

In Vivo Hepato-Protective Properties of the Essential Oils of *Boswellia papyrifera* (Del.) Hochst (Burseraceae) and *Ruta chalepensis* L. (Rutaceae)

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Abstract

Objective: *Boswellia papyrifera* (Del.) Hochst and *Ruta chalepensis* L. are aromatic plants used in the Republic of Djibouti, both as food and medicine. The importance of essential oils of these two species in the treatment of certain pathologies such as inflammations and infections has led to an assessment of their composition in volatile organic compounds and their hepato-protective properties on rats poisoned by carbon tetrachloride (CCl₄).

Methods: The chemical composition of essential oils studied was determined by GC-MS. The hepato-protective properties of the essential oils of *Boswellia papyrifera* and *Ruta chalepensis* were assessed through inhibition of hepato toxicity in rats by CCl₄ poisoning. The hepato-protection of essential oils was estimated by measuring biochemical and hematological parameters. **Results:** Essential oils of *Boswellia papyrifera* and *Ruta chalepensis* reduced the blood level of transaminases and triglycerides at the dependent dosage, and restored liver proteins (0.27 g/l) to levels similar to those rats not poisoned by CCl₄. Blood levels of uric acid, urea, creatinine and HDL have also been restored to values similar to those of non-poisoned rats. The chemical composition of the essential oils studied shows that *Boswellia papyrifera* contains incensole acetate (43.76%) and isoincensole (18.42%), and that *Ruta chalepensis* contains menthyl acetate (29.8%) and piperitone (22.22%). **Conclusion:** The studies carried out have shown that the essential oils of *Boswellia papyrifera* and *Ruta*

chalepensis have a hepato-protective potential similar to that of sylimarin (reference hepato-protective substances). The results show that the essential oils of *Boswellia papyrifera* and *Ruta chalepensis* could present good prospects in the formulation of phytomedicines for the management of liver diseases.

Keywords

Essential Oils, Hepatoprotection, Tetrachloride, Djibouti

1. Introduction

The liver participates in the metabolism of the endogenous and exogenous substances of the body, and plays a role in the detoxification and elimination of certain substances such as alcohol, paracetamol, carbon tetrachloride, tetracyclines, and oral contraceptive pills of the body [1]. Exposure of the liver to xenobiotics, alcohol and some drug molecules, malnutrition, and certain forms of infection, can cause damage to the liver tissue, resulting in changes in its functions [2]. Liver diseases are one of the leading causes of mortality and morbidity of animal and human species worldwide. Approximately 20,000 cases of human death due to liver disorders are reported worldwide every year [3]. Oxidative stress plays an important role in the loss of liver function. Indeed, induction of hepatitis in the animal model is most often carried out by pro-oxidant substances such as alcohols and carbon tetrachloride [4], and is also carried out by high dosages of paracetamol which are converted by cytochrome P450 enzymes into toxic metabolites such as N-acetyl-p-benzene-o-quinoneimine which causes oxidative stress and decreased glutathione peroxidase [5]. It has been proved through numerous studies that the molecules used in the treatment of liver disorders have antioxidant properties. This is the case of sylimarin isolated from the species *Silybium marianum*, which is used in the composition of a large number of products used in the treatment of hepatic disorders [6]. Plants are reservoirs of bioactive compounds, and are widely used by traditional medicine in the treatment of liver diseases [7]. *Boswellia papyrifera* (Del.) Hochst and *Ruta chalepensis* L. are aromatic species whose resin for one and leaves for the other are traditionally used in the Republic of Djibouti and Ethiopia as healing, anti-inflammatory, stimulating immune defenses, and anti-microbial [8], and as cosmetic products for perfuming the body and as dietary product for flavoring certain beverages. Studies carried out on the above mentioned two species have shown the richness of essential oil of *Boswellia papyrifera*, in terpenic compounds. They have also shown the presence of terpene and polyphenolic compounds (coumarin, flavonoid).

They also showed the presence of terpenic compounds and polyphenolic compounds (coumarin, flavonoids) in the essential oil of *Ruta chalepensis* [9]. The objective of this study is to determine the terpenic content of essential oils

of *Ruta chalepensis* and *Boswellia papyrifera* to determine their antioxidant effect through hepatoprotection in Wistar strain rat.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

Plant samples composed of *Boswellia papyrifera* wood and *Ruta chalepensis* leaves were collected in March 2014, respectively in the Arta district (11.52'N 42.84'E) and in Randa (11.8'N 42.6'E) in the Tadjourah district. Both species collected were identified by Prof. MAHA Kordofani (Botanist) University of Khartoum (Sudan). Specimens were deposited in the herbarium of Djibouti's Study and Research Center (DSRC) under the numbers 40 and 2 respectively.

2.1.2. Laboratory Animals

Male WISTAR rats, 200 to 250 grams body weight, aged 3 to 4 months were obtained from the animal house of Joseph Ki Zerbo University. The animals were kept in a 12 hour-cycle of brightness and 12 hour darkness with free access to food (cereal mix granules enriched with 29% protein) and water. All methods of animal use in this work have been in accordance with the International Protocol for the Use of Laboratory Animals [10].

