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HABERLEA RHODOPENSIS: A PLANT WITH MULTIPLE PHARMACOLOGICAL ACTIVITIES

R. Radev[1]*, L. Peychev[3], G. Lazarova[2], K. Sokolova[1], Z. Tsokeva[1], St. Radev[1], D. Rukanova[2]

[1]Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[2]Department of Microbiology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[3]Department of Pharmacology, Facultyof Medicine, Medical University, Plovdiv, Bulgaria

ABSTARCT

The total leaf extract from the resurrection plant Haberlea rhodopensis was screened for some pharmacological properties. Antioxidant activity was assessed by determining the total (Mg-Zn and Mn) superoxide dismutase (SOD) activity according to the method of Sun et al. Antibacterial tests were performed by the disk-diffusion method. The tests for explicit memory we conducted using the Ugo Basile (Italy) equipment: Shuttle box, Step trough, Step down and Open field. The results showed that the extract demonstrated higher SOD activity compared to the referent compound Troxol, an antibacterial activity against tested pathogenic strains markedly directed towards Gram positive microorganisms, and improved to a greater extent short memory compared to long memory. These facts can be explained by the existence of some phytochemicals such as phenol compounds. The present study has preliminary character and further ones will be developed to confirm our results and identify the compounds responsible for this bioactivity.

Key words: resurrection plant, antioxidant activity, antibacterial activity, memory changes

INTRODUCTION

Regardless of the large quantity of synthetic drugs used in contemporary medicine, the interest toward drugs of natural origin among drug producers, physicians and the population is constantly increasing. Natural remedies, especially herbal ones, are taking up a bigger portion of the pharmaceutical market. This is due to the fact that during their phylogenesis the plants have built complex biosystems for production of various phytochemical substances and those systems are, in many aspects, superior to the methodological abilities of today's synthetic chemistry. In most cases, the advantages of a natural drug from plants are fewer side effects, better patient tolerance and relatively lower cost.

***Correspondence to**: *Radoslav Radev, Ph.D, Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria, Armeiska 11 St., 6000 Stara Zagora, Bulgaria, Tel.: +359888986966, E-mai adddress: radoslav.radev@abv.bg*

From this point of view, we turn our attention to the weakly studied from a medical perspective plant Haberlea rhodopensis Friv. (HR) – rare Balkan endemic relict, belonging to the family Gesneriaceae. HR possesses a unique biological ability to fall in anabiosis for a long time when there are unfavorable conditions and to restore back to normal when the conditions become appropriate. That ability connects it to the so-called "resurrection plants". Previous basic studies have explained the mechanism of anabiosis. The bulk of the research has focused mainly on the thermo- and photostability of the photosythesis system of the herb in different conditions (1-6), as well as on the role and the activity of the constituent antioxidant enzymes of the plant (7-10). Until recently the data about the phytochemistry of HR in the literature was scarce. Different studies showed presence of lipids and polysaccharides, flavonoids, tannins, carbohydrates (11, 12). The increased interest in HR and the contemporary methods of analysis have led to a more complete characterization of the biologically active

ingredients of the plant. In 2011 Berkov et al. found more than one hundred compounds in a methanolic HR leaf extract (saccharides, phenolic acids, sterols, glycerides, amino acids, fatty acids, etc.) (13). The profile of the phenolic constituents of HR has also been investigated (14).

The small group of the so-called „ resurrection plants" is a unique model for the apprehension of the mechanisms of anabiosis and attracts the interest of biologists and phytochemists.Yet, HR and the other specimen from the Gesneriaceae family have not been studied from a medical point of view (15). According to natives, HR has been used for treatment of Paronichya contagiosa by being added to the food of the animals. Additional investigations in the specialized literature showed that this is probably the polyetiologic disease Paronychia contaginosa ovium, whose current treatment includes different antibacterial agents and local antiseptics.

Recent studies explore the cytogenetic activity of total extract of HR and demonstrate an immunomodulatory and antimutagenic effect (16, 17).

Motivated by the prospect of HR being a source of bioactive compounds with putative application in phytoterapy in human and veterinary medicine, we made a preliminary evaluation of some of its pharmacological activities. We worked in three directions: determining the antioxidant potential, testing the antimicrobial activity and identifying the influence of the extract on the processes of learning and memory in rats.

AIM

The aim of the recent study is to explore a total extract of HR for the presence of antioxidant, antibacterial activity, as well as to test its influence on the process of learning and memory in experimental animals as a stepping stone towards investigating the possible medicinal application of HR.

