Antidepressant Effects of Apocynum venetum Leaves in a Forced Swimming Test

Veronica BUTTERWECK,* Sansei NISHIBE,b Tsutomu SASAKI,c and Masaru UCHIDAc

Institute of Pharmacology and Toxicology, WWU-Muenster,* Domagkstrasse 12, 48149 Muenster, Germany, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido,b Ishikari-Tobetsu, Hokkaido 061–0293, Japan, and Tokiwa Phytochemical Co., Ltd.,c 158 Kinoko Sakura, Chiba 285–0801, Japan.

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An extract of the leaves of Apocynum venetum L. (Apocynaceae) markedly shortened the immobility time of male rats in a forced swimming test (FST) in a dose range of 30—125 mg/kg, indicating a possible antidepressant activity. This effect was comparable to that of the tricyclic antidepressant imipramine (20 mg/kg). Neither imipramine (20 mg/kg) nor the Apocynum extract in various doses (30, 60, 125 mg/kg) produced any overt behavioural change or motor dysfunction in the open field test. This result confirms the assumption that the antidepressant effect of an Apocynum extract in the FST is specific. Further, it can be speculated that this effect might be related to hyperoside and isoquercitrin which are major flavonoids in the extract.

Key words Apocynum venetum; leave extract; antidepressant effect; flavonoid

Apocynum venetum L. (Luobuma in Chinese, Apocynaceae) is a wild shrub widely distributed in mid and northwestern China. Since ancient times the leaves of A. venetum have been used in traditional Chinese medicine for the treatment of hypertension, nephritis and neurasthenia.1) Previous papers describe the diuretic,2) antihyperlipemic3) and sedative4) effects of the plant. The leaves of A. venetum are also used in China as an anti-aging agent. The leaves of A. venetum had a strong inhibitory effect in a lipid-peroxidation assay.5)

Flavonoids are polyphenolic compounds that are ubiquitously present in foods of plant origin. Flavonoids are capable of modulating the activity of enzymes and affect the behaviour of many cell systems, suggesting that the compounds may possess significant antihapetotoxic, antiallergic, anti-inflammatory, antioestropotropic and even antitumour activities.6)7) Interestingly, the list of plant flavonoids did not include effects on the central nervous system (CNS) up to 1990 when Paladini et al. described the anxiolytic potency of chrysin (5,7-dihydroxyflavone) and apigenin (5,7,4'-trihydroxyflavone).8) Since then, a great deal of research has been done in this field. Recently, it was shown that a flavonoid fraction obtained from a crude extract of Hypericum perforatum (St. John’s wort) was remarkably active in a forced swimming test (FST).9) Isolates obtained from this fraction including hyperoside, isoquercitrin, miquelianin and quercitrin were also active in the FST. These data gave the first evidence that some natural flavonoids possess antidepressant activity.

In the present study it was therefore of interest to investigate the effects of an extract of A. venetum leaves (Apocynum extract), containing an amount of 2.1% hyperoside and 2.7% isoquercitrin, in the FST. The FST was chosen as this test is a valid animal model to detect antidepressant compounds.10)

MATERIALS AND METHODS

Animals Male CD rats (230—250 g, Charles River WIGA, Sulzfeld, Germany) were used. The animals were kept in a 12 h light/dark cycle at a constant temperature of 25±1 °C with free access to food (altromin 1324) and tap water. Animals were divided randomly into control and experimental groups. Animal experiments were officially approved by the Regierungspräsident, Münster (A 38/93).

Preparation of Apocynum Extract Leaves of Apocynum venetum (100 g) were refluxed for 1 h in aqueous ethanol (70% v/v, 60 ml) twice and the combined alcoholic extract was evaporated to dryness (28 g). The extract (13.5 g) was dissolved in hot water (200 ml), and adjusted to pH 3.0 with sulfuric acid, then filtered. The filtrate was chromatographed on DIAION HP-20 (3.6 cm i.d.×18 cm) and eluted with water (200 ml) and then aqueous ethanol (70% v/v, 200 ml). The aqueous ethanol fraction was collected and evaporated to dryness to obtain Apocynum extract (4.2 g).

