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Pharmacological activities of *Hypericum scabrum L*

B. ESLAMI[1], S.F. NABAVI[2], S.M. NABAVI[3], M.A. EBRAHIMZADEH[4], M. MAHMOUDI[4]

[1]Department of Biology, Islamic Azad University, Qhaemshahr Branch, Qhaemshahr (Iran)
[2]National Elites Foundation of Iran, Tehran (Iran)
[3]Department of Biology, College of Sciences, University of Mazandaran, Babolsar (Iran)
[4]Pharmaceutical Sciences Research Center, School of Pharmacy and Traditional and Complementary Medicine Research Center, Mazandaran University of Medical Sciences, Sari (Iran)

Abstract. – *Objectives:* *Hypericum* spp. (H.) has been used in traditional medicine for their sedative effect for many years. In spite of many works on this genus, little is known about *H. scabrum*. In this work antidepressant and its protective effect against hypoxia-induced lethality were evaluated.

Materials and Methods: **Antidepressant and its protective effects against hypoxia-induced lethality were evaluated. Antidepressant activity was determined by forced swimming test (FST) and tail suspension test (TST). Antihypoxic activities were determined by asphytic, haemic and circulatory hypoxia models in mice. Also, hydrogen peroxide scavenging activity and cumene hydroperoxide (CuOOH) induced hemolysis also were investigated.**

Results: **At all tested doses *H. scabrum* significantly and dose dependently reduced the immobility periods in FST and TST. Also, extract showed statistically significant antihypoxic activities in three asphytic, haemic and circulatory hypoxia models in mice. The extract showed moderately good scavenging activity with IC_{50} = 518.8±20.7 µg ml^{-1}. Extract inhibited significantly CuOOH induced hemolysis in red blood cells (RBC).**

Conclusions: ***H. scabrum*** **aqueous extract showed remarkable antihypoxic and antidepressant effects thus, lend pharmacological justification to the use of the plant extract by traditional medicine practitioners. Mechanism of antidepressant activity of extract may be through nitric oxide pathway.**

Key Words:

Antidepressant activity, Antihypoxic activity, *Hypericum scabrum*, Cumene hydroperoxide, Antihemolysis.

Introduction

Despite the immense technological advancement in modern medicine, many people from all over the world still rely on traditional medicine and medicinal plants for their daily healthcare needs because they are safe[1]. *Hypericum* (*Hypericaceae*) (H.) genus which contains more than 400 species occurs throughout the world and is well represented in the Mediterranean and the Near East Areas distributed across tropic and subtropic regions, as well as across Europe, Asia, Africa and North America[2,3]. Recently, there has been increasing interest in the genus *Hypericum*, because it is a source of a variety of compounds[2]. Modern studies have been focused on the activity of extracts of these plants against certain viruses and bacteria and on their possible applications as medicines for various diseases[4]. Many reports have been published for antimicrobial, antifungal, antiviral, antioxidant, antidepressant and anticonvulsant activities of *Hypericum* species[2,5-9]. Previous reports showed that *H. scabrum L* has antimicrobial activity[10]. Also, it used to its sedative effect and has antiseptic, antidiarrhea, antihemorrhoid, antieczema, antipsoriasis, anthelmintic and antifungal activities[11]. Essential oil composition, fatty acid composition, antimicrobial and antiulcerogenic activities of *H. scabrum* have been reported[2,4,8,9,12].

In order to scientifically evaluation of some ethnomedical uses of *H. scabrum*, its antidepressant activity and protective effect against hypoxia-induced lethality in mice were investigated.

Materials and Methods

Chemicals

Phenytoin sodium, cumene hydroperoxide (CuOOH) and hydrogen peroxide were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Sodium nitrite and sodium fluoride were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade or purer.

Corresponding Author: Mohammad Ali Ebrahimzadeh, Ph.D; e-mail: zadeh20@yahoo.com

Plant Material and Preparation of Freeze-Dried Extract

H. scabrum (*Hypericaceae*) aerial part was obtained in summer of 2008 from Golestanak area, central Elburz Mountain, northern of Iran. The sample was authenticated by Dr. Bahman Eslami and the voucher specimen (No. HS132) has been deposited in the Sari School of Pharmacy Herbarium. Plant material was dried under dark conditions at room temperature for 10 days. The dry material was milled, obtaining 2-5 mm particles and then extracted by water for 24 h at room temperature. The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper and repeated three times. The resulting extracts were concentrated over a rotary vacuum at 40°C until a crude solid extract was obtained which then was freeze-dried (MPS-55 Freeze-drier, Cperon, Korea) for complete solvent removal (29%).

