

Short Communication

Effect of leaves of *Apocynum venetum* L. on the activity of MAO in mice

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The purpose of this study was to evaluate the effect of leaves of *Apocynum venetum* L. (AV) on the monoamine oxidase (MAO) activity in mice whole brain. After 7 days oral administration, we assayed spectrophotometrically the activity of MAO. The ethanolic extract of leaves of *A. venetum* (EAV) showed a tendency to inhibit MAO activity in animal brain. However, the aqueous phase of EAV (EAV-4) significantly inhibits the MAO activity. The effect of fluoxetine on activity of MAO was not marked. The aqueous phase of ethanolic extracts of AV significantly inhibited the activity of MAO, which might be the active part of antidepressant effect of AV.

Key words: *Apocynum venetum* L., monoamine oxidase, monoamines.

INTRODUCTION

Monoamine oxidase (MAO), an important enzyme in the metabolism of a wide range of monoamine neurotransmitters, including noradrenaline, dopamine, and 5-hydroxytryptamine (5-HT), plays a crucial role in depression and age-related disorders. MAO exists in two forms, A and B. MAO-A is more important than MAO-B in the metabolism of the major neurotransmitter monoamines. MAO-A inhibitors have been accepted to treat depression (Knoll, 1997; Wouters, 1998; Toshiharu, 2004). *Apocynum venetum* L. (AV) is a wild shrub widely distributed in central and northwestern China and it has been used in traditional Chinese medicine for the treatment of hypertension, nephritis and neurasthenia (Takako et al., 2004). Previous papers described the antidepressant effects (Veronika et al., 2001, 2003) of the plant. However, the mechanism of this action is not clear. MAO assay may be available to elucidate the antidepressant action of AV.

MATERIALS AND METHODS

Apparatus, reagents and medicinal materials

3K30 laboratory centrifuges (Sigma USA); UV-2800 UV

spectrophotometer (Unico, Shanghai); 5-HT was purchased from Sigma (USA); Benzylamine (Fluck Switzerland). All other reagents used in the study were of analytical grade. AV was collected in April from Shandong province of China.

Animals

Male Kunming mice weighing 18 to 22 g were purchased from the Experimental Animal Center of Wuhan University and were housed in plastic cages. They were housed in a quiet room under a 12 h light/12 h dark cycle at 25±2°C for 5 days before experimentations. All the animals were given standard food and water *ad libitum*, except during observation periods.

Preparation of extracts

360 g leaves of AV were lixiviated with alcohol to obtain powdery extracts of AV (EAV). The powder was suspended in water and extracted by petroleum ether, acetic ether and n-butanol, respectively. After the extraction, the aqueous phase was dried to powder (EAV-4, 31.9 g).

Drug administration

The animals were randomized into control and experimental groups and divided into four groups (10 animals each). Group 1 was administered with normal saline (0.9% NaCl solution). Groups 2 and 3 were administered with EAV and EAV-4 at the doses of 1500 and 960 mg/kg, respectively. Group 4 were administered with fluoxetine at the dose of 26 mg/kg.

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Table 1. Effects of EAV, EAV-4 and fluoxetine on activity of MAO-A in the mouse whole brain (mean \pm S.E.M).

Group	Dose (mg/kg)	Number of mice	MAO A activity (nmol/mg protein·h)	MAO A inhibition (%)
Control	10	10	34.6 \pm 3.9	
Fluoxetine	26	10	34.0 \pm 1.4	1.7
EAV	1500	10	23.0 \pm 2.0*	33.7
EAV-4	960	10	19.3 \pm 0.9	44.2

*P<0.05 when compared with control groups.

Table 2. Effects of EAV, EAV-4 and fluoxetine on activity of MAO-B in the mouse whole brain (mean \pm S.E.M).