2.1.3. Reagents

The reagents used for the various tests of this study consist mainly of: Carbon tetrachloride (CCl₄), diethyl ether, sodium carboxymethyl cellulose (CMC), LABKIT (Spain), SPINREACT, S.A.U, (Barcelona), silymarin. The reagents cited were used to treat animals on the one hand, and to measure biochemical and hematological parameters on the other.

2.2. Methods

2.2.1. Plant Extract Preparation

Essential oils obtained from the hydro-distillation of *Boswellia papyrifera* wood and *Ruta chalepensis* leaves.

2.2.2. Acute Systemic Toxicity

The acute oral toxicity test of essential oils of *Boswellia papyrifera* wood and *Ruta chalepensis* leaves was carried out following the OECD-423 procedure (Organization for Economic Cooperation and Development) with some modifications [11]. Wistar rats (n = 6) were used for the test. Increasing doses of essential oils of *Boswellia papyrifera* and *Ruta chalepensis* (500 mg/kg, 1000 mg, 1500 mg/kg, and 2000 mg/kg) were administered orally to rats kept starved for 12 hours with access to water. After the extracts were administered, the number of rats that died after 24 hours was counted. Surviving rats were observed for seven days to identify potential signs of toxicity. The LD₅₀ of the essential oils of *Boswellia papyrifera* and *Ruta chalepensis* was estimated from the log-probit regression line of the mortality percentage as a function of the natural logarithm of

the administered extract dose [12].

2.2.3. Hepato-Protective Activity

The *in vivo*, hepato-protective activity of the essential oils of *Boswellia papyrifera* and *Ruta chalepensis* was performed according to the modified protocol described by Srinivasan (2007) [13].

2.2.4. Treatment of Animals

Male Wistar rats (220 g to 280 g) are gathered into seven groups of six and receive the compounds orally, and according to body mass as described below for seven days.

Group I (normal): 1 ml Na cellulose carboxymethyl (CMC) 0.5%.

Group II (Control-): 1ml of CMC 0.5%.

Group III: 100 mg/kg/day silymarin (S+).

Group IV: 200 mg/kg/day *Boswellia* essential oil (He1).

Group V: 300 mg/kg/day *Boswellia* essential oil (He2).

Group VI: 100 mg/kg/day *Ruta* essential oil (H1).

Group VII: 200 mg/kg/day *Ruta* essential oil (H2).

On the eighth day, rats in different groups except group I were intraperitoneally intoxicated with carbon tetrachloride (CCl₄) at 1.59 g/Kg body mass. Twenty-four hours later, all rats were anesthetized with diethyl ether, and blood was collected by cardiac puncture, and stored in heparinized and dry tubes, to measure biochemical and hematological parameters related to oxidative stress caused by CCl₄.

2.2.5. Determination of Biochemical Parameters

Blood levels in transaminases (ASAT and ALAT), triglycerides, uric acid, urea, creatinine, HDL, and protein were determined using the assay kits LABKIT (Spain), SPINREACT S.A.U (Barcelona).

2.2.6. Leucocyte Count

The morphological number of leucocytes was determined by digitization/differential (Panotico-commercial), microscope analysis (Zeiss room 100), and relative count, which provided the percentage of cells. The leucocyte blood content analysis was performed in comparison with the data [14].

2.2.7. Determination of the Chemical Composition of the Samples

1) Analytical parameters

Quantitative analyses of the essential oil of *Ruta chalepensis* and *Boswellia papyrifera* were carried out by gas chromatography combining flame ionization (FID), coupled with a mass spectrometer, and carried out using the Varian 3900 apparatus (Varian, Ruisseau de la Noix, CA, USA). For this purpose, a DB-5 capillary apolar column (Scientist J & W, Folsom, USA) 30 m long and 0.25 mm in diameter, and with a stationary phase thickness of 0.25 µm, was used. Helium was used as a carrier gas with a constant flow rate of 1.0 ml/min. The injector and detector temperatures have been set at 250°C and 300°C respectively. The

analysis temperature has been programmed from 40°C to 300°C, with a continuous increase at a rate of 4°C/min. The column was then recycled at 300°C for 30 minutes. The quantity of each essential oil injected was between 0.5 and 1 µl HE 10% v/v (pentane). A 1:100 split was applied. Identification was performed with retention indices and compared to n-alkane retention indices from C5 to C24 [15]. The compounds were identified using the NIST 2005 spectral base, as well as Kovatz data and indices from the literature. Each analysis was performed at least three times.

2) Statistical analysis

The one-way ANOVA (Analysis of Variance) statistical analysis, followed by Tukey's Multiple Comparison Test, was performed using GraphPad prism 5 statistical software for window, GraphPad Software Inc. The results were expressed as mean ± standard deviation (S.D.) or mean ± standard error of the mean (S.E.M.). Differences for $p < 0.05$ were considered statistically significant.

