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Effect of *Rhazya stricta* Decne on Monoamine Oxidase and Cholinesterase Activity and Brain Biogenic Amine Levels in Rats

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Abstract

The effect of treatment with the medicinal plant *Rhazya stricta* Decne, on monoamine oxidase (MAO) and cholinesterase activity, and on the concentration of brain biogenic amines was studied in rats.

R. stricta extract, at doses of 0·2 and 0·5 g kg^{-1}, significantly ($P < 0·05–0·01$) increased the hepatic and cerebral activity of MAO by 36–127%. The higher doses used (2·0 and 8·0 g kg^{-1}) produced smaller (10–26%) and statistically insignificant increases in MAO activity in liver and brain. Cholinesterase activity in blood, liver and brain was not significantly influenced by treatment with *R. stricta*. The concentrations of the measured biogenic amines (noradrenaline, adrenaline, 5-hydroxytryptamine and dopamine) were significantly lowered in rats treated with *R. stricta*.

The observed increase in MAO activity may be responsible for the lowered biogenic amines levels and may, in part, be responsible for the pharmacological effects of *R. stricta* extract in rats.

Rhazya stricta Decne (family Apocynaceae) is one of the most widely used medicinal plants in the Arabian Peninsula. The leaves of *R. stricta* are often used in folk medicine to treat numerous unrelated diseases including diabetes mellitus, inflammatory conditions, gastrointestinal ailments and helminthiasis.

Previously, *R. stricta* extract was shown to have CNS-depressing properties in mice and rats, including sedation, antidepressant-like action in the forced-swimming test and decreased motor activity (Ali et al 2000a). The plant extract was also shown to have a centrally-mediated hypotensive action (Tanira et al 2000). An attempt to explore the neurochemical basis of these actions revealed that *R. stricta* leaf extract (and fractions thereof) induced complex effects on rat brain tribulin (Ali et al 1998a), and had no significant effect on brain amino acids (Ali et al 2000b).

To obtain further information on the possible neurochemical changes induced by *R. stricta*, we studied the effect of treatment with *R. stricta* leaf extract on monoamine oxidase (MAO) and cholinesterase activity in rats. These enzymes are responsible for the inactivation of a number of highly active neurotransmitters that participate in the control of CNS activity as well as blood pressure. MAO is responsible for the enzymatic deamination of primary, secondary and tertiary amines, whereas, cholinesterase is responsible for cleavage of acetylcholine. We also studied the effect of *R. stricta* extract on biogenic amine levels (noradrenaline, adrenaline, 5-hydroxytryptamine (5-HT) and dopamine) in brain.

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Materials and Methods

Drugs and chemical

All the chemicals used were of analytical reagent grade and obtained from BDH (Poole, UK) or Sigma (St Louis, MO) unless otherwise stated.

Animals

Male Wistar rats (230–250 g) from the Desert and Marine Environment Research Centre, United Arab Emirates University Animal House were used.

Animals were housed in groups of six under standard conditions of temperature ($22 \pm 2^{\circ}C$), humidity (50–60%), and light (artificial light from 0600 to 1800 h). They were given standard pelleted diet (Abu Dhabi Animal Feed Factory) and had free access to water.

Procedures involving rats and their care were conducted in accordance with international laws and policies (EEC Council directives 86/609; NIH Guide for the Care and Use of Laboratory Animals, NIH Publications no. 85-23).

Plant material

R. stricta was collected from Umm Ghafa, Al Ain district and authenticated at the National Herbarium of the United Arab Emirates University, where a voucher specimen was deposited.

Preparation of the lyophilized extract

The leaves of *R. stricta* were air-dried in the shade and coarsely powdered. The powdered material (150 g) was macerated with distilled water (250 mL) and allowed to stand for 12 h with occasional shaking. The extract was filtered, and the filtrate was freeze-dried using a Christ-B1-16 freeze-dryer.

Preparation of the hydrochlorinated alkaloidal fraction

The coarsely powdered leaves were extracted with 95% alcohol using a Soxhlet extraction apparatus. The alcoholic extract was evaporated under vacuum to give an alcoholic residue, which was suspended in water and acidified with 10% glacial acetic acid and extracted with chloroform. This chloroform fraction contained weakly basic alkaloids and neutral compounds.

The remaining aqueous solution was basified using NH_4OH and the pH was adjusted to between 9 and 11. The alkaline aqueous layer was extracted with chloroform to give a chloroform fraction of strongly basic alkaloids.

The chloroform layer was evaporated to dryness and redissolved in ether. Hydrogen chloride gas was bubbled through the ether layer to give a precipitate of the alkaloidal salts as hydrochloride.

