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Toxicological Studies on Aucubin (I)

Acute Toxicities and Effects on Blood Serum Enzymes

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Aucubin의 독성연구 (I)

급성독성 및 혈청효소에 미치는 영향

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Aucubin, an iridoid glucoside which was previously reported to exhibit liver-protective activities against CCl_4 and α-amanitin induced liver damages, was subject to toxicological studies. To measure the lethal dose, the doses of 100 mg/kg, 300 mg/kg, 600 mg/kg and 900 mg/kg were administered intraperitoneally to experimental mice. No death was observed 24 hrs later, but serum GOT and alkaline phosphatase activities were decrased slightly at the doses of 300 mg to 900 mg/kg, and the triglyceride contents were slightly increased. To investigate acute toxicity of aucubin itself, multiple dosages(20 mg/kg, 40 mg/kg and 80 mg/kg for four times a week) were injected intraperitoneally into mice, then serum enzymes activities and chemistries were assayed; no significant change of the enzyme activities of alkaline phosphatase, GPT, GOT in the test groups were observed in comparison with those of the control group, and the contents of triglyceride, glucose, urea nitrogen and total proteins in the test group serums appeared to be almost same levels as those of the control group were. Histological examiation on liver biopsy samples indicated no gross changes between the control group and the test group were noted. Therefore, aucubin appears to be apparantly low toxic substance and its minimum lethal dose in mouse seems to be more than 0.9 g.

Introduction

In the course of searching for liver-protective agents from medicinal plants, we found that seven plants out of 45 plants so far screened exhibited most significant protective activities againist liver damage induced by CCl_4 intoxication in mice; they were *Alisma orientale*(Alismataceae), *Atractylodes japonica*(Compositae), *Cyperus rotundus*(Cyperaceae), *Gentiana scabra* (Gentianaceae), *Plantago asiatica*(Plantaginaceae), *Polygonatum japonicum*(Liliaceae) and *Aucuba japonica*(Cornaceae).[1~6] Among them, two plants, *P. asiatica*,, and *A. japonica* were

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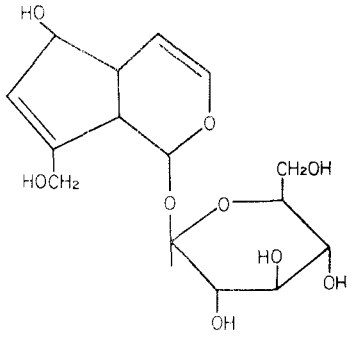

Fig. 1. Structure of aucubin.

selected for further studies and aucubin, an iridoid glucoside being present in both plants, were isolated.[7] Thereafter, the aucubin has been reported to be one of active principle being responsible for liver-protective activities against liver damages produced by CCl_4 and α-amanitin intoxications in mice.[8,9]

We also found that aucubin itself exhibited some degree of inhibitory effects on liver RNA and protein biosyntheses, but not much potent.[8,10] Subsequently it was suggested that such inhibitory effect of aucubin could be a possible mechanism of liver-protective activities,[8,9] because aucubin counteracted the severe depressions of liver RNA and protein biosyntheses caused by CCl_4 and α-amanitin intoxications.

In addition, aucubin has been higly recommended to be a potential antidote for fatal mushroom poisoning caused by noxious *Amanita phalloides*. Because the administration of aucubin achieved easily 50% complete recovery from α-amanitin poisoning in mice, even when aucubin administration was withheld for 12 hrs after α-amanitin challenge.[9] With this connection, assessing the safety of aucubin as a potential drug is of interest to us and the present study aims to conduct toxicological studies on aucubin, investigating a possible acute toxicity and other adverse effects on blood serum enzymes and chemistry.

Experimental

Materials: Aucubin was isolated from *Aucuba japonica* (Cornaceae) as reported elsewhere.[10,11] Mice (ICR, male, weighing 20±2 g) were supplied by the Animal Care and Breeding Laboratory in Seoul National University.