3. Results

3.1. Acute Systemic Toxicity

In this study, the acute systemic toxicity of *Boswellia papyrifera* and *Ruta chalepensis* essential oils via the oral route was assessed. *Boswellia*'s essential oil at dosage limit of 500 to 2000 mg/kg, showed no signs of toxicity and mortality. In contrast, *Ruta chalepensis* essential oil at the various dosages used (500 mg/kg to 2000 mg/kg) had recorded mortality cases after 24 hours (Figure 1). The observation of surviving animals after the 24-hour waiting period showed some signs of toxicity, characterized by locomotion difficulty and eye redness. Determination of acute oral toxicity of *Ruta chalepensis* essential oil, calculated from the dosage ranges of 500 mg/kg to 2000 mg/kg, gave an $LD_{50} = 1016 \pm 6.7$ mg/kg.

3.2. Biochemical Parameters

This study was conducted to assess the effect of *Boswellia* essential oils wood and *Ruta* leaves on carbon tetrachloride (CCl_4) induced hepatotoxicity. CCl_4 injected intravenously into rats produced hepatotoxicity, which was manifested by an increase in transaminase (ALAT and ASAT) levels, serum triglycerides, and a decrease in protein content in liver tissues (Table 1). A significant increase in transaminase and triglyceride content was observed in untreated rats and injected with CCl_4 (control-) compared with the normal group ($p < 0.05$). However, there was a significant reduction in transaminase (ALAT and ASAT) levels in rats treated with *Boswellia* and *Ruta* essential oils, and sylimarin (reference hepatoprotective substance), compared with rats not treated and intoxicated with CCl_4 ($p < 0.05$). Essential oils from *Boswellia* and *Ruta* resulted in a significant decrease in serum-dependent dosage in transaminases content. Essential oils of *Boswellia* (He1) and *Ruta* (H1), as well as sylimarin at 100 mg/kg, showed a level of ASAT similar to that of the normal group ($p > 0.05$); *Ruta* oil (H2) had a level of ALAT similar to that of the normal group ($p > 0.05$). The triglyceride

Acute systemic toxicity of *Ruta chalepensis*

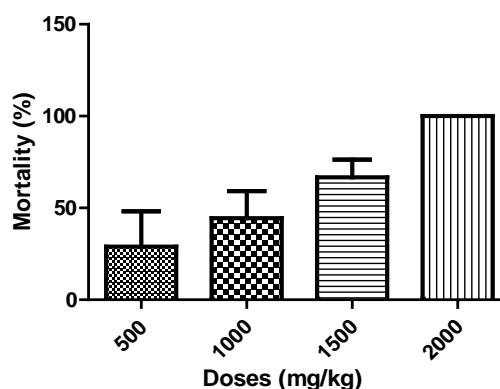


Figure 1. Acute systemic toxicity of *Ruta chalepensis* essential oil.

Table 1. Serum content of transaminases, triglycerides and proteins.

	ASAT (U/l)	ALAT (U/l)	Tgly (mmol/L)	Proteins T. (g/l)
Normal	24.75 ± 2.74 ^b	16.5 ± 1.83 ^c	148.4 ± 0.99 ^d	0.27 ± 0.06 ^e
CTL-	48.91 ± 0.91 ^c	47.15 ± 3.98 ^e	464.3 ± 23.68 ^e	0.04 ± 0.01 ^e
S+	32.41 ± 2.42 ^b	11.79 ± 0.91 ^b	159.2 ± 10.85 ^d	0.13 ± 0.02 ^d
He1	22.39 ± 3.3 ^b	21.81 ± 0.91 ^d	113.4 ± 4.30 ^c	0.16 ± 0.02 ^d
He2	14.14 ± 2.74 ^a	7.66 ± 0.91 ^a	38.85 ± 4.30 ^a	0.66 ± 0.04 ^b
H1	31.82 ± 2.74 ^b	48.33 ± 3.29 ^e	101.3 ± 6.16 ^c	0.27 ± 0.02 ^c
H2	24.16 ± 0.03 ^b	18.86 ± 0.91 ^c	77.07 ± 3.56 ^b	1.13 ± 0.05 ^a

Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), triglyceride (Tgly). Data are means ± SEM (n = 3), Values showing by the same letter are not significantly different (p > 0.05) from another in the same column.

tenor of serum in rats treated with silymarin and essential oils from *Boswellia* and *Ruta* showed a significant decrease compared with the control group (-) (P < 0.05). The effect of *Boswellia* and *Ruta* essential oils on serum triglycerides in rats intoxicated with CCl₄ was dependent dosage; and the group of rats treated with silymarin had a triglyceride content similar to that of the normal group (P > 0.05).

Table 2 shows a significant increase in urea, uric acid, and creatinine levels, and a significant decrease in HDL, serum content in control rats (-) compared with the normal group (P < 0.05). Note: A similarity between the serum content of silymarin-treated rats (100mg/kg), *Boswellia* essential oil (dose 1 and 2), *Ruta* essential oil (dose 2) and the normal group (P > 0.05); A similarity between the serum creatinine content of rats treated with silymarin, *Boswellia* essential oil (dosage 1), *Ruta* essential oil (dosage 1) and that of the normal group (P > 0.05); A similarity between serum HDL cholesterol content of rats treated with silymarin and the normal group (P > 0.05). There was a significant difference between the uric acid content of groups rats treated with silymarin, *Boswellia* and *Ruta* essential oils, and that of the normal group (P < 0.05); however, no significant differences (P > 0.05) were observed between serum uric acid content in the

Table 2. Uric acid, urea, creatinine and HDL.