Experimental design of the enzyme activity study

Rats (n = 35) were randomly divided into five equal groups, and were given *R. stricta* lyophilized extract orally at doses of 0·2, 0·4· 2·0 and 8·0 g kg^{-1} for three consecutive days. A control group received distilled water in the same volume. At 24 h after the last dose, the rats were weighed, lightly anaesthetized with diethyl ether and quickly decapitated. Blood was collected in heparinized tubes and 1 mL was used for the estimation of cholinesterase activity in erythrocytes. Another 2 mL was centrifuged at 900 *g* for 10 min at 5°C to obtain plasma, which was also used for cholinesterase measurement.

Estimation of enzyme activity

The preparation of liver and brain tissues for the estimation of MAO activity was carried out as described by Krajl (1965). The method depends on the flurometric determination of 4-hydroxyquioline that is formed from the substrate kynuramine, a substrate for both MAO type A and B. The measurement of cholinesterase activity was carried out using commercial kits (Sigma, St Louis, MO).

Estimation of brain biogenic amines

Tissue sample preparation. Rats were given *R. stricta* strongly basic alkaloidal fraction orally in a dose of 200 mg kg^{-1}. After 1 h the rats were decapitated and the brains were collected. Catecholamine tissue content was measured in whole brain tissue, and separately measured in the cerebrum, cerebellum, pons and medulla and the remaining part of the brain.

The brain tissue was weighed, homogenized in 0·1 M perchloric acid (10% w/v) at 2–4°C and then centrifuged at 5400 *g* for 20 min at 2°C. The supernatant was aspirated for HPLC analysis.

HPLC system

A Waters HPLC system consisting of a pump (model 510), electrochemical detector (ECD model 460) and a manual injector (model U6K) with a Waters catecholamines column (250 mm in length, 45 mm i.d.) was used. The mobile phase was 0·15 mM sodium EDTA, 0·1 M citric acid, 1·0 mM D-1-*n*-butylamine and 10% methylalcohol and the flow rate was 1 mL min^{-1}. The ECD setting was 0·5 v potential, filter: 1, s: 2, range: 0·2 nA, offset: 1·5 nA.

Calibration procedure

Standard biogenic amines solution (Waters) was run to establish the column integrity, mobile phase and retention times. The solution comprised 120 pg noradrenaline, 60 pg adrenaline, 120 pg dopamine

and 60 pg dihydroxybenzylamine (internal standard). To 25 μL of this standard solution, 1 ng 5-HT was added in 10 μL. The total injection volume was 35 μL.

Test sample preparation

Each sample was mixed with internal standard and then injected undiluted. The injection volume was as follows: undiluted sample (25 μL) + 5 μL internal standard + 5 μL 0·02% sodium metabisulphite. In many cases, the biogenic amine contents were so high that it was necessary to dilute the sample (1 : 4) before injection. The catecholamine concentration was calculated as follows:

catecholamine concentration (mg tissue)$^{-1}$ = concentration of calibrator/peak area ratio of calibrator with internal standard × peak area ratio of sample with internal standard × dilution factor/ weight of tissue

Statistical analysis

Values are reported as mean ± s.e.m. Statistical differences between the groups were determined by an analysis of variance, followed by Dunnett's test (Montgomery 1991). A value of $P < 0.05$ was considered significant.

Results

Accuracy of HPLC assay

Figure 1 represents typical traces of standards mix and sample injections in two traces. The intra- and inter-assay coefficient of variation percentage was 2·30–5·70% and 4·89–8·97%, respectively.

Effect of R. stricta *on enzyme activity*

The effects of the various doses of *R. stricta* lyophilized extract on MAO activity in liver and brain of rats are shown in Table 1. Doses of 0·2 and 0·5 g kg^{-1} produced a significant increase in the hepatic and brain activity of MAO ranging from 36 to 127% ($P < 0.05$–0.01). Higher doses of the extract (2 and 8 g kg^{-1}) produced a smaller and statistically insignificant increase (10–26%) in MAO activity. Cholinesterase activity in blood, liver and brain of rats with was not significantly affected by *R. stricta* any of the doses used (data not shown).

Effect of R. stricta *on brain catecholamine levels*

The *R. stricta* alkaloidal fraction has been shown to contain the active ingredient in *R. stricta* leaf lyophilized extract (Ali et al 1998b; Tanira et al 2000). The changes induced by the *R. stricta* alkaloidal fraction on brain catecholamine concentrations are shown in Figure 2. *R. stricta* caused a reduction in the levels of all the measured biogenic amines. The reduction was most notable and highly significant in the whole brain tissue. Other parts of the brain, such as cerebrum and cerebellum showed similar changes. The adrenaline concentration was the most affected being reduced to less than 2% of the control level. The whole brain tissue showed statistically significant ($P < 0.01$) reductions in all biogenic amines concentrations (adrenaline 99%, noradrenaline 80%, dopamine 90% and 5-HT 87%). The pons and medulla were least affected. The cerebrum and the remaining part of the brain tissue showed a variable response but significant reductions were observed with noradrenaline in the cerebellum (72%) and dopamine in the cerebrum (69%).

