A Survey of the Response of Medicinal Plants on Drug Metabolism

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Introduction

It is well known that drug metabolizing enzyme activities of the liver may be affected by the treatment of the animals with a variety of environmetal factors such as drugs, insecticides, polycyclichydrocarbons and food additives[1]) as well as naturally occuring compounds[2]). Therefore, inducers and inhibitors of drug metabolizing enzymes may be expected to cause marked changes in the pharmacological and toxicological activity of drugs.

Although modern medicine of today, when medicinal science and chemical science are highly developed, is using many synthetic drugs, a number of crude plant materials used by the ancients are still employed alone or in combination as drugs in much the same manner or sometimes in combination with modern synthetic drugs by today's medicinal practitioners especially in oriental countries.

Recently, in this Institute, a survey was initiated to find out whether the constituents of widely employed medicinal plants affect the activity of drug metabolizing enzymes, thereby modifying the intensities of the therapeutic or toxicologic responses of other drugs.[2,3]).

As a result at least 29% of the plant materials tested were suggested to affect the drug metabolism.

Result and Discussion

Effect of plant extracts on barbiturate-induced hypnosis and strychnine mortality: Table I shows the screening result of one hundred and forty one plant matrials belonging to 127 genera and 62 families which have been most frequently prescribed in chinese medicine. In the animal experiment male mice were used. As a test standard drug, hexobarbital was used since the duration of its action in the body is known to be regulated largely by the levels of liver microsomal drug metabolizing enzymes,[4]) that oxidize and inactivate it and since most of the known inhibitors and inducers alter the duration of its action. And the duration of hypnotic response of hexobarbital has been used as an index of the rate of drug metabolism.

In the screening test for enzyme inhibitors, mice were pretreated with a single intraperitoneal injection of 500mg/kg of methanol extracts suspended in 0.5% CMC. When the extracts were toxic the dose was reduced. Thirty minutes after the pretreatment of the extract, 50mg/kg of hexobarbital sodium was injected intraperitoneally and then the duration of sleep induced by hexobarbital was measured.

In the screening test for inducers, mice were given the extracts once a day for three days and forty eight hours after the last dose of the materials, 100mg/kg of hexobarbital

Table I. Effects of botanical drugs on hexobarbital sleeping time and strychnine mortality in mice.

Plant name	Plant[a] part	Prolongation			Shortening			Strychnine[c] mortality (%)
		Dose (mg/kg)	Percent of control	Signif.	Dose (mg/kg)	Percent of control	Signif.	
Alismataceae								
Alisma Plantago var. *parviflorum*	rz	500	108.6	N.S.	500	116.0	N.S.	
Amaranthaceae								
Achyranthes japonica	rt	500	213.5 161.0	P<0.001 P<0.05[b]	500	86.4	N.S.	90.0
Anacardiaceae								
Rhus javanica	ga	15	168.4 141.4	P<0.01 P<0.01	15	119.7	N.S.	100.0
Apocynaceae								
Nerium indicum	lf	60	121.6	N.S.	60	124.0	N.S.	
Araceae								
Acorus gramineus	rz	500 250	455.5 255.8	P<0.01 P<0.001	500	107.0	N.S.	100.0
Arisaema amurense var. *serratum*	rz	500	123.8	N.S.	500	84.9	N.S.	
Pinellia ternata	tb	500	88.0	N.S.	50	101.9	N.S.	
Araliaceae								
Acanthopanax spinosus	rt-bk	50	113.6	N.S.	50	124.0	N.S.	
Aralia continentalis	rt	500	100.0	N.S.	500	121.6	N.S.	
Kalopanax pictum	bk	125	101.0	N.S.	125	113.7	N.S.	
Panax ginseng	rt	500	106.8	N.S.	500	81.8	N.S.	
Tetrapanax papyriferum	sm	500	114.0	N.S.	500	114.5	N.S.	
Aristolochiaceae								
Asiasarum heterotropoides var. seoulensis	wp	500	133.1	N.S.	500	94.5	N.S.	
Asclepiadaceae								
Cynanchum wilfordii	rt	500	94.8	N.S.	500	94.6	N.S.	
Aspidaceae								
Dryopteris crassirhizoma	rz	250	162.3 158.3	P<0.02 P<0.01	250	90.3	N.S.	80.0
Campanulaceae								
Adenophora remotiflora	rt	125	95.2	N.S.	60	124.5	N.S.	
Platycodon grandiflorum	rt	125	118.1	N.S.	125	81.3	N.S.	
Caprifoliaceae								
Lonicera japonica	fl	500	93.0	N.S.	500	110.1	N.S.	
Caryophyllaceae								
Dianthus chinensis	wp	250	83.6	N.S.	250	179.9 139.0	P<0.01 P<0.01	
Gypsophyla oldhamiana	rt	25	139.3	N.S.	25	101.6	N.S.	
Melandrium firmum	wp	125	112.4	N.S.	125	169.6 164.9	P<0.05 P<0.01	
Combretaceae								
Terminalia chebula	fr	62.5	89.1	N.S.	62.5	120.2	N.S.	
Compositae								
Arctium lappa	sd	500	99.3	N.S.	500	117.6	N.S.	
Artemisia vulgaris var. *indica*	lf	125	125.6	N.S.	125	96.2	N.S.	