The extract contained 2.1% hyperoside and 2.7% isoquercitrin, respectively. HPLC analytical conditions were as follows: Column: SHISEIDO CAPCELL PAKC18 (UG) 4.6 mm i.d.×150 mm, detector at 330 nm, mobile phase: 0.1% TFA in water/0.1%TFA in acetonitrile 85:15, flow rate: 1.0 ml/min. Equipment: Waters 600 with a Waters 2487 UV detector. Hyperoside appeared at 13.5 min and isoquercitrin at 14.7 min. Authentic hyperoside and isoquercitrin were purchased from Funakoshi (Tokyo, Japan).

Drug Treatment Imipramine-HCL was purchased from Sigma (Deisenhofen) and dissolved in deionized water. For all experiments, powdered Apocynum extract (drug/extract ratio: 25/2 prepared by Tokiwa Phytochemical Co., Ltd., Chiba, Japan) was used. Apocynum extract was dissolved homogeneously in water by sonication. Control animals received deionized water only. All substances were administered orally by gavage.

FST after Acute Pre-treatment The purpose of this experiment was to investigate whether an Apocynum extract possesses antidepressant activity in the FST. The administered dose range of the extract was chosen in the same manner as that of St. John’s wort extract9 since Apocynum extract contains an almost equal amount of flavonoids to those of St. John’s wort extract. Subsequently, a group of 40 animals was subdivided as follows: 8 animals were treated with imipramine.

* To whom correspondence should be addressed. e-mail: butterw@uni-muenster.de
were treated with imipramine (20 mg/kg, p.o.), 24 with Apocynum extract (125, 250, 500 mg/kg, p.o., 8 animals/group), and 8 animals received vehicle (deionized water). Test solutions were administered 24, 5 and 1 h before the test. This procedure has been found to produce more marked pharmacological effects than a single application 1 h before the test.11)  

**FST after Repeated Treatment** This experiment was designed to validate the results of the acute experiment. The administered dose range of Apocynum extract was chosen in the same manner as that of St. John’s wort extract9) since Apocynum extract contains an almost equal amount of flavonoids to those of St. John’s wort extract. Consequently, a group of 40 animals was subdivided as follows: 8 animals were treated with imipramine (20 mg/kg, p.o.), 24 with Apocynum extract (30, 60, 125 mg/kg, p.o., 8 animals/group), and 8 animals received vehicle (deionized water). Test solutions were administered once daily over a period of 14 d. Administration was performed between 3—4 p.m. every day. A dosage of 30 mg/kg imipramine was chosen for the acute study because former experiments indicated that dosages below 30 mg were only partially active; for chronic treatment 20 mg/kg proved to be an active dosage without any toxic effects, whereas higher doses of imipramine showed toxic effects in the chronic treatment (Butterweck, unpublished results).

**Measurement of Immobility** This experiment was performed using the FST according to the method published by Porsolt et al.12) Male rats were placed in a plexiglass cylinder (40×18 cm i.d.) containing a column of 17 cm of water at 25±1°C. According to Porsolt the rats learned in a pretest session of 15 min that they could not escape from the cylinder. In the test period, 24 h later, the animals were exposed to the experimental conditions for 5 min. A rat was judged to be immobile whenever it remained floating in the water, in an upright position, making only small movements to keep its head above the water.

The FST for both treatment paradigms was performed between 1—3 p.m. and recorded by a video camera. The tapes were evaluated by an observer who was not informed about the kind of treatment each animal had received.

