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Original Article

Phytochemical characterization and preclinical studies of *Rhodiola heterodonta dry* extract

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ABSTRACT

The dry extract of Rhodiola heterodonta rhizome was studied for pharmacological active compounds and tested for acute/chronic toxicity, analgesic and antihypoxic activity. Using HPLC-MS it is shown, that the dry extract kept main active compounds of plant source. No chronic toxicity at dose 20 to 200 mg/kg and no histopathological change of key internal organs tissue at these doses were observed. LD_{50} at *per os* administration observed as 3550 mg/kg.

Key words: dry extract of *Rhodiola heterodonta*, acute and chronic toxicity, analgesic and adaptogenic activity

INTRODUCTION

The herbal compositions of *Rhodiola* ssp and extract from these extensively used in folk medicine plants are high effective remedy with broad desired activities such as adaptogenic, immune stimulation etc. In mountain area of Central Asia grows *Rhodiola heterodonta* [2]. Close pharmacological properties of this plant with more known *Rhodiola rosea* (golden root) and easy accessibility for industrial scale creation of herbal medicinals are reason for it phytochemical and pharmacological investigation [3]. Aim of this work was identification of phenylethanoid constituents in *dry Rhodiola heterodonta* rhizome extract and study of it pharmacological activity. Daily life of contemporary human, especially the ones living in big cities and megapolises, is full of distressing situations and conditions related, in particular, to environment, nutrition, work and rest conditions, bad habits, low physical activity. All these reduce compensatory capacity of organism and lead initially to functional and then to organic changes of various organs and systems. Thereby in prevention of diseases significance of adaptogens and dietary supplements of various brands is increasing [8, 9]. Elaboration of preparation of this group produced from local raw materials and determination of the most effective one is an actual task. An example of plant which can be used as raw material for effective dietary supplement is *Rhodiola heterodonta* – perennial plant growing in Uzbekistan and seen in most mountainous regions of Central Asia. Roots and rootstocks of this plant contain polyphenols and in particular proantocyanidines. The research also indicated low toxicity of preparations obtained from this raw material. There are other types of allied species showing tonic, adaptogenic and other valuable properties. The most common type is *Rhodiola rosea*. The extract of Rhodiola heterodonta may be an alternative to a certain degree.

MATERIAL AND METHODS

Plant material: Rhizomes of *Rhodiola heterodonta* (Hook et Thoms) Boriss. Were harvested from Ugam mountain ridge Tashkent region, Uzbekistan in 2014. Identification of species specificity carried out by prof. A.Y.Ibragimov, department of pharmacognosy of Tashkent Pharmaceutical Institute.

Preparation of dry extract: Air dried rhizome of *Rhodiola heterodonta* was grinded (particle sizes 2-3 mm) and extracted 3 days with 75% aqueous ethanol at proportion 1 g plant: 5 ml solvent by infusion method at room temperature. Extract filtered through 4 layers of tissue filter and dried using rotary evaporator at bath temperature 40 ^{o}C.

Animal: For experiments healthy outbred white mice weighting 20±2 g of both sexes have been selected and used. The animals were procured and

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housed in the animal house of Tashkent Pharmaceutical institute. They were kept under standard hygienic conditions, at 25±3 °C, relative humidity 60±10 %, with 12 hour day/night cycle, with food and water *ad libitum* according to the governmental rules of Republic of Uzbekistan.

Analgesic activity: Analgesic activity of experimental animals given 100 mg/kg of dry extract (*per os*) was studied by tail compression test in mice [5] and characterized as decreasing of squeal reaction of test animal appears during pressure performed with air bulb.

Antihypoxic activity: The antihypoxic effect of dry extract was studied on asphyxial hypoxia model. The tested dry extract was administered at a dose of 40 mg/kg intraperitoneal as a 1% water suspension, 15 min prior to the experiment. Animals after administration of testing doses were placed into hermetic bottles of 200 cm^3. Latency of hypoxic time (min) was compared with the control, water-treated group.

Acute toxicity: Acute toxicity was carried out on mice, LD_{50} calculated according modified Litchfield and Wilcoxon graphical method [10]. Single dose of *R.heterodonta* water suspension 2500 - 5000 mg/kg administered *per os* mode and lethality was considered after 14 day.