MATERIAL AND METHODS

H.R was collected from different locations within its natural habitat in the Plovdiv region, Bulgaria. The plant leaves were air-dried at a room temperature and then were roughly pulverized and stored in airtight containers until needed. A total extract from HR leaves macerated for 48 hours in 70% water-ethanolic solution with subsequent distillation of the ethanol in vacuum vaporizer to a drug/liquid phase proportion of 5:1, was used.

1. The antioxidant effect was assessed by determining the total (Cu-Zn and Mn) superoxide dismutase (SOD) activity according to the method of Sun et al (18), based on the inhibition of NBT (nitro – blau – tetrazol) reduction by the hypoxanthine- xanthine oxidaze system as a superoxide generator. One unit of SOD activity was defined as the enzyme amount causing 50 % inhibition in the NBT reduction rate in the absence of SOD. SOD activity was expressed as Units per gram hemoglobin. Spectrophotometer Specol 11 was used to measure the extinction at 560 nm wave. Extracts in concentration 1:1 and 1:2 were used and compared to a referent compound TroloxTM (water- soluble vitamin E analog), whose antioxidant activity is 81 U SOD per 1 mg

2. The antimicrobial activity of the extract to standard strains of Staphylococcus aureus (S. aureus ATCC 25923; MSSA S.aureus ATCC29213 (Methicillin-sensitive), MRSA S. aureus ATCC 39592 (Methicillin-resistant), E. coli ATCC25922 and P. aeruginosa ATCC27853, and clinical isolates of S. aureus 707 and 1707, has been tested. The testing was done by the disc-diffusion method using filter paper discs impregnated with 5 mcl of the solution in differing concentration of the initial extract: undiluted or diluted 1:1 or 1:2. A 24-hour culture of the examined strains with microbe density of 0,5 on the McFarlan scale was inoculated on Mueller Hinton plates (a standard solid environment for antibiogrammes). Discs impregnated with differing concentration of the extract were applied to the surface of Mueller Hinton plates. The inoculated cultures were incubated for 24 hours at 350C. After the incubation the diameter of the zones of bacterial growth inhibition around the discs were measured in mm.

3. 24 sexually immature (age 40-45 days), male albino rats Wistar strain (130-140 g) were used to determine the effect on behavioral reaction and memory. The animals were randomly divided into three groups, with each group containing 8 animals. Extracts in concentration 1:3 was used. **Group 1** served as the control group (placebo) and received orally (by stomach sond) 2ml distilled water. **Group 2** received extract of HR – 1ml/kg. **Group 3**

received extract of HR – 5ml/kg. Administration of the compounds began seven days prior to the experiments and continued 22 days after the start of the tests. Every seven days the body weight of the rats was measured in order to determine the physical development of the young rats.

The training and the tests on the behavioral reactions and memory of the animals were carried out by apparatus from Ugo Basile (Italy): shuttle box; step- through and step-down and open field.

The programs for training and memory of the rats start every day, 30 min after the inoculation of the studied compounds. The experiments with the open field test, registering horizontal and vertical moves start on the first, fifth and twelfth day and immediately continue in the Shuttle box. The training session in the Shuttle box lasts five days. We registered active avoidance, passive escapes, latency time and intertrial crossings. Short memory was tested daily and long memory was tested on the seventh day after the end of the training. The training with the Step-through and step-down lasted three days. We registered the latency time and the number of trained animals. The long term memory test was carried out on the fifth day after the end of the training.

The results from the experiments were processed with the statistical package Instat 2.0 for experimental medical studies.

RESULTS AND DISCUSSION

Antioxidant Activity

The results of the in vitro antioxidant activity data showed higher SOD activity for 1 ml extract 1 (305.4±8.4 U SOD) and for 1ml extract 2 (373.3±12.2), compared to the referent compound TroloxTM . **(figure 1)**