Measurements of lethal dose: To measure the lethal dose of aucubin in mice, one of four doses; 100 mg/kg, 300 mg/kg, 600 mg/kg and 900 mg/kg, was injected intraperitoneally into each mouse and 24 hrs later numbers of dead mice in each group were noted. Each group consisted of 10 mice. Then blood was withdrawn from each mouse by cardiac puncture. Serum was immediately separated by centrifugation (3,000 RPM, 1,495×g for 3 min). Serum was stored in a freezer at 20°C before assay.

Acute short-term toxicity - Effects on blood serum enzymes and chemistries: All mice were divided into four groups; one control group received i.p. physiological saline and test groups received i.p. aucubin dissolved in saline in a dose of 20 mg/kg, 40 mg/kg and 80 mg/kg. On day 1, 3, 5, and 7, each mouse in test grous was i.p. administered aucubin and mice in control group received saline on same days. Each group consisted of 18 mice and on day 1, 3, 5, and 7, three mice from each group were sacrificed and blood was withdrawn by cardiac puncture, and serum was separated as mentioned in the above. And the following serum enzymes and the contents of important biomolecules were assayed by the ABA 200 autoanalyzer (Abbottt Laboratory, Diagnostic Division, Irving, TX 75061, U.S.A.); alkaline phosphatase,[12] glutamic pyruvic transaminase,[13] glutamic oxaloacetic transaminase,[14] triglyceride,[15] urea nitrogen,[16] total protein[17] and glucose[18] were measured. In addition, on day 1 and 7,

Table I. Dose-schedule and effects on serum enzymes and chemistry.

Treatments(mg/kg)	Day 1*	Day 3*	Day 5*	Day 7*
Control(saline)	Saline	Saline	Saline	Saline
Aucubin 20	Liver biopsy and			Liver biopsy and
40	Serum enzymes**	Serum enzymes	Serum enzymes	Serum enzymes
	assay	assay	assay	assay
80				

* Aucubin for the test groups and 0.9% saline for the control group were administered. intraperitoneally. Each group consisted of 18 mice, and on specified days three mice were sacrificed for the liver biopsies and the serum enzymes assay.

** Serum enzymes; alkaline phosphatase, SGPT, SGOT, triglyceride, BUN, total protein, and glucose.

liver biopsy samples were prepared for histopathological observations.

Results and Discussion

Acute toxicity and measurement of lethal dose: To measure a single lethal dose of aucubin each mouse in four groups was intraperitoneally injected a single dose out of four doses, 100 mg/kg, 300 mg/kg, 600 mg/kg and 900 mg/kg of aucubin dissolved in saline. Twentyfour hrs later, numbers of lethalities were counted, but no death was observed. They were apparently active as healthy mice.

As the data shown in Table II, the minimum lethal dose of aucubin appears to be more than 900 mg/kg in mice, indicating this iridoid compound being low toxic. However, when the doses of aucubin were increased, the serum enzymes activites of SGOT and alkaline phospatase were gradually decreased, and the triglyceride contents were slightly incrased. These data indicate that aucubin may cause some adverse effects at higher dose. Regarding this, it should be noted that aucubin itself has some inhibitory effects on liver RNA and protein syntheses *in vivo*.[8~10] Therefore such inhibitory effects of aucubin might influence serum enzymes activities and triglyceride content. It should be pointed out that the optimal dose exhibiting maximum therapeutic effiicacy appeared to be about 100 mg/kg for a single intraperitoneal administration,[8,9] therefore the doses of 300 mg/kg to 900 mg/kg are exceedingly high doses over the optimal range. Further study on determining the therapeutic indice will be needed to clarify the dose dependant activities between pharmacological and toxicological aspects. The results obtained from the acute toxicity study indicate the aucubin possesses low acute toxicity. With respect to such low acute toxicity of aucubin, it is worthwhile to refer to Leveau and Durand's

Table II. Acute toxicity and measurement of a single lethal dose of aucubin.

Treatment	Dose(mg/kg)	Number of death	SGOT(IU/L)	Alkaline phosphatase(IU/L)	Triglyceride(mg/dL)
Aucubin	100	0	261	236	no test
	300	0	194	157	35
	600	0	179	135	39
	900	0	162	—	51

Each group consisted of 10 mice.

report, in which they observed that the extract of *A. japonia* fruit was about 20 g/kg for minimum lethal dose to mice[19]. Taking this into account, our observation appears to be in good agreement with it, because the fruits contain high amount of aucubin.

