INACTIVATION OF FORMOSAN SNAKE VENOMS IN VIVO BY ALLANTOIN, THE CHEMICAL COMPONENT OF ARISTOLOCHIA RADIX

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The effects of allantoin in inactivating Formosan Snake venoms in vivo were investigated in this study. Constant amount of allantoin was injected intramuscularly into one leg of a mouse beforehand, later within 24 hours the same amount of allantoin was injected again into the same leg, and then constant amount of snake venom was injected into the other leg. Results, mortality of the mice showed a considerable degree of variation. In general, allantoin in vivo was effective against Elapid venoms (Naja naja atra and Bungarus multicinctus), but not effective against Crotalid venoms (Trimeresurus mucrosquamatus, Agkistrodon acutus and Trimeresurus gramineus). More than 0.1 ml of allantoin saline buffer solution containing 500 micrograms of allantoin were effective against Elapid venoms, but less than 0.1 ml were not effective against them.

Key words: *Inactivation, Formosan snake venoms, in vivo, allantoin, Aristolochia radix.*

In our previous studies (4), we found that the crude extract of Aristolochia radix in vitro was very effective against venoms of Formosan snakes of family of Crotalidae (Trimeresurus granineus, Trimeresurus mucrosquamatus, Agkistrodon acutus), but not effective against venoms of family of Elapidae (Bungarus multicinctus, Naja naja atra). It needs further studies to see whether or not Aristolochia radix extract can inactivate Formosan snake venoms in vivo. Allantoin is one of the most important chemical component of Aristolochia radix (2), and it has been used topically in suppurating wounds and resistant ulcers (3), It is highly probable that Allantoin may inactivate snake venoms. Therefore we have tried to observe the inactivating effect of Allantoin against Formosan snake venoms in vivo.

Materials and Methods

The dried root Aristolochia Shimadai Hay was extracted with ethylalcohol (EtOH) and concentrated according to the process shown in the following chart. After concentrating in vacuum, the EtOH extract became a dark brown liquid. It was refrigerated for several

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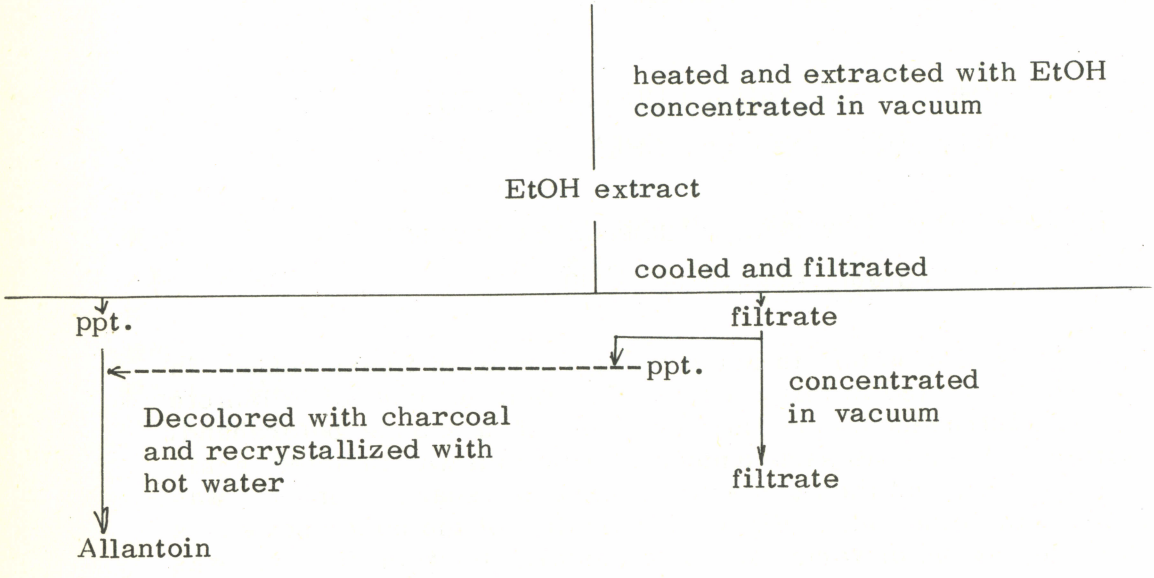
Chart 1
Dried root of Aristolochia Shimadai Hay.



days, and white needle crude crystals were obtained. The filtrate was again concentrated to one fourth of its volume in vacuum, and similar precipitate was produced. When this was mixed with the first crystallized product, it was decolored with charcoal and recrystallized in hot water, then allantoins the colorless column crystals were obtained. Their melting point 230°C (decomp.)