Group	Dose (mg/kg)	Number of mice	MAO-B activity (nmol/mg protein·h)	MAO-B inhibition (%)
Control	10	10	25.0 \pm 2.8	
Fluoxetine	26	10	24.2 \pm 3.0	3.2
EAV	1500	10	22.6 \pm 2.3*	9.6
EAV-4	960	10	17.2 \pm 0.9	31.2

*P<0.05 when compared with control groups.

MAO assay in mouse whole brain

Preparation of enzyme liquid

Mouse brain mitochondrial fraction was prepared following the procedure described previously (Veronika et al., 2003) with some modification. Briefly, the mitochondrial fraction suspended in 10 vol. of cold sodium phosphate buffer (0.2 M, pH 7.4), was mingled twice (20 s each with alternation of 30 s) with ultrasonic (59 KHz). The mixture was centrifuged at 1 000 \times g for 10 min at 0°C and the pellet was discarded.

The resultant supernatant was centrifuged at 17 000 \times g for 30 min at 4°C and the pellets were re-suspended and stored at -30°C for MAO analysis within 3 days.

Determination of the concentration of protein

Protein concentration was estimated by the Coomassie brilliant blue method (Chun et al., 2008) using bovine serum albumin as the standard. Then the protein concentration was adjusted to 1 mg/ml.

MAO assay

MAO activity was assayed spectrophotometrically as described previously (Charles and McEwen, 1977; Yu and Kong, 2002). The assay mixtures contained 0.3 ml 4 mM 5-HT or 8 mM benzylamine as specific substrates for MAO A and B, respectively, 0.5 ml solution of the mitochondrial fraction and 0.2 M sodium phosphate buffer (pH 7.4) up to a final volume of 3 ml.

The reaction was allowed to proceed at 37 to 40°C for 1 h and stopped by adding 1 M HCl (0.6 ml), the reaction product was extracted with 4 ml of butyl acetate (for MAO-A assay) or cyclohexane (for MAO-B assay), respectively. The organic phase was measured at wavelength of 280 nm for MAO- A assay and 242 nm for MAO- B assay with spectrophotometer, respectively. Blank samples were prepared by adding 0.3 ml 4 mM 5-HT or 8 mM benzylamine after the reaction and worked up subsequently in the same manner.

Monoamines assay

The amounts of monoamines in various brain tissues were quantitatively measured by spectrofluorometer. NE and DA tissue levels of monoamines were expressed in terms of nanograms per gram of tissue and determined by calibration curves. Calibration curves and correlation coefficients based on absorbance- concentration relationship of standard substance were established by means of linear regression analysis:

NE: 0.999, $y = 3.2223x + 0.035$; DA: 0.998, $y = 0.293x + 0.451$;
5-HT: 0.999, $y = 4.8967x + 4.4147$.

RESULTS

Statistical analysis

Values are given as mean \pm S.E.M. (standard error of mean) and significances calculated using one-way analysis of variance following Dunnett's T3 test. A probability level of 0.05 or less was accepted as significant.

Effects of EAV and EAV-4 on MAO A and B activities in mouse whole brain

The effects of EAV and EAV-4 for 14 days on the MAO A and B activities in mouse whole brain were shown in Tables 1 and 2. The MAO A and B activities in normal group were 34.6 \pm 3.9 nmol/mg protein·h and 25.0 \pm 2.8 nmol/mg protein·h, respectively. Oral administration of EAV at the doses of 1500 mg/kg provided 33.7% (MAO-A) and 9.6% (MAO-B) inhibition. Fluoxetine at the dose of 26 mg/kg showed a tendency to reduce the MAO A (1.7%) and B (3.2%) activity. The effects of EAV and fluoxetine were not significant in the study. However, EAV-4

Table 3. Effects of EAV, EAV-4 and fluoxetine on levels of NE, DA and 5-HT in striatum and hippocampus (mean \pm S.E.M).