Multiple doses and effects on serum enzymes and chemistries: We could not observe any lethality by a single administration of aucubin at dose of 900 mg/kg, therefore, we employed low doses and multiple admnistrations. The dose-schedule were shown in Table I. All mice were divided into four groups; they were one control group and three test groups. On day 1, 3, 5 and 7, the control group received only physiological saline, and each mouse in the test

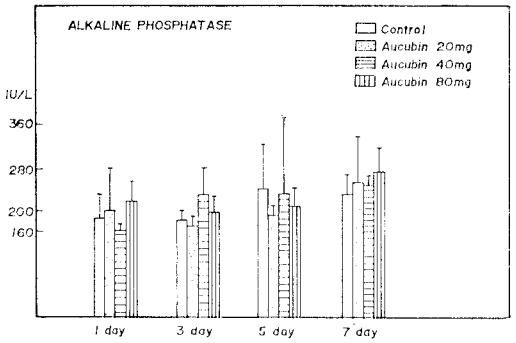

Fig. 2-1. Alkaline phosphatase activities in serum.

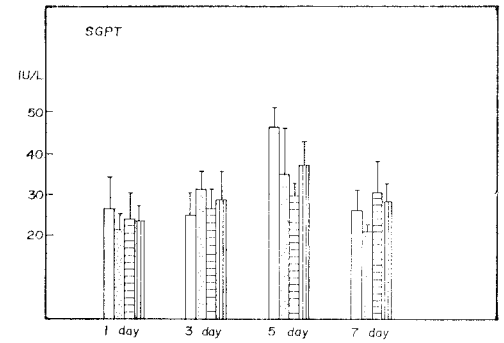

Fig. 2-2. Serum glutamic pyruvic tranaminase activities.

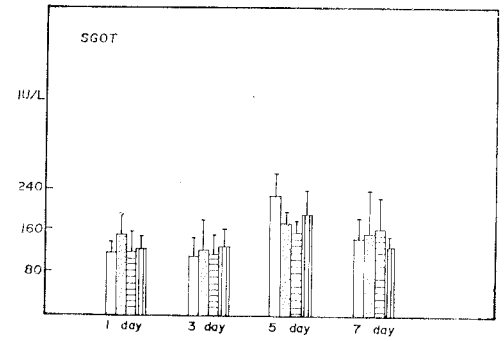

Fig. 2-3. Serum glutamic oxaloacetic transaminase activities.

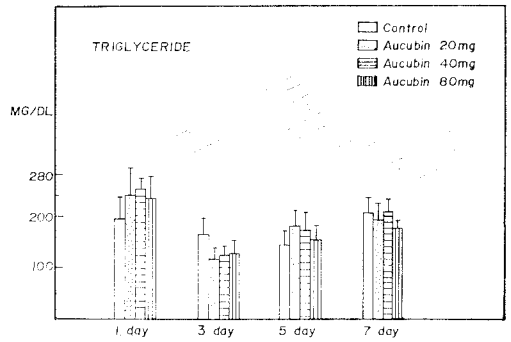

Fig. 2-4. Triglyceride content in serum.

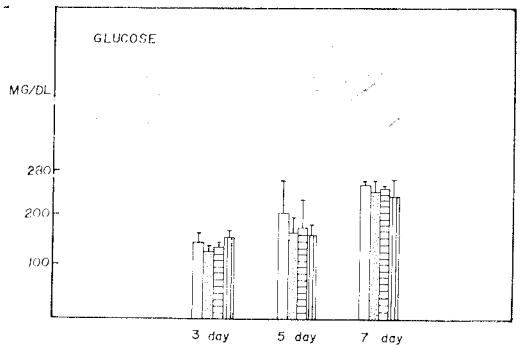

Fig. 5. Glucose content in serum.