Plant name	Plant[a] part	Prolongation			Shortening			Strychnine[c] mortality (%)
		Dose (mg/kg)	Percent of control	Signif.	Dose (mg/kg)	Percent of control	Signif.	
Aster tartaricus	rt	125	92.0	N.S.	62.5	103.9	N.S.	
Atractylodes japonica	rz	500	169.9 169.0	P<0.01 P<0.001	500	116.3	N.S.	70.0
Carthamus tinctorius	fl	500	107.1	N.S.	500	98.6	N.S.	
Chrysanthemum indicum	fl	500	104.1	N.S.	500	101.6	N.S.	
Echinops latifolius	rt	500	121.5	N.S.	500	163.2 155.0	P<0.02 P<0.01	
Echinops setifer	wp	250	122.3	N.S.	250	111.5	N.S.	
Inula helenium	rt	500	120.0	N.S.	500	87.2	N.S.	
Siegesbeckia pubescens	wp	125	115.0	N.S.	125	186.4 129.4	P<0.02 P<0.05	
Taraxacum platycarpum	wp	500	100.8	N.S.	500	83.8	N.S.	
Convolvulaceae								
Cuscuta japonica	sd	125	87.5	N.S.	125	121.2	N.S.	
Pharbitis Nil	sd	5	103.0	N.S.	1	130.3	N.S.	
Cornaceae								
Cornus officinalis	fr	500	153.9 213.1	P<0.01 P<0.001	500	116.4	N.S.	40.0
Cruciferae								
Brassica alba	sd	500	117.5	N.S.	500	89.4	N.S.	
Raphanus sativus	sd	500	98.4	N.S.	500	101.9	N.S.	
Cucurbitaceae								
Tricosanthes kirilowii	sd	250	112.8	N.S.	250	118.3	N.S.	
Cupressaceae								
Biota orientalis	sd	500	82.9	N.S.	500	96.4	N.S.	
Cyperaceae								
Cyperus rotundus	rz	500	84.7	N.S.	500	83.2	N.S.	
Scripus maritimus	tb	250	114.3	N.S.	250	127.5	N.S.	
Ephedraceae								
Ephedra sinica	wp	250	119.4	N.S.	250	110.0	N.S.	
Equisetaceae								
Equisetum hiemale var. *japonicum*	wp	500	126.8	N.S.	500	93.0	N.S.	
Eucommiaceae								
Eucommia ulmoides	bk	500	96.1	N.S.	500	91.4	N.S.	
Euphorbiaceae								
Croton tiglium	sd	1	118.0	N.S.	1	107.0	N.S.	
Flacourtiaceae								
Hydnocarpus sp.	sd	500	140.4 215.9	P<0.05 P<0.001	500	107.6	N.S.	90.0
Gentianaceae								
Gentiana scabra	rt	250	120.0	N.S.	250	103.3	N.S.	
Gramineae								
Coix Lachryma-jobi	sd	250	102.1	N.S.	125	120.5	N.S.	
Phyllostachys reticulata	wd	500	122.3	N.S.	500	111.5	N.S.	
Iridaceae								
Belamcanda chinensis	rz	500	184.9 233.0	P<0.001 P<0.01	500	121.6	N.S.	100.0