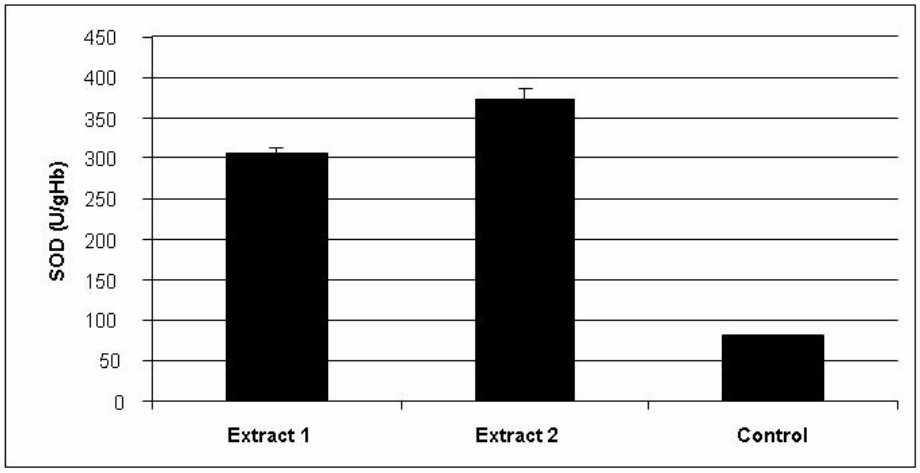


Figure 1 Antioxidant activity of Haberlea Rhodopensis

The obtained results supported the existing data of the high activity of SOD and other antioxidant enzymes in HR and in other species from the Gesneriacea (8, 9). We suggested that the increased SOD activity was probably caused by the presence of a high content of phenol compounds in the total extract of HR, which play an important role in the mechanism of anabiosis (7, 11). In the scientific literature, the presence of phenolic compounds in the plant's extracts is associated with antioxidant activity (19, 20). Several parallel studies established high phenol content and a linear relationship between the antioxidant activity and the phenolic content of HR, indicating that the phenolic compounds are major contributors to antioxidant properties (13, 14). The antioxidant activity and the free radical scavenging is likely a mechanism of radio protective effect demonstrated by HR extract. The HR pretreatment at the different doses reduced the radiation-induced oxidative stress by significantly preserving the SOD and other antioxidant enzymes. (21, 22)

Antimicrobial activity

The results from the conducted experiments are presented in **table 1**. Our results show convincingly that HR has an antibacterial effect towards the tested pathogenic strains, as well as towards the undiluted and diluted 1:1 and 1:2. This effect is markedly directed towards Gram-positive microorganisms – S. aureus, including MSSA and MRSA. Although to a smaller degree, it shows an activity against some Gram – negative strains such P.

aeruginosa and E. coli (ATCC 25922), which corresponds with the well known fact that Gram- negative microorganisms have increased activity due to their morphological and other characteristics, such as a triple structure of the cell wall. This antibacterial activity may be attributed to the glycosides and flavonoids, contained in the plant. The antimicrobial activities of various plants have been reported by many researchers (23). The research on the therapeutic use of natural products is on the increase and this may be caused by the resistance of microorganisms to many orthodox antibiotics. To the best of our knowledge, there are no scientific reports on the antibacterial activity of HR and we first reported the antibacterial activity of those species. The present study proves the antibacterial activity of the extract, but it is impossible to tell for sure whether the extract acts like an antibiotic or an antiseptic. The present study has preliminary character and can be extended by determining the minimal inhibitory concentration (MIC) of the HR extract itself or of separate active principles.

Table 1. *Zones of bacterial growth inhibition in different tested strains (mm)*

Bacterial strains	Disk-undilution extract	Disk-dilution extract 1:1	Disk-dilution extract 1:2
S. aureus ATCC 25923	14	10	10
MSSA *S. aureus* ATCC29213	20	14	12
MRSA S. aureus ATCC39592	18	15	14
S. aureus clinical isolates 707	16	14	12
S. aureus clinical isolates 1707	18	14	12
E. coli ATCC25922	8	-	-
P. aeruginosa ATCC27853	12	-	-

Effect on central nervous activity

On the fifth day of the training in the Shuttle box, the rats from the second group showed significant difference in active avoidances in comparison with placebo – 1,69±0,14 v/s PL 0,93±0,19; p<0.05 **(figure 2)**. On the fifth day, the animals from the third group increased considerably the number of escapes in comparison with placebo – 6,10±0,52 v/s Pl 4,25±0,6; p<0,05 **(figure 3)**. There were no statistically significant differences as to the indicated latency time and intertrial crossings among the experimental groups and placebo.

