FBG, and CK levels. FBG reached the highest value (630 mg/dL) in DM animals, while supplementation with CPRO decreased the serum FBG. Table 5 represents the biochemical parameters of antioxidant enzymes, liver function, lipid oxidation, tumor markers, and free radicals of DM animals and offspring. DM increased alanine and aspartate transaminase. Liver bilirubin and albumen contents, as well as the activities of α-L-fucosidases and arginase, increased in DM animals. Antioxidant enzymes (i.e., superoxide dismutase, catalase, glutathione *S*-transferase, peroxidase, and reductase) showed a reduction in DM mothers and offspring. Fig. 4 shows photomicrographs from Comet assay for the livers. DM animals and offspring showed increased stretching of apoptotic cells, while in the CPRO-treated group, normal cell content was shown. DNA fragments were detected in the livers of DM animals and offspring, but there was some amelioration upon CPRO supplementation (El-Beeh et al., 2019).

Tocols are in vivo lipid radical scavengers and play a key role in the maintenance of cell membrane integrity (Gugliandolo, Bramanti, & Mazzon, 2017). Tocols maintain the membrane structure, limit lipid oxidation, and prevent inflammation in the neuroglia and hippocampal neurons (Galli et al., 2017). CPRO contained high levels of tocols and phenolics, which have unique antioxidative effects and play a role as antioxidant agents in treating DM and cardiovascular diseases (El-Beeh et al., 2019; Wang et al., 2018).

5.4 Hepatoprotective activity

El-Hadary, Elsanhoty, and Ramadan (2019) studied the hepatoprotective impact of CPRO against carbon tetrachloride (CCl_4)-induced toxicity in rats. Using concentrations of CPRO (100, 200, and 400 mg/kg, p.o.), only 400 mg/kg decreased the body weight. Based on a 24-h toxicity investigation, the LD_{50} of CPRO was 5780 mg/kg. The defensive aptitude of