Five thousands micrograms of allantoin were dissolved in one ml of the saline buffer solution. The saline buffer solution was prepared in 0.2 M phosphate buffer at pH 6.2. Various amounts of the dried Formosan snake venoms were prepared for one hour incubation at 37°C before intramuscularly injection into the legs of the white mice (d. d. strain) weighing 18-20 gm. One hundred and ninety two micrograms of Naja naja atra venom were dissolved in one ml of the saline buffer solution; one hundred and seventy six micrograms of Bungarus multicinctus in one ml; Eleven hundreds micrograms of Trimeresurus mucrosquamatus in one ml; Seventeen hundreds and twenty micrograms of Agkistrodon acutus in one ml; and nine hundreds and sixty micrograms of Trimeresurus gramineus in one ml.

Inactivating tests in vivo were conducted by injecting 0.1 ml or various amounts of allantoin intramuscularly into one leg of a mouse beforehand, later within 24 hours injection of the same amount of allantoin again into the same leg, and then at the same time injection of various amounts of snake venom in 0.1 ml into the other leg of the same mouse. Inactivating tests in vitro were conducted by injecting 0.1 ml of mixed solution of a certain amount of allantoin and snake

venom intramuscularly into the legs of the mice at the same time.

Results

Inactivation of Formosan Snake Venoms in Vivo by Allantoin:

Thirty mice were used as an experimental group. Each mouse of the experimental group was injected intramuscularly into one leg in a volume of 0.1 ml of allantoin saline buffer solution beforehand, later within 24 hours injection of the same volume of allantoin solution into the same leg again and then at the same time injection of 0.1 ml of each venom saline buffer solution into the other leg. Each mouse of the control group was injected intramuscularly into one leg in a volume of 0.1 ml of venom solution once.

Data on inactivation of Formosan snake venoms in vivo by allantoin were summarized in Table 1, which indicated that Elapid venoms (Naja naja atra and Bungarus multicinctus) were inactivated by allantoin in vivc but Crotalid venoms (Trimerusurus mucrosquamatus, Agkistrodon acutus and Trimeresurus gramineus) were not inactivated.

Table 1. Inactivation of Venoms in Vivo by Allantoin

Venoms / Mice	Naja naja atra		Bungarus multicinctus		Trimeresurus mucrosquamatus		Agkistrodon acutus		Trimeresurus gramineus	
	M	X^2	M	X^2	M	X^2	M	X^2	M	X^2
Control group	23/30	($p<0.005$)	24/30	14.1 ($p<0.005$)	25/30	0.42 ($0.1<p<0.9$)	23/30	1.01 ($0.1<p<0.9$)	21/30	0
Experimental group	9/30		10/30		23/30		26/30		21/30	

Mortality(M): Numerator indicates the number of mice which died.
Denominator indicates the number of mice used.

X^2 in the table is the value of chi-square calculating from experimental group compared with control group. (1)

Inactivation of Formosan Snake Veenoms in Vitro by Allantoin:

Ten mice were used as an experimental group and another ten mice as a control group. Each mouse of the experimental group was injected intramuscularly in a volume of 0.1 ml of allantoin solution mixed with venom into the leg. Each mouse of the control group was injected only 0.1 ml of venom solution intramuscuarly into the leg once.

Data on inactivation of venoms in vitro by allantoin were summarized in Table 2, which indicated that all of the venoms were not significantly inactivated.

Table 2 Inactivation of Venoms in Vitro by Allantoin

Venoms / Mice	Naja naja atra		Bungarus multicinctus		Trimeresurus mucrosquamatus		Agkistrodon acutus		Trimeresurus gramineus	
	M	X^2	M	X^2	M	X^2	M	X^2	M	X^2
Control group	8/10	0.27	9/10	2.4	8/10	0.266	7/10	0.225	9/10	1.25
Experimental group	7/10	$(0.1 < p < 0.9)$	6/10	$(0.1 < p < 0.9)$	7/10	$(0.1 < p < 0.9)$	6/10	$(0.1 < p < 0.9)$	7/10	$(0.1 < p < 0.9)$

Quantiatively Inactivating Effects of Allantoin against Naja naja Venom in Vivo:

In order to observe the quantitative effects of allantoin against Naja naja atra venom, one hundred and fourty mice were divided into seven groups. Each group was consisted of twenty mice. One group was the control, the other six were the experimental groups. Each mouse in each experimental group was injected in different volume of allantoin solution such as 0.0125 ml, 0.025 ml, 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml intramuscularly into one leg beforehand, later within 24 hours injection of the same volume of allantoin solution into the same leg again, and then at the same time injection of 0.1 ml of Naja naja atra venom solution into the other leg. Each mouse of the control group was injected intramuscularly into one leg in a volume of 0.1 ml of Naja naja atra venom solution.

