Pharmacology Biochemistry and Behavior, Vol. 59, No. 3, pp. 671–675, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00464-4

Effect of Extract of *Rhazya stricta*, a Traditional Medicinal Plant, on Rat Brain Tribulin

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Received 7 March 1997; Revised 4 August 1997; Accepted 4 August 1997

ALI, B. H., A. K. BASHIR, M. O. M. TANIRA, A. E. MEDVEDEV, N. JARRETT, M. SANDLER AND V. GLOVER. *Effect of extract of* Rhazya stricta*, a traditional medicinal plant, on rat brain tribulin*. PHARMACOL BIOCHEM BEHAV. **59**(3) 671–675, 1998.—*Rhazya stricta* leaves, which have both antidepressant and sedative properties in animal models, are widely used in folk medicine in the Arabian peninsula. In this study, the effects of oral administration of leaf extracts on rat brain tribulin levels [endogenous monoamine oxidase (MAO) A and B inhibitory activity], were determined. In an acute study, low doses brought about an increase in MAO A inhibitory activity, while intermediate doses caused a significant reduction. The highest doses had no significant effects on activity. There were no significant effects on MAO B inhibitory activity at any dose. Subchronic administration (21 days) caused a significant decrease in MAO A inhibitory activity, most prominent at low dosage, and an increase in MAO B inhibitory activity. Acute intramuscular administration also resulted in a similar pattern. Such paradoxical effects were at least partially explained when different extracts of the leaves were used; a weakly basic chloroform fraction caused an increase in MAO A inhibitory activity, whereas butanol extracts brought about a decrease. These fractions had no significant effects on MAO B inhibitory activity. The findings show that *Rhazya stricta* leaves contain at least two different components that affect MAO inhibitory activity in opposite directions. It may be that the antidepressant and sedative actions of the plant are explicable in terms of these different components. © 1998 Elsevier Science Inc.

Brain Tribulin Monoamine oxidase Stress *Rhazya stricta*

RHAZYA STRICTA Decne (Family Apocynaceae), a plant growing commonly in the Arabian peninsula, is used in local folk medicine to treat diabetes mellitus, certain inflammatory conditions, and helminthiasis. Extensive phytochemical studies exist (4,5) showing that its leaves contain alkaloids with a β-carboline nucleus (akuammidine, rhaziminine, and tetrahydrosecamine). The plant also contains some flavonoids (Bashir, unpublished data).

In animal experiments, lyophilized crude leaf extracts appear to be of low toxicity and without mortality in doses of up to 8 g/kg (12). They have both sedative (1,3) and antidepressant-like activity in the forced swimming test (2).

In the present study, we have 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 the rat brain. It is well established that MAO inhibition, particularly of MAO A, has antidepressant effects (9), and it was, therefore, of interest to determine whether the antidepressant action of *R. stricta* is linked to any change in level of endogenous MAO inhibitor(s).

Tribulin is the name given to endogenous MAO inhibitory activity, present in urine and tissues of both humans and animals (7). Several components have been identified to date, including some selective inhibitors of MAO A (10) and isatin, a selective inhibitor of MAO (8).

As the lyophilized extract used in the above experiments contains several active components, complicating the interpretation of observed results, we attempted to separate the ef-

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fects of different fractions of crude extract on tribulin activity and identify the fraction(s) responsible for augmenting or attenuating the two types of inhibition. We also attempted to find out whether the gut flora is involved in the action of the orally administered plant fractions by comparing their effect in orally and parenterally treated rats. Some of the results reported here have been briefly communicated (13).

METHODS

Animals

Male Wistar rats weighing 230–250 g were used. They were housed in groups of six under standard temperature (22 ± 2°C), humidity (50–60%) and light conditions (artificial light from 0600–1800 h). They were given standard pelleted diet (Abu Dhabi Animal Feed Factory) and water ad libitum.

