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Sedative and anxiolytic-like activities of the moss *Rhodobryum ontariense* water extract in rodents: a preliminary study

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Abstract – This is the first study designed to explore behavioral effects of the water extract of the moss *Rhodobryum ontariense* (ROE). Experimental adult animals received intraperitoneal injections of ROE (50-400 mg/kg; *vs.* saline) 45 min before behavioural evaluation. Motor effects of ROE were estimated in male mice using the open field test (OFT), whereas the elevated plus maze (EPM) model of anxiety was applied on male rats. Single ROE treatments significantly decreased motor activity of mice in OFT ($p<0.001$) by reducing both the percentage of activity time and the distance travelled in 15 min. Also, ROE in rats significantly increased ($p<0.05$) the percentage of time spent in open arms and the number of open-arm-entries during 5 min, which was similar to the actions of diazepam in rats (1 mg/kg). These results demonstrated that ROE produced motor sedation and anxiolytic-like effects in rodents.

Elevated plus maze / Open field test / *Rhodobryum ontariense* / Water extract / Sedation

INTRODUCTION

Mosses are one of three bryophyte phyla, which are among the rifest and the most tenacious overland plants. Besides their poor nutritional evaluation, they have been widely used as medicinal plants, especially in China (Asakawa, Ludwiczuk & Nagashima, 2012). Traditional Chinese medicine suggests that some mosses of the genus *Rhodobryum* (Bryaceae) may be useful to treat hypertension, cardiopathy and neurasthenia, mostly as crude drugs and in the form of medicinal teas (Harris & Yang, 2009). In consequence of such suggestions, our research group have recently investigated the chemical composition and biological activity of the previously unexplored species *Rhodobryum ontariense* (Kindb.) Kindb.,

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Abbreviations: EPM, elevated plus maze; OFT, open field test; ROE, water extract of *Rhodobryum ontariense*; SHR, spontaneously hypertensive rats.

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which is widely distributed (Pejin, Sabovljevic, Tesevic & Vajs, 2012b; Pejin *et al.*, 2011a; Pejin *et al*., 2012 a, c, d). Both *in vitro* and *in vivo* studies on spontaneously hypertensive rats (SHR) suggested antihypertensive potential of the water-based extract of *R. ontariense* (ROE) (Pejin *et al*., 2011b, 2012e). This study was intended to expand the biomedical research of ROE on the behavioural effects. The aim of this survey has been to investigate a possible sedative action of ROE, which was claimed by indigenous communities for two other *Rhodobryum* species, namely *R. giganteum* and *R. roseum* (Harris, 2008). In addition, ROE was evaluated for its anxiolytic-like activity. For these purposes, two behavioural rodent models were used: open field test (OFT) on mice and elevated plus maze (EPM) on rats.

MATERIAL AND METHODS

Plant material

The sample of *Rhodobryum ontariense* (Kindb.) Kindb. originated from the Fraser's Hill (Malaysia, August 2010). A voucher specimen has been deposited in the Herbarium of the Institute of Biological Sciences, Faculty of Science, University of Malaya (KT Yong 7635).

Preparation and analysis of water extract of R. ontariense

Before extraction the moss was carefully inspected for contaminants: soil and plant material were completely removed. The gametophyte tips were used for the extraction. Briefly, air-dried parts of *R. ontariense* (90 g) were ground and extracted with hot water for 30 min. The extract (tea) was filtered and concentrated by lyophilisation to give the residue (the yield, 10%) which was stored at –20°C for further use.

Animals and experimental design

Male adult HAN mice (7-8 weeks, weighing 27-35 g) were used in the OFT. Three-month-old male Wistar albino rats, weighing 200-240 g, were employed in the EPM. The animals from our breeding stock (the vivarium of the Institute for Biological Research, Belgrade) were housed in groups of 6 *per* cage under controlled conditions (room temperature 22-25 °C, humidity 40-70%, 12 h light-dark cycle, food and water *ad libitum*). The maintenance of animals and experimental protocols were in accordance with the Official Institutional Guide for Experimental Work on Animals, adjusted to the European Communities Council Directive (86/609).

The rodent experimental groups consisted of 6 animals each. Freshly prepared saline (0.9% NaCl) solutions of ROE were injected intraperitoneally (*i.p.*) to mice (50 mg, 100 mg, 200 mg or 400 mg in 10 ml of saline *per* kg b.w.) or rats (50 mg, 100 mg, 200 mg in 2 ml/kg of saline). Saline injections were used in control groups, while diazepam was applied as referent drug in EPM (*i.p.*; 1 mg/kg b.w.).

