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Analgesic effects of rosemary essential oil and its interactions with codeine and paracetamol in mice

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Abstract. – OBJECTIVE: The use of herbal medicinal products in the management of pain has been increasing steadily in recent years, often in combination with conventional analgesics, which can induce significant interactions. In traditional medicine, rosemary was used as mild analgesic, for relieving renal colic pain and dysmenorrhea. The aim of our study was to examine analgesic effects of rosemary essential oil and its pharmacodynamic interactions with codeine and paracetamol in mice.

MATERIALS AND METHODS: The identification and quantification of chemical constituents of the essential oil isolated from air-dried aerial parts of rosemary were carried out by GC/FID and GC/MS. The hot plate test was performed on NMRI mice by placing them individually on hot plate and assessing their response to the thermal stimulus.

RESULTS: In this research, we identified 29 chemical compounds of the studied rosemary essential oil, and the main constituents were 1,8-cineole, camphor, and α-pinene. Administration of investigated essential oil increased significantly the latency time of animal response to heat-induced pain between 20th and 50th minute of the test, when compared to saline-treated group. Rosemary essential oil in the dose of 20 mg/kg was shown to be more efficient than in the dose of 10 mg/kg, in combinations with both codeine and paracetamol.

CONCLUSIONS: Our findings support the use of rosemary in the management of pain and indicate a therapeutic potential of rosemary essential oil in combination with analgesic drugs. The mechanisms involved in analgesic effects of rosemary essential oil and the potential influence on cytochromes and drug metabolism should be more in-depth investigated.

Key Words:
Rosmarinus officinalis, Essential oil, Antinociceptive, Hot plate, Interaction.

Introduction

The use of herbal medicinal products to relieve pain has been increasing steadily in recent years because they are often perceived as being natural and therefore harmless[1,2]. Herbal medicines are often taken in combination with conventional drug therapies and some of their pharmacologically active ingredients might interact with synthetic drugs[3]. Drugs that are substrates for metabolism mediated by cytochrome P450 (CYP) enzymes are particularly subject to herb-drug interactions, which can be attributed to a large extent to CYP polymorphism and ability of many herbal compounds to induce or inhibit these enzymes[4].

Rosemary (*Rosmarinus officinalis L., Lamiaceae*) is widely cultivated all over the world as an ornamental and aromatic plant, and has been commonly used for flavoring food, but also for different medicinal purposes. In traditional medicine, rosemary was used as mild analgesic, for relieving renal colic pain, dysmenorrhea, respiratory disorders, due to its antispasmodic properties[5,6]. Recently, essential oil isolated from rosemary and monoterpenes as its main active compounds have been of great interest due to their various health benefits and therapeutic effects. According to the recommendation of European Medicines Agency (EMA) from 2010, rosemary essential oil (REO) can be used for treating dyspepsia and mild spasmodic disorders of the gastrointestinal tract, as well as an adjuvant in the relief of minor muscular and articular pain and in minor peripheral circulatory disorders[7]. Besides, the experiments conducted with REO have demonstrated its several notable pharmacological effects, such as an-

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tioxidant and antimicrobial[8], anti-inflammatory and antinociceptive[9], antidepressant[10], cognition-enhancing[11], among others.

On the other hand, there are several well-established interactions of rosemary preparations with different drugs, such as antibiotics, anxiolytics and anticoagulant medicines, in terms of potentiation of their activity[3,12]. Given that REO was found to exert analgesic effects in different experimental models of nociception, its potential interactions with analgesics may be assumed. Besides, it was demonstrated that REO and its main components induce catalytic activities of microsomal enzymes, particularly CYP2B, but also slightly the activity of UDP-glucuronosyltransferase (UGT) and, therefore, may interact with drugs metabolized by cytochromes or UGT[13,14].