Data on quantitatively inactivating effects of allantoin against Naja naja atra venom in vivo were summarized in Table 3, which indicated that more than 0.1 ml of allantoin solution showed significant inactivating effect against Naja naja atra venom, but less than 0.1 ml did not show any significant effect.

Table 3 Quantitatively Inactivating Effects of Allantoin against Naja naja Atra in Vivo

Mice / Mortality	Control group	Experimental groups: Volume of Allantoin Solution Injected					
		0.0125 ml	0.025 ml	0.05 ml	0.1 ml	0.2 ml	0.3 ml
Mortality	17/20	15/20	12/20	11/20	7/20	7/20	8/20
X^2		0.625 $(0.1 < P < 0.9)$	3.13 $(0.05 < p < 0.1)$	4.3 $(0.025 < p < 0.05)$	10.42 $(p < 0.005)$	10.42 $(p < 0.005)$	8.64 $(p < 0.005)$

Quantitatively Inactivating Effects of allantoin against Bungarus Multicinctus Venom in Vivo:

All procedures were the same as the above stated.

Data on quantitatively inactivating effects of allantoin against Bungarus multicinctus venom in vivo were summarized in Table 4, which also were almost the same result as Table 3. More than 0.1 ml of allantoin solution showed a significant inactivating effect against Bungarus multicinctus venom, but less than 0.1 ml did not show any significant effect.

Table 4 Quantitatively Inactivating Effects of Allantoin against Bungarus Multicinctus Venom in Vivo

Mice / Mortality	Control group	Experimental groups					
		Volume of Allantoin Solution injected					
		0.0125 ml	0.025 ml	0.05 ml	0.1 ml	0.2 ml	0.3 ml
Mortality	18/20	14/20	14/20	12/20	6/20	6/20	8/20
X^2		2.5 (0.1>p>0.9)	2.5 (0.1>p>0.9)	4.8 (0.025 p>0.05)	15 (p 0.005)	15 (p 0:005)	10.8 (p 0.005)

Discussion

In our previous studies (4), we found that the crude extract of Aristolochia radix in vitro was very effective against Crotalid venoms, but not effective against Elapid venoms. On the contrary we found that allantoin, one of the most important chemical component of Aristolochia radix, in vivo was very effective against Elapid venoms, but not effective against Crotalid venoms in the study. These results suggest that the crude extract of Aristolochia radix in vitro might be a useful treatment for bites by Crotalid snakes and allantoin in vivo might be good for treatment of Elapid snake bites.

Okonogi et al(5) reported that 5% solution of tannic acid could neutralize many snake venoms in vitro by production of precipitates. We reported in the previous studies(4) that pH of 15% Aristolochia crude extract solution mixed with venom were 4.1, It suggests that the Aristolochia extract solution containing some acids may neutralize the crotalid snake venoms as well as tannic acid in vitro, Kumar and Krishnan(6) found that an allosteric protein separated from guanine deaminase of rat liver inhibited by allantoin. We suppose that allantoin might inhibit toxic activities of elapid snake venoms in vivo similar to that it inhibit an allosteric protein in rat liver.

Li etal (2) reported that two most important chemical components of Aristolochia radix were obtained in crystal forms, they were allantoin and Aristolochic acid. We already investigated the effects of allantoin against Formosan snake venoms. Therefore it needs further study to investigated the effects of aristolochic acid against Formosan snake venoms.

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中 文 摘 要

臺灣馬兜鈴根化學成分尿囊素 (ALLantoin) 在活體內對於臺灣蛇毒解毒之研究

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本實驗研究臺灣馬兜鈴根主要化學成分，尿囊素 (ALLantoin) 在活體內對臺灣蛇毒解毒之效果。對照組係分別注射 0.1 ml 各種濃度及種類之蛇毒。實驗組分別預先注射含有 500 μg 尿囊素溶液 0.1 ml 在小白鼠一邊的後腿肌肉然後在 24 小時內再注射同量的尿囊素溶液在同一邊的後腿肌肉，同時注射 0.1 ml 各種濃度及種類蛇毒在另外一邊的後腿肌肉。經 24 小時後觀察其死亡率比較。

結果顯示臺灣馬兜鈴根主要化學成分尿囊素在活體內對於臺灣毒蛇蝙蝠科如飯匙倩，雨傘節蛇毒的效果有，而對於響尾蛇科如龜殼花、百步蛇及赤尾鮐蛇毒解毒的效果沒有。含有 500 μg 在 0.1 ml 容積以上時尿囊素對於蝙蝠蛇毒解毒之效果顯著，但是 0.1 ml 容積以下時沒有效果。