	Uric acid. (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	HDL (mg/dl)
Normal	2.16 ± 0.17 ^d	6.41 ± 1.1 ^a	0.41 ± 0.01 ^a	1.67 ± 0.24 ^c
CTL-	2.63 ± 0.18 ^e	14.88 ± 1.61 ^b	0.66 ± 0.02 ^{bc}	1.3 ± 0.11 ^b
S+	1.28 ± 0.1 ^a	7.95 ± 1.14 ^a	0.48 ± 0.11 ^a	1.6 ± 0.11 ^c
He1	1.14 ± 0.04 ^a	9.67 ± 0.32 ^{ab}	0.54 ± 0.03 ^{ab}	1.9 ± 0.05 ^d
He2	1.50 ± 0.02 ^b	6.59 ± 0.86 ^a	0.66 ± 0.03 ^{bc}	1.42 ± 0.05 ^b
H1	1.55 ± 0.04 ^b	12.32 ± 1.15 ^b	0.57 ± 0.05 ^{ab}	1.8 ± 0.106 ^d
H2	1.76 ± 0.04 ^c	7.95 ± 1.32 ^a	0.59 ± 0.03 ^b	0.94 ± 0.03 ^a

Data are means ± SEM (n = 3), Values showing by the same letter are not significantly different (p > 0.05) from another in the same column.

group of rats treated with sylimar and that of the group treated with *Boswellia* essential oil (dosage 1).

3.3. Leucocytes Count

Blood leukocyte counts of rats treated with *Boswellia papyrifera* (dosage 1 and 2) and *Ruta chalepensis* (dosage 1 and 2) oils are shown respectively in **Figure 2** and **Figure 3**. Compared to the reference values of total white blood cells in whole blood, male rats aged 8 - 16 weeks [14], we noted that: abnormal values of Eosinophil (11.03% ± 2.5% and 5.36% ± 0.99%) in rats treated with *Ruta Chalepensis* essential oil (dosage 1 and 2), abnormal monocyte values of (7% ± 00% and 6.74% ± 1.7%), respectively, in rats treated with essential oils of *Boswellia papyrifera* (dosage 2) and *Ruta chalepensis* (dosage 1) compared with reference values. There was also an abnormal lymphocyte count in rats treated with *Ruta chalepensis* essential oil (dosage 1) compared with reference values (66.6%- 90.3%).

3.4. Chemical Composition of Essential Oils

Analyzes of the chemical composition of essential oils of *B. papyrifera* and *R. Chalepensis* by gas chromatography method, coupled with MS (GC-MS), are represented in **Table 3**.

The results show that 43 volatile organic compounds have been identified in *B. papyrifera* essential oil and 49 constituents in the essential oil of *R. chalepensis* representing 99.86% and 99.21% of their essence, respectively. Essential oil of *B. papyrifera* is very rich in oxygen compounds (89.57%), mainly oxygenated diterpenes (69.84%). The main major compounds of *B. papyrifera* are: incensole acetate (43.76%), isoincensole (18.42%), incensole (5.58%), 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (3.10%), n-hexyl hexanoate (3.26%) and neryl acetate (2.84%). Most of these compounds are comparable to those found by [15]. The essential oil of *R. chalepensis* is rich in oxygen compounds (87.26%), mainly oxygenated mono terpenes (79.56%). The main compounds are: Menthyl acetate (29.80%), Piperitone (22.22%), Thymol, methyl ether (14.80%), β-Caryophyllene (6.90%), Linalool (6.21%), Nootkatone (3.32%) and Incensole acetate (2.2%) 67%).

Table 3. Chemical composition of essential oils.

N°Pic	IR	Composés	<i>B. papyrifera</i> (%)
1	942	α -Pinène	0.24
2	966	β -Pinène	0.12
3	1020	Limonène	0.07
4	1022	Eucalyptol	0.24
5	1063	n-Octanol	0.13
6	1344	Terpinyl acetate	0.29
7	1375	Neryl acetate	2.84
8	1387	n-Hexyl hexanoate	3.26
9	1406	n-Decyl acetate	1.94
10	1418	(Z)-Caryophyllène	0.78
11	1454	α -Humulène	0.27
12	1456	allo-Aromadendrène	0.75
13	1475	γ -Muuroène	0.67
14	1479	Germacrène D	0.75
15	1480	Isocaryophyllène	1.8
16	1483	β -Eudesmène	0.15
17	1485	2-Isopropenyl-4a,8-dimethyl- 1,2,3,4,4a,5,6,8a-octahydronaphtalène	1.7
18	1509	γ -Cadinène	0.79
19	1511	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiène	3.1
20	1524	δ -Cadinène	0.17
21	1528	1,2,4a,5,6,8a-Hexahydro-1- isopropyl-4,7-dimethylnaphtalène	0.12
22	1533	Elemicine	0.27
23	1572	Acide laurique	0.25
24	1579	Hexyl caprylate	0.87
25	1587	Ledol	0.47
26	1604	Cedrenol	0.14
27	1607	1,5,5,8-Tetramethyl-12-oxabicyclo-[9.1.0]dodeca-3,7-diène	0.56
28	1609	γ -Eudesmol	0.47
29	1634	τ -Cadinol	0.23
30	1636	δ -Cadinol	2.92
31	1649	α -Eudesmol	0.23
32	1651	α -Cadinol	0.46
33	1654	β -Eudesmol	0.48
34	1662	α -Bisabolol	0.34