Chronic toxicity: Chronic toxicity was studied on white outbred mice weighing 18-22 grams. Experiment carried out using *Rhodiola heterodonta* dry extract in the form of 1% water suspension administering orally. The animals were divided into 4 groups. Group 1 was administered 20 mg/kg; group 2 – 50 mg/kg and group 3 – 200 mg/kg of above suspension daily dose, calculated on anhydrous extract during one month. Group 4 was administered water in equal amount. At the end of experiment the mice were killed under ether anesthesia by decapitation. Samples of organs such as stomach, small and large intestine, liver, kidneys, adrenals, spleen, lungs, heart, pancreas, aorta and brain were isolated. All the organs and tissues in were further investigated histologically - morphologically. Tissue samples were processed with conventional histological methods, the sections were stained with haematoxylin and eosin and examined under the Leica-Biomed, Germany microscope.

All of obtained digital data were statistically processed in Excel with Student criteria. Significance value was $p \leq 0.05$.

RESULTS AND DISCUSSION

The dry extract of Rhodiola heterodonta prepared as described [6] was studied for determination characteristic constituents. Main biological active constituents - phenylethanoids were determined using LC-MS [Figure 1].

Identification of phenylethanoids in MS-scanning mode [Figure 2] allows identifying 9 compounds in dry extract. These compounds are: (1)-1.93 min 260$[M+H]^+$, rhodiocyanoside A , M=259; (2) - 2.44 min 915$[M+H]^+$, dimer of EGCG, M=914; (3) - 3.14 min 459$[M+H]^+$, rosavin, M=458; (4) - 3.68 min 465$[M+2(18)+H]^+$, rosarin, M=428; (5) - 4.75 min 337$[M+2(18)+H]^+$, salidroside, M=300; (6)- 5.40 min 171$[M+32+H]^+$, tyrosol, M=138; (7) - 6.38 min 465$[M+18+H]^+$, heterodontoside, M=446; (8) - 7.31 min 315$[M+H]^+$, viridoside, M=314; (9) - 8.26 min 465$[M+18+H]^+$, mongrhoside, M=446.

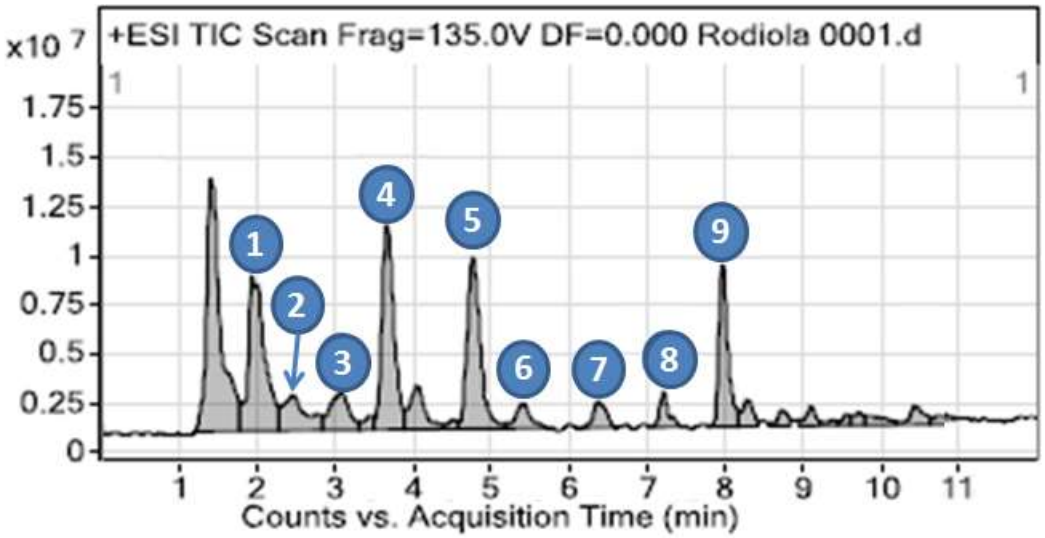

Figure 1. Total ion chromatogram of dry Rhodiola heterodonta extract, Positive ESI mode.

Quantitative determination of identified compounds was carried out by RP-HPLC [Figure 3]. Studied Rhodiola heterodonta extract contains 14.50 ±2.5 % of total phenylethanoids and cyanoglycoside (rhodiocyanoside). Content of oligomeric proantocyanidins (epigallocathechin gallate – EGCG and dimer of EGCG) was at trace amount (0.75±0.07%). The results confirm that main pharmacological important constituents are kept in dry extract.The acute toxicity of dry extract one experimental animals revealed LD_{50} value of 3550 (2920 ± 4200) mg/kg. The result indicates low acute toxicity of dry extract and reconcile with published in the literature data [1,4]. The chronic toxicity at tested doses (20-200 mg/kg) shown no toxic effects and no histopathological change of key internal organs tissue at these doses were observed. Comparative analysis of morphology of internal organs of all the groups having taken the Rhodiola heterodonta preparation indicates that the histological structure of internal organs is similar to the control group and no pathological change was observed.