Plant name	Plant[a] part	Prolongation			Shortening			Strychnine[c] mortality (%)
		Dose (mg/kg)	Percent of control	Signif.	Dose (mg/kg)	Percent of control	Signif.	
Labiatae								
Anisomeles indica	lf	500	117.5	N.S.	250	79.8	N.S.	
Elsholtzia patrini	wp	500	113.5	N.S.	500	107.4	N.S.	
Elsholtzia splendeus	wp	500	123.4	N.S.	500	117.2	N.S.	
Leonurus sibiricus	wp	500	122.2	N.S.	500	121.8	N.S.	
Mentha arvensis var. *piperasens*	lf	500	107.4	N.S.	500	103.9	N.S.	
Nepeta japonica	wp	500	220.8	P<0.01	500	122.6	N.S.	90.0
			188.2	P<0.01				
Perrila nankinensis	lf	125	459.1	P<0.001	125	120.0	N.S.	100.0
			278.7	P<0.01				
Phlomis umbrosa	rt	500	117.1	N.S.	500	116.8	N.S.	
Prunella vulgaris	wp	500	103.6	N.S.	500	120.2	N.S.	
Scutellaria baicalensis	rt	500	144.0	P<0.02	500	115.5	N.S.	100.0
			174.8	P<0.01				
Lardizabalaceae								
Akebia quinata	lg	500	102.0	N.S.	250	119.2	N.S.	
Lauraceae								
Cinnamomum cassia	bk	500	122.3	N.S.	500	120.4	N.S.	
Lindera strychnifolia	rt	500	407.3	P<0.01	500	93.9	N.S.	100.0
			423.3	P<0.001				
Leguminosae								
Albizzia julibrissin	bk	250	124.7	N.S.	250	115.3	N.S.	
Astragalus membranaceus	rt	500	107.8	N.S.	500	116.1	N.S.	
Cassia occidentalis	sd	500	218.2	P<0.01	125	115.1	N.S.	90.0
			230.1	P<0.01				
Dolichos Lablab	sd	500	113.3	N.S.	500	83.7	N.S.	
Glycyrrhiza uralensis	rt	500	247.4	P<0.001	500	73.9	P<0.05	80.0
			166.7	P<0.01		75.0	P<0.05	
Pueraria Thunbergii	fr	500	95.0	N.S.	125	78.2	N.S.	
Sophora japonica	fl	250	93.0	N.S.	250	103.3	N.S.	
Liliaceae								
Anemarrhena asphodeloides	rz	125	117.9	N.S.	125	90.5	N.S.	
Asparagus lucidus	rt	500	88.9	N.S.	500	107.3	N.S.	
Fritillaria verticillata var. *thunbergii*	rz	500	120.8	N.S.	125	82.8	N.S.	
Liriope platyphylla	rz	500	91.5	N.S.	500	124.1	N.S.	
Polygonatum japonicum	rz	500	84.3	N.S.	500	117.7	N.S.	
Smilax china	rz	500	118.5	N.S.	50	192.9	P<0.001	
						165.0	P<0.05	
Loganiaceae								
Strychnos ignatii	sd	500	164.4	P<0.001	500	103.0	N.S.	90.0
			151.0	P<0.05				
Magnoliaceae								
Magnolia obovata	bk	250	102.3	N.S.	250	209.8	P<0.01	
						164.3	P<0.001	
Schizandra chinensis	fr	500	175.7	P<0.05	500	101.3	N.S.	80.0
			222.7	P<0.001				
Menispermaceae								
Sinomenium acutum	rt	60	88.0	N.S.	60	99.3	N.S.	

