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Introduction
In recent years, many plant extracts were analyzed and their positive effects on the human body were proven. The interest of the scientist to the herbal remedies has grown considerably, because of their harmless effect. They are preferable as therapeutic agents to reduce inflammation and pain. According to the European Medicines Agency (EMA) from 2010, rosemary is a traditional herbal medical product and can be used for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract (EMA, 2010). Rosemary essential oil (RO) can be used for treating as an adjuvant in the relief of minor muscular and articular pain and in minor peripheral circulatory disorders (EMA).

RO consists of different active substances– some of them are presented in high concentrations, and other compounds are presented in traces. The effect of this natural product is a result of the synergetic action of all substances (Faixova & Faix, 2008). The antibacterial, antifungal and anti-oxidant effects of RO have been described from many authors (Santoyo et al., 2005; Bozin et al., 2007). Some rosemary constituents have analyzed in vitro and in vivo as a potential anticancer agents (Huang et al., 1996). Its ability to reduce DNA damages and tumor formations determine the possible application as the chemo preventative agent (Singletary et al., 1996). Its anti-inflammatory and analgesic potential of RO was proven (Takaki et al., 2008; Raskovic et al., 2015). The application of rosemaryin folk medicine as spasmolytic was

Rosemary oil reduces electrical field stimulated tetanic muscles contractility

ABSTRACT
Rosemary oil is an essential oil extracted from Rosemary officinalis. It consists of more than 50 compounds with different biological activities. Empirically it is used in therapy for many years. Rosemary oil is analyzed in vivo and in vitro as many of its effects - antimicrobial, antitumor, antioxidant etc. are known. We cannot find suggested reports concerning the Rosemary oil effect on contractility of striated muscles. The aim of this study was to investigate the influence of Rosemary oil on maximal tetanic contraction provoked by electrical field stimulation. We use abdominal transversal muscles strips isolated from guinea pigs. The tetanic muscles force, was provoked by means of repeated multipulse electrical field stimulation, square-wave pulses of supramaximal intensity (60V) and 0.5 ms in duration were applied at frequency of 50 Hz for 3s followed by 7s pause. The concentration effect curve of the action of Rosemary oil (1.5×10^-6 M=1.5×10^-3 M) on this type electrical field stimulated - muscle activity was plotted on a statistical graph. During our observation the effect of Rosemary oil on contractile properties of abdominal muscles and calculated half maximal effective concentration (EC50). Our study revealed that myorelaxant activity of the essential oil in this concentration is time-dependent and after application in the interval of 25 minutes the contractile ability is decreased to 5.5% within comparison with the initial one. Our investigation is a first report on an action of Rosemary oil on the guinea pig's striated muscles strips.

Key words: Rosemary oil, electrical field stimulation, straight muscle contractility
RESEARCH ARTICLE

analyzed and antispasmodic activity on the smooth muscles contractions was proven. The authors induced contractions on muscles strips from ileum by KCl and demonstrated the involvement of calcium channels in this activity (Ventura-Martínez et al., 2011). However, there is no scientific evidence concerning the RO effect on contractility of abdominal transversal muscles and mechanism of its action is unknown. This determines our interest to RO as a potential product for reduction of tetanic muscle contraction.

Materials and Methods

Ethics statement

All experiments (in vivo and in vitro) were approved by the Bulgarian Food Safety Agency and the Ethics Committee of the Medical University of Plovdiv, Bulgaria.

In vitro striated muscle experiments

Animals and tissue preparations

Fifteen male Guinea pigs (300±350) g were kept under standard laboratory conditions (temperature 22±1°C, humidity 45% and 12-h light cycle). All in vitro experiments were approved by the Bulgarian Food Safety Agency.

Male Guinea pigs were euthanized and transversus abdominis striated muscles (ASM) were isolated. Preparations were obtained while cutting the muscle tissue in strips (20.0±1.5 mm length, 3.0±0.5 mm width). The samples were immediately rinsed and cooled (4°C) preparation solution. The muscle strips were isometrically fixed in individual organ baths containing 15 mL modified Krebs’ solution (KS) with temperature 35.5±0.25°C and constantly oxygenated with 95% O₂ and 5% CO₂. The preparations were connected to an isometric force transducer (TRI 201, LSi LETICA; Pnlabs.l., Barcelona, Spain). Preparations were allocated to the organ baths in random manner and muscle tension (7 mN) was applied to achieve isometric recording. They were allowed in an equilibration period of 20 minutes.

For inducing contractile activity, we used electrical field stimulation (EFS) through platinum electrodes. The electrodes were connected to both sides of each strip and to electronic stimulator (EFS-PZ03, C-optic, Bulgaria). Tetanic contractions were induced by pulse with intensity 60V; duration 0.5 ms and frequency 50 Hz which determine conditions, similar to direct (muscle) stimulation (MS). Shiina et al. (2010) and Su et al. (2012) conducted experiments with EFS and similar to our parameters. The mechanical muscles activity was recorded according to experimental protocol represented previously (Ivanov et al., 2011) with 1 ms interval of discretisation.