Since first isolated from *Papaver somniferum L.* in 1832, codeine has been used as an analgesic, antitussive and antidiarrhoeal drug. For its analgesic effect, codeine is regarded as a prodrug that is metabolized through O-demethylation to morphine by CYP2D6 and N-demethylation to norcodeine by CYP3A4. Codeine, morphine and norcodeine can be further glucuronidated. The majority of the analgesic effect of codeine is attibuted to the potent μ-receptor agonist morphine[15]. Genetic polymorphism of CYP2D6 is common and can affect therapeutic response of codeine. Poor CYP2D6 metabolizers are at risk of reduced or abolished analgesic effects of codeine, while people who are ultra-rapid metabolizers are at greater risk of opiate related side-effects when given codeine at commonly used therapeutic doses[16].

Paracetamol (acetaminophen) is one of the most widely used drugs for the treatment of both acute and chronic pain. It has a unique position among analgesic drugs, having a spectrum of action similar to that of NSAIDs, but with negligible anti-inflammatory and antirheumatic activities. The mode of action of paracetamol has still not been fully elucidated, but there are some evidences supporting a central analgesic effect. It is now generally accepted that it inhibits COX-1 and particularly COX-2 through metabolism by the peroxidase function of these isoenzymes[17,18]. Recently, it was discovered that paracetamol may act as a prodrug by triggering the CB_1 receptor - mediated effects of the cannabinoid system. At therapeutic doses, majority of paracetamol is conjugated with glucuronic acid and, to a lesser extent, with sulphate or cysteine. A fraction usually ranging from 5 to 15% is oxidized by CYP2E1, CYP1A2, CYP3A4, and CYP2A6 subfamilies of cytochromes P450, resulting in the formation of the highly reactive and hepatotoxic N-acetyl-p-benzoquinoneimine (NAPQI), which is quickly conjugated with glutathione to form non-toxic cysteine and mercapturic acid conjugates[19]. Hepatic enzymes induction may increase paracetamol hepatotoxicity, and therefore the potential for interactions of paracetamol with herbal medicinal products should be carefully considered.

Based on the above mentioned facts, the aim of our study was to examine analgesic effects of REO and its pharmacodynamic interactions with codeine and paracetamol in mice.

Materials and Methods

Plant Material and Chemicals

Aerial parts of cultivated plants of rosemary were obtained from the Institute for Studies on Medicinal Plants, Dr Josif Pancic, Belgrade, in 2010. A voucher specimen of the plant (Rosmarinus officinalis L. 1753 subsp. officinalis No 2-1746, det.: Goran Anackov) was confirmed and deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Natural Sciences, University of Novi Sad. The essential oil, used in our experiments, was isolated from the obtained plant material.

Paracetamol was purchased from Sigma-Aldrich (St Louis, MO, USA). Codeine hydrochloride was obtained from Fampharm (Kruševac, Serbia).

Isolation and Analysis of Essential Oil

The essential oil was isolated from air-dried aerial parts of rosemary by hydrodistillation, according to the procedure of the European Pharmacopoeia 4[20]. N-hexane was used as a collecting solvent, which was afterward removed under vacuum from the obatined essential oil.

The identification and quantification of chemical constituents of the essential oil were carried out by gas chromatography coupled with flame ionization detection (GC/FID) and mass spectrometric detection (GC/MS). GC/FID analysis was performed using a Hewlett-Packard HP 5890 series II chromatograph equipped with an autosampler and a split/splitless injection system. The capillary column used in this study was HP-5 (25 m × 0.32 mm; film thickness of 0.52 μm), coupled to the flame ionization detector (FID). The injector and detector temperatures were set at

250°C and 300°C, respectively, and the column temperature was programmed from 40 to 260°C at a rate of 4°C/min. The flow rate of hydrogen as a carrier gas was 1 ml/min. A sample of 1% solution of the oil in ethanol (1 µl) was injected in split mode (split ratio, 1:30). GC/MS analysis was carried out using a Hewlett-Packard HP G1800C series II GCD system under the same analytical conditions as in GC/FID. The column HP-5MS (30 m × 0.25 mm; film thickness 0.25 µm) and helium as a carrier gas were used in this analysis. The system was operated in electron ionization (EI) mode at 70 eV, in the mass (m/z) range 40-450 Da.