Continued

35	1666	β -Bisabolol	0.24
36	1959	Cembrène A	1.29
37	2004	Verticilla-4(20),7,11-triène	0.62
38	2126	Nezukol	0.11
39	2150	Incensole	5.58
40	2152	Isoincensole	18.42
41	2189	Incensole acetate	43.76
42	2260	Incensole oxide	1.4
43	2329	Incensole oxide acetate	0.57
Total identified compounds			99.86
Hydrocarbon monoterpenes			0.43
Oxygenated monoterpenes			8.7
Hydrocarbon Sesquiterpenes			7.95
Oxygenated Sesquiterpenes			11.03
Hydrocarbon diterpenes			1.91
Oxygenated diterpenes			69.84

IR: Retention index relative to C5-C24 n-alkanes on column DB-5. %: Percentage of each component in the essential oil.

N° Pic	RI	Components	<i>R. chalepensis</i> (%)
1	942	α -Pinene	0.07
2	966	β -Pinene	0.04
3	990	β -Myrcene	0.17
4	1020	Limonene	0.38
5	1101	Linalool	6.21
6	1111	Thujone	0.11
7	1123	trans p-2,8-Menthadien-1-ol	0.03
8	1146	Camphor	1.29
9	1170	cis-Sabinol	0.14
10	1187	p-Cymen-8-ol	0.60
11	1193	α -Terpineol	0.08
12	1198	Myrtenol	0.04
13	1221	Verbenone	0.02
14	1231	Thymol, methyl ether	14.80
15	1241	Pulgeone	0.03
16	1249	Linalyl acetate	2.21
17	1250	Piperitone	22.22
18	1275	Bornyl acetate	0.17

Continued

19	1286	Isobornyl acetate	0.15
20	1291	Menthyl acetate	29.80
21	1293	Thymol	0.04
22	1306	δ -Octalactone	0.21
23	1321	1-Methyl-4-(1-methyl-ethenyl)-	0.79
24	1364	Eugenol	1.41
25	1390	β -Cubebene	0.50
26	1418	(Z)-Caryophyllene	0.15
27	1426	β -Caryophyllene	6.90
28	1427	β -Gurjunene	0.41
29	1475	γ -Muurolene	0.05
30	1481	β -bisabolene	0.10
31	1490	β -Selinene	0.04
32	1495	Cadina-1,4-diene	0.68
33	1501	α -Muurolene	0.36
34	1515	γ -Curcumene	0.05
35	1524	δ -Cadinene	0.09
36	1547	Elemol	0.32
37	1558	(E)-Nerolidol	0.03
38	1568	Isogenol acetate	0.02
39	1578	Spathulenol	0.11
40	1638	α -Muurolol	0.02
41	1642	epi- α -Muurolol	0.02
42	1654	β -Eudesmol	0.13
43	1666	β -Bisabolol	0.78
44	1740	Chamazulene	0.54
45	1814	Nootkatone	3.32
46	1860	Dibutyl Phthalate	0.02
47	2037	Dehydroabietadiene	0.60
48	2111	8, β -Hydroxysandaracopimarane	0.27
49	2176	Incensole acetate	2.67
		Total identified compounds	99.21
		Hydrocarbon monoterpenes	1.45
		oxygenated monoterpenes	79.56
		Hydrocarbon sesquiterpenes	9.90
		Oxygenated sesquiterpenes	4.76
		hydrocarbon diterpenes	0.60
		Oxygenated diterpenes	2.94

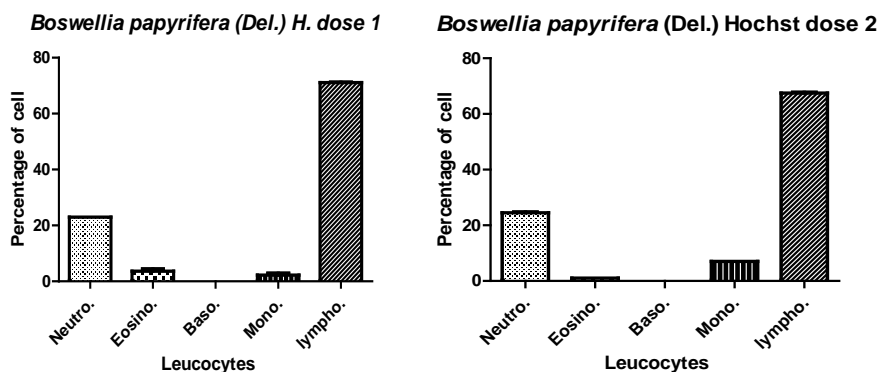


Figure 2. Blood white cell counts of rats treated with *Boswellia p.* essential oil dosage 1 and 2.

