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The Effect of *Rhazya stricta* Decne, a Traditional Medicinal Plant, on the Forced Swimming Test in Rats

B. H. ALI,* A. K. BASHIR* AND M. O. M. TANIRA†

**Desert and Marine Environment Research Centre,*
†Department of Pharmacology, Faculty of Medicine & Health Sciences,
United Arab Emirates University, Al Ain, United Arab Emirates

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ALI, B. H., A. K. BASHIR AND M. O. M. TANIRA. *The effect of* Rhazya stricta *Decne, a traditional medicinal plant, on the forced swimming test in rats*. PHARMACOL BIOCHEM BEHAV **59**(2) 547–550, 1998.—Immobility induced by forced swimming is well known as an animal model of depression. Using this paradigm, we have, in the present work, tested the possibility that the medicinal plant *Rhazya stricta*, which has previously been found to affect the monoamine oxidase inhibitory activity in rat brain, may have an antidepressant-like action. Rats were pretreated with various doses (0.025–6.4 g/kg) of the lyophilized extract of the plant leaves, or with desipramine (10, 20, and 40 mg/kg) and were subjected to the forced swimming test. The results indicated that the plant extract produced a biphasic (bell-shaped) effect on the immobility time. The lower doses (0.1, 0.2, and 0.4 g/kg) elicited a highly significant and inversely dose-dependent decrease in immobility time, and the higher doses (0.8, 1.6, and 6.4 g/kg) showed a dose-dependent decrease in immobility time. Under the same experimental conditions desipramine (20 and 40 mg/kg) produced dose-dependent significant decreases in immobility time. Following administration of *R. stricta* (6.4 g/kg) the immobility time recovered progressively with time, and 4 h after its administration the immobility time was about 70% of the control level (statistically insignificant). It is concluded that *R. stricta* extract [or component(s) thereof] may possess an antidepressant-like effect. © 1998 Elsevier Science Inc.

Antidepressant Desipramine Forced swimming test *Rhazya stricta*

Rhazya stricta Decne (family Apocynaceae) is a shrub with a smooth central stem and dense semierect branches (16). The plant grows commonly in the Arabian Peninsula and is used in local folk medicine practices to treat diabetes mellitus, certain inflammatory conditions, and helminthiasis. Extensive phytochemical studies have been published on this plant [see (4) and references therein]. The plant leaves contain alkaloids with β-carboline nucleus (akuammidine, rhaziminine, and tetrahydrosecamine) (4). *R. stricta* has also been shown to contain two flavonoids, isorhamnetin 3-(6-dirhamnosylgalactoside)-7-rhamnoside, and 3-(6-rhamnosylglalactoside)-7-rhamnoside (Bashir, unpublished data). However, relatively little work has been reported on the pharmacological and toxicological properties of the plant (2,5,13,15). While testing the antihyperglycemic effect of *R. stricta*, a quiescent behavior in treated mice was noticed shortly after giving the plant extract, suggesting that this may have depressed the activity of the central nervous system (CNS). This possibility was tested and confirmed (2,3).

We have recently investigated the effect of acute and subchronic administration of different doses of *R. stricta* extract on monoamine oxidase (MAO) A and B inhibitory components of tribulin in rat brain. It was found that the plant's extract produced variable and paradoxical effects on both MAO A and MAO B inhibitory components (14). It is well established that MAO inhibition, particularly of MAO A, has antidepressant effects (10), and it was, therefore, of interest to determine whether the changes in the level of endogenous MAO inhibitor(s) can be seen in a biological test for antidepressants, viz the forced swimming test. This test has been shown to possess excellent predictive and construct validity [for reviews, see (11,17–19)].

Requests for reprints should be addressed to Dr. B. H. Ali, Desert and Marine Environment Research Centre, UAE University, P.O. Box: 17777, Al Ain, United Arab Emirates.

METHOD

Animals

One hundred and thirty-three male Wistar rats weighing 150–155 g were obtained from our University Animal House and were housed in groups of seven under standard temperature (22 ± 2°C), humidity (50–60%), and light conditions (artificial light from 0600 to 1800 h). They were given standard pelleted diet (Abu Dhabi Animal Feed Factory) and water ad lib.

Procedures involving animals and their care were conducted in conformity with international laws and policies (EEC Council directives 86/609, OJL 358, 1 December, 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publications No. 85-23, 1985).