Identification of essential oil constituents was performed by comparison of obtained mass spectra and retention indexes with those of reference compounds or those from mass spectra libraries and literature data. The quantitative analysis provided the percentage composition of the essential oil components, calculated by FID peak area normalization method.

Animals and Treatment

Experiments were carried out on adult, sexually mature NMRI mice of both sexes, weighing 25-35 g, which were obtained from the Veterinary Institute Novi Sad, Serbia. Animals were housed in standard laboratory cages at a controlled temperature (23 ± 1°C) and humidity (55 ± 1.5%) under standard circadial rhythm (day/night), with free access to pelleted food and water. Animal care and experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals edited by Commission of Life Sciences, National Research Council (USA). The experimental procedures were approved by Ethical Committee for Animal Use in Experiments of the University of Novi Sad (No. 01-153/6-2).

All animals were divided into 8 experimental groups, each containing 6 individuals, and treated as follows:

- **ConS:** control group, saline solution p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of saline solution
- **REO:** REO (20 mg/kg) p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of REO
- **Cod:** saline solution p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of codeine
- **Par:** saline solution p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of paracetamol
- **CodR10:** REO (10 mg/kg) p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of codeine
- **CodR20:** REO (20 mg/kg) p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of codeine
- **ParR10:** REO (10 mg/kg) p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of paracetamol
- **ParR20:** REO (20 mg/kg) p.o. for 7 days, where the last dose on the 7th day was applied 30 minutes before i.p. administration of paracetamol

Applied daily doses of REO for mice were 10 mg/kg and 20 mg/kg. Recommended human daily dose of REO of 40 mg/day for a male of approximately 70 kg weight[7,21] was adapted for the experimentation on mice. Each animal received appropriate dose of REO in the volume of 10 ml of emulsion per kg of body weight, by per os gavage. The tested compounds, codeine hydrochloride (30 mg/kg) or paracetamol (60 mg/kg), were administered intraperitoneally (i.p.) 30 min after the last REO intake. The control group of animals received an equivalent volume of saline solution. All experiments were carried out during the daytime.

Hot Plate Test

The hot plate test was performed by placing mice individually on hot plate and assessing their response to the thermal stimulus. The temperature of the metal plate enclosed by plexiglas walls was maintained at 52.5°C. The response time was measured in seconds at which the animal licked or flinched one of the hind paws, or jumped off the plate. To prevent tissue damage, a cut-off time was used as a double value of latencies measured before drug application. Response latencies were first determined two times before the application of the tested compound in order to determine a pre-treatment response for each mouse, and then 5, 10, 15, 20, 30, 40, 50, 60 minutes following the drug administration. After responding or reaching the cut-off time, mice were removed from the plate. Analgesic effect determined in seconds was expressed as percentage of prolongation of measured reaction time compared to control reaction time[22].

Statistical Analysis

The level of significance between the groups was assessed with the Student's *t*-test for small independent samples using MedCalc 9.2.0.1 software. All data are expressed as a mean ± standard deviation (SD). A value of $p < 0.05$ was considered to be statistically significant.

Results

The essential oil obtained from rosemary had a pale yellow color and a strong odor, and the obtained yield of the essential oil was 1.03% (v/w in dry matter). The total number of identified chemical constituents was 29, representing 99.87% of the total oil content. As presented in Table I, the isolated essential oil contains a complex mixture of 95.10% of monoterpenes and 4.77% of sesquiterpenes. It was found to be composed mainly of oxygenated monoterpenes (63.88%), followed by monoterpene hydrocarbons (31.22%) and sesquiterpene hydrocarbons (4.77%). The major compounds that were identified and quantitated by GC/FID and GC/MS were 1,8-cineole (43.77%), camphor (12.53%), α-pinene (11.51%), β-pinene (8.16%), camphene (4.55%), and β-caryophyllene (3.93%).

In our study, REO (20 mg/kg) increased significantly the latency time of animal response to heat-induced pain between 20th and 50th minute of the test, when compared to saline-treated group (Table II). Codeine and paracetamol were used as antinociceptive reference drugs. We

Table I. Chemical composition of the rosemary essential oil.