Dosage (mg/kg)	Striatum			Hippocampus		
	NE (ng/g)	DA (ng/g)	5-HT (ng/g)	NE (ng/g)	DA (ng/g)	5-HT (ng/g)
Control	1035.9 \pm 95.2	2010.9 \pm 201.0	251.1 \pm 23.6	1791.8 \pm 146.0	1924.8 \pm 181.4	148.7 \pm 93.4
Fluoxetine 25	1103.9 \pm 168.4	1464.6 \pm 290.4	453.6 \pm 30.0**	1696.1 \pm 171.4*	1985.3 \pm 124.6	430.7 \pm 35.8*
EAV 80	1118.5 \pm 95.4*	1434.5 \pm 129.4*	167.7 \pm 16.6*	1585.8 \pm 108.0	1963.2 \pm 146.1*	313.8 \pm 65.1
EAV-4 80	1736.6 \pm 89.8**	2392.9 \pm 203.2*	428.1 \pm 40.2	2555.6 \pm 110.0*	2019.5 \pm 167.3*	281.8 \pm 90.4*

Values are mean \pm S.E.M. expressed as ng/g tissue of 10 animals in each group. DA = dopamine; NE = norepinephrine; 5-HT = 5-hydroxytryptamine. * P < 0.05. ** P < 0.01.

(960 mg/kg) was significantly exhibited to inhibit MAO A and B, producing 44.2 and 31.2% inhibition, respectively.

Effects of fluoxetine and extracts of AV on NE, DA and 5-HT in striatum and hippocampus

The content of NE, DA and 5-HT in the striatum and hippocampus after treatments is shown in Table 3. Compared with control, the treatment in three groups leads to a moderate increased level of NE in striatum, but in hippocampus only EAV-4 shows significant alteration. Interestingly, levels of DA were all modified with drug treatment in hippocampus tissues and we also discovered that it showed an important improvement to level of DA (2392.9 \pm 203.2 ng/g). Such results seem to reflect a contrasted relationship between levels of NE and DA in striatum and hippocampus, respectively. After administration, the level of 5-HT were increased in two inspected brain tissues except for EAV in striatum.

DISCUSSION

To our knowledge, these results are the first to demonstrate that leaves of *A. venetum* L. inhibited significantly the activity of MAO in the model described earlier. This may be the part reason why *A. venetum* L. can significantly infect the level of monoamine (MA) neurotransmitters. Presently, most of antidepressants are administrated clinically, which based on monoamine hypothesis (Olivier and Eric, 2006). It's the cardinal antidepressive mechanism that these antidepressants inhibit reabsorption and degradation of MA to elevate the concentration of MA at synaptic cleft. Monoamine neurotransmitters, including 5-hydroxytryptamine (5-HT), noradrenaline (NE) and dopamine (DA), are considered as critical factors in the pathogenesis of depressive disorder. 5-HT is the moderator on affection, somnus, memory, vigilance and appetite etc. And insufficiency of NE may result in mental depression. Frequently, low level of 5-HT, NE appears in the encephalon and neurolymph of patients with depressive disorder. MAOs are the specific inactivator of monoamine neurotransmitters (5-HT, NE and DA). Inhibition on MAOs could increase monoamine neurotransmitters in brain and it's considered

the approach to moderate depressive symptoms. Flavones are the major ingredients in *A. venetum* L. (Guangting et al., 2008) and the inhibition activity of flavones on MAOs is reported in previous research (Veronika et al., 2001). Since monoamine neurotransmitters are very important to the mechanism of depressant, our finding may be available to explain the potentiality of the antidepressant activity of *A. venetum* L. Flavones are the major ingredients in *A. venetum* L. and the inhibition activity of flavones on MAOs is reported in previous research, so further study will focus on the active flavone compounds in *A. venetum* L. against MAOs.

Moreover, AV is also used as an anti-aging agent (Veronika et al., 2003). MAO-B is very important in the aging process. So, our finding may be also useful to explain why AV has anti-aging activity.

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