Plant Material and Extraction Preparation

The plant was collected from Umm Gafa, Al Ain district in February 1994, and authenticated at the National Herbarium of the U.A.E. University, where a voucher specimen was deposited.

The leaves were air dried in the shade and coarsely pulverized, and the resultant powder (200 g) was macerated with distilled water (3 liters) for 16 h at room temperature, with occasional shaking. The extract was filtered, and the filtrate obtained was lyophilized using a freeze dryer. The final lyophilized product constituted about 18.3% of the original material. Aqueous solutions were prepared freshly from the same lyophilized product and used in all tests. The aqueous extract was always administered orally in a volume of 4 ml/kg body weight. An HPLC fingerprinting of the plant extract has been documented in this laboratory.

Forced Swimming Test

The forced swimming test, described by Porsolt et al. (12) was used as modified by Benvenga and Leander (6). Rats were placed singly and forced to swim in a Plexiglas cylinder with the following dimensions: 45 cm height, 27 cm internal diameter, filled with water, maintained at 28–29°C to a height of 24 cm. This ensured that the rat's feet did not touch the floor of the vessel and that it did not escape from the vessel. Two sessions were conducted, an initial 15-min training session followed 24 h later by a 5-min test session. Following training sessions rats were removed from the cylinders, towel dried, and placed under a lamp for 5 min. They were then returned to the home cage for testing the next day. A rat was judged to be immobile whenever it remained floating in the water, without struggling, in an upright position making only very small movements necessary to keep its head above water. Scoring was made by an observer unaware of the drug treatment. The measure of immobility served as a quantitative measure of the animal's behavioral despair, where it is assumed that the animal has given up hope of escaping from the confines of the vessel (12). Each animal was used only once. In accordance with the suggestion of Abel (1), fresh water was used for each animal to minimize any effect of the soiled water from the previous rat (possibly containing "alarm pheromones") inducing agitation and decreased motility.

The test was carried out in a room with no other nontreated animals in the same or adjacent room to avoid giving rise to signals or odours that may affect nontreated animals (7).

Rats were trained in the forced swimming test for 15 min (first session) as described above, and 23.5 h later they were treated with the plant extract or desipramine (see below). Thirty minutes thereafter, a 5-min session in the forced swimming test was carried out.

Effect of R. stricta

In this experiment, the forced swimming test was conducted on rats orally pretreated 30 min earlier with 0.9% NaCl (2 ml/kg) (control) or with the lyophilized extracts of *R. stricta* at doses of 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 6.4 g/kg (test). In another experiment, rats were treated with *R. stricta* (6.4 g/kg) and forced to swim 0.5, 1, 2, 3, and 4 h after extract administration.

Effect of Desipramine

Desipramine was dissolved in 0.9% NaCl and injected intraperitoneally (IP) into rats at doses of 10, 20, and 40 mg/kg, given in a volume of 1.7 ml/kg. Thirty minutes thereafter each rat underwent a 5-min test session in the forced swimming test.

Drugs and Chemicals

Desipramine was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical reagent grade.

Statistical Analysis

Values reported are means ± SEM (number of observations). Differences between groups were analyzed by a one-way analysis of variance followed by Scheffe's test using the computer program (Statview 5-1). A p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

As shown in Table 1, the immobility time in *R. stricta*-treated rats exhibited a bimodal (bell-shaped) significant reduction, compared to the control ($F = 12.2$, $p < 0.05$). The reduction was most marked (and almost maximal) at dose of 0.1 g/kg and 6.4 g/kg. The "recovery" from the effect of *R. stricta* (6.4 g/kg) was studied 0.5, 1, 2, 3, and 4 h after its administration, and the results (Fig 1) showed that with time there was a gradual loss of the plant effect manifested as a progressive increase in the immobility time. However, 4 h after treatment

TABLE 1

EFFECT OF *RHAZYA STRICTA* AND DESIPRAMINE ON IMMOBILITY TIME DURING FORCED SWIMMING TEST IN RATS

Treatment	Immobility Time (s)
Control	
2 ml/kg 0.9% NaCl	135.00 ± 10.33*
R. stricta	
0.025 g/kg	128.9 ± 5.7
0.05 g/kg	64.4 ± 10.1*
0.1 g/kg	0.45 ± 0.03*
0.2 g/kg	21.82 ± 1.75*
0.4 g/kg	93.16 ± 12.19*
0.8 g/kg	29.88 ± 3.50*
1.6 g/kg	0.14 ± 0.11*
6.4 g/kg	0.09 ± 0.05*
Desipramine	
10 mg/kg	118.4 ± 14.2
20 mg/kg	93.6 ± 8.1*
40 mg/kg	59.4 ± 2.7*

Values are mean ± SEM ($n = 7$).
*$p < 0.05$ (compared to control).

