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Behavioral effects of dried methanolic extract from *Desmodium pulchellum* in rats

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ผลของสารสกัดด้วยเมทานอลจากสมุนไพรเกล็ดปลาช่อนต่อพฤติกรรมของหนูขาว

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จากที่มีรายงานว่าสมุนไพรเกล็ดปลาช่อน *(Desmodium pulchellum)* มีฤทธิ์เป็นยาได้ กลุ่มผู้ทดลองนี้จึงเริ่มทดสอบสรรพคุณของ D. *pulchellum* ในแง่ฤทธิ์ต่อพยาธิใบไม้ตับและฤทธิ์ทางเภสัชวิทยาอื่น ๆ และได้รายงานไปแล้วว่าน้ำสกัดจากส่วนต่าง ๆ ของพืชโดยเฉพาะอย่างยิ่งจากเปลือกรากให้ฤทธิ์กระตุ้นและกดการหายใจ เพิ่มความดันโลหิต ลดการเคลื่อนไหวของหนู ในการทดลองนี้ได้สกัดสารที่มีฤทธิ์ออกมาและพบว่าสารสกัดด้วยเมทานอลจากเปลือกรากให้ผลต่าง ๆ ดั่งเช่นที่ได้รายงานไว้ และเมื่อศึกษาสารสกัดนี้โดยใช้ความเข้มข้นต่างกัน พบว่ามีผลต่อพฤติกรรมของหนูซึ่งคล้ายกับที่เกิดจาก serotonin กล่าวคือมีการส่ายศีรษะเท้าตะปบพื้น ขาหลังเหยียด สารนี้ยังเสริมฤทธิ์ของ tryptophan ในการเหนี่ยวนำพฤติกรรมดังกล่าว และในทำนองกลับกันฤทธิ์เสริมนี้ถูกยับยั้งได้ด้วย cyproheptadine (ซึ่งเป็น serotonin antagonist) ผลนี้เป็นแนวทางบ่งไปถึงการเป็นยาต้านภาวะซึมเศร้า (depression) เมื่อทำการทดสอบหาฤทธิ์ดังกล่าวในหนูถีบจักรที่ถูกบังคับให้ว่ายน้ำโดยเปรียบเทียบกับ imipramine เห็นว่าสารสกัดเมทานอลนี้มีฤทธิ์ต้านอย่างอ่อนเท่านั้น

Desmodium pulchellum **has been reported to be a traditional remedy of schistosomiasis. Our previous pharmacological studies demonstrated a decreased locomotion, an increased blood pressure, and an increased respiration in rats treated with aqueous extracts from various parts of the herb, especially the root bark. This prompted us to isolate the active principles responsible for such activities. In this experiment, the root bark was extracted by maceration using a series of solvents. Dried methanolic extract was found to possess the above mentioned effects. At a dose of 30 mg/kg (p.o) induced stereotyped behaviors (paw padding, tail-flick, hind-limb extension) in rats. Higher doses caused convulsion and death within an hour. These**

similar behaviors induced by tryptophan, were potentiated by the extract. This potentiation was antagonized by cyproheptadine, a serotonin antagonist. This evidence of action mediated-through serotonergic system indicates that the extract may become a useful antidepressant. The speculation was tested on behavioral despaired-mice which was forced to swim. A weak antidepressant effect compared to an antidepressant, imipramine, was observed.

Introduction

Desmodium pulchellum (Phyllodium pulchellum) is an indigenous plant in Thailand and is abundant in Khon Kaen. The plant is used by Chineses for traditional remedy of schistosomiasis (1). Some villagers in the North-Eastern of Thailand claimed that the herb is effective in treatment of liver fluke infection (from interviews). Although chemical constituents of the plant grown in other countries have already been elucidated (2,3,4), pharmacological effects of the plant have not been thoroughly explored. Recently, Chitcharoenthum et al. (5) have shown a marked pharmacological effects of the plant, especially, the root bark. This prompted us to investigate chemical compositions as well as pharmacological effects of the plant grown in Khon Kaen. Solvent extraction and chromatographic techniques were employed in the isolation. Pharmacological tests were carried out using concious rats and mice.