Compounds	RT-FID[a]	RT-MS[b]	RRT[c]	Percentage content (% m/m)
Monoterpene hydrocarbons				31.22
Tricyclene	12.640	6.82	0.579	0.23
α-Thujene	12.777	7.00	0.585	0.13
α-Pinene	13.100	7.19	0.600	11.51
Camphene	13.737	7.62	0.629	4.55
Sabinene	14.697	8.47	0.673	0.05
β-Pinene	14.887	8.50	0.681	8.16
β-Myrcene	15.292	9.04	0.700	0.99
α-Phellandrene	15.955	9.43	0.730	0.19
δ3-Carene	16.212	9.62	0.742	0.13
α-Terpinene	16.456	9.85	0.753	0.14
p-Cymene	16.782	10.13	0.768	1.23
Limonene	16.970	10.25	0.777	2.80
γ-Terpinene	18.178	11.30	0.832	0.92
α-Terpinolene	19.416	12.31	0.889	0.19
Oxygenated monoterpenes				63.88
1,8-cineole	17.124	10.43	0.784	43.77
Linalool	19.746	12.82	0.904	0.46
Camphor	21.845	14.30	1.000	12.53
Isoborneol	22.318	14.71	1.022	0.53
Borneol	22.655	15.05	1.037	2.97
Terpinen-4-ol	23.059	15.44	1.056	0.56
α-Terpineol	23.548	15.93	1.078	1.53
γ-Terpineol	23.802	16.17	1.090	0.40
Bornyl acetate	27.185	19.14	1.244	1.13
Sesquiterpene hydrocarbons				4.77
α-Copaene	30.598	22.03	1.401	0.12
Longifolene	31.866	22.93	1.459	0.18
β-Caryophyllene	32.240	23.41	1.476	3.93
α-Humulene	33.406	24.45	1.529	0.36
Germacrene D	34.029	25.18	1.558	0.08
δ-Cadinene	35.528	26.59	1.626	0.10
Total identified				99.87
Number of compunds identified				29

[a]RT-FID - retention time in GC/FID system; [b]RT-MS - retention time in GC/MS system; [c]RRT - relative retention time with respect to camphor.

Table II. Response latency times to the termal stimulus in seconds.

Time [min] Group	0	5	10	15	20	30	40	50	60
ConS	10.9 ± 2.8	11.1 ± 2.5	9.9 ± 2.2	12.5 ± 3.4	10.1 ± 1.8	10.9 ± 2.8	10.3 ± 1.7	11.6 ± 1.6	11.9 ± 2.5
REO	10.9 ± 2.8	12.9 ± 3.3	13.8 ± 7.7	15.2 ± 3.0	14.2 ± 3.2*	14.7 ± 5.2	14.2 ± 6.5	15.6 ± 3.1*	14.4 ± 3.9
Cod	10.9 ± 2.8	20.1 ± 8.7*	20.8 ± 12.8*	21.1 ± 14.2	15.9 ± 5.1*	13.7 ± 4.8	15.4 ± 5.8*	15.7 ± 3.0*	14.5 ± 3.9
Par	10.9 ± 2.8	10.3 ± 1.1	12.4 ± 3.9	11.7 ± 2.3#	11.1 ± 3.8	11.6 ± 2.5	12.4 ± 1.8*	13.3 ± 2.3	12.1 ± 5.1

All values are expressed as mean ± standard deviation. *Significantly different from ConS group; #Significantly different from REO group; $p < 0.05$.

showed that analgesic effect of REO was slightly higher than that of paracetamol, but significantly lower than that of codeine, especially from 5th to 20th minute of the experiment.

As shown in Figure 1, the administration of codeine in combination with REO in the dose of 20 mg/kg significantly supressed the termal pain response of animals, and induced more pronounced analgesic effect when compared to mice treated only with codeine. On the other hand, REO in the dose of 10 mg/kg slightly lowered codeine analgesic effect.