Stomach mucosa is well preserved; glandular cells are without specific changes. The section shows all the layers of stomach being well preserved. The mucosa is well defined due to numerous prolonged tube-like structures – fundal glands of stomach. The integumentary epithelium is formed by a single layer of glandular epithelium. This epithelium forms stomach pits which close up with glands. At the cellular level the body and fundus of stomach is composed of numerous parietal cells. Histological-morphological analysis of stomach of experimental animals which were administered 20 - 200 mg/kg of the preparation shows that the tissues are comparable morphologically to control animals, and that Rhodiola heterodonta preparation does not cause any dystrophic changes.

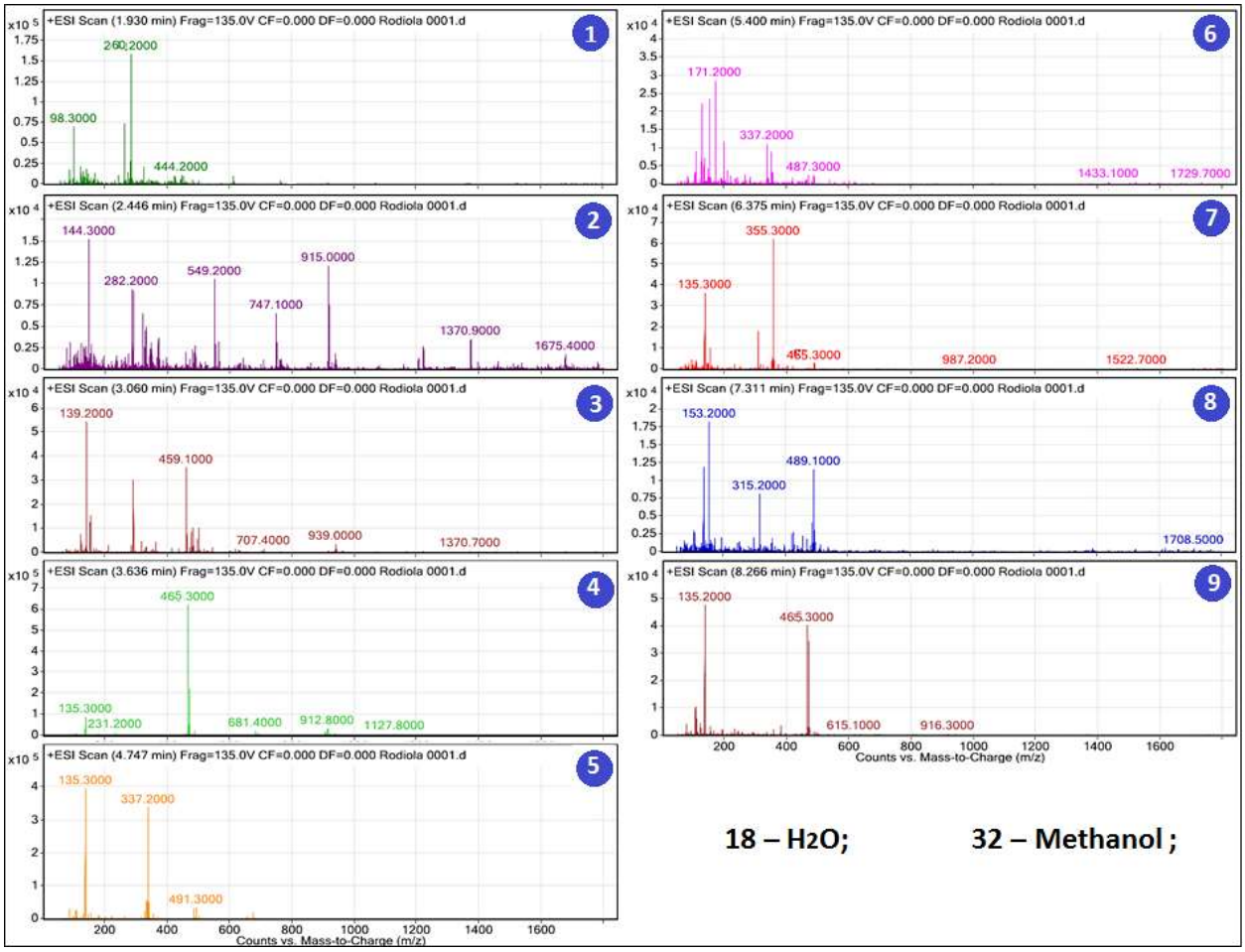

Figure 2. Mass spectrums for peaks of dry *R. heterodonta* chromatogram. Peak numbers same as in Figure 1.