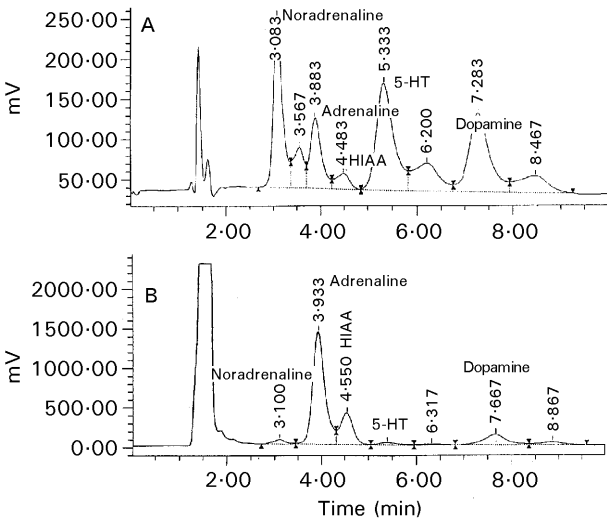

Figure 1. Typical HPLC chromatograms of biogenic amines standards mix (A), and of a whole rat brain tissue sample (B).

Discussion

R. stricta leaf extract caused an increase in liver and brain MAO activity which was not dose-dependent in rats. Lower doses produced a significant increase in the hepatic and cerebral MAO activity, whereas higher doses did not significantly influence the enzyme activity. This pattern of activity, with regard to dose relationship, is similar to that reported for the concentration of tribulin in rat brain (Ali et al 1998a), and to that in the forced-swimming test results (Ali et al 1998b). In both of those studies a biphasic mode of action was observed. However, the present results do not agree with the dose-dependent activity of the plant

Table 1. Monoamine oxidase (MAO) activity in liver and brain of rats treated orally with *Rhazya stricta* lyophilized extract for three consecutive days.

Organ	Control	*R. stricta* dose ($g\,kg^{-1}$)			
		0·2	0·5	2·0	8·0
Liver	5·25 ± 0·33	7·15 ± 0·31*	11·90 ± 1·11**	6·03 ± 0·48	6·59 ± 0·44
Brain	1·55 ± 0·13	2·57 ± 0·18*	3·00 ± 0·26**	1·95 ± 0·21	1·70 ± 0·17

Data are mean ± s.e.m., n = 7. MAO activity is expressed as μL mol 4-hydroxyquinoline (g tissue)$^{-1}$ h^{-1}. The control group received distilled water. *$P < 0.05$ and **$P < 0.01$ significantly different from respective controls.

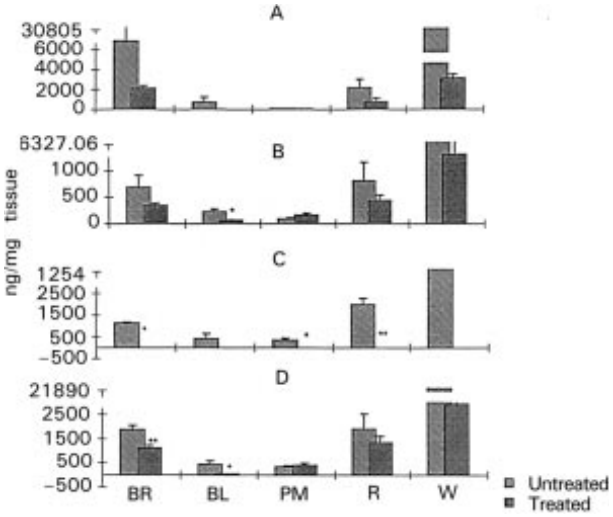

Figure 2. Effect of *Rhazya stricta* alkaloidal fraction on rat brain biogenic amines.

extract observed on blood pressure (Tanira et al 2000).

The reasons for these complex effects that fail to show simple dose–response curves are not fully understood, but they indicate the possible presence of different active ingredients in the extract, contributing to the changes observed at the different doses acting on different biological processes. This is supported by the number of compounds that are isolated from *R. stricta* leaf (Atta-ur-Rahman 1986).

The statistically significant reduction in the concentrations of brain biogenic amines may be related to the observed increase in MAO activity in rats. The changes in biogenic amine concentrations mediated by changes in MAO activity may play a role in the reported CNS activity of *R. stricta*. The lack of a significant effect of *R. stricta* on cholinesterase activity suggests that the published pharmacological activities of the plant extract may not directly involve cholinesterase or its substrates.

Further work on the effect of various components of *R. stricta* leaf extract on the synthesis, concentration and turnover of different biogenic amines and metabolites is necessary to determine the functional significance of these effects.

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