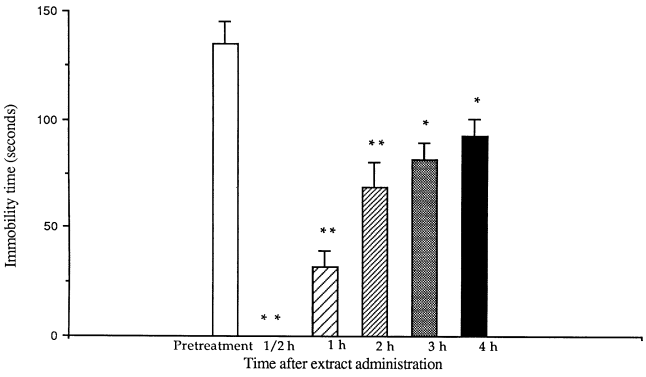

FIG. 1. Immobility time (s) in the forced swimming test measured in control rats and in rats treated with *Rhazya stricta* extract (6.4 g/kg) 0.5, 1, 2, 3, and 4 h before the test. Each column and vertical bar represent mean SEM ($n = 7$ rats). Asterisks denote significant differences from the control ($*p < 0.05$; $*p < 0.01$). Immobility time 0.5 h after extract administration averaged (0.1 s) and is not shown for convenience.

with the extract the immobility time was still significantly lower than in the untreated animals (about 70% of the control's).

For comparative purposes, and to confirm the validity of the test, rats were pretreated with desipramine. The results of this experiment (Table 1) showed that desipramine (10, 20, and 40 mg/kg) produced progressive decreases in the immobility time, which was statistically significant at the two higher doses used ($F = 20.1$, $p < 0.05$). Our results with desipramine are similar to those reported by several authors before [e.g., (9)].

Previously we have shown that the plant extract given at doses that bracket the present ones (2–8 g/kg) produced sedation (2) and decreased spontaneous motor activity (3). In the forced swimming test, however, the plant extract induced a significant reduction in the immobility time, an effect opposite to that observed in the motor activity cage. This indicates that the sedation and decreased motor activity were not involved in the action seen in the forced swimming test, and that the effect on immobility in the forced swimming test was central rather than peripheral in origin. Thus, a false positive effect is not possible. It was of interest to note that at a low dose (0.1 g/kg) and high doses (1.6 and 6.4 g/kg), the extract treatment produced almost complete cessation of motility in the forced swimming test. Treatment with antidepressant drugs usually reduce immobility time but do not eliminate it completely. The reason for this apparently unusual result is uncertain. The treated rats remained in the water continously and actively swimming, and struggling to survive. No sign of seizure was seen in these animals in the water. However, other relevant measures in the forced swimming test (e.g., time of struggling and frequency of diving) could have yielded some useful information on the behavior of treated rats in the water, but were not measured in the present study.

The mechanisms by which the extract produced its bimodal response is currently unknown. However, the present results were obtained with a lyophilized extract that may contain several active compounds (see the introductory paragraphs). The observed effect in this work may be due to one or more of these compounds. In a recent unpublished work we have found that it was possible to separate the fractions that increase MAO A inhibitory components from those that decrease it. Further studies with pure compounds isolated from the extract are warranted. Also, the neurochemical changes that occur in the brains of treated animals need to be investigated. In this respect we have already found that acute or subchronic treatment with *R. stricta* has significant and complex effect on the level of tribulin (endogenous MAO inhibitor/BZP receptor ligand) and did not affect the concentrations of brain amino acids (unpublished data). The role of other possible central neurotransmitters that may be involved in the observed effects, as well as the interaction with adrenergic and serotoninergic antagonists (8) are currently being studied.

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