Animals

Swiss albino mice with either sex (20-24 g, Institute Pasteur of Iran) were used in this study. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23±3°C with a 12-h light/12-h dark cycle (light on from 06:00 to 18:00 h). Each animal was tested once. All of the experiments conducted between 10:00 and 14:00 h. All the experimental procedures were conducted in accordance with the NIH guidelines of the Care and Use of Laboratory Animals.

Screening of Antidepressant Activity

Forced Swimming Test

The male mice were dropped into glass cylinder (20 cm in height/12 cm in diameter) containing 8-cm-deep water at 24-25ºC and left for 6 min. The duration of immobility during the final 4-min interval of the swimming test was measured[13,14]. Control groups were treated with normal saline. Other groups received an *i.p.* injection of extract (62.5, 125, 250, 500 mg/kg) in normal saline solution and imipramine (15 mg/kg), 1 h before the experiment.

Tail Suspension Test (TST)

Male mice are housed in plastic cages for at least 10 days prior to testing in a 12 h light cycle with food and water freely available. Animals are transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Groups of 10 animals received an *i.p.* injection of extract (125, 250, 500 mg/kg) in saline solution and imipramine (15 mg/kg), 30 min before the experiment. For the test the mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility is recorded for periods of 5 min. Mice are considered immobile when they hang passively and completely motionless for at least 1 min[15].

Anti Hypoxic Activity

Asphyctic Hypoxia

Animals were subjected to hypoxia by putting them individually in a tightly closed 300 ml glass container which was placed under water in an aquarium of 25°C. The animals had convulsions and died from hypoxia. The latencies for death were recorded[16]. The animals died approximately 2 min following convulsions. Mice received single *i.p.* injections of different doses of extract (7.75, 15.5, 31 and 62 mg/kg) and phenytoin (50 mg/kg) as 30 min before they were subjected to hypoxia. Another control group was treated with normal saline.

Haemic Hypoxia

Twenty four mice were divided into four groups each containing six mice. Control group was treated with normal saline. Thirty minutes after *i.p.* administration of extract 31.25, 62.5, 125, 250 and 500 mg kg^{-1}, $NaNO_2$ (360 mg kg^{-1}) was applied *i.p.* to mice and antihypoxic activity was estimated as the latent time of evidence of hypoxia in minutes [17].

Circulatory Hypoxia

Twenty four mice were divided into four groups each containing six mice. Groups were treated with normal saline. Thirty minutes after i.p. administration of extract 31.25, 62.5, 125, 250 and 500 mg kg^{-1}, NaF (150 mg kg^{-1}) was applied *i.p.* to mice and antihypoxic activity was estimated in minutes as the latent time of evidence of hypoxia[18].

Scavenging of Hydrogen Peroxide

The ability of the extract to scavenge hydrogen peroxide was determined according to our recent-

ly published paper[19]. Extract (0.1-3.2 mg ml^{-1}) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM in phosphate buffer, pH 7.4). The absorbance of H_2O_2 at 230 nm (UV-Visible EZ201, Perkin Elmer, Waltham, MA, USA) was determined after 10 minutes against a blank solution containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging was calculated as follows: % Scavenged $[H_2O_2] = [(A_o - A_1)/A_o] \times 100$ where A_o was the absorbance of the control and A_1 was the absorbance in the presence of extract or standard.

CuOOH-Induced Hemolysis

Red blood cells (RBC) were isolated from male Wistar rats and suspended in balanced phosphate buffered saline (PBS) to obtain a 1% RBC suspension. Aliquots (3.5 ml) were incubated at 37°C for 210 min in the presence of 50 µM CuOOH (in ethanol) and the cellular integrity determined turbidimetrically at 710 nm at 30 min intervals[20]. The extracts (dissolved in ethanol, final concentrations 50 µg/ml) were preincubated for 30 min with RBC before the addition of CuOOH. Percentages hemolysis were determined setting as a 100% hemolysis the absorbance value determined in RBC suspensions sonicated for 5 s at 50% power.

Statistical Analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test for multiple comparisons. Antihypoxic activity was expressed relative to the control and was compared by Student's paired *t*-test. Results were considered significant at $p<0.05$. The IC_{50} values were calculated from linear regression analysis.