Procedures involving animals and their care were conducted in conformity with international laws and policies (EEC Council directives 86/609, OJL 358, 12 December, 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 1996, and authenticated at the National Herbarium of the UAE University, where a voucher specimen was deposited.

The leaves were air dried in the shade and coarsely pulverized; the resulting powder (200 g) was macerated in distilled water (3 L) for 16 h at room temperature, with occasional shaking. The extract was filtered and freeze dried. The final lyophilized product constituted about 18.3% of the original material. Aqueous solutions were prepared freshly from it and used in all tests. The aqueous extract was always administered orally in a volume of 4 ml/kg body weight. Control rats received the same volume of distilled water. An HPLC "fingerprint" of the aqueous plant extract was prepared using 25 μl injections. Details of this procedure are given in the legend to Fig. 1. The fingerprint was done to enable standardization of different leaf extracts between laboratories.

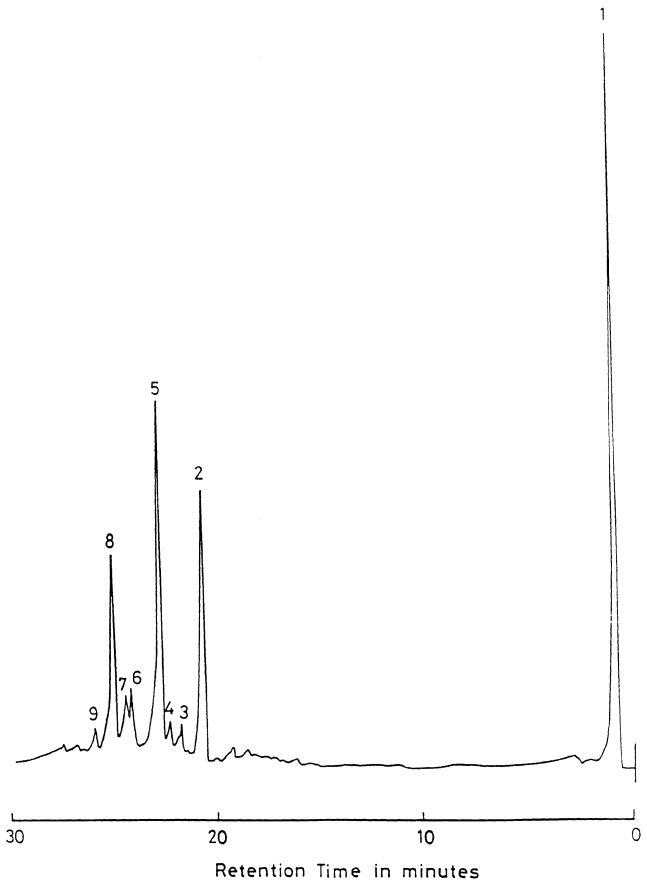

FIG. 1. High-performance liquid chromatograph of *Rhazya stricta* lyophilized extract on Nova-Pack C_{18} column (3.9 × 150 mm, 4 μm), using gradient elution (MeOH:H_2O). UV detection was at 254 nm; flow rate: 1.2 ml/min. The numerals 1–9 represent the major peaks of the extract, except peak number 8, which represents the internal standard caffeine.

Fractionation of R. Stricta

Powdered leaves (2 kg) were extracted with alcohol (95% v/v), using a Soxhlet extraction apparatus. The alcoholic extract, when evaporated under vacuum, gave a residue of 320.6 g. This residue (200 g) was suspended in water (600 ml), acidified with 10% glacial acetic acid (200 ml) and extracted with chloroform (5 × 500 ml), to give a chloroform fraction containing weakly basic alkaloids, and neutral compounds (64.7 g). The remaining aqueous solution was made alkaline with ammonium hydroxide and the pH adjusted to 9–11. This alkaline aqueous layer was extracted with chloroform (5 × 500) to give a chloroform fraction containing strongly basic alkaloids (9.7 g). The aqueous layer (after chloroform extraction) was further extracted with *n*-butanol (4 × 500 ml) to give the butanol fraction. The remaining aqueous volume was the aqueous fraction (57.8 g). All fractions were evaporated and taken up in distilled water before use.