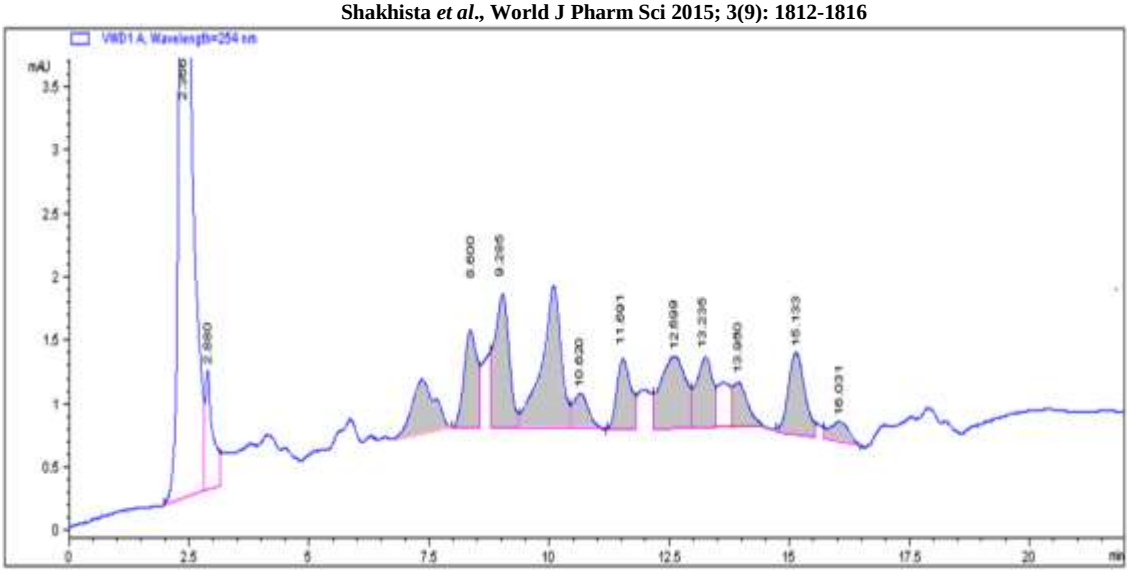

Figure 3. HPLC of dry *R. heterodonta* extract.

Small intestine has pronounced villi and crypts, and enterocytes with a rim. The crypts of large intestine are deep, layered with mucoid cells, rift of the crypts is distended and filled with mucoid secretion. Enterocytes have cylindrical form and bright pink cytoplasm, enlongated nuclei located basally. Comparative study of histo-morphology of small intestine of experimental animals that received Rhodiola heterodonta preparation at the 20-50 mg/kg and 200 mg/kg dose and of control animals revealed that they have common structure and we did not detect any significant variations. Liver of experimental animals did not shown any noticeable morphological changes, hepatic plate and hepatocytes are well preserved, many binucleatedhepatocytes, sinusoid hemocapillaries are moderately distended, stellate reticuloendotheliocytes have usual structure.

Renal corpuscule, proximal and distal parts of Henle's loop did not have any change. Moreover, chronic toxicity study did not reveal significant morphological changes of structure in the studied organs. Based on the research results and on the fact that low toxicity is confirmed by other publications, suggested preparation may be considered safe therapeutic measure and be recommended for clinical application. The dry *R.heterodonta* extract tested at 100 mg/kg *per os* dose display significant analgesic effect after 30 min of administration at 226 mm and after 90 min at 253 mm of Hg column, whereas in control group animal react for pain at pressure 93 mm. The pain sensitivity decreased on 126 and 153% respectively.

Thereby the results of the research indicated that the extract of Rhodiola heterodonta has analgesic effect which may suggest presence of adaptogenic properties. The Intraperitoneal administration of dry *R. heterodonta* extract at a dose of 40 mg/kg significantly enhance the survival time of mice in normobaric hypoxia model. Survival time of experimental animals after administration of testing doses was 23.20 ± 2.10 min, whereas control group (given distilled water) survival time was 17.20 ± 1.50 min. Latency of hypoxic time of dry extract administered animals increased for 6 min ($p<0.05$) compared with the control group. The antihypoxic activity of studied dry extract which stipulated salidroside content [7] is one of significant pharmaceutical properties of *R. heterodonta*.

CONCLUSIONS

The experiments showed that the dry extract of *Rhodiola heterodonta* kept main pharmacological important constituent of plant source. In dry extract are identified 9 active phenylethanoids - factors responsible for adaptogenic and enhancing the body's resistance to stress and fatigue. Study of acute/chronic toxicity, analgesic and antihypoxic activities allows consider of investigated dry extract as potential remedy for treatment above disorders.

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