**Measurement of Locomotor Activity** In order to see whether changes in immobility were associated with changes in motor activity as described by Porsolt et al.11) for amphetamine and caffeine, animals treated with the various substances were tested for activity in an open field. For measurement of locomotor activity naive rats were placed in an open field apparatus. The open field apparatus was an arena 70 cm in diameter divided into 18 approximately equal areas. Hand-operated counters were employed to score locomotion (number of line crossings within 5 min) and rearing frequencies (number of times an animal stood on its hind legs). In this experiment the animals received the same drugs and doses as used for the measurement of immobility after repeated treatment. For open field observations, each rat was individually placed in the center of the arena 15 h after the last treatment and its behavioural parameters were recorded for 5 min. The open field apparatus was washed with a detergent solution before each behavioural test to eliminate possible odor clues left by previous subjects. To minimize circadian changes in the rat open-field behaviour, control and experimental animals were intermixed. Open field observations were made between 8—9 a.m.

**Statistics** All data were expressed as the mean±S.E.M. Group mean differences were ascertained with analysis of variance (ANOVA). Multiple comparisons among treatment means were checked with the Fisher-PLSD. The results were considered significant if the probability of error was <5%.

**RESULTS**

As shown in Fig. 1, immobility was significantly reduced after acute pre-treatment by imipramine (30 mg/kg) and Apocynum extract (125 mg/kg). No effects after acute administration were observed in the FST for the higher doses of the extract (250, 500 mg/kg). However, 14 d after daily pre-treatment, the extract markedly reduced immobility at a dosage of 30 and 125 mg/kg (Fig. 2). This effect was comparable to that of the tricyclic antidepressant imipramine (20 mg/kg), although the effect of the synthetic substance was more pronounced. The dosage of 60 mg/kg of the extract slightly reduced immobility, but the effect was not significant. Neither imipramine (20 mg/kg) nor the Apocynum extract in various doses (30, 60, 125 mg/kg) produced any overt behavioural change or motor dysfunction in the open field test (Table 1). In fact, none of the drugs increased open field activity.

![Fig. 1. Activity of Apocynum Extract in the FST after Acute Treatment](image)

Test solutions were administered orally 24, 5 and 1 h before the test. n=8; *p<0.05 vs. control.

![Fig. 2. Activity of Apocynum Extract in the FST after Repeated Treatment](image)

Test solutions were administered orally once daily over a period of 14 d. n=8; *p<0.05 vs. control.
mission and whether antidepressant effects are only present most appropriate to identify diverse antidepressant treat-
ment period of 14 d was comparable to that of the tricyclic antidepressant imipramine (20 mg/kg), although the effect of the synthetic substance was more pronounced after repeated treatment. Neither imipramine (20 mg/kg) nor the \textit{Apocynum} extract at various doses (30, 60, 125 mg/kg) produced any overt behavioural change or motor dysfunction in the open field test, so that the observed effects in the FST were not due to a stimulation of locomotor activity. The results of the FST after repeated treatment and after additional open field tests confirms the assumption that the antidepressant effect of an \textit{Apocynum} extract in the FST is specific. Further, it can be speculated that this effect might be related to hyperoside and isouqueritrin, as both flavonoids recently showed antidepressant activity in the FST.\textsuperscript{9} A further reason for this assumption is that the effective extract doses used in the present study correlate well with the effective hyperoside and isouqueritrin doses applied in the previous study by Butterweck \textit{et al}.\textsuperscript{9} In detail, Butterweck \textit{et al}. showed that pure hyperoside significantly shortened immobility time in the FST in a dose range of 0.6—1.3 mg/kg, whereas the effects of doses below 0.6 mg/kg and above 1.3 mg/kg were not significantly different from control in St. John’s wort extract.\textsuperscript{9} Pure isouqueritrin was also active in the FST at a dosage of 0.6 mg/kg.\textsuperscript{9} This amount of hyperoside as well as of isouqueritrin is approximately reached with 30 mg/kg of the \textit{Apocynum} extract, which was active in the FST after repeated treatment. The fact that 125 mg/kg of the \textit{Apocynum} extract was also active in the FST after acute and repeated treatment leads to the assumption that this extract probably contains other substances with possible antidepressant potential. Further investigations in this direction are necessary to confirm this hypothesis.

Acknowledgments These study results were followed by application for a Japanese Patent (Application No. 2000-334122). We thank Dr. S. Seo of Tokiwa Phytochemical Co., Ltd., for supporting our Patent application.

REFERENCES