Extraction

Desmodium pulchellum was identified by Samghang Homcheon, a plant taxonomist of Biology department, Khon Kaen University. The schedule of extraction was as followed.

Desmodium pulchellum 's root (1.72 kg)

↓

(ต้นเกล็ดปลาช่อน)
root bark (860 g)
air-dried over night
chopped into pieces

Extraction
1. petroleum ether (40-60 °), room temp., 7 days
2. dichloromethane, room temp., 7 days
3. methyl alcohol, room temp., 7 days
4. water, 80 °C 6 h

↓

Filtrate from 1-3 : evaporated to dryness under reduced pressure in water bath at 60 °C

↓

dried substances
- petroleum ether (1.3 g)
- dichloromethane (5.2 g)
- methanol (50.7 g)

testing for pharmacological activities (Muckda Chitcharoenthum)

separation, purification and identification (Parinya Theramongkol)

Behavioral observations

1. Dose response of dried methanolic extract on behavior of rats.

Male Sprague-Dawley rats of average weight 183±14.5 g (mean±SD) were orally fed solution of dried methanolic extract from *Desmodium pulchellum* or normal saline solution. Four groups of 5 rats were fed either 30, 300 and 1,500 g/kg bw of the dried methanolic extract, and 1 ml normal saline/200 g bw. Behavior of the rats was observed at 5, 10, 20, 30, 40, 60 min. after the administration of the test substance. Each observed parameter was counted for 1 min-period.

2. Interaction of dried methanolic extract on tryptophan-mediated behavioral changes in rats.

Male Sprague-Dawley rats were injected intraperitoneally with 10 mg/kg tranylcypromine (Trany 1.). At 20 minutes later these rats were injected 50 mg/kg L-tryptophan (Tryp.) intraperitoneally. The solution of dried methanolic extract 3 mg/kg (Ext.) were fed orally by intragastric tube at 15 min after tryptophan-administration. Three groups of five rats were treated according to the following schedule:

Treatment	Body weight (±s.e.m)	time 0	20	35 min
control	183.0±4.8	Tranyl.	Tryp.	NS 1 ml
Treatment 1	223.0±14	NS 0.2 ml	NS 0.2 ml	Ext.
Treatment 2	214.0±15	Tranyl.	Tryp.	Ext.

Behavior of rats was observed for 2 to 18 h after treatments. Each observed behavior was taken 1 min at each time point.

3. Antagonistic effects of cyproheptadine against the potentiating action of dried methanolic extract and tryptophan.

Rats recieved Treatment 2 as mentioned in the previous experiment were given cyproheptadine (4.7 mg/kg) orally at either 10 min before or after the administration of dried methanolic extract in 3 and 2 rats, respectively.

4. Effects of dried methanolic extract on mice in "Behavior despair" test (6)

Five groups of 6 to 7 albino mice (20 to 30 g) were treated either normal saline (0.2 ml/20 g body weight, p.o), the dried methanolic extract (4.5 and 45 mg/kg;p.o), the most polar part of the dried methanolic extract separated from a silica gel column (27.5 mg/kg, p.o), or 20 mg/kg (i.p.) imipramine. Total mobility time was recorded on untreated (4 days) and on treated day at 20 min after the administration of the tested solutions. The increase in total mobility time during the test session was calculated from the difference of mobility time of individual mice on treated and on untreated day. The observation was taken for 5 min when mice was put in a beaker (4L) containing 25°C water for 3/4 of the beaker. The observer had been trained to time mobility and did not know which treatment the individual mice had received.

Animals, solution and chemicals

Rats were bought from Saraya, Mahidol University and were kept in the animal house of Faculty of Medicine, Khon Kaen University. Mice were bred and taken care of by the staff of the animal house, Faculty of Medicine.

