생 약 학 회 지
Kor. J. Pharmacog.
13(1):14~19(1982)

Toxicological Evaluation of Medicinal Plants Used for Herbal Drugs (II)

Acute Toxicity and Effects on DNA Biosynthesis in Bone Marrow Cells and Hemoglobin Content in Blood

Il-Moo Chang,* Young-Soo. Kim and Byung-Hoon Han

Natural Products Research Institute, Seoul National University

한국산 생약의 약리작용 및 독성연구 (제 2 보)

급성 독성 및 골수세포의 DNA생합성에 미치는 영향

장 일 무*·김 영 수·한 병 훈

서울대학교 생약연구소

Potential toxicity of 15 medicinal plants used for herbal drugs, which were also described as being tonic for hematopoietic system or being toxic for the system in a oriental book "Dong Ee Bo Gam", were evaluated in mice. Six plants among 15 plants tested appeared to exhibit acute toxicity along with bone marrow depression or with abnormally enhancing the ^{3}H-thymidine incorporation into DNA biosynthesis in bone marrow cells. Six plants were *Paeonia albiflora, Pharbitis nil, Cemphalia lapidescens, Scutellaria baicalensis, Akebia quinata* and *Glycyrriza uralensis.*

Introduction

There have been unique medical care systems available in Korea for many years, that is, the oriental medical doctors in addition to ordinary western style medical practitioners who are similar to medical doctors in U.S.A. or in many other industrialized countries, have legally practiced in this country. Consequently, two entirely different higher medical education systems, which are the oriental medical schools and the western type medical schools, have been also operated parallely.

The oriental medical doctors frequently prescribe herbal drugs for their patients as decoction form or other preparation like extract. At present, it is beyond the subject whether such two paralleled medical care system and education bodies are indeed necessary and beneficial for public health in general.

Nonetheless, it is rather desirable and needed to make proper safety assessment of herbal drugs prescribed by the oriental doctors. Because, in terms of modern scietnific pharmacology and toxicology, no significant progress for the evaluation of those medicinal plants with respect to pharmacologic efficacy has been

* Reprint requests should be addressed to Dr. I.M. Chang

made yet. Previously, the authors reported the part of results obtained from the toxicity studies with 30 species of medicinal plants elsewhere, which have been frequently prescribed by the oriental medical doctors.[2-5]

With connection of preceeding work, at present, 15 species of medicinal plants which were described as tonic plants for hematopoietic system or as toxic plants for the system in a famous oriental medical text named in "Dong Ee Bo Gam", in fact this book has been used as a textbook for the oriental medical school till now, were selected to investigate their potential toxicity in experimental mice.[1] All plants were extracted in methanol-water solution and were orally administered (see the Material and Methods).

For the criteria of measuring the acute toxicity, and potential toxicity in bone marrow cells, comparisons of body weight change between test group and control group, the measurements of the incorporation rate of ^{3}H-thymidine into DNA in bone marrow cells and hemoglobin content in blood were employed.

Materials and Methods

Preparation of Plant Extract

All plants sample were purchased from local herb drug store, and they were identified taxonomically with respect to systemic biology in our institute. Each of dried plant samples, 500g, was extracted with methanol-water solution (90:10, v/v %) under reflux for 3 hrs. Then the extract solution was evaporated under reduced pressure at 50° to complete dryness. Dried residue was dissolved in physiological saline, or in case of difficult dissolution, few drops of Tween 80 were added to make homogenious suspension for oral administration.

Acute Toxicity

All mice were divided into tests and control group. Each group consisted of 5 mice. Male ICR mouse, 20±2g, of body weight, each received orally one of doses of 600mg/kg, 400 mg/kg, and 200mg/kg of plant extracts daily for 4 days. Mice in control group received each orally 0.2ml of physiological saline daily for 4 days. After two days of lag period (days on 5 and 6), on day 7, body weight changes between the control and test group were measured. Significant acute toxicity could resulted from severe loss of body weight (larger than 30%).