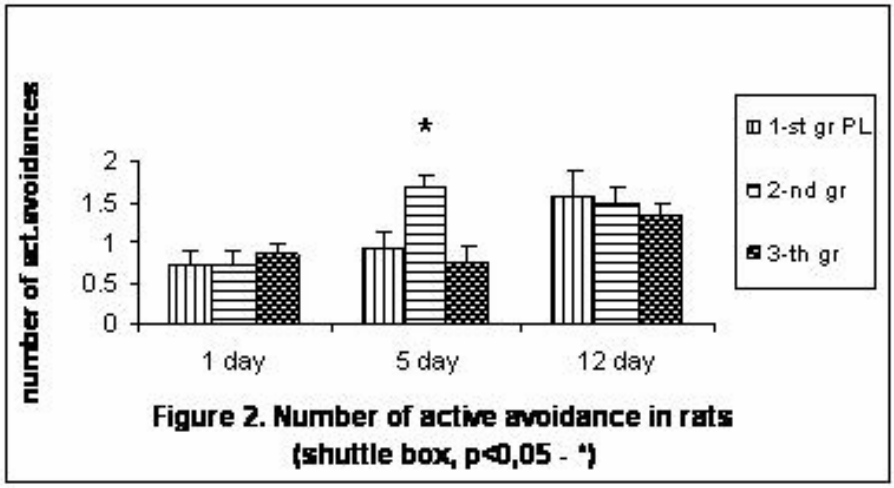
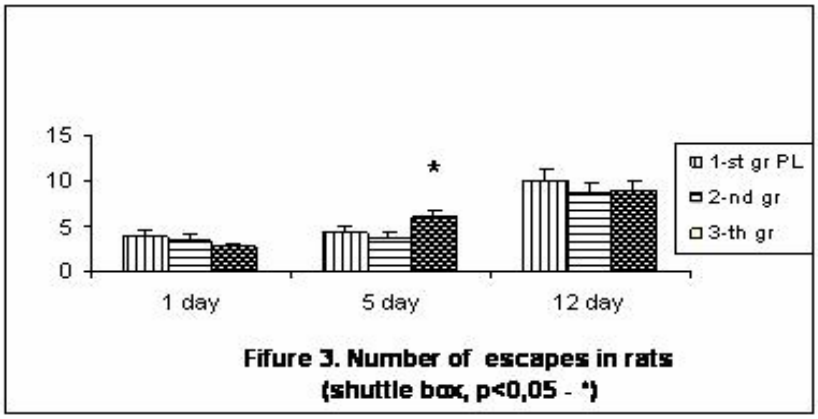


Figure 2. Number of active avoidance in rats (shuttle box, p<0,05 - *)



Fifure 3. Number of escapes in rats (shuttle box, p<0,05 - *)

The latency time of the rats treated with HR in the equipment Step- trough on the second and eleventh days of the training exhibited a tendency to shorten in comparison with placebo, but that difference was not statistically significant **(figure 4)**. Regarding to the latency time of the rats in the Step- down apparatus no statistically significant difference between the experimental groups and placebo were established. Rats from the second and third group, which were treated with HR, gained equal body mass as animals from the placebo group.

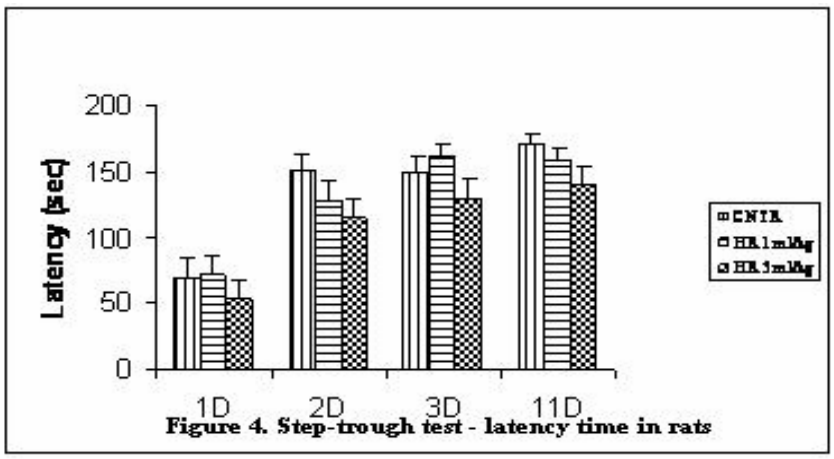


Figure 4. Step-trough test - latency time in rats

There were no statistically significant differences among the groups in respect to the horizontal and vertical movement in conditions of open field.

Long-term administration of HR extract is effective in enhancing rats' short memory and does not influences long memory. This facilitates the creation conditional reflexes among rats. The animal's locomotor activity and willingness to explore in open field remain unchanged under the influence of HR.

CONCLUSION

In our study HR showed in vitro antioxidant and antimicrobial properties as well as positive influence on short memory in rats, indicating the potential for discovery of antioxidant, antibacterial, and other pharmacological principles.The observed activities encourage further studies to confirm our results and determine the types of phytochemical compounds responsible for the different effects of these species.

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