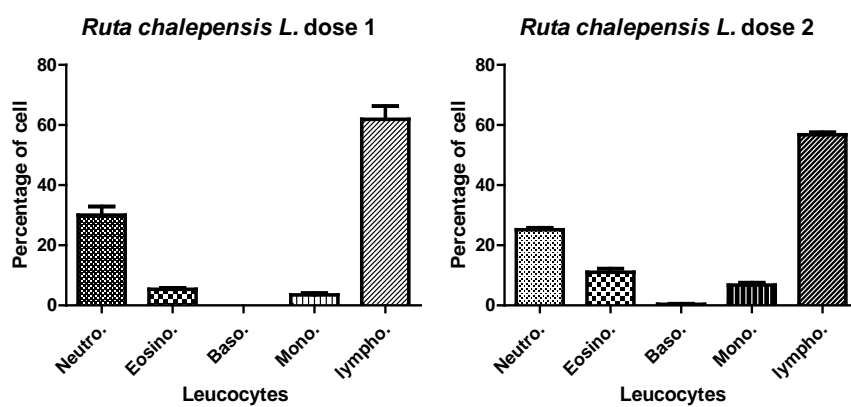


Figure 3. Blood white cell counts of rats treated with *Ruta chalepensis* oil dosage 1 and 2.

4. Discussion

The acute toxicity study of *Boswellia papyrifera* and *Ruta* essential oils showed that *Boswellia papyrifera* oil at a maximum dosage of 2000 mg/kg does not cause mortality in rats. Oral LD₅₀ is considered in this case to be greater than 2000 mg/kg, and thus allows the essential oil of *Boswellia chalepensis* to be classified as a very slightly oral-toxic oil in rats, according to the scale of [16]. Daily dosages of 200 mg/kg and 300 mg/kg were considered tolerable for antioxidant and hepato-protective tests. Our results are consistent with those of Abdallah *et al.* [17] that showed the very low toxicity of *Boswellia papyrifera* resin gum in rats.

Of all hepatotoxic substances, carbon tetrachloride (CCl₄) is one of the most commonly used biochemical and pathological substance [13]. The hepatotoxicity of CCl₄ is based on its reductive dehalogenation catalyzed by cyt 450 in the endoplasmic reticulum of the hepatic cell, leading to the formation of an unstable radical complex, CCl₃·. CCl₃· is a highly reactive chemical species that can attack microsomal lipids, leading to their peroxidation [18]. The metabolism of CCl₄ to CCl₃·, after absorption by living organisms, may also affect other vital organs such as kidneys, lungs, testicles, brain and blood [19]. This study was conducted to highlight the hepato-protective properties of *Boswellia* and *Ruta* essential oils in rats intoxicated by CCl₄ through their antioxidant capacity.

Intoxication of rats by CCl_4 resulted in increased transaminases (ALAT and ASAT) and serum triglycerides on the one hand, and decreased liver protein content on the other hand. Transaminases and triglycerides are highly sensitive markers used in the diagnosis of liver diseases. During hepatotoxicity, enzymes contained in the cytosol of liver cells are released into the bloodstream and can be detected by assay in blood serum [20]. However, a significant reduction ($P < 0.05$) in serum transaminase levels in sylimarin-treated rats was noted, and essential oils from *Boswellia papyrifera* (dosage 1 and 2) and *Ruta chalepensis* (dosage 1) were more or less similar to those of non-poisoned batches. Sylimarin is a protective hepatochemical widely used in Europe for the management of various hepatobiliary diseases [21]. Restoration of transaminase content and triglyceride content by *Boswellia* and *Ruta* essential oils indicates their protective hepato-protective property towards CCl_4 .

The intoxication of rats with CCl_4 also resulted in a very significant increase in uric acid, urea, creatinine levels ($P < 0.05$), and a decrease in HDL content, compared with non-poisoned rats. However, the level of uric acid, urea, creatinine, and HDL was restored in rats treated with sylimarin and essential oils from *Boswellia papyrifera* and *Ruta chalepensis* to a level close to the non-poisoned group ($P > 0.05$). Increasing serum levels in uric acid and urea is an indicator of nephrotoxicity caused by CCl_4 . Our observations are consistent with those of [22], which showed a significant increase in uric acid, urea, and creatinine levels in rats intoxicated with CCl_4 compared to the non-intoxicated group ($P < 0.05$). Increased levels of biomarkers of renal function can be attributed to severe damage in glomeruli and tubules by the metabolized form ($\text{CCl}_3\cdot$) of CCl_4 [23]. The results show a restoration of the level of uric acid, urea, and creatinine in rats treated with sylimarin, and *Boswellia* and *Ruta* essential oils, to a level close to the normal ($P > 0.05$), indicating a nephroprotective effect. It is noted, however, that *Ruta* essential oil at the daily dosage of 100 mg/kg has no effect on the restoration of urea content at a level close to the normal in rats intoxicated by CCl_4 .

