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Protective action against Amanita poisoning by iridoid glucoside, aucubin

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Aucubin, an iridoid glucoside, exhibits significant protective activities against Amanita poisoning in beagle dogs. The post-injection of aucubin helps beagles survive from lethal poisoning caused by Amanita virosa. Protective activities of aucubin result from primarily preventing hepatic injury caused by Amanita poisoning, and is partly due to a protective affect of aucubin on the depression of m-RNA biosynthesis in the liver caused by α-amanitin intoxication.

Key words: Aucubin, antidote, Amanita poisoning, hepatic injury, beagle dog.

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Aukubin, iridoidní glukosid působí významně protektivně při otravě Amanita u laboratorních psů (beagle). Injekční aplikace aukubinu podaná laboratorním psům po otravě letální dávkou Amanita virosa způsobila jejich přežití. Protektivní účinek aukubinu vyplývá z jeho primární prevence jaterního poškození a částečně i chrání před poklesem biosyntézy m-RNA v játrech, kterou způsobuje otrava alfa amanitinem.

INTRODUCTION

Over the past decades, many efforts have been made to search for an antidote for deadly *Amanita* poisoning. Various compounds such as cytochrome C, pencillin G, thioctic acid and silibinin (Vögel et al. 1984) have been reported to exhibit some degree of potency for the treatment of *Amanita* poisoning (Floersheim 1987), but their clinical efficacies have been controversial (Piqueras 1989). Previously, we reported that an iridoid glucoside, aucubin, markedly increased survival rate in mice intoxicated with α-amanitin (Chang 1984). based on this finding, the present study aims to evaluate the protective potency of aucubin as an antidote in beagle dog that had ingested aqueous extract of *Amanita virosa.*

MATERIALS AND METHODS

Isolation of aucubin

Aucubin (Fig.1) was isolated from fresh leaves of *Aucuba japonica* Thunb. (*Cornaceae*) (Trim and Hill 1952).

Preparation of aqueous extract of *A. virosa.*

An aqueous extract of the mushroom was prepared and contents of toxins were analysed by the method reported previously (Yamaura et al. 1981). One gram of fresh mushroom contained 1.06 mg of α-amanitin and 0.64 mg of phalloidin.

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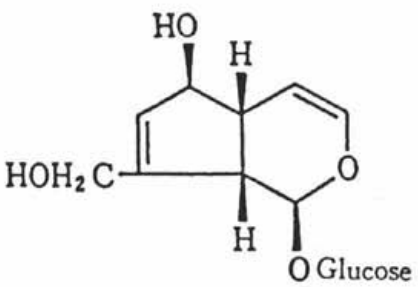

Aucubin $C_{15}H_{22}O_9$
mol. wt. 346.33

Fig. 1. The structural formula of aucubin (C15H22O9, mol. wt. 346.33) isolated from *Aucuba japonica* Thunb.

Administration routes of aqueous extract of the mushroom and aucubin

Animals and treatment

Each of two male beagle dogs (9.8 – 1.6 kg) were used as a survival study. Two groups received orally a lethal dose (0.4 g fresh mushroom/kg body weight) of aqueous extract of the mushroom. Treated group was administrated intravenously with a single dose (100 mg/kg) of aucubin 30 min, 2 hrs and 4 hrs after the administration of *A. virosa* extract (aucubin treated dog). The other dogs served as the control and received only aucubin (100 mg/kg).

Assay of blood and urinary parameters

Contents of serum glucose and glutamic-oxaloacetic transaminase (SGOT) activity was assayed by the method using commercial kit. Other serum enzymes were carried out to measure the extent of glucose and protein excretion on 1st, 2nd and 3rd day using Urinary Analyzer (Ames Co. USA).