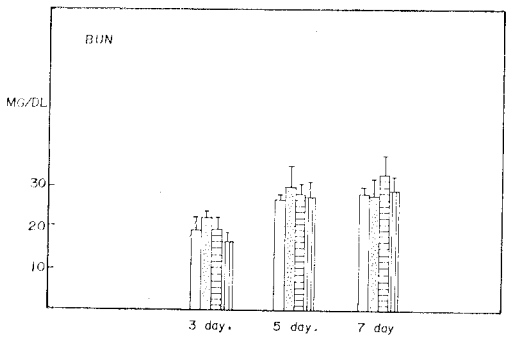

Fig. 2-6. Urea nitrogen content in serum.

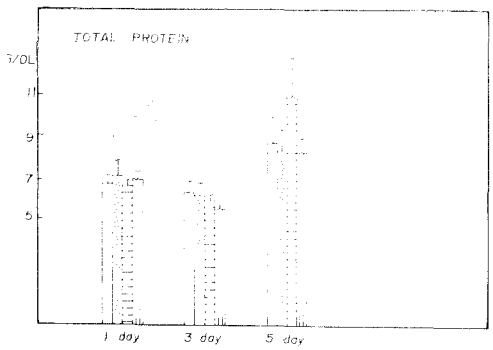

Fig. 2-7. Total proteins content in serum.

groups intraperitoneally received one dose out of three doses (20 mg/kg, 40 mg/kg and 80 mg/kg) on the same days as the control group received saline. Thus each mouse in the test groups received each dose four times during a week period. On day 1, 3, 5, 7, three mice from each group were sacrificed and the serum enzymes activities and chemistries were assayed. These enzymes activities and biomolecules contents like glucose, serum protein and urea nitrogen have been known to be sensitive to various toxic effects on liver function.

As the results were depicted by figures 2-1 to 2-7, the enzymes activities of alkaline phosphatase, GPT and GOT in the test groups were not significantly altered in comparison with those of the control group. And they appeared to be in normal range as were the control group, indicating that aucubin itself (under the dose-schedule employed for the experiment) might not give rise a significant toxic effect on liver.

It is well known that many toxic substances like CCl_4 and α-amanitin intoxication manifest usually the increase of triglyceride content, hypoglycemia and severe depressions of protein biosyntheses. Therefore the levels of triglyceride, glucose, urea nitrogen and total proteins in serum were measured and the data were shown in Fig. 2-4, 2-5, 2-6 and 2-7. We could not observed any significant changes in the contents of triglyceride, glucose, urea nitrogen and total proteins in the test groups in comparison with those of the control group. Although aucubin itself possesses some inhibitory activities of RNA and protein biosyntheses, the contents of total protein in serums of the test group were not appreciably decreased, which indicated that the dosages of aucubin employed in the experiments might be low to exhibit liver toxicity.

Histological examinations of liver biopsy samples: Liver biopsies were undertaken on day 1 and day 7 as shown in Table II and samples were prepared as reported previously.[6,7] Histological examinations were made under optical microscope. Microscopic pictures of biopsy samples for the control and two test groups which received the dosages of aucubin, 20 mg/kg and 80 mg/kg were shown in Fig. 3-1, 3-2 and 3-3. As the Fig. 3-1 shows, the liver biopsy sample of control group mouse appeared to be normal state. In the case of liver biopsy samples of test groups, no gross changes were observed, except few regenerating cells were located in the samples of those which received the dosage of 80 mg/kg of aucubin. The results from the histological examination, therefore, indicated that aucubin did not apparently cause liver damage at the dosages employed in this experiment.

Summarilly, on the basis of the results so far obtained, aucubin appeares to be virtually low toxic substance because its minimum lethal dose seems to be more than 0.9 g. When multiple doses of aucubin (20 mg, 40 mg and 80 mg/kg, 4 times/week) were administered, no significant acute toxic effects on serum enzymes (alkaline phosphatase and transaminases) and chemistries (triglyceride, glucose, urea nitrogen, and total proteins contents) were observed.