Plant name	Plant[a] part	Prolongation			Shortening			Strychnine[c] mortality (%)
		Dose (mg/kg)	Percent of control	Signif.	Dose (mg/kg)	Percent of control	Signif.	
Moraceae								
Morus bombycis	rt-bk	500	104.8	N.S.	500	89.8	N.S.	
Myristicaceae								
Myristica fragrans	sd	125	208.9 265.4	P<0.01 P<0.001	125	81.2	N.S.	80.0
Myrtaceae								
Eugenia caryophyllata	fl	125	96.0	N.S.	125	119.5	N.S.	
Nymphaceae								
Euryale ferox	sd	500	107.8	N.S.	500	128.3	N.S.	
Oleaceae								
Forsythia viridissima	fr	500	88.7	N.S.	500	114.6	N.S.	
Orchidaceae								
Dendrobium officinale	wp	500	168.2 221.9	P<0.05 P<0.02	230	77.5	N.S.	70.0
Gastrodia elata	rz	500	108.3	N.S.	125	109.7	N.S.	
Palmae								
Areca catechu	sd	500	101.6	N.S.	250	116.1	N.S.	
Papaveraceae								
Cordalis ternata	tb	500	101.1	N.S.	250	80.2	N.S.	
Piperaceae								
Piper nigrum	fr	50	235.1 253.0	P<0.001 P<0.001	125	69.1 52.0	P<0.05 P<0.01	0.0
Piper retrofractum	fr	125	287.2 302.5	P<0.01 P<0.01	125	55.2 46.2	P<0.01 P<0.01	10.0
Polygonaceae								
Polygala tenuifolia	rt	25	156.6 168.1	P<0.05 P<0.05	5	77.0	N.S.	80.0
Polygonum cuspidatum	rz	500	110.5	N.S.	500	113.7	N.S.	
Rheum undulatum	rz	500	92.0	N.S.	500	85.0	N.S.	
Ranunculaceae								
Aconitum ciliare	rt	125	168.7 168.0	P<0.001 P<0.02	125	92.7	N.S.	80.0
Cimicifuga heracleifolia	rz	500	154.5 217.7	P<0.02 P<0.01	500	108.1	N.S.	90.0
Clematis manshurica	rt	500	121.6	N.S.	500	112.9	N.S.	
Lycoctonum pseudolaeve	rt	125	95.6	N.S.	100	116.7	N.S.	
Paeonia albiflora	rt	500	100.2	N.S.	500	116.5	N.S.	
Paeonia moutan	rt-bk	500	103.6	N.S.	250	119.8	N.S.	
Paeonia ovata	rt	500	102.5	N.S.	500	104.6	N.S.	
Rhamnaceae								
Zizyphus vulgaris var. *spinosus*	sd	500	116.5	N.S.	500	116.4	N.S.	
Rosaceae								
Chaenomeles sinensis	fr	500	111.3	N.S.	500	101.4	N.S.	
Crataegus pinatifida	fr	500	110.6	N.S.	500	120.2	N.S.	
Prunus ansu	sd	250	113.1	N.S.	250	110.2	N.S.	
Prunus mume	fr	500	192.3 130.6	P<0.001 P<0.01	500	86.4	N.S.	80.0

Plant name	Plant[a] part	Prolongation			Shortening			Strychnine[c] mortality (%)
		Dose (mg/kg)	Percent of control	Signif.	Dose (mg/kg)	Percent of control	Signif.	
Prunus persica	sd	500	93.8	N.S.	500	110.6	N.S.	
Rubus coreanus	fr	500	119.2	N.S.	500	97.0	N.S.	
Sanguisorba officinalis	rt	500	108.3	N.S.	125	127.5	N.S.	
Rubiaceae								
Gardenia jasminoides	fr	500	109.9	N.S.	500	96.5	N.S.	
Rubia akane	rt	500	190.1	P<0.01	500	129.5	N.S.	100.0
			216.2	P<0.001				
Rutaceae								
Citrus aurantium	pc	500	288.6	P<0.001	250	108.3	N.S.	90.0
			143.6	P<0.01				
Citrus unshiu	pc	500	104.5	N.S.	500	106.9	N.S.	
Evodia rutaecarpa	fl	500	86.8	N.S.	500	114.7	N.S.	
Phellodendron amurense	bk	125	98.0	N.S.	125	123.0	N.S.	
Poncirus trifoliata	fr	500	562.9	P<0.001	500	75.9	P<0.05	100.0
			418.0	P<0.01		70.9	P<0.02	
Sapindaceae								
Euphoria longana	al	500	120.8	N.S.	500	112.8	N.S.	
Scrophulariaceae								
Picrorrhiza kurroa	rz	500	107.3	N.S.	500	90.7	N.S.	
Rehmannia glutinosa	rt	500	84.9	N.S.	500	82.1	N.S.	
Solanaceae								
Lycium chinense	fr	500	107.4	N.S.	500	99.0	N.S.	
Lycium chinense	rt-bk	500	121.6	N.S.	500	91.4	N.S.	
Stemonaceae								
Stemona japonica	rt	250	124.7	N.S.	250	115.5	N.S.	
Taxaceae								
Torreya nucifera	sd	250	131.5	N.S.	250	122.2	N.S.	
Typhaceae								
Typha orientalis	fl	500	102.5	N.S.	500	115.3	N.S.	
Umbelliferae								
Angelica dahurica	rt	500	514.9	P<0.0001	500	48.3	P<0.001	100.0
			487.6	P<0.001		55.9	P<0.001	
Angelica gigas	rt	500	516.7	P<0.0001	500	58.4	P<0.05	100.0
			517.9	P<0.0001		59.8	P<0.05	
Angelica koreana	rt	500	649.4	P<0.0001	500	53.4	P<0.001	100.0
			518.0	P<0.0001		63.0	P<0.01	
Angelica tenuissima	rt	500	135.6	N.S.	500	117.2	N.S.	
Anthriscus sylvestris	rt	500	119.3	N.S.	500	119.1	N.S.	
Bupleurum falcatum	rt	500	122.9	N.S.	250	120.2	N.S.	
Cnidium officinale	rz	500	117.2	N.S.	500	91.2	N.S.	
Siler divaricatum	rt	500	104.8	N.S.	500	88.7	N.S.	
Valerianaceae								
Patrinia scabiosaefolia	rt	125	159.0	P<0.01	125	344.2	P<0.001	80.0
			186.0	P<0.01		230.9	P<0.001	
Verbenaceae								
Vitex rotundifolia	fr	500	311.0	P<0.001	500	108.5	N.S.	90.0
			227.9	P<0.01				