We demonstrated that analgesic effect of REO was comparable to those of paracetamol, alone and in combinations with REO (Figure 2). The administration of REO in the dose of 20 mg/kg with paracetamol significantly prolonged the reaction time of animals provoked by heat stimuli, when compared to both saline- and paracetamol-treated group, with a maximal response observed between 30th and 50th minute of the test.

Discussion

In this research, we determined chemical composition of the studied REO, its analgesic activity, and its potential to interact with opiod analgesic codeine and non-opioid analgesic paracetamol. Two main chemotypes of essential oils isolated from rosemary have been reported considering the chemical composition. The main component of

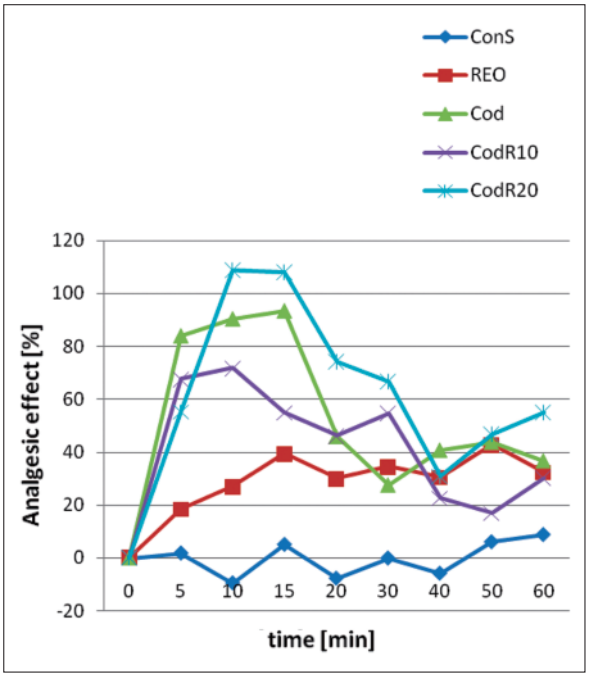

Figure 1. Interactions of REO with codeine. Analgesic effect is expressed as percentage of prolongation of measured reaction time compared to control reaction time.

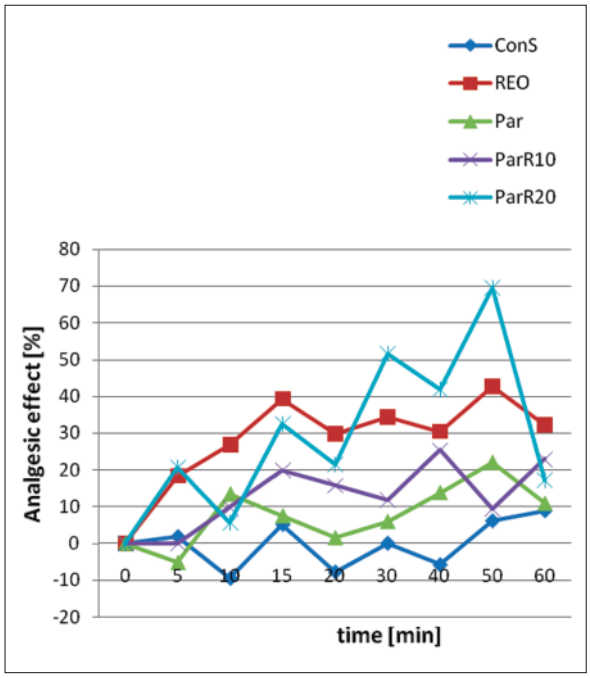

Figure 2. Interactions of REO with paracetamol. Analgesic effect is expressed as percentage of prolongation of measured reaction time compared to control reaction time.

the Tunisian, Turkish, Moroccan and Italian oils is 1,8-cineole with usually over 40%, whereas most Spanish, French and Greek oils have 1,8-cineole, α-pinene and camphor with approximately equal ratios (20-30%)[8]. The main constituents in the rosemary essential oil investigated in our study were 1,8-cineole (43.77%), camphor (12.53%), and α-pinene (11.51%), and therefore it can be categorized in the Morocco/Tunisian type. It should be noted that there are several factors that contribute to significant variations in the chemical composition of rosemary essential oils, including the geographic origin, part of the plant, season of harvesting, hence the phenological stage of the plant, and also the essential oil isolation method[23]. All effects of REO should be, therefore, carefully examined, considering the chemical composition of the investigated oil.