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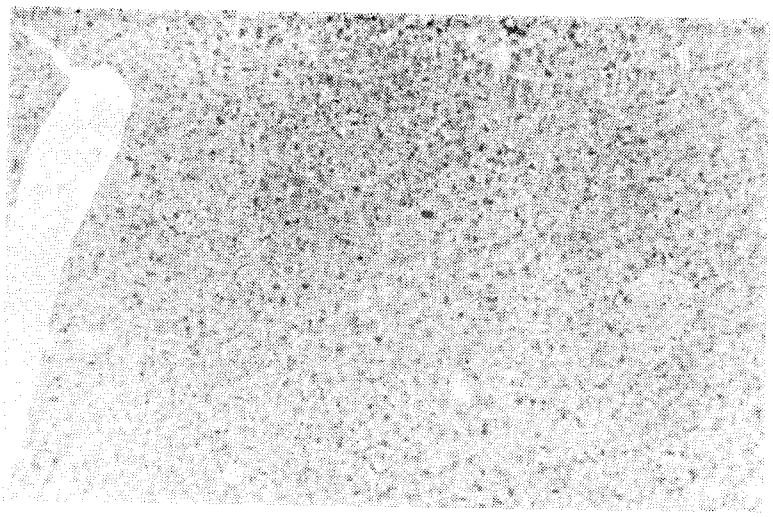

Fig. 3-1. Histologicl observations of liver biopsy. Biopsy taken from a liver of the control mouse on day 7 (Saline treated).

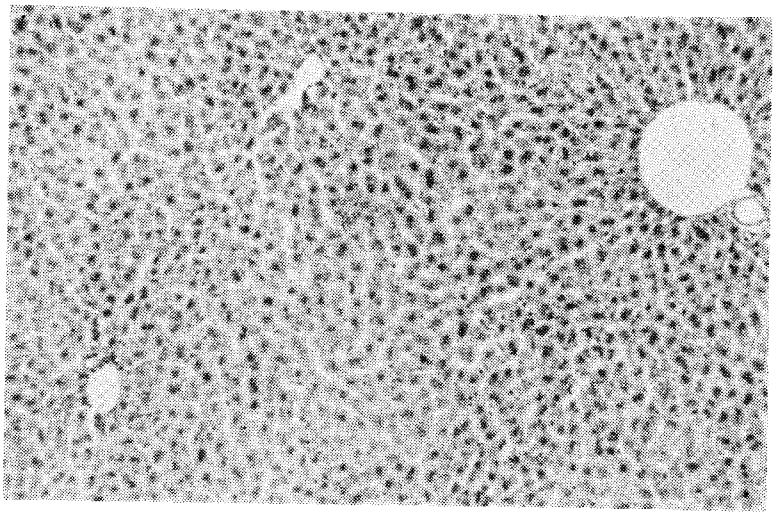

Fig. 3-2. Test group which received the dosage of 20 mg/kg(See, Table I).

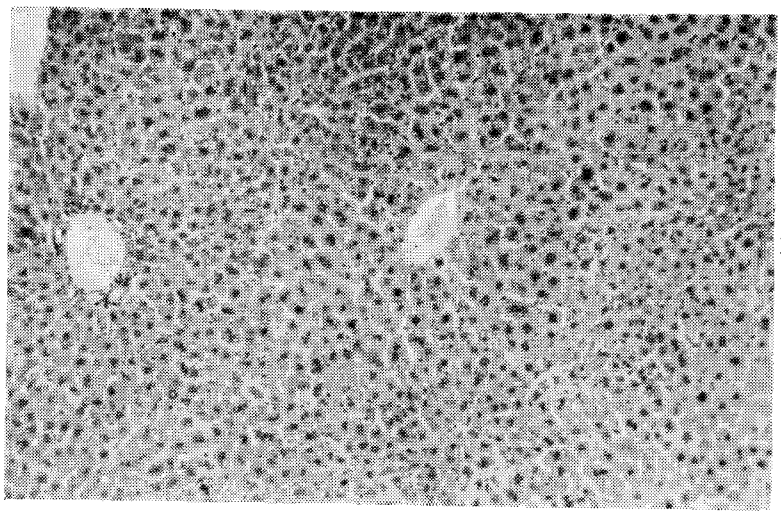

Fig. 3-3. Test group which received the dosage of 80 mg/kg(See, Table I).

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