Measurement of the incorporation rates of ratio-labelled adenosine into polyadenylated sequence of m-RNA precursor in mice liver

Male ICR mice (20-22 g) were used. Each specified time comprised four groups; control (saline), aucubin only-treated, α-amanitin only-treated, and α-amanitin plus aucubin-treated group in which each mice received a single dose of aucubin (80 mg/kg) 1 hr after α-amanitin intoxication (0.1 mg/kg) except the mice of the saline-control group. At each specified time interval, each mouse in the four group (3 mice/group) was injected intraperitoneally with [8-^{14}C]-adenosine (1 μCi/mouse; specific activity, 55 mCi/mmole) and radio-labelling was allowed for 1 hr. the isolation of liver polyribosomes (Lee and Brawerman 1971a), the extraction of RNA portion from polyribosomes (Perry et al. 1972), and the adsorption of m-RNA on nitrocellulose filters (Millipore) (Lee et al. 1971b). The radio activities on nitrocellulose filter discs were measured in a liquid scintillation spectrometer (Tricarb 1000, Packard Co., USA). The radioactivities per mg of extracted RNA were expressed as a percentage of the control values.

RESULTS AND DISCUSSION

After about 20 hrs, the *Amanita* poisoning and aucubin treated dogs started vomiting and discharging stools covered with mucus. At 48 hrs, the *Amanita* poisoning dogs discharged stools with blood stains and the final outcome was death in the end of 72 hrs. However, we did not observe such toxic symptoms and death in aucubin treated dogs.

As shown in Fig. 2, a rapid depletion of serum glucose was observed during a 3 days period following intoxication in the *Amanita* poisoning dogs, but in the aucubin treated dogs, however, the level of glucose decreased slightly and then recovered to the normal range after 3 days.

Time course change of SGOT in the indicators if liver damage was shown in Fig. 3. SGOT increased markedly from 24 hrs, and a continuous increase of SGOT was observed during a 3 days period and it's level was about 250 times of the normal range at 3rd day. In the aucubin treated dogs, the level of SGOT was only about 40 times of the normal range after 3 days, and decreased gradually and returned to nearly normal range at 14th day. The other parameters indicate the extent of liver damage were shown in Table 1. In the aucubin treated dogs, their values were lower than that of the *Amanita* poisoning dogs.

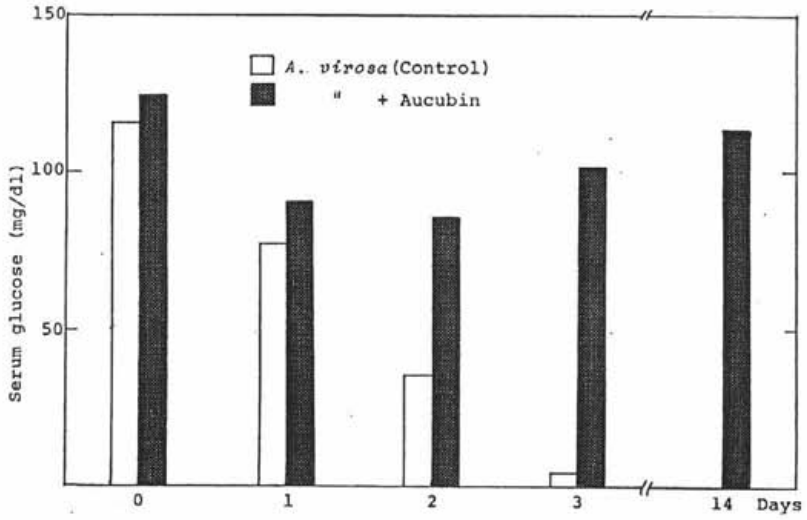

Fig. 2. Effect of aucubin of glucose in dogs after intoxication. Control was administered orally 4.0 g/kg of *A. virosa*. Aucubin was i.v. injected with a single dose (100 mg/kg) at 30 min, 3 hrs and 6 hrs after administration of the mushroom.