Treatment

First experiment. Three oral doses of lyophilized extract of *R. stricta* were used (2, 4, and 8 g/kg). Control rats were treated with the same volume of distilled water. Animals were sacrificed 45 min after treatment. Animals were killed in all experiments 20 min after treatment, by cervical dislocation and decapitation. Brains were rapidly removed from the skull and immediately frozen at −80°C prior to analysis within 4–5 weeks.

Second experiment. Rats were treated orally with doses of 0.25, 0.5, and 2 g/kg of the lyophilized extract for 21 consecutive days and then sacrificed 24 h after the last dose.

Third experiment. Groups of rats were given orally distilled water (the controls), or either 0.2 or 2 g/kg of crude ethanolic extract, chloroform fraction (strongly basic), chloroform fraction (weakly basic), butanol fraction, or aqueous fraction. Rats were killed 45 min after the treatment.

Fourth experiment. Four groups of rats were used. The first was injected intramuscularly (IM) with distilled water (3 ml/kg), and the second, third, and fourth with lyophilized extract in a single dose of 10, 50, or 250 mg/kg, respectively.

Tribulin Assay

Frozen brains were weighed, thawed, and used for assay of MAO-A and MAO-B inhibitory components of tribulin as described previously (11). Briefly, brains were homogenized

TABLE 1

THE INFLUENCE OF ACUTE ORAL ADMINISTRATION OF LYPHILIZED EXTRACT OF *RHAZYA STRICTA* ON MAO A AND MAO B INHIBITORY COMPONENTS OF RAT BRAIN TRIBULIN

Groups of Animals (Treatment)	MAO A Inhibition (% per g wet wt)	*p*	MAO B Inhibition (% per g wet wt)	*p*
Control	29.0 ± 2.8		35.3 ± 2.8	
0.5 g/kg	39.0 ± 2.8	<0.01*	33.5 ± 2.8	NS
2 g/kg	1.0 ± 0.7	<0.001	29.8 ± 2.1	NS
8 g/kg	34.2 ± 2.5	NS†	38.8 ± 2.5	NS

Values in the table are means ± SEM ($n = 6$).

,†Symbols shows significance of differences between 0.5 and 2 g/kg and 2 and 8 g/kg†, both $p < 0.001$.

in 2 M HCl and centrifuged, to give a clear supernatant. This was extracted with 2 vol of ethyl acetate and the extract dried down under nitrogen. The residue was reconstituted to 0.1 M phosphate buffer, pH 7.4 and washed with heptane. Tribulin activity in the phosphate buffer was quantified by bioassay, using human placenta as a source of MAO A, and human platelets as a source of MAO B (11).

Materials

The solvents chloroform, acetic acid, ethanol and *n*-butanol were all reagent grade and obtained from BDH, Poole, UK. Other chemicals were obtained from the same source and were analytical reagent grade.

Statistical Analysis

Values reported are means ± SEM ($n = 5.6$). Differences between the means of the different groups were assessed by the two-tailed Student's *t*-test. A *p*-value greater than 0.05 was not considered significant. For the multiple comparisons used in Table 3, a one-way ANOVA was used with a Bonferoni correction.

RESULTS

The results are summarized in Tables 1, 2, 3, and 4. Acute administration of the lyophilized extract of *R. stricta* resulted in a biphasic effect on the MAO A inhibitory component of tribulin (Table 1). There was a significant increase at 0.5 g/kg but administration of a higher dose (2 g/kg) was followed by virtual disappearance of MAO A inhibitory activity. After a dose of 8 g/kg, there was little effect compared with control values. None of these doses of *R. stricta* extract produced any significant change in MAO B inhibitory activity. Three independent acute studies gave similar results.