Effect on DNA Biosynthesis in Bone Marrow Cells

The incorporation rate of ^{5}H-thymidine into acid insoluble portion in bone marrow cells

Table I. Dose-schedule.

	Days						
	1	2	3	4	5	6	7
Control	0.9% Physiological saline (0.2ml/mouse/day)						Ⅰ) Weight changes measurement Ⅱ) Hb number
600, 400, 200(mg/kg)	Total methanol-water extracts (0.2ml/mouse/day)						Ⅲ) DNA synthesis in bone marrow cells *in vivo*

* Each group consists of five mice.
* All extracts were orally administered.
* DNA synthesis in bone marrow cells *in vivo*.
1) ^{3}H-Thymidine (10μci/mouse) was i.p. injected and pulse-labeled for 15 min.
2) Protein determination (Lowry method)
* Body weight change: (Average T-Average C) g on day 7.

Table II. Acute toxicity and effects on DNA biosynthesis in born marrow cells and hemoglobin content in blood.

Plant name (Family name) Part for use	Dose (mg/kg)	Schedule	Body weight change on day 7 (T-C)g	Hb number control	(g/100ml) Test	C.P.M./5mg control	Protein in test	Bone marrow T/C (%)
Daphne genkwa	600	$QD_1 \times 4$	−1.30	20.6±1.1	20.0±1.9	1,455	898	62
(Thymelaceae)	400		−0.18		20.0±1.3		1,229	84
Flos(芫花)	200		1.05		20.1±0.9		1,309	90
Strychnos ignatii	600	$QD_1 \times 4$	−4.98	20.6±1.1	19.7±1.3	1,455	1,391	96
(Loganiaceae)	400		−4.78		19.9±1.5		1,591	109
semen(寶豆)	200		−4.57		19.9±1.1		1,842	127
Paeonia albiflora var. typica	600	$QD_1 \times 4$	−6.50	20.6±1.1	19.0±1.0	1,455	602	41
(Paeoniaceae)	400		−6.05		19.3±1.0		695	48
Radix(赤芍藥)	200		−4.28		19.6±0.6		1,334	92
Scutellaria baicalensis	600	$QD_1 \times 4$	−6.00	20.6±1.1	19.2±1.4	1,455	3,458	238
(Labiatae)	400		−5.68		19.3±1.1		2,974	204
Radix(黃芩)	200		−4.03		19.6±0.5		2,188	150
Pharbitidis nil	600	$QD_1 \times 4$	−8.55	20.6±1.1	19.0±0.4	1,455	911	63
(Convolvulaceae)	400		−5.05		19.3±0.5		1,306	90
Semen(索牛子)	200		−1.67		20.1±0.6		1,482	102
Oemphalia lapidescens	600	$QD_1 \times 4$	−5.39	20.6±1.1	19.6±0.2	1,455	537	37
(Agaricaceae)	400		−3.09		20.1±0.6		757	52
Sclerotium(雷丸)	200		−1.02		20.4±0.3		1,070	74
Akebia quinata	600	$QD_1 \times 4$	−7.59	20.6±1.1	18.0±0.4	1,455	1,021	?
(Lardizabalaceae)	400		3.05		19.8±0.3		1,937	1,933
Liguum(木通)	200		1.41		19.2±0.6		1,804	124
Glycyrrhiza uralensis	600	$QD_1 \times 4$	−6.13	20.6±1.1	18.9±1.5	1.455	2,685	185
(Lequminosae)	400		−5.10		18.9±0.4		3,282	226
Radix(甘草)	200		−2.85		18.9±0.8		3,582	246
Plantago asiatica	600	$QD_1 \times 4$	1.67	20.6±1.1	20.2±0.6	1,455	485	33
(Plantaginaceae)	400		1.57		20.1±0.6		723	50
Semen(車前子)	200		1.63		20.1±0.4		879	60
Atractylodes japonica	600	$QD_1 \times 4$	4.03	20.6±1.1	20.1±0.5	1,455	2,363	162
(Compositae)	400		3.47		19.8±0.9		2,141	147
Rhizoma(蒼朮)	200		2.85		19.6±0.5		1,880	129
Asiasarum sieboldii	600	$QD_1 \times 4$	−2.74	20.7±0.7	19.0±0.5	1,390	1,326	95
(Aristolochiaceae)	400		−2.61		19.1±0.8		1,325	95
Radix(細辛)	200		0.09		19.2±0.1		1,162	84