Open-field test (OFT)

OFT is a standard behavioral test (Vogel, 2008), which was performed to evaluate the effects of the four doses of ROE on spontaneous motor activity of mice. It was implemented daily between 10 a.m. and 1 p.m. Immediately after the injection, each animal was positioned in the center of the plastic open-box ($40 \times 40 \times 30$ cm). There were four separated cages placed in the test room with acoustic isolation and dim illumination (indirect 2×40 W light). During the 45 min post-injection, the mice were allowed to accommodate to the environment. Subsequently, in the next 15 min, motor activities of up to four animals were registered by web-camera positioned above the cages, which was connected to PC and controlled by ANY-maze software (ANY-maze Video Tracking System 4.30, Stoelting Co., USA). This software was also used to register and calculate two parameters chosen to illustrate animal locomotion: (1) percentage of time when animals were moving in space, and (2) total distance that animal bodies travelled in space (in m).

Elevated plus maze (EPM)

Anxiolytic-like activity of ROE was examined by EPM, according to the described procedure (Pellow, Chopin, File & Briley, 1985). The apparatus for EPM was positioned in a sound attenuated room with a diffuse illumination. It was made of blue acrylic and consisted of two open (50×10 cm) and two closed arms (50×10 cm) with 40 cm walls, connected by a central platform (10×10 cm); the open arms were opposite to each other and the cross platform was elevated to a height of 50 cm. The experimental procedure was performed on the 5 experimental groups of 6 rats, 45 min after the treatment, whereas each rat was being placed in the central square of the maze, facing one of the enclosed arms. Behavior was recorded for the next 5 min with a video camera mounted vertically above the apparatus. Two parameters were automatically scored and analysed by ANY-maze software: (1) the number of entries into the open arms, and (2) the time of permanence in the open arms, which was subsequently reformulated in the percentage of time spent in the open arms. The apparatus was cleaned (10% ethanol) after each trial to remove any trace of odor.

Statistical analysis

Raw data from both behavioral tests, collected and calculated by AnyMaze software, were submitted to the GraphPad Prism software (v 4.00 for Windows, GraphPad Software, San Diego, USA) for statistical analysis. These data were analysed by one-way ANOVA, followed by Bonferroni's multiple group comparison test, if overall differences were significant ($p < 0.05$).

RESULTS

Effects of ROE in OFT on mice

The effects of the acute treatment with ROE on mice behaviour in OFT are presented on Fig. 1. For the period between 45 and 60 min after the treatment, one-way ANOVA indicated strong influences of ROE on both parameters that

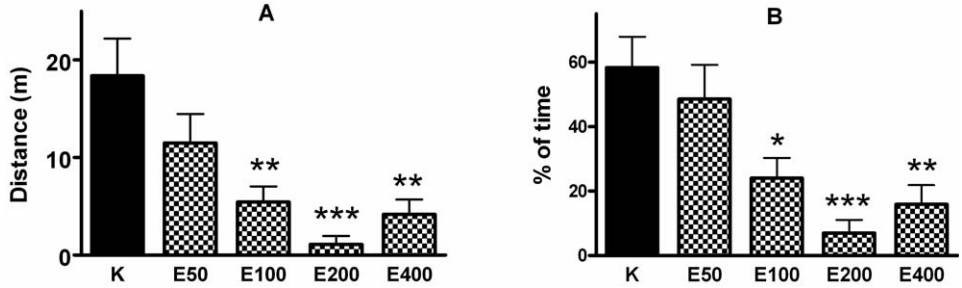

Fig. 1. Effects of ROE on male mice in OFT. Four groups of animals (E50, E100, E200, E400) were intraperitoneally injected with ROE (50-400 mg/kg body wt.) dissolved in saline. Control group (K) received only saline. (**A**) Distances (in m) travelled during 15 min; (**B**) Percentages of time when mice were in ambulation. Each bar represents mean ± S.E.M. (n = 6). *, p<0.05; **, p<0.01; ***, p<0.001 *vs.* control group, by Bonferroni's multiple group comparison test.

represent motor behaviour of the animals: the distance travelled /$F(4,25)=8.18$, $p=0.0002$/, and the percentage of the 15-min-period when animals were moving in space /$F(4,25)=8.10$, $p=0.0002$/. Post-hoc analysis detected the greatest reduction of both parameters ($p<0.001$) after the 200 mg/kg dose. This treatment induced almost 17-fold reduction of the distance travelled and about 7-fold decrease of the percentage of activity time. The higher dose (400 mg/kg), as also the lower dose (100 mg/kg) of ROE, provoked significant ($p<0.01$), but smaller effects on the both OFT parameters (Fig. 1). The lowest dose applied (50 mg/kg) was without significant effects.