The results of this study have also shown that CCl_4 may affect liver lipid metabolism (cholesterol level). The results clearly show that CCl_4 causes a significant reduction in cholesterol level (HDL). However, there was a significant increase in cholesterol (HDL) levels in rats treated with dosage 1 of essential oils. However, there was a significant increase in cholesterol (HDL) levels in rats treated with dose 1 of essential oils from *Boswellia* and *Ruta*, compared with rats intoxicated by CCl_4 ($P < 0.05$). Similar results were obtained by [24] which showed that consumption of *Salvia officinalis* L. leads to an increase in HDL-c content by the mechanism of suppression of cholesterol biosynthesis. The high leucocyte rate in rats treated with *Ruta c.* essential oil, and intoxicated with CCl_4 , compared with reference values, indicates a high immune response, and therefore an increased inflammatory process. However, in rats treated with *Boswellia papyrifera*, leucocytes were more or less close to normal values. Results indicate

that *Boswellia*'s essential oil has anti-inflammatory properties compared to *Ruta chalepensis*. Pharmacological data indicate that *Boswellia papyrifera* essential oil has anti-inflammatory, immunostimulating and anti-infectious properties [25]. Majority compounds such as incensole acetate identified in *Boswellia p.* essential oil, and Menthyl acetate identified in *Ruta c.* essential oil, may justify the protective hepatoprotective power observed in rats intoxicated with CCl₄. Indeed, it has been proven that the resin of species of the genus *Boswellia*, is rich in incensole acetate derivative, and is traditionally used in many Asian and European countries, for the management of inflammatory situations [26]. In recent years, the antioxidant properties of the resin of the genus *Boswellia* have been proven [26]. [26] showed that incensole acetate, a major compound of the genus *Boswellia* resins, can inhibit the release of pro-inflammatory cytokines through inhibition of inflammation-inducing genes such as NF- κ B-inflammatory inflammation. Chemical analysis of *Ruta*'s essential oil has shown that it is rich in mono-terpene compounds. Studies have shown that essential oils rich in mono terpenes are natural antioxidants and are active against certain forms of cancer [27]. It is also established that most drugs used for the treatment of liver diseases are antioxidants [28].

5. Conclusion

Essential oils of *Boswellia papyrifera* and *Ruta chalepensis* have hepato-protective properties similar to sylimarin. With regards to the chemical compositions of these oils, a bio-guided study of fractions extracted from both plants would isolate the hepato-protective compound in order to develop a new formulation of phytomedicine for the treatment of liver disorders in both humans and animals.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] James, L.P., Mayeux, P.R. and Hinston, J.A. (2003) Acetaminophen-Induced Hepatotoxicity. *Drug Metabolism and Disposition*, **31**, 1499-1506. <https://doi.org/10.1124/dmd.31.12.1499>
- [2] Mroueh, M., Saab, Y. and Rizkallah, R. (2004) Hepatoprotective Activity of *Centaurium erythraea* on Acetaminophen-Induced Hepatotoxicity in Rats. *Phytotherapy Research*, **18**, 431-433. <https://doi.org/10.1002/ptr.1498>
- [3] Akhil, B., Pankaj, K. and Soni, M.L. (2011) Potent Herbal Hepatoprotective Drugs:

A Review. *Journal of Advanced Scientific Research*, **2**, 15-20.

- [4] Tirkey, N., Pilkhwal, S., Kuhad, A. and Chopra, K. (2005) Hesperidin, A Citrus Bioflavonoid, Decreases the Oxidative Stress Produced by Carbon Tetrachloride in Rat Liver and Kidney. *BMC Pharmacology*, **5**, Article No.: 2. <https://doi.org/10.1186/1471-2210-5-2>
- [5] Parmar, S.R., Patel, H.V. and Kalia, K. (2010) Hepatoprotective Activity of Some Plants Extract against Paracetamol Induced Hepatotoxicity in Rats. *Journal of Herbal Medicine and Toxicology*, **4**, 101-106.
- [6] Luangchosiri, C., Thakkinstian, A., Chitphuk, S., Stitchantrakul, W., Petraksa, S. and Sobhonslidsuk, A. (2015) A Double-Blinded Randomized Controlled Trial of Silymarin for the Prevention of Antituberculosis Drug-Induced Liver Injury. *BMC Complementary and Alternative Medicine*, **15**, Article No.: 334. <https://doi.org/10.1186/s12906-015-0861-7>
- [7] Madani, H., Talebolhosseini, M., Asgary, S. and Naderi, G.H. (2008) Hepatoprotective Activity of *Silybum marianum* and *Cichorium intybus* against Thioacetamide in Rats. *Pakistan Journal of Nutrition*, **7**, 172-176. <https://doi.org/10.3923/pjn.2008.172.176>
- [8] Foster, S. and Tyler, V.E. (1999) Tyler's Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies. The Haworth Press, New York, 97-99. <https://doi.org/10.4324/9780203819692>
- [9] Attou, A. (2011) Contribution à l'étude phytochimique et activités biologiques des extraits de la plante *Ruta chalepensis* (Fidjel) de la région d'Ain Témouchent. Mémoire soutenue publiquement en vue de l'obtention du diplôme de Magister en biologie, Université de Tlemcen, Tlemcen, 119 p.
- [10] Zimmermann, M. (1983) Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals. *Pain*, **16**, 109-110. [https://doi.org/10.1016/0304-3959\(83\)90201-4](https://doi.org/10.1016/0304-3959(83)90201-4)
- [11] Ecobichon, D.J. (1997) the Basis of Toxicology Testing. RC Press, New York, 4386 p.
- [12] Carvalho, J.C.T., Sertie, J.A.A., Barbosa, M.V.J., Patricio, K.C.M., Caputo, L.R.G., Sarti, S.J., Ferreira, L.P. and Bastos, J.K. (1999) Anti-Inflammatory Activity of the Crude Extract from the Fruits of *Pterodon emarginatus* Vog. *Journal of Ethnopharmacology*, **64**, 127-133. [https://doi.org/10.1016/S0378-8741\(98\)00116-0](https://doi.org/10.1016/S0378-8741(98)00116-0)
- [13] Srinivasan, R., Chandrasekar, M.J.N., Najan, M.J. and Suresh, B. (2007) Antioxidant Activity of *Caesalpinia digyna* Root. *Journal of Ethnopharmacology*, **113**, 284-291. <https://doi.org/10.1016/j.jep.2007.06.006>
- [14] Mary, L.A. and Charles, B. (2008) Clinicals Laboratory Parameters for Crl:CD (SD) Rats, 14 p.
- [15] Adams, R.P. (2007) Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing, Carol Stream, IL.
- [16] Hodge, A.C. and Sterner, J.H. (1980) In études de toxicité: Quelques données fondamentales. (A. K. DONE) TEMPO MEDICAL Afrique N°7.
- [17] Abdallah, E.M., Khalid, A.S. and Ibrahim, N. (2009) Antibacterial Activity of Oleo-Gum Resins of *Commiphora molmol* and *Boswellia papyrifera* against Methicillin Resistant *Staphylococcus aureus* (MRSA). *Scientific Research and Essays*, **4**, 351-356.
- [18] Wei, Y.H. (1998) Oxidative Stress and Mitochondrial DNA Mutations in Human Aging. *Proceedings of the Society for Experimental Biology and Medicine*, **217**,