Results

Antidepressant Activity

H. scabrum, at all tested doses (125, 250 and 500 mg/kg) significantly and dose dependently reduced the immobility period respect to control in FST ($P<0.001$). Imipramine showed significant anti-immobility activity with minimum immobility time of 88±5.4 s vs. control (164.2±9.3 s) ($P<0.001$). The extract at 500 mg/kg showed more potent antidepressant activity than imipramine ($P<0.01$, Table I). *H. scabrum*, at all tested doses (62.5, 125 and 250 mg/kg) significantly and dose dependently decreased the immobility time respect to control in TST ($P<0.001$). Imipramine showed significantly decreased in immobility period 74.2±7.3 s vs. control (157.8±12 s) ($P<0.001$, Table I). Extract at the dose of 250 mg/kg showed more potent antidepressant activity than imipramine ($P<0.05$).

Antihypoxic Activity

Statistically significant antihypoxic activities were established in the experimental model of asphytic, haemic and circulatory hypoxia in mice. The effect was dose-dependent (Table II). Extracts showed significant antihypoxic activity in asphytic model. At 7.75 mg/kg it prolonged latency for death (33.60±1.90 vs. 26.78±1.84 min for control groups, $P<0.01$). In other two antihypoxic models, extracts showed statistically significant activity at 31.25 mg/kg respect to control groups ($P<0.001$).

Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water[17]. The extract was capable of scavenging H_2O_2 in a concentration-dependent manner. The extract showed moderately good scavenging activity.

Table I. Effect of *H. scabrum* aqueous extract on duration of immobility during Forced Swimming Test (FST) and Tail Suspension Test (TST).

Group	Dose (mg/kg)	Duration of immobility (s), FST	Duration of immobility (s), TST
Control	–	164.2 ± 9.3	157.8 ± 12.0
Extract	62.5	–	127.32 ± 13.2**
Extract	125	126.4 ± 13.9**	79.2 ± 4.5**
Extract	250	99.8 ± 5.3**	66.3 ± 5.9**
Extract	500	78.2 ± 6.6**	–
Imipramine	15	88 ± 5.4**	74.2 ± 7.3**

Values are Means ± SD, (N = 10), **$P<0.001$.

Table II. Anti-hypoxic activities of *H. scabrum* in asphyctic, haemic and circulatory hypoxia in mice.

Asphyctic hypoxia (min)	Sodium fluoride test (min)	Sodium nitrite test (min)	Doses (mg/kg)	Group
26.78 ± 1.84	9.37 ± 1.32	9.52 ± 1.63		Control
33.60 ± 1.90**	–	–	7.75	Extract
46.28 ± 2.09***	–	–	15.5	Extract
56.18 ± 2.88***	10.24 ± 0.73***	11.16 ± 1.46***	31.25	Extract
63.65 ± 2.61***	11.58 ± 0.84***	12.15 ± 1.59***	62.5	Extract
–	14.50 ± 1.43***	15.48 ± 1.33***	125	Extract
–	17.14 ± 1.59***	18.42 ± 1.97***	250	Extract
–	20.26 ± 2.07***	22.36 ± 1.95***	500	Extract
88.47 ± 3.56***	–	–	50	Phenytoin

Data are Means ± SD (n = 10). $^{**}P < 0.01$,$^{***}P < 0.001$ respect to control.

IC_{50} was 518.8±20.7 µg ml^{-1}. The IC_{50} for vitamin C and quercetin were 21.4±0.12 and 52.0 ± 3.11 µg ml^{-1}, respectively.

The antioxidant activity of the extracts was confirmed in rat erythrocytes (RBC) exposed to CuOOH, by measuring the erythrocyte membrane resistance to free radical-induced hemolysis. When control RBC were incubated with extracts (50 µg ml^{-1}), no significant hemolysis was observed within 3 h, thus to exclude any membrane-perturbing effect of the compounds. In RBC exposed to CuOOH (Figure 1), hemolysis started after 30 min incubation and reached a plateau between 180 and 210 min (90-100% hemolysis).