Table II. (*Continued*)

Plant Name (Family name) part for use	Dose (mg/kg)	Schedule	Body weight change on day 7 (T-C)g	Hb number control	(g/100ml) Test	C.P. M./ 5mg control	Protein in test	Bone marrow T/C (%)
Rehmannia glutinosa	600	$QD_1\times4$	0.46	20.7±0.7	20.8±0.5	1,390	748	54
(Scrophulariaceae)	400		0.42		20.7±0.2		919	66
Rhizoma(熟地黃)	200		−0.06		20.4±0.2		975	70
Liriope muscari	600	$QD_1\times4$	−0.08	20.7±0.7	20.6±0.4	1,390	1,536	111
(Liliaceae)	400		−0.45		20.4±0.6		1,406	101
Tuber(麥門冬)	200		−0.53		20.2±0.6		1,331	96
Coptis japonica	600	$QD_1\times4$	0.42	20.7±0.7	20.7±0.8	1,390	1,878	135
(Ranunculaceae)	400		0.39		20.5±0.6		1,629	117
Rhizoma(黃蓮)	200		0.17		20.4±0.4		1,220	88
Euphorbia pekinensis	600	$QD_1\times4$	0.54	20.7±0.7	21.0±0.5	1,390	1,538	111
(Euphorbiaceae)	400		0.02		20.6±0.5		1,474	106
Semen(續隨子)	200		−0.87		20.5±0.3		1,279	92

was employed for the measure of potential toxicity in bone marrow cells, which was caused by administrating plant extracts. Details in method were reported previously elsewhere.[6]

Briefly, after decapitation of experimental mouse, bone marrow cells were collect by aspirating physiological saline through femoral bone. On day 7, ^{3}H-thymidine, 10 μ Ci/mouse (Sp. Act. 47 Ci/mmole) was i.p. injected and pulse-labeling was continued for 15 min.

The bone marrow cells were washed with ice cold 5% TCA fourtimes and with 95% ethyl alcohol one time. This acid insoluble portion was completly dissolved in tissue solublizer, Protosol (New England Nuclear, MA. U.S.A.), then it was thoroughly mixed with scintillation fluid. Radioactivities were measured in the scintillation spectormeter, Nuclear Enterpirze, Model NE 8310. Protein content in bone marrow cells was measured by the method of Lowry.[7]

Hemoglobin Content

Blood was collected from jugular vein, and hemoglobin content was measured by hemoglobinometer (Super. & Gim, Co.).

Results and Discussion

Among 15 species of medicinal plants tested, 7 plant extracts reduced body weight markedly. The extracts causing more than 30% loss of body weight compared with that of average control mouse body weight appeared to be *Strychnos ignatii* (600mg, 400mg, and 200mg/kg), *Paeonia albiflora* (600mg, 400mg, 200mg/kg), *Scutellaria baicalensis* (600mg, 400mg, and 200mg/kg), *Pharbitidis nil* (600mg, and 400mg/kg), *Oemphalia lapidescens* (600mg/kg) and *Glycyrrhiza uralensis* (600mg, 400mg/kg), whereas the extract of *Atractlylodes japonica* (600mg/kg) exhibited gaining of body weght more than 30% compared with that of control group.

As the data shown in Table 2, the effect on DNA biosynthesis in bone marrow cells appeared to be two different ways of the incorporation of ^{3}H-thymidine into DNA: The one was

inhibited and the other was enhanced by the plants extracts. The enhancement of ^{3}H-thymidine incorporation into DNA along with marked body weight loss was considered as potential toxicity by administration of plant extracts.