- 53-63. <https://doi.org/10.3181/00379727-217-44205>
- [19] Ozturk, F., Ucar, M., Ozturk, I.C., Vardi, N. and Batcioglu, K. (2003) Carbon Tetrachloride Induced Nephrotoxicity and Protective Effect of Betaine in Sprage Dawley Rats. *Urology*, **62**, 353-356. [https://doi.org/10.1016/S0090-4295\(03\)00255-3](https://doi.org/10.1016/S0090-4295(03)00255-3)
- [20] Brent, J.A. and Rumack, B.H. (1993) Role of Free Radicals in Toxic Hepatic Injury II. Are Free Radicals the Cause of Toxin-Induced Liver Injury? *Journal of Toxicology: Clinical Toxicology*, **31**, 173-196. <https://doi.org/10.3109/15563659309000384>
- [21] Flora, K., Hahn, M., Rosen, H. and Benner, K. (1998) Milk Thistle (*Silybum marianum*) for the Therapy of Liver Disease. *American Journal of Gastroenterology*, **93**, 139-143. <https://doi.org/10.1111/j.1572-0241.1998.00139.x>
- [22] Makni, M., Chtourou, Y., Barkallah, M. and Fetoui, H. (2012) Protective Effect of Vanillin against Carbon Tetrachloride (CCl₄)-Induced Oxidative Brain Injury in Rats. *Toxicology and Industrial Health*, **28**, 655-662. <https://doi.org/10.1177/0748233711420472>
- [23] Khan, A.R., Khan, R.M. and Sahreen, S. (2012) CCl₄-Induced Hepatotoxicity: Protective Effect of Rutin on P53, CYP2E1 and the Oxidative Status in Rat. *BMC Complementary and Alternative Medicine*, **12**, Article No.: 178. <https://doi.org/10.1186/1472-6882-12-178>
- [24] Carla, M.S., Alice, A.R., Marisa, F.A., Cristovao, F.L., Manuel, F.F. and Cristina, P.W. (2009) Sage Tea Drinking Improves Lipid Profile and Antioxidant Defenses in Humans. *International Journal of Molecular Sciences*, **10**, 3937-3950. <https://doi.org/10.3390/ijms10093937>
- [25] Van Vuuren, S.F., Kamatou, G.P.P. and Viljoen, A.M. (2010) Volatile Composition and Antimicrobial Activity of Twenty Commercial Frankincense Essential Oil Samples. *South African Journal of Botany*, **76**, 686-691. <https://doi.org/10.1016/j.sajb.2010.06.001>
- [26] Moussaieff, A., Shein, N.A., Tsenter, J., Grigoriadis, S., Simeonidou, C., Alexandrovich, A.G., et al. (2008) Incensole Acetate: A Novel Neuroprotective Agent Isolated from *Boswellia carterii*. *Journal of Cerebral Blood Flow & Metabolism*, **28**, 1341-1352. <https://doi.org/10.1038/jcbfm.2008.28>
- [27] Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. and Etherton, T.D. (2002) Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer. *The American Journal of Medicine*, **113**, 71S-88S. [https://doi.org/10.1016/S0002-9343\(01\)00995-0](https://doi.org/10.1016/S0002-9343(01)00995-0)
- [28] Feher, J., Csomos, G. and Vereckei, A. (1986) Free Radical Reaction in Medicine. Springer-Verlag, Berlin, 107. <https://doi.org/10.1007/978-3-642-83104-1>