Concentration-response curve for Rosemary oil

The normal contractile activity was recorded when muscle contraction (MS) was applied after the equilibration period to preparations. RO in concentration 1,5μM was added to the organ baths and the change in the contractile activity was recorded for a 5-min period. The strips were washed out with KS before adding higher concentration. RO concentrations 15μM; 0.15mM; 1.5mM were studied and a concentration - effect curve was obtained. The cut-off time for each experiment was 45 minutes after muscle isolation.

Effect of Rosemary oil on the amplitude of tetanic muscle contraction

The normal tetanic MS was obtained by 3s electrical stimulus (constant supramaximal intensity 60V; duration 0.5 ms; frequency 50 Hz) and was compared to activity which was achieved after 25min exposure of the strips on 0,15mM RO.

Drugs and solutions

Rosemary oil was purchased from Dullberg comp, Hamburg, BRD. The preparation solution contains Na⁺ (143 mmol/L); K⁺ (5.84 mmol/L) and Ca²⁺ (3.7 mmol/L). Composition of KS: Na⁺ - 143 mmol/L; K⁺ - 5.84 mmol/L; Ca²⁺ - 2.5 mmol/L; Mg²⁺ - 1.19 mmol/L; Cl⁻ - 133 mmol/L; HCO₃⁻ - 16.7 mmol/L; H₂PO₄⁻ - 1.2 mmol/L and glucose - 11.5 mmol/L.

Statistics

Skeletal muscle activity: Data are presented as mean and standard error (SEM). Normal distribution was tested with one sample Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) and Tuckey post hoc test were used in case of normal distribution. In other case non-parametric Wilcoxon signed rank test was used. The number of tested preparations is given as n. Results were considered significant at P<0.05.

Results

We evaluated the effects of RO in concentrations of 1.5 μM (n=7; P>0.05); 4.5 μM (n=8; P>0.05); 30 μM (n=8; P>0.05); 0.3 mM (n=8; P<0.05); 0.75 mM (n=8; P<0.05); 1.05 mM (n=9; P<0.05) and 1.5 mM (n=7; P<0.05) on the

98

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maximal muscle force, generated by ASM. The maximal muscle force after MS in absence of RO was taken as 100% and the IC50 of RO was calculated (Figure 1). The maximal muscle force of preparations after MS was reduced with 50 % when 0.15 mM RO was present in the medium. We found no significant difference in the duration of muscle twitches before and after treatment with RO (data not shown).

The application of stimuli with high frequency (50 Hz) evokes a tetanic muscle contraction. In 10 min presence of 0.15 mM RO in the medium the maximal muscle force (10.35 ± 0.54 mN) was reduced to 8.46 ± 1.35 mN.

The application of stimuli with high frequency and long lasting duration of 0.5 ms evokes a tetanic muscle contraction. In the background of 0.15 mM RO, applied for 20 min, the maximal muscle force (10.35 ± 0.54 mN) was reduced to 5.76 ± 0.40 mN (Figure 3 - top). If 0.15 mM RO presences in the medium for more than 25 min we can detect only a little alteration of the normal muscular tonic activity. The muscle response to the electrical stimulus 500 µs 60 V 50Hz can be calculated as a 3.5% - rise of the tonic striated muscle force (Figure 3 - bottom).

To check striated muscle condition after application of RO we studied effect of MS on the maximal muscle force generated by AMS after 3h application of 0.15 mM RO and 6 times tissue-bath solution replacement. Results on Figure 4 showed relative the same maximal striated muscle force, which is an indication for unaffected muscle tissue in case of long treatment with RO - Figure 4.
Increasing the time duration of RO action at the same other conditions lead to a reduction of the response amplitudes, which could be explained by intensifying the repolarization and the release of various mediators of intramural nerve structures. It is known that a fundamental prerequisite for muscle relaxation is the reduction of intracellular \( \text{Ca}^{2+} \) concentration. This reduction is possible in the inhibition of transmembrane \( \text{Ca}^{2+} \) transport, decreased release of \( \text{Ca}^{2+} \) from intracellular calcium stores, activation of different types of \( \text{Ca}^{2+} \) pumps, as well as the synchronized activation of several of the said processes (Widmaier et al., 2010).

Due to the main application of essential oils in aromatherapy massage it will be important to prove their ability to penetrate through the skin and the existence of the local effect. But action on skeletal muscles \textit{in vitro} is different for different oils (Lis-Balchin, 2005). The chemical diversity of the RO constituent determines the diversity of their potential to affect various biological structures and processes. Since there are no literature data RO to penetrate muscle cells probably the described phenomenon is connected with influence on the intramural nerve structures or the membrane receptors. Such interference could explain the change in the power of electrostimulation responses observed following administration of RO - responsive \( \text{Ca}^{2+} \) current.

**Conclusion**

We characterized the contractile properties of abdominal transversal muscles. The present results suggest that Rosemary essential oil depresses force development, probably acting as a calcium channel blocker. It is the first report on a new mechanism of action of \textit{Rosmarinus officinalis} on the guinea pig striated muscles. We speculated that the RO may offer powerful and effective therapies for muscular spasms, tissue repair and myorelaxation.

**References**