Table 2 shows that subchronic treatment of rats with two lower doses of extract (0.25 and 0.5 g/kg) reduced MAO A and increased MAO B inhibitory activities. There were no significant effects at the highest dose used (2 g/kg). The lower doses had an effect on MAO A inhibitory activity similar to that following acute administration of 2 g/kg, while 2 g/kg given chronically had an action equivalent to that of 8 g/kg given acutely.

Table 3 shows the effects of the different extracts and fractions *Rhazya stricta* on MAO A and MAO B inhibitory activities. None of the fractions had a significant effect on MAO B inhibitory activity. Neither the crude ethanolic nor strongly basic extracts, in the two doses used, significantly affected the MAO A inhibitory component. The aqueous extract had no effect either. However, the weakly basic chloroform fraction at both doses (0.2 and 2 g/kg) increased the MAO A inhibitory component by 58 and 45%, respectively. In contrast, the butanol fraction, at doses of 0.2 or 2 g/kg, significantly decreased the MAO A inhibitory component by 38 and 75%, respectively.

The effect of IM administration of lyphilized extract of *R. stricta* on MAO A and MAO B inhibitory components is shown in Table 4. There was a dose-related decrease in MAO A inhibitory component amounting to about 44% ($p < 0.001$),

TABLE 2

THE EFFECT OF SUBCHRONIC ORAL ADMINISTRATION OF LYOPHILIZED EXTRACT OF *R. STRICTA* ON MAO A AND MAO B INHIBITORY COMPONENTS OF RAT BRAIN TRIBULIN

Group of Animals (Treatment)	MAO A Inhibition (% per g wet wt)	*p*	MAO B Inhibition (% per g wet wt)	*p*
Control	31.7 ± 3.3		50.5 ± 5.6	
0.25 g/kg	16.0 ± 3.8	<0.02	90.2 ± 3.6	<0.001*
0.5 g/kg	19.8 ± 4.3	NS	88.8 ± 3.4	<0.001†
2 g/kg	25.5 ± 6.0	NS	41.4 ± 4.0	NS

Values are means ± SEM ($n = 6$).

*,†Symbols shows significant differences from control.

TABLE 3

THE EFFECTS OF ORAL ADMINISTRATION OF VARIOUS EXTRACTS AND FRACTIONS OF *RHAZYA STRICTA* ON MAO A AND B INHIBITORY COMPONENTS OF RAT BRAIN TRIBULIN

	Group of Animals (Treatment)	Dose (g/kg, PO)	MAO A Inhibitory Component % per g net wt	MAO B Inhibitory Component % per g net wt
1	Control		26.7 ± 1.7 (10)	50.2 ± 2.9 (6)
2	Crude ethanolic extract	0.2	21.4 ± 0.7 (5)	46.7 ± 2.3 (6)
3	Crude ethanolic extract	2.0	26.5 ± 2.8 (6)	45.0 ± 1.3 (6)
4	Strongly basic alkaloidal fraction	0.2	28.8 ± 2.7 (5)	45.8 ± 2.0 (6)
5	Strongly basic alkaloidal fraction	2.0	30.6 ± 4.1 (5)	46.4 ± 2.2 (5)
6	Weakly basic alkaloidal fraction	0.2	42.2 ± 5.5 (5)	44.8 ± 2.0 (6)
7	Weakly basic alkaloidal fraction	2.0	38.6 ± 4.1 (5)	50.0 ± 2.8 (6)
8	Butanol fraction	0.2	16.6 ± 2.7 (6)	44.5 ± 3.4 (6)
9	Butanol fraction	2.0	6.8 ± 1.7 (5)	48.3 ± 2.1 (6)
10	Aqueous fraction	0.2	29.0 ± 6.0 (5)	55.5 ± 2.8 (6)

Values are means ± SEM.

MAO A inhibitory components.

One-way ANOVA with Bonferoni adjustment between group $p = 0.0003$.

Significance values of differences between groups. Group 1/9 0.015; 2/6 0.024; 3/9 0.041; 4/6 0.003; 5/9 0.005; 6/8 0.008; 6/9 0.000; 7/9 0.003; 9/10 0.018.