Effects of ROE in EPM on rats

EPM was applied to investigate possible anxiolytic-like effects of acute ROE application on rats in comparison to diazepam (1 mg/kg). As shown in Fig. 2, this influence was significant for the number of rat entries into open arms [$F(4,25)=5.31$, $p=0.003$], and for the percentage of time spent in open arms [$F(4,25)=4.68$, $p=0.006$]. It is evident (Fig. 2) that the two higher doses of ROE

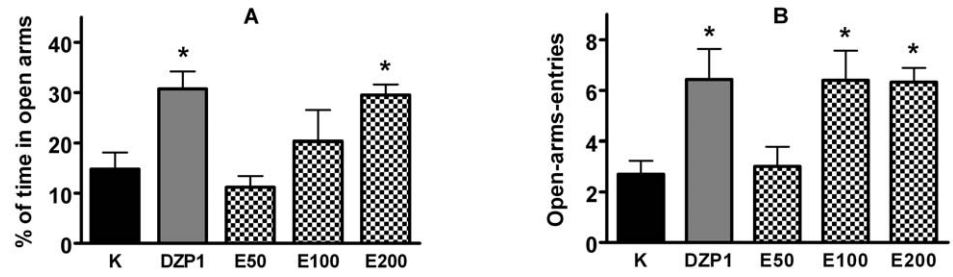

Fig. 2. Effects of ROE on male rats in EPM. Animals received intraperitoneal injection of either ROE (50-200 mg/kg body wt.; E50, E100 and E200 groups), diazepam (1 mg/kg; DZP1) or saline (K). (**A**) Percentages of time spent in the open arms; (**B**) Numbers of entries in the open arms during 15 min. Each bar represents mean ± S.E.M. (n = 6). *, p<0.05; *vs.* control group, by Bonferroni's multiple group comparison test.

(100 and 200 mg/kg) almost equally increased the number of entries in open arms (about 2.5 times), which was similar to the effects of diazepam, whereas only 200 mg/kg of ROE extended the percentage of time spent in the open arms.

DISCUSSION

This is the first experimental study that presents behavioural effects of *R. ontariense*. The results of the behavioural tests in rodents suggest that ROE contains at least one active principle, which may suppress motor activity of mice in a manner that could be expressed as sedation. It appears to be the unique experimental evidence for the sedative potency of any of *Rhodobryum* species, as far as we know, outside the claims of the traditional Chinese medicine for this moss genus *Rhodobryum* (Asakawa, Ludwiczuk & Nagashima, 2012). We do not consider this sedation as the consequence of a general hypotension induced by ROE, since this chance has not been corroborated by our previous study, which has shown that ROE (100 mg/kg) quickly normalises arterial blood pressure and reduces pulse pressure only in the group of SHR rats, without any effects on the rats with normal blood pressure (Pejin *et al*., 2011b).

The peculiarity of the ROE action on mice in OFT is an absence of the linear dose-activity relation. It seems that the inhibition of motor action exhibits the dose-activity pattern of an inverted U-shape. This evidence suggests that there are components of ROE which at their higher doses may counteract a reduction of motor activity. The results of EPM exhibit that ROE may also have an anxiolytic-like activity, whereas the pattern of this dose-activity curve appears to be more linear in relation to the sedative actions. Unfortunately, we could not evaluate the effects of a higher (400 mg/kg) dose of ROE on rats in EPM, as we were on mice in OFT, due to the limitations of the extract amounts*, i.e.* the herbal availability for the preparation. Therefore, we were not able to test a possibility if the anxiolytic-like activity of ROE may be reduced with the higher doses of the extract.

In conclusion, this study indicates that acute ROE application induced motor sedation and anxiolytic-like effects in rodents, which were partially dose-related, since the effects of the increasing ROE doses on OFT parameters in mice exhibited U-shaped forms. Considering the marked behavioural effects of ROE in our experimental study, it would be reasonable to extend the analyses of this extract and to identify its active principle(s), which remains our scope for the next period.

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