Hepatotoxicity of an essential oil of *Salvia officinalis* L. - - an *in vitro* study using freshly isolated rat hepatocytes

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Introduction

Sage (*Salvia officinalis* L.) is a popular Mediterranean aromatic herb that is cultivated worldwide. It is used since ancient times as a medicinal herb for treating a variety of ailments, such as to drying up the flow of mother`s milk, in reducing saliva secretion, as an anhidrotic to control night sweats associated with illness, in treating menopause problems and has a reputation for memory enhancement . It is also commonly used for flavouring and seasoning of foods, most of their properties being due to essential oils. Sage extracts have also been reported to have antioxidant effects and excellent properties in inhibiting lipid peroxidation.

Sage derivatives continue to be important components of contemporary phytopharmaceuticals, although their potentially toxic effects have not received much attention.

Methods

In this study an essential oil (EO) obtained by hydrodistillation of aerial parts of *Salvia officinalis* L. plants (12 mg/g dry weight) harvested in April 2000, cultivated in Arouca experimental farms in northern Portugal, was characterised by GC and GC-MS analyses [1]. The constituents of this essential oil are presented in table I.

The effects of the mixture on liver was investigated in freshly isolated rat hepatocytes in suspension, where it was used in concentrations of 0, 0.08, 0.4, 2 and 10 µl/ml. Hepatocyte isolation was performed by collagenase perfusion as described by Moldéus *et al.* [2]. The hepatocytes were incubated in suspension with the studied concentrations of EO, being *tert*-butylhydroperoxide (t-BHP) 1mM as positive control. After 30 min of incubation, aliquots were taken out for measurement of the following: lactate dehydrogenase (LDH) leakage as an indicator of cell death, the thiobarbituric acid reactive substances (TBARS) assay as an indirect lipid peroxidation indicator, and reduced (GSH) and oxidised glutathione (GSSG) as indicators of the redox status of the cells by the methods described in [3].

Table I - Essential oil composition of *S. officinalis* aerial parts obtained by hydrodistillation.