The solution of dried methanolic extract was prepared by dissolving in warm water and filtered. The stock solution was kept in a refrigerator (4°C) for use no longer than a month.

Following chemicals; tranylcypromine (Sigma), L-tryptophan (Sigma) and imipramine (Sigma), were used in this experiment Results.

1. Dose response of dried methanolic extract on behavior of rats.

Behavioral changes in rats appeared within 5 min after the administration of solution from dried methanolic extract. Initially, behavioral changes was paw padding and decreased locomotion. These were followed by tail flicking, hind-limb extension, then, convulsion and death. Some rats had mild convulsion during 2 h but became more severe and died within 18 h after the extract administration. Table 1. shows that the methanolic extract increases paw padding in rats,which is significant at 1,500 mg dose ($MSE=252.375$, $F(3, 16)=39.838$, $p<0.0001$). Locomotion of the rats, both verticle and horizontal, is significantly decreased by 300 and 1,500 mg dose of the extract. ($MSE=4.025$, $F(3, 16)=15.615$, $p<0.0005$; $MSE=9.575$, $F(3, 16)=5.65$, $p<0.01$, respectivety). Tail flicking is also noticed within 10 min or more after administration of the extract. A significant tail-flick is observed at the highest dose of the

extract. Rats that shown tail flicking usually progress to the state of hind-limb extension. High doses of about 300 mg/kg or more of the extract causes convulsion and rats exhibited convulsion usually die within 2 to 18 h-period of observation.

2. Interaction of dried methanolic extract on tryptophan-mediated behavioral changes in rats.

After 20 min of tryptophan administration, the rats exhibited behavioral changes such as head-weaving, paw padding, horizontal locomotion and tail flicking (Table 2). There was no rat in this group died during the 2 h period of observation. On the other hand, rats treated with 3 mg/kg methanolic extract showed a slight effects of these behaviors. The combined treatment of tryptophan and 3 mg/kg methanolic extract had potentiating effects which are characterized by the progression of stereotyped behaviors to fetal effects, tail flicking, convulsion and death.

3. Antagonistic effects of cyproheptadine on the potentiating action of dried methanolic extract and tryptophan

Five rats received combined treatment of solution from dried methanolic extract (3 mg/kg) and tryptophan (50 mg/kg) were treated with cyproheptadine (4.7 mg/kg). Two of these rats which had developed paw padding, jerking or convulsion within 10 min after the administration of the extract, cyproheptadine decreased the severity of the extract's effects (Table 3). Another three rats which were received cyproheptadine (1 mg/kg) at 10 min before the extract administration did not show tail flicking, jerking and convulsion within an hour period of observation.

4. Effects of dried methanolic extract on mice in "Behavior despair" test

Total mobility time taken in 5 min was recorded on the untreated and treated day at 20 min after treatment. The increase in total mobility time of individual mice between treatment and untreatment day was compared among 5 treatments. Table 4 shows a similar control mobility time of these groups of rats on the untreated day (MSE = 2959.02, F = 0.22, $p>0.9$) and a significant (MSE = 3477.38, df4, 28, F = 3.65, $p<0.02$) increases in mobility time by drug treatment. Rats treated with imipramine show a significantly longer mobility time than the controls and rats treated with 4.5 mg/kg dried methanolic extract. All the extract treatment prolonged mobility time but are not significantly difference from the controls. A purified polar part of the methanolic extract from a silica gel column also exerts a slight antidepressant action similar to the methanolic extract but at 40% lower dose. All doses of the extract used in this experiment did not affect gripping of the mice nor did it cause any behavioral changes.