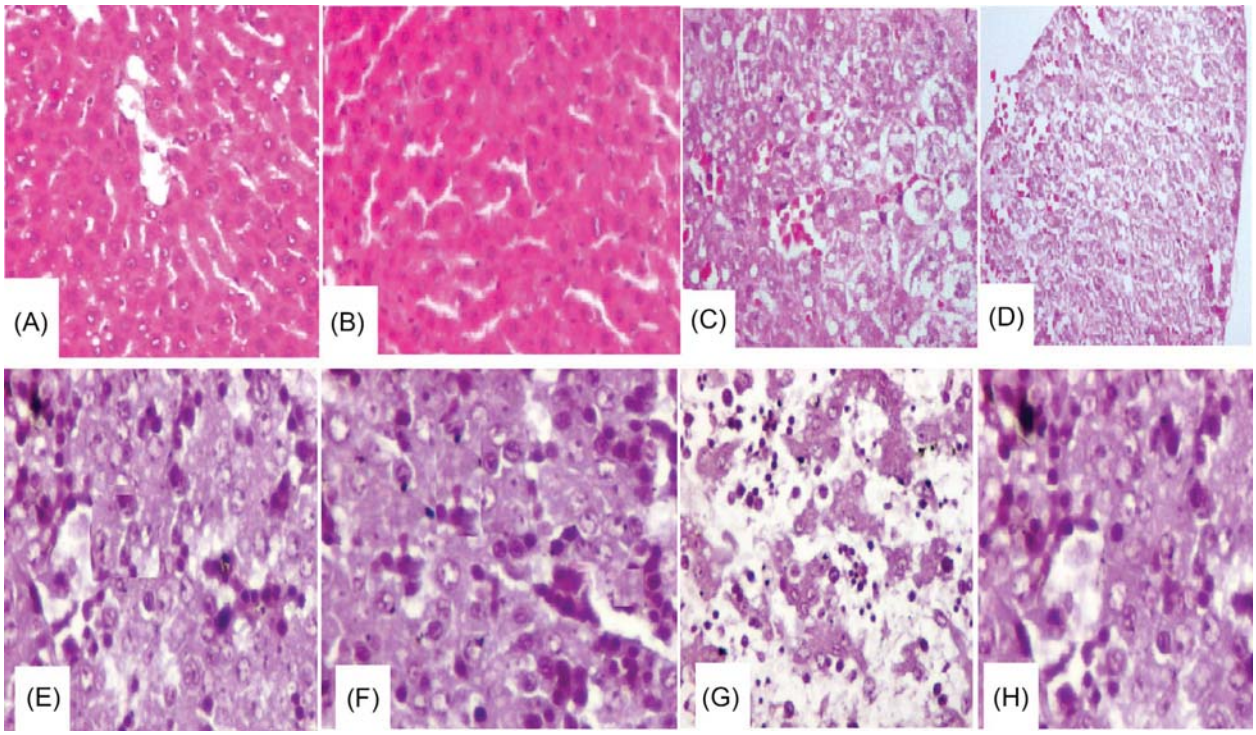

FIG. 3 Photomicrographs of transverse histological sections of maternal and offspring liver (sections stained with hematoxylin and eosin H&E × 400) (El-Beeh, Aljabri, Orabi, Qari, Ramadan, 2019). (A) Control group showing a normal arrangement of hepatic cells in cords around the central vein. (B) CPRO-treated group showing a normal arrangement of hepatocytes. (C) Experimental diabetic group (group D) showing degeneration of hepatic cells. (D) Experimental D-CPRO group. (E) Control pups liver showing normal hepatocytes. (F) Liver of pups maternally CPRO-treated group showing a normal arrangement of hepatocytes. (G) Liver of offspring group (D) showing degeneration of hepatic cells. (H) Liver of offspring of diabetic mothers treated with CPRO (D-CPRO) showing some degree of recovery.

TABLE 4 Biochemical profiles in the serum of nondiabetic and diabetic animals treated with CPRO (El-Beeh et al., 2019).

	FBG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	TC (mg/dL)	CK (U/L)
Control pregnant (C)	238	52	41	33	30	87
CPRO-treated group (CPRO)	245	57	40	32	34	88
Diabetic group (D)	630	110	39.2	52	45	348
Combined diabetic and CPRO-treated group (D-CPRO)	367	65	57.3	47	42	153

CPRO was validated by determining serum ALT, AST, and ALP. Serum markers levels were reduced upon CPRO supplementation at low dose. In addition, CPRO attenuated the increased levels of AST, ALT, and ALP enzymes, and caused a recovery toward normalization. Table 6 represents the CPRO impact on the protein profiles (albumin (A), globulin (G), A/G ratio, and total protein) of CCl_4-adminstrated animals. The impact of CPRO on the kidney function markers is also shown in Table 6. CCl_4 resulted in an increase in creatinine, urea, and uric acid levels, while CPRO administration decreased urea, creatinine, and uric acid amounts (El-Hadary et al., 2019). CPRO administration prevented the harmful impacts of CCl_4, indicating that CPRO might attenuate lipid per-oxidation resulted from CCl_4. Bioactive phytoconstituents might be the cause of CPRO protective potential. The mechanism of CPRO hepatoprotection might be due to its antioxidant activities. CPRO is rich in essential fatty acids. MUFAs were reported to reduce "bad" LDL-C and retain "good" HDL-C. CPRO also

TABLE 5 Antioxidant markers and enzymes levels in the livers of mothers and offspring in nondiabetic and diabetic rats treated with CPRO (El-Beeh et al., 2019).

	Mother				Offspring			
	C	CPRO	D	D-CPRO	C	CPRO	D	D-CPRO
MDA (nmol/g tissue)	10.47	10.27	19.24	12.37	11.37	10.37	22.4	12.87
H_2O_2 (mmol/g tissue)	166.09	156.19	380.03	199.65	164.0	154.0	231.6	195.6
SOD (U/g tissue)	19.25	19.2	10.48	17.37	25.35	25.35	12.88	15.3
CAT (U/g tissue)	362.08	362.01	323.20	330.24	379.1	379.7	333.87	360.86
GSH (mg/g tissue)	1.85	1.82	0.58	0.99	2.91	2.99	1.60	2.68
GST (U/g tissue)	87.51	86.51	144.8	113.6	81.99	80.09	368.33	220.93
GSPase (U/g tissue)	41.05	42.05	25.29	32.57	45.1	45.91	24.9	25.7
ALT (U/mL)	1.25	1.24	2.11	1.11	1.05	1.14	2.01	1.01
AST (U/mL)	29.7	30.5	45.85	33.99	28.79	31.5	44.85	35.99
Albumin (mg/dL)	12.43	13.3	23.56	15.29	13.43	13.9	25.56	14.29
Bilirubin (mg/dL)	1.02	1.05	1.4	1.31	1.52	1.55	1.04	1.30
Arginase (U/L)	4.08	4.0	50.6	10.75	4.8	3.9	45.6	10.75
α-L-fucosidase (U/L)	21.81	20.5	45.09	25.5	22.8	22.5	46.19	25.9

MDA, malondialdhyde concentration; *H_2O_2*, hydrogen peroxide; *SOD*, superoxide dismutase activity; *CAT*, catalase activity; *GSH*, glutathione reduced concentration; *GST*, glutathione-*S*-transferase activity; *GSPase*, glutathione peroxidase activity; *ALT*, alanine transaminase; *AST*, asparate transaminase.