Peak nº	Compound name	%	µg/g dry wt
1	1-Butyl acetate	0,03	3,6
2	cis-2-Methyl-3-methylene-hep-5-ene	1,01	98,8
3	*trans*-2-Methyl-3-methylene-hep-5-ene	0,11	11,6
4	Tricyclene	0,03	2,0
5	α-Thujene	0,24	23,1
6	α-Pinene	1,00	97,9
7	Camphene	0,62	59,3
8	Sabinene	0,48	47,4
9	**β-Pinene**	**4,02**	**395,8**
10	Myrcene	0,67	66,2
11	*n*-Decane	tr	1,4
12	α-Phellandrene	0,03	3,0
13	α-Terpinene	0,16	15,0
14	*P*-Cymene	0,08	7,1
15	Limonene	0,44	43,6
16	**1,8-Cineole**	**7,98**	**1070,9**
17	***cis*-β-Ocimene**	**2,84**	**278,8**
18	*trans*-β-Ocimene	0,62	60,8
19	γ-Terpinene	0,35	33,8
20	*trans*-Sabinene hydrate and/or cis-Linalool oxide	0,22	28,4
21	Not identified	0,03	2,4
22	Terpinolene	0,13	12,9
23	*n*-Undecane	0,13	17,2
24	Linalool		
25	**α-Thujone**	**32,10**	**4311,3**
26	**β-Thujone**	**2,96**	**396,3**
27	α-Campholenal	0,03	2,9
28	Not identified	0,20	26,9
29	**Camphor**	**2,18**	**291,5**
30	Not identified	0,02	2,0
31	*(cis-3-)*-Pinanone	0,08	10,7
32	**Borneol**	**2,10**	**282,6**
33	Pinocamphone isomer (T)	0,03	3,5
34	4-Terpineol	0,19	26,3
35	α-Terpineol	0,17	23,6
36	Not identified	0,03	4,7
37	Not identified	tr	1,5
38	Not identified	0,04	5,4
39	Bornyl acetate and/or Isobornyl acetate	0,24	32,7
40	*cis*-Sabinyl acetate	0,06	7,3
41	Not identified	0,03	3,0
42	δ-Terpineol (T) (?)	tr	1,2
43	δ-Elemene	0,08	6,7
44	*trans*-Carvyl acetate		
45	Not identified		
46	*cis*-Carvyl acetate	tr	1,2
47	Neryl acetate	0,08	10,5
48	β-Bourbonene + Geranyl acetate	0,06	7,6
49	**β-Caryophyllene**	**7,70**	**759,9**
50	Aromadendrene or α-Guaiene (?)	0,09	9,2
51	**α-Humulene**	**9,03**	**889,0**
52	*allo*-Aromadendrene	0,09	8,9
53	Germacrene D isomer #3	0,21	20,3
54	Germacrene D	0,26	25,6
55	α-Selinene	0,16	15,8
56	δ-Cadinene	0,26	26,3
57	Caryophyllene oxide and/or Globulol	0,35	45,2
58	**Viridiflorol**	**10,94**	**1462,4**
59	Widdrol (?)	0,53	70,9
60	*trans*-α-Bergamatol acetate or (E), cis, epi-β-Santalol acetate (?)	0,07	11,3
61	Not identified	0,05	5,6
62	**Manool**	**8,05**	**806,1**
63	Not identified	0,32	39,2
64	Not identified	0,03	3,3
	Grouped components	**%**	**µg/g dry wt**
	Monoterpene hydrocarbons	12,86	1259,4
	Oxygen-containing monoterpenes	48,31	6489,8
	Monoterpenyl esters	0,40	54,6
	Sesquiterpene hydrocarbons	17,88	1761,7
	Oxygen-containing sesquiterpenes	11,82	1578,5
	Sesquiterpenyl esters	0,07	11,3
	Oxygen-containing diterpenes	8,05	806,1
	n-Alkanes	0,13	18,6
	Others	0,48	59,3
	total	100	12039,3

tr - trace amounts

Results

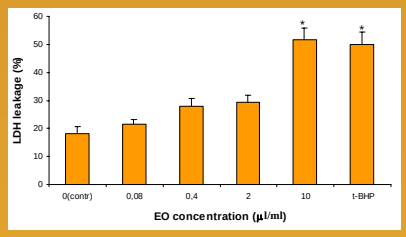

Figure 1 - Effect of essential oil of *S. officinalis* on cell death expressed by LDH leakage in hepatocyte suspensions after 30 min of exposure (means ± SEM). n=5. Significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test. Differences were considered significant when P<0.05. * P<0.001 when compared with control.

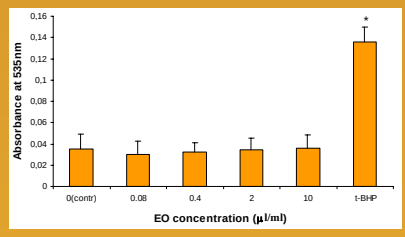

Figure 2 - Effect of essential oil of *S. officinalis* on lipid peroxidation expressed by TBARS formation in hepatocyte suspension after 30 min of exposure (means ± SEM). n=5. For statistics see figure 1. * P<0.001 when compared with control.

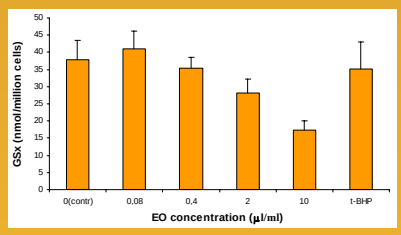

Figure 3 - Effect of essential oil of *S. officinalis* on hepatocyte suspensions GSx (total glutathione) content after 30 min of exposure (means ± SEM). n=5. For statistics see figure 1.