In hot plate assay 7-day treatment with REO (20 mg/kg) induced significant analgesic effects in mice, which is in accordance with results of previous studies. Analgesic activity of REO was confirmed in different nociceptive experimental models. The treatment with REO inhibited paw edema induced by carrageenan in rats in a dose-dependent manner, significantly reduced the number of writhing movements induced by the i.p. administration of acetic acid, and produced an inhibition during both early (neurogenic pain) and the late phase (inflammatory pain) of the formalin test, suggesting antinociceptive and anti-inflammatory activity of REO[24]. Besides, REO has also produced a dose-dependent antinociceptive effect in the pain-induced functional impairment model in the rat (PIFIR model)[25]. In addition to REO, analgesic effect was confirmed for different monoterpenes that constitute more than 90% of the essential oils[26], but also for the ethanol extract of rosemary[27] and its major constituent carnosol[28], and triterpenes fractionated from the rosemary extract[29].

The observed analgesic effect of REO in hot plate test in our study indicates its central mechanism of analgesia, since both paw licking and jumping are supraspinally integrated responses[30]. Serotonergic and opioid endogenous systems were suggested to be involved in the mechanism of action as an antinociceptive of the essential oil isolated from rosemary[25]. Furthermore, the GABAergic system may be another possible route involved in the pharmacological activity of rosemary[27].

Several studies using different nociceptive in vivo models demonstrated analgesic activity of many monoterpenes[31]. All three major constituents of REO in our study (1,8-cineole, camphor and α-pinene) were shown to exert analgesic effects in acetic acid-induced writhing test in mice[32]. The mechanisms of antinociceptive effects of monoterpenes are still to be elucidated, but are suggested to involve different members of the TRP channel family, as confirmed for camphor[33]. It is generally assumed that acyclic monoterpenes modulate the opioid system, while monocyclic and bicyclic monoterpenes produce analgesic effects mostly by peripheral pathways[26]. The potent analgesic and anti-inflammatory activity of 1,8-cineole, as a dominant component of REO, was shown to be mediated through the inhibition of COX enzymes and suppression of cytokines (TNF-α and IL-1β) production as well[34].

Although it possesses analgesic activity, REO can also influence catalytic activities of cytochromes and, thus, efficacy of other analgesic drugs. REO rich in 1,8-cineole was found to induce catalytic activities of microsomal enzymes, particularly CYP2B, but also slightly the activity of UDP-glucuronosyltransferase (UGT), and therefore may interact with codeine and paracetamol[14]. The main REO component 1,8-cineole also increased the levels of cytochromes CYP2B1 and 3A2[13]. It was demonstrated that water extracts of rosemary significantly increased activity of CYP2E1, 1A2 and 3A, which are involved in formation of hepatotoxic metabolite of paracetamol[35,36]. As shown in Figures 1 and 2, the administration of REO in combination with codeine and paracetamol exerted distinct effects when applied in different doses. REO in the dose of 20 mg/kg was shown to be more efficient than in the dose of 10 mg/kg, in combinations with both codeine and paracetamol, which suggests that dose determines whether REO will predominantly induce cytochromes or act as analgesic and have additive effect with administered antinociceptive drugs. This is in agreement with previous findings that many monoterpenes do not exhibit dose-dependent effects and that it is necessary to find the most appropriate dose range that shows effectiveness[37].

Conclusions

REO possesses centrally acting analgesic properties, as determined in hot plate assay, although a more in-depth evaluation of the mechanisms involved should be investigated. Our find-

ings support the use of rosemary in the management of pain, but also indicate a therapeutic potential of REO in combination with analgesic drugs. Considering nonlinear dose-response relationship of most monoterpenes, the appropriate dose of REO has to be determined in order to obtain an improved therapeutic effect without adverse reactions due to interactions.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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