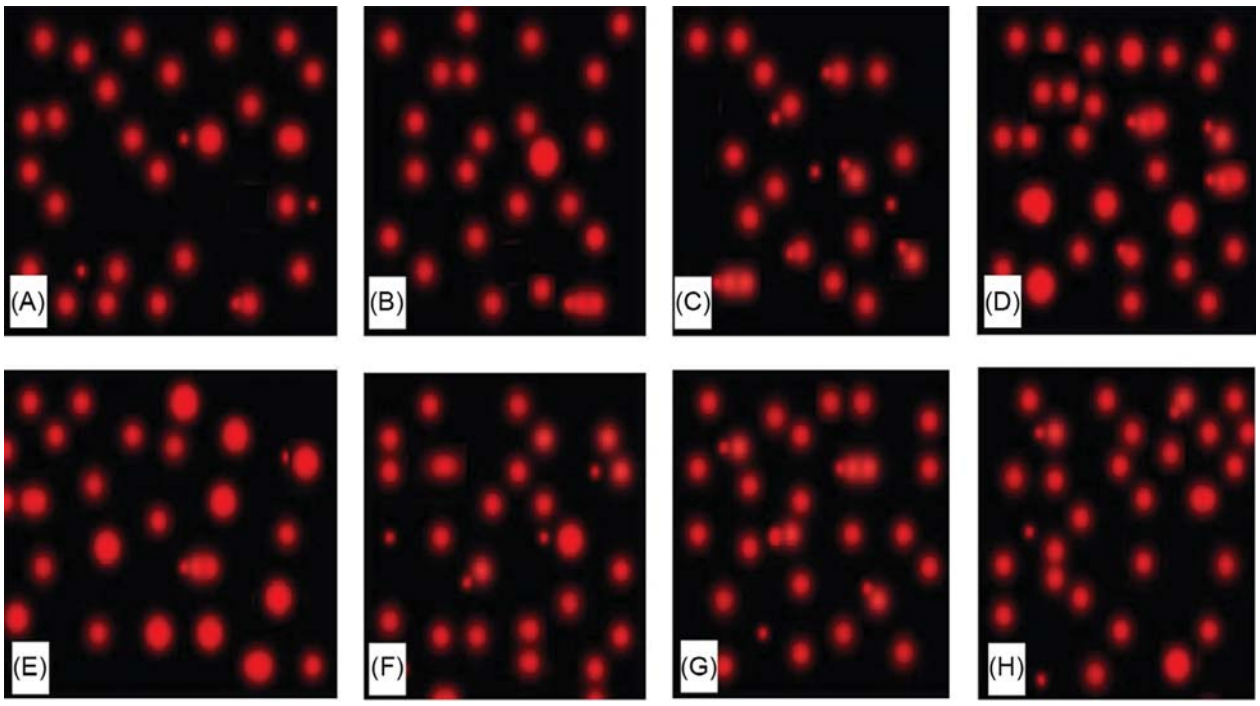

FIG. 4 Photomicrograph of Comet cells of the mothers and their offspring liver of rats treated with CPRO (El-Beeh et al., 2019). (A) Control group showing a normal structure of the liver cells. (B) RO-treated rats showing a normal structure of the liver. (C) Experimental diabetic group (D) showing increased stretching of apoptic cells. (D) Experimental D-CPRO group showing some amelioration. (E) Control offspring group showing a normal structure of the liver cells. (F) CPRO-maternally treated offspring group showing a normal structure of the liver. (G) Experimental maternally diabetic offspring (D) showing increased stretching of apoptic cells. (H) Experimental D-CPRO treated offspring group showing amelioration traits.

TABLE 6 Effect of CPRO on kidney function indicators and protein profile in CCl_4-induced injury in rats.

Treatment	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	T-protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio
Negative control (normal)	0.716	25.3	3.63	6.50	3.78	2.72	1.40
Positive control (CCl_4)	1.85	56.3	7.93	5.40	3.01	2.38	1.31
CPRO (100 mg/kg) + CCl_4	0.69	28.3	3.30	6.60	3.78	2.81	1.34
CPRO (200 mg/kg) + CCl_4	0.66	28.3	3.42	6.66	3.91	2.74	1.43

contains high amounts of unsaponifiables (tocols, sterols, and phenolics). Healthy oils rich in phenolic compounds play an important role in preventing several diseases (Ramadan, 2013; Ramadan, Kinni, Seshagiri, & Mörsel, 2010).

6 Edible and nonedible applications of cold pressed *R. officinalis* oil

CPRO is a promising healthy oil rich in phytonutrients (i.e., PUFAs, MUFAs, tocols, and phenolic compounds), and exhibits unique antiradical, antioxidant, and antimicrobial traits. CPRO could be used in novel pharmaceutical, cosmetic, and edible applications. Recently, Mahgoub et al. (2019) studied the effects of CPRO on growth performance, biostimulating health, and intestinal bacterial populations in Japanese quail. The addition of CPRO increased the body weight and the body weight gain of birds. CPRO administration showed an increase in metabolic hormones levels and serum total protein, while it reduced serum TC, LDL-C, 8-hydroxy-2′-deoxyguanosine, and protein carbonyl levels. In addition, CPRO increased antioxidant enzymes and reduced lipids oxidation in quail liver. Supplementation with CPRO also reduced the populations of total cultural bacterial count, *E. coli*, coliforms, and *Salmonella* spp.

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