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Toxicity and Biological Activity of Extracts from *Stichopus japonicus*

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Abstract □ The toxicity of water extracts from intestine parts (digestive tract and respiratory tree) of Korean *Stichopus japonicus* was determined using mouse units and more purified substance decreases the amplitude of contraction of guinia pig atria *in vitro:* showes negative chronotropic and ionotropic effects in the spontaneously beating guinea pig atria.

Keywords □ *Stichopus japonicus*, Mouse unit, Negative chronotropic and ionotropic effects, Guinea pig atria.

From the great diversity of marine organisms, it might be expected that new chemical compounds with useful pharmacological action could be isolated from them, and intensive studies have been focussed on marine natural products.[1-2]

We recently reported that California dorido nudibranchs contain a new hypotensive N^1-methylisoguanosine riboside named doridosine, isolated from the digestive glands of *Anisodoris nobilis*.[3-4] In connection with the sea animal, we have tried to isolate some biologically active substances from Korean *Stichopus japonicus* and now report that toxic substance occurs in Korean *Stichopus japonicus* and that the pharmacological properties show both negative chronotropic and ionotropic effects in isolated guinea pig atria. Pooled intestine parts (digestive tract and respiratoratory tree, 680g, wet weight) of *Stich*opus *japonicus* which were collected at Pusan area were homogenized in methanol (1,000ml) and centrifuged twice at 5°C at 5,000×g for 20min. Methanol extracts concentrated were suspended in chloroform (150ml), which was extracted with pure water (100ml×3) three times. The aqueous layer was lyophilized to give crude material (designated A, 22g). The toxicity of water extracts (A) was determined by injection into mice. Volumes of 0.1 to 1.0ml were injected intraperitoneally into adult white mice weighing 20 to 30g with the results shown in Table I.

The crude solution (A) was dialysed through Sepctrapor membrane tubing (No. 1, molecular weight cut off 6,000-8,000) to remove polymers. Dialyzates prepared as above were chromatographed twice on 3,8×50cm columms of Bio-Gel

Table I: Toxicity of extracts of intestine system.

Dose(ml)	Mouse Wgt.(g)	Time to death(h)	Toxicity Mouse unit[a] (mg/20g)
0.2	27	survived	—
0.4	28	20	65
0.4	30	18	54
0.8	32	6	34
0.8	26	5	35
0.6	22	7	46
		Mouse unit: 49mg/20g (average)	

a) A mouse unit is defined to be approximate amount which can kill a mouse of 20g weight for 20h after intraperitonial injection into a mouse.

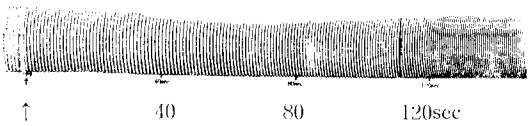

Fig. 1: Effects of extract of *Stichopus japonicus* on spontaneously beating guinia pig atria. Sample solution added at arrow to an 10ml tissue bath.

P-2 (Bio-Rad., Solvents; H_2O: Pyridine: AcOH =500:12:1, pH=6.0). Three fractions (designated B,C,D, R*f*: Ca. 0, 0.4, 0.8 respectively on silica gel TLC., solvent system; t-BuOH:H_2O: AcOH=40:10:2:1) were collected. Only fraction C (150mg) was active as shown in Fig. 1.

It has both negative chronotropic and ionotropic effects in the isolated spontaneously beating guinea pig atria. About forty per cent decrease in amplitude is produced by a concentration 100μg/ml of the C fractionation from Bio gel P-2 column. (statham Model P 23 AC)[1],[2] The amplitude decreased in guinea pig atria soon increased probably due to some impurity in sample solution. The substance C, which showes one spot on silica gel. TLC (t-; BuOH EtOAc:H_2O:AcOH=20:5:1:1), but two or three spots appeared on silica gel tlc. (iPrOH;NH_4 OH:H_2O=7:3:1) or on the two demensional silica gel TLC. (AUCELF 250μm, Analtech. 10× 10cm, solvent:MeCN:0.1M NH_4OAc=6:4, Sat. $(NH_4)_2SO_4$:0.1M NaOAc:i-PrOH=79:19:2) The spots are close to those of adenosine N^1 methyl isoguanosine, but not identical.

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