Discussion

Extracts significantly reduced the immobility period in both FST and TST. They represent the behavioral despair models, claimed to reproduce a condition similar to human depression. The

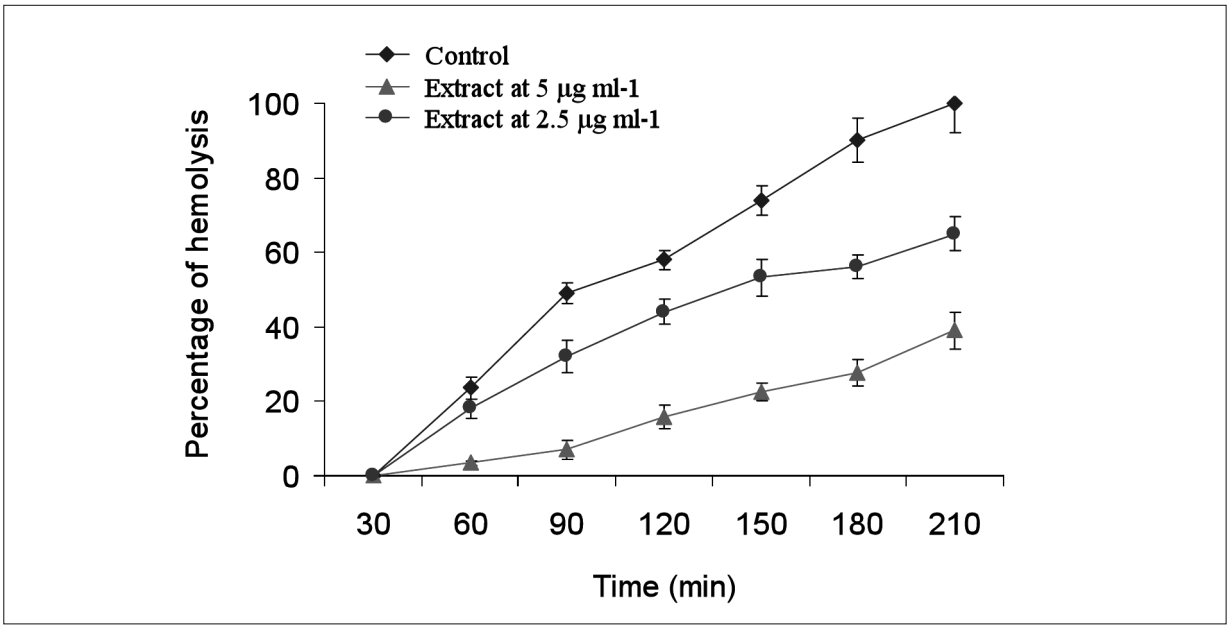

Figure 1. Protective effect of *H. scabrum* on red blood cell hemolysis induced by CuOOH (50 µM). Values are the means ± SD.

tests are based on the observation that animals, following initial escape oriented movements, developed an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behavior or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli[21]. Pretreatment with extracts and imipramine, exhibited a significant and dose dependent inhibition of immobility time FST and TST. So, the escape-directed behaviors with minimal immobile posture showed by extracts treated mice may be due to its attenuating effect in endogenous depression. TST is a reliable and rapid screening method for antidepressants including those involving the serotonergic system[22]. It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity[23]. TST detects the anti-immobility effects of a wide array of antidepressants, including tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, electro-convulsive shock, and even atypical antidepressants. Thus, the activity of *H. scabrum* could involve one of the mechanisms of the established agents as described above. Antidepressant activity of quercetin has been reported previously[24]. Potent central nervous system (CNS) depressant activities of extracts maybe result of high quercetin and flavonoid contents of these species.

There are literature data that administration of sodium fluoride increases the blood histamine content and decreases the oxygen carrying capacity[25]. A significant protective effect on other forms of hypoxia such as hypobaric hypoxia has been reported by *Ginkgo biloba* that contains flavonoids[26]. Our results may be supported by other literature data that flavonoids increase cerebral blood flow and possess antihypoxic activity[17]. The mechanism of this protective action may be due in part to the antioxidant activity of quercetin[27,28]. The extracts showed a very good protective effect against the hypoxia. It produced significant and dose dependent effect on all model of haemic asphytic and circulatory hypoxia (Table II).

Scavenging of H_2O_2 by extracts be attributed to their phenolics, and other active components which can donate electrons to H_2O_2, thus neutralizing it to water[19]. The extract was capable of scavenging H_2O_2 in a concentration dependent manner. IC_{50} for H_2O_2 scavenging activity was 518.8±20.7 μg ml^{-1}. The IC_{50} values for ascorbic acid and butylated hydroxyanisole (BHA) were 21.4 and 52.0 μg ml^{-1}, respectively. Although H_2O_2 itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems.

Conclusion

H. scabrum aqueous extract showed remarkable antihypoxic and antidepressant effects thus, lend pharmacological justification to the use of the plant extracts by traditional medicine practitioners. It is promising for further pharmacological and biochemical experiments, which will be focused on evaluating the precise mechanism of these activities.

Acknowledgements

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