Discussion

Dried methanolic extract from *Desmodium pulchellum* induced behavioral changes; paw padding, tail-flick, hind-limb extension or convulsion, within 5 to 20 min after the extract administration. These behaviors are also inducible by tryptophan or serotonin (4). Potentiation on these behavioral changes by the combined treatment of the extract and tryptophan (the serotonin precursor), and antagonism of cyproheptadine against the patentiating effects indicate that the extract mediates the action through the increased activity of serotoninergic system. The study of chemical composition in the plant by other investigators has shown several tryptamine bases such as dimethyl tryptamine (2) and bufotenine (3) which are known to exert serotonin-like effects (7). Evidently, a substance exerts the effects may be used effectively in the treatment of depression (8). However our

results showed that the extract and its purified substances have only a mild antidepressant effect compared to that of imipramine. A more detail study in dose-response of the extract on animal models of depression should be furtherly examined.

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Table 1. Effects of dried methanolic extract on behavior of rats. Each figure represents mean±s.e.m from 5 rats.

Time after treatment	**5-10 min**	**5-15 min**		**10-20 min**	**during 2 h**	**within 18 h**
Behaviors	**paw padding (times/min)**	**locomotion vertical horizontal (times/min) (ft/min)**		**tail flicking (times/min)**	**convulsion (# rats)**	**death (# rats)**
control	0.0±0.0[a]	7.2±1.1[a]	7.6±2.4[a]	0.0±0.0	0	0
dried extract (mg/kg) per os 30	1.4±0.9[a]	4.8±1.3[a]	4.0±0.9[ac]	0.0±0.0	0	0
300	22.0±12.4[a]	0.2±0.2[b]	0.8±0.5[bc]	4.4±2.0	4*	4*
1500	95.2±6.8[b]	0.0±0.0[b]	0.6±0.6[bc]	21.2±2.1*	5**	5**

different alphabet denotes pair of groups significantly diffferent at 0.05 level by Student Newman-Keul test. after analysis of variance (ANOVA)
*$p<0.05$, **$p<0.01$ denotes significant difference from the control by Fisher's Exact test

Table 2 Potentiating effects of dried methanolic extract on tryptophan-mediated behavioral changes in rats. Each figure represents mean±s.e.m from 5 rats.

	Behavioral effects				
	head weaving	**forepaw padding**	**locomotion**	**tail-flick**	**death**
control	52.0±23.7	15.0±4.7	16.2±10.4	2.0±2.2	0
treatment 1	11.0±8.1	0.4±0.4	11.6±7.3	0.0	0
treatment 2	103.2±71.0	12.0±12.5	17.6±6.5	22.0±8.0*	4**

control = tranylcypromine + tryptophan
treatment 1 = methanolic extract
treatment 2 = control + treatment 1
*$p<0.01$, **$p<0.005$ (Fisher's Exact test)

Table 3 Antagonistic effect of cyproheptadine on behavioral changes induced by dried methanolic extract and tryptophan

			antagonistic effect of cyproheptadine			
time after extract adm. (min)		**10**	**20**	**30**	**40**	**60**
Cyproheptadine administered at 10 min. after dried methanolic extract						
rat # 1	paw padding	80/min	22	50	30	5
	tail-flick	32/min	0	0	0	
rat # 2	jerking, convulsion	15/min	rest	rest	rest	rest
Cyproheptadine administered at 10 min. before dried methanolic extract						
rat # 3,4,5	tail-flick	0	0	0	0	0
	convulsion	0	0	0	0	0

Table 4 Total increased mobility time. Each figure represents mean±s.e.m from n rats.

Treatment		**# rats**	**Total mobility time (sec.)**	
			control	**increases**
control		6	92.2±27.7	25.7±10.2 a
Imipramine	20 mg/kg	6	89.0±20.4	138.7±34.2 b
methanolic extract	4.5 mg/kg	7	98.0±20.8	31.3±15.2 ab
	45 mg/kg	7	90.4±28.4	77.1±31.4 ab
purified substance from methanolic extract				
	27.5 mg/kg	7	92.0±15.3	70.0±21.1 ab

increased mobility time = mobility time of a rat on treated day (minus) mobility time of that rat on untreated day

different alphabet denotes pair of groups significantly different at 0.05 level (Student Newman-Keul)