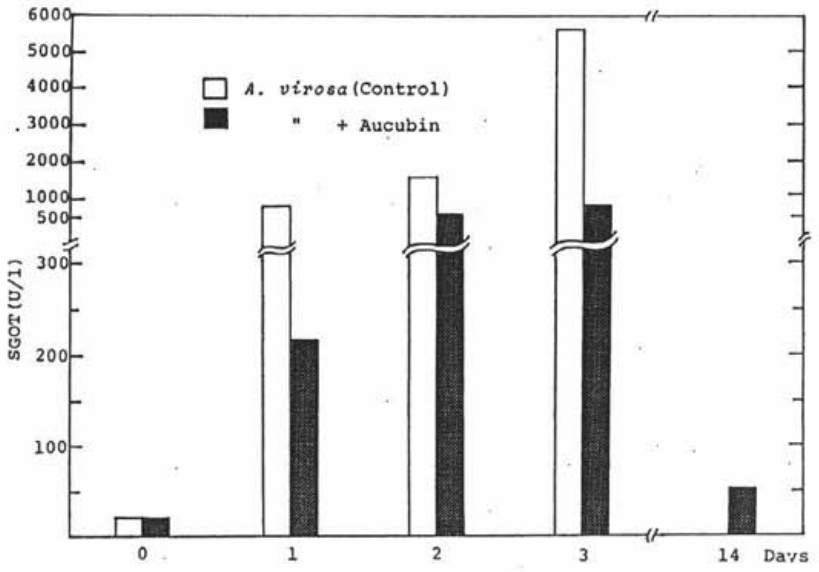

Fig. 3. Effect of aucubin of SGOT in dogs after intoxication. Control was administered orally 4.0 g/kg of *A. virosa*. Aucubin was i.v. injected with a single dose (100 mg/kg) at 30 min, 3 hrs and 6 hrs after administration of the mushroom.

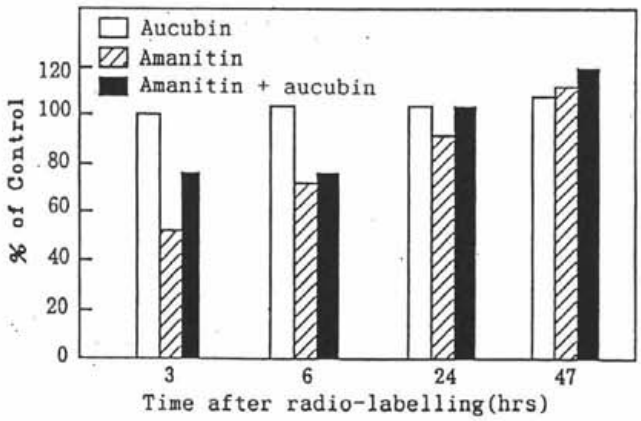

Fig. 4. Effect of aucubin on the incorporation rate of radio-labelled adenosine into polyadenylated sequence of m-RNA precursor in mouse liver.

Tab. 1 Effects of aucubin on components and enzymes in blood.

	GPT (u/l)	γ-GTP (u/l)	LDH (u/l)	BUN (u/l)	CRN (mg/dl)
A. virosa	8800	60	3700	62	0.8
A. virosa + acubin	110	11	752	38	0.5

Tab. 2 Excretion of glucose and protein into urine. Urine was collected for 24 hrs.

	Glucose (mg/dl)		Protein (mg/dl)	
	A. virosa		A. virosa	
Days	Control	+Acubin	Control	+Acubin
1	60	3700	62	0.8
2	11	752	38	0.5
3				

Excretion of glucose and protein into urine was shown in Table 2. Their levels were lower in the aucubin treated dogs than that of the *Amanita* poisoning dogs.

On the other hand, the results of histological examination also support biochemical effect. Massive liver cell necrosis was observed in the liver of *Amanita* poisoning dogs, but we can't see it in the liver of the aucubin treated dogs.

On the glycogen granules in the liver specimens, no glycogen granules were found in the liver of the *Amanita* poisoning dogs. In contrast, we could see glycogen granules in the liver of the aucubin treated dog.

It's well known that α-amanitin inhibit eucaryotic RNA polymerase II. Consequently, the formation of m-RNA precursors is blocked and cellular functions are impaired. We study how the aucubin influenced the liver's m-RNA biosynthesis in mice. As a measure of m-RNA biosynthesis, we used the degree of incorporation rates of radioactively labelled adenosine into the polyadenylated sequence of m-RNA precursors (Fig. 4). The incorporation rate was reduced to 54% of the control level after 3 hrs in the α-amanitin group. In the aucubin treated group, the incorporation rate was 75% of the control level after 3 hrs, and then it reached the control level at 24 hrs. These results suggested that the aucubin treatment prevents the depression of m-RNA biosynthesis in the liver by α-amanitin.

Such biochemical activity of aucubin may account in art of the protective activities against hepatic injury caused by *Amanita* poisoning.

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