TABLE 4

THE EFFECT OF INTRAMUSCULAR ADMINISTRATION OF LYOPHILIZED EXTRACTS OF *RHAZYA STRICTA* ON MAO A AND MAO B INHIBITORY COMPONENTS OF RAT BRAIN TRIBULIN

Group of Animals (Treatment)	MAO A Inhibition (% per g wet wt)	*P*	MAO B Inhibition (% per g wet wt)	*p*
Control	25.5 ± 1.2		44.5 ± 3.3	
10 mg/kg (I.M.)	14.3 ± 1.9	<0.02	45.7 ± 2.9	NS
50 mg/kg (I.M.)	17.8 ± 2.1	NS	54.6 ± 2.0	≤0.05
250 mg/kd (I.M.)	21.5 ± 2.3	NS*	62.2 ± 5.3	<0.02

Values are means ± SEM ($n = 6$).

Extract was administered 20 min before decapitation.

*Significance of differences in tribulin activity compared with the 10 mg/kg dose.

40% ($p < 0.001$) and 16% ($p < 0.001$). At doses of 50 and 250 mg/kg, the extract brought about a significant increase in MAO B inhibitory component by 23% ($p < 0.05$) and 40% ($p < 0.02$), respectively.

DISCUSSION

The present findings show clearly that lyophilized extracts of *R. stricta* have marked effects on rat brain tribulin levels. However, the findings presented here are complex and fail to show simple dose–response curves, pointing to the possibility of different components contributing to the changes observed at different doses. A striking biphasic action on MAO A inhibitory activity was observed when low acute dosage of extract (0.5 g) caused potentiation but intermediate dosage (2 g) inhibition. It is possible that such changes in activity contribute to any antidepressant, sedative, or anxiolytic effects the drug may possess, although further work is needed to separate and quantify these actions more precisely.

The subchronic study showed that treatment of rats with *R. stricta* at lower dosage had an effect on MAO A inhibitory activity similar to that observed with the acute administration of 2 g/kg, suggesting that component(s) of *R. stricta* extract may accumulate within the brain. Table 3 clearly showed that different fractions of the crude ethanolic extract (which was itself without effect on tribulin activity) produced distinct effects. It is possible to separate the fraction(s) that increase tribulin (MAO A inhibitory component) from those that decrease it; the butanol fractions, given at a dose of 0.2 g/kg, resulted in a highly significant decrease in the MAO A inhibitory component of tribulin, whereas the weakly basic chloroform fraction produced an increase.

It was of interest that none of the orally administered fractions affected the MAO B inhibitory component in acute experiments. This was in contrast to the intramuscularly administered lyophilized extract, which increased this component in rat brain. It is possible that the gut flora inactivates the component of the fraction affecting tribulin B.

We do not yet know whether the changes in tribulin reported here have functional effects in themselves or whether they are the cause or consequence of other changes in the central nervous system or some different system. Even so, the ability of the various fractions of *R. stricta* to differentially affect the two tribulin component groups in the brain may well have therapeutic implications. It is possible that an increase in the MAO A inhibitory component contributes to any antidepressant effects (2) of the plant, and a decrease to any sedative effects (1).

These results provide a new example of how it may be possible to explain the action of folk medicines in terms of conventional biochemistry or pharmacology. In general, an increase in tribulin activity has been found to be associated with behavioral activation, and a reduction with sedation. Anxiogenic agents such as pentylenetetrozole and yohimbine (6,7), for example, have been shown to increase the MAO A inhibitory component of tribulin in rat brain, while having no effect on the B component. Benzodiazepines have been shown to cause a reduction. The ability demonstrated here by different extract fractions to increase or decrease endogenous MAO A inhibitory activity may well have therapeutic implications.

ACKNOWLEDGEMENTS

A. M. was partly supported by the Royal Society and by the Russian Foundation for Fundamental Research.

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