Because the disturbance of deoxynuclotide precursors' pool may cause such abnormally enhanced incoration rate of ^{3}H-thymidine.

In addition, most plant extracts possessing acute toxicity owing to severe weight loss together with inhition or enhancement of DNA synthesis in bone marrow cell showed also reduced hemoglobin contents in this experiment. The plant extracts exhibiting potent inhibitory effect on the incorporation rate of ^{3}H-thymidine into DNA in bone marrow cells appeared to be; *Daphne genkwa* (600mg/kg), *Paeonia albiflora* (600mg, and 400mg/kg), *Pharbitidis nil* (600mg/kg), *Oemphalia lapidescens* (600mg and 400mg/kg) *Plantago asiatica* (600mg, 400mg, and 200mg/kg), and *Rehmannia glutinosa* (600mg, 400mg/kg), whereas the plant extracts, *Scutellaria balcalensis* (600mg, 400mg and 200mg/kg), *Akebia quinata* (400mg /kg), *Glycyrrhiza uralensis* (600mg, 400mg and 200mg/kg), *Atractylodes japonica* (600mg and 400mg/kg) and *Coptis japonica* (600mg/kg) appeared to enhance the incorporation rate of ^{3}H-thymidine into DNA biosynthesis in bone marrow cells.

Most of these plant extracts possessing either inhibitory or enhancing activities of DNA biosyntheses in bone marrow cells exhibited reduced hemoglobin content in blood shown in Table 2.

Interestingly, one plant extract, *Atractylodes japonica* exhibited gaining of body weight compared with that of control group and enhancing DNA biosynthesis in bone marrow cells along with no significant variation of hemoglobin content in blood. So it may be of worthwhile to investigate further with this plant extract whether it resulted from tonic action as described in "Dong Ee Bo Gam" with respect to the pharmacological efficacy in the field of the oriental medicine.

Without significant weight loss under the dose-schedule employed, the following plant extracts, *Plantago asiatica*, *Rehmannia glutinosa*, and *Coptis japonica* appeared to exhibit some degree of inhibitory or enhancing activities of DNA biosyntheses in bone marrow cells. However, it is uncertain whether these plant may possess some cummulative toxicity or chronic toxicity, although they were specified as slight toxic plant extracts in this experiment.

In general, most of tonic herbal drugs as decoction form have been orally administer longer than two weeks according to the oriental medicinal drug regimen. With this connection, instead of administrating relatively low dose of extract during long duration (longer than two weeks as did for human patients), relatively high doses, if they are converted to human dose, were employed throughout the experiment.

In summary, six plants among 15 plants tested appeared to exhibit acute toxicity along with bone marrow depression or abnormally enhancing the ^{3}H-thymidine incorporation into DNA biosynthesis. Regarding the results, although present study appeared to be a preliminary toxicity screening, more systemic toxicity studies are desirable for safety assessment of the herbal drugs for human use.

Acknowledgement: Authors are grateful to Dr. Hyung-Joon Chi who identified kindly our medicinal plants samples.

〈Received April 22, 1982〉

References

1. Huh Joon: "Dong-Ee-Bo-Gam" published in 1613 A.D. in Yi dynasty.
2. Chang, I.M. and Chi H.J.: *Kor. J. Pharmacog.*, **12**, 125 (1981)
3. Chang, I.M. and Yun(Choi) H.S.: *Kor. J. Pharmacog.*, **10**, 79 (1979)
4. Yun(Choi) H.S. and Chang I.M.: *Kor. J. Pharmacog.*, **11**, 41 (1980)
5. Woo, W.S., Lee, E.B. and Chang I.M.: *J. Pharm. Soc. Korea.*, **21**, 37 (1977)
6. Bear J.L., Gray Jr. H.B., Rainen L., Chang, I.M., Howard R., Serio G. and Kimball A.P.: *Cancer Chemother. Rept.*, (part 1) **59**, 611 (1975)
7. Lowry O.H., Rosebrough N.J. Farr A.L. and Randall R.J.: *J. Biol. Chem.*, **1193**, 265 (1951)