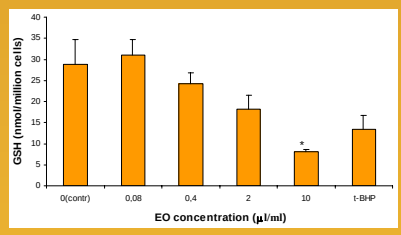

Figure 4 - Effect of essential oil of *S. officinalis* on hepatocyte suspensions GSH content after 30 min of exposure (means ± SEM). n=5. For statistics see figure 1. * P<0.01 when compared with control.

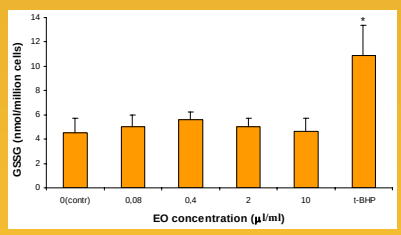

Figure 5 - Effect of essential oil of *S. officinalis* on hepatocyte suspensions GSSG content after 30 min of exposure (means ± SEM). n=5. For statistics see figure 1. * P<0.05 when compared with control.

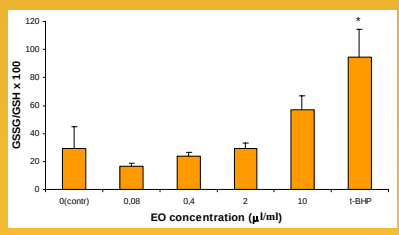

Figure 6 - Effect of essential oil of *S. officinalis* on hepatocyte suspensions GSSG/GSH×100 ratio after 30 min of exposure (means ± SEM). n=5. For statistics see figure 1. * P<0.01 when compared with control.

Discussion and Conclusions

Our results show that:

- *S. officinalis* essential oil (EO) had no toxic effects on rat hepatocyte suspensions when used in low concentrations (0.08 – 2 µl/ml).
- A concentration of 10 µl/ml caused significant LDH leakage and decrease of GSH, indicative of cell damage.
- By contrast with t-BHP-induced toxicity which is the result of both GSH depletion, GSSG increase and lipid peroxidation, the cell death induced by the EO at 10 µl/ml was not mediated by lipid peroxidation

GSH was possibly recruited to act as a nucleophilic scavenger of some compounds and their metabolites with electrophilic properties, via chemical mechanisms. The consequent GSH depletion may be one of the causes for the loss of viability observed with the high concentrations of EO, once it is known that the decrease of this cellular antioxidant can impair the cell's defence against toxic compounds and may lead to cell injury and death.

On the other hand, the cellular death indicated by LDH leakage can be due to a solvent effect of the essential oil, because it is composed mainly by hydrophobic compounds (table I). These compounds may exert a direct action on cellular membranes by disturbing the physico-chemical properties of the bilayer which could culminate with the disruption of cellular volume and cell death.

Concluding, our results show that care should be taken when using essential oils of sage in the food industry or as alternative medicines because of their potentially toxic effects on the liver. However, more studies should be carried out to elucidate the mechanisms of toxicity of this essential oil and determine the active compound(s) in the mixture.

References

[1] Santos-Gomes P.C., Fernandes-Ferreira M. (2001), *Organ- and season-dependent variation in the essential oil composition of Salvia officinalis L. cultivated at two different sites.* J. Agric. Food Chem. 49: 2908-16

[2] Moldéus P., Högberg J., Orrenius S. (1978), *Isolation and use of liver cells.* Methods in Enzymology, 52: 60-71

[3] Fernandes, E.R., Carvalho, F.D., Remião, G., Bastos, M.L., Pinto, M.M., Gottlieb, O.R. (1995*), Hepatoprotective activity of xanthones and xanthonolignoids against tert-butylhydroperoxide-induced toxicity in isolated rat hepatocytes – comparison with silybin*. Pharmaceut. Res. 12: 1756-60