Plant name	Plant[a] part	Prolongation			Shortening			Strychnine[c] mortality (%)
		Dose (mg/kg)	Percent of control	Signif.	Dose (mg/kg)	Percent of control	Signif.	
Zingiberaceae								
Amomum xanthioides	sd	500	184.5 218.5	P<0.001 P<0.01	250	118.8	N.S.	100.0
Curcuma aromatica	rz	500	226.8 211.8	P<0.001 P<0.02	500	100.0	N.S.	100.0
Curcuma zedoaria	rz	250	370.6 308.8	P<0.001 P<0.01	250	80.5 61.7	P<0.05 P<0.05	100.0
Zingiber nigrum	sd	500	119.2	N.S.	500	119.5	N.S.	
Zingiber officinale	rz	500	96.8	N.S.	500	103.2	N.S.	
Zygophyllaceae								
Tribulus terrestris	fr	500	117.8	N.S.	250	112.6	N.S.	

a) al, aril; bk, bark; fl, flower; fr, fruit; lf, leaf; pc, pericarpium; rt, root; rz, rhizome; sd, seed; sm, stem; tb, tuber; wd, wood; wp, whole plant; rt-bk, root-bark; ga, gall.
b) Retested value
c) Mortality in untreated control mice was 50%.

sodium was injected and then the sleeping time was measured. The plants which gave positive results were newly recollected and retested for confirmation of the activity.

For the extracts which were positive in screening test for inhibitors the strychnine mortality test was again carried out, because prolonging effects on sleeping time induced by hexobarbital do not necessarily verify their enzyme inhibitory action.

Some prolonging effects might result from the simple potentiation action of a depressant without altering the rate of hexobarbital oxidation. Inhibitors of drug metabolizing enzyme may cause an increase not only in the activity of depressant, hexobarbital but also in the activity of stimulant, strychnine. In the strychnine mortality test 1.2mg/kg of strychnine nitrate was injected 30 min after pretreatment with the extracts and the number of the animals dying within 30 min was recorded. At this dose, strychnine nitrate caused tonic convulsion and 50% mortality.

The present screening result showed that some plant extracts possessed prolonging or shortening effects on barbiturate induced hypnosis. From the result, medicinal plants used in chinese medicine are classified into four categories:

1) The plants prolonging the action of hexobarbital

2) The plants both prolonging and shortening the action of hexobarbital

3) The plants not affecting the action of hexobarbital

4) The others (the plants causing liver damage)

The plants prolonging the action of hexobarbital: Twenty-six species gave the significant prolongation of barbiturate-induced hypnosis without showing shortening effect in the induction screening test. Most plants (except one species) showed an increase in strychnine mortality by a single administration.

Therefore these plants were suggested to contain drug metabolizing enzyme inhibitors. As a matter of fact, fractionation of *Acori graminei* Rhizoma resulted in isolation of asarone.[5] *Corni* Fructus which was ineffective in strychnine mortality test, are suggested that

the activity of the extract is independent of the drug metabolizing enzyme system.

The plants both prolonging and shortening the action of hexobarbital: The plants belonging to this category were eight species. It is well known that a variety of compounds produce biphasic alterations in the metabolism of drugs. They block the oxidation of drugs during the first phase and stimulate the oxidation during the second phase.[6] For example, many inhibitors of the drug metabolism such as SKF-525A enhance the activity of the microsomal drug metabolizing enzymes 48 hours after the administration: on the other hand, many inducers such as glutethimide inhibit enzyme activity within six hours after the administration.[6,7]

Therefore the plants belonging to this category may be expected to cause marked changes in pharmacological and toxicological action of drugs. Among these plants, *Angelica koreana* was investigated for active principles. We succeeded in isolating imperatorin, isoimperatorin, oxypeucedanin and prangolarin as active

Table II. Effects of natural coumarins and the related compounds (30mg/kg, ip) on the hexobarbital-induced sleeping time in mice

Compounds	Prolongation		Shortening	
	Percent of control	Signif.	Percent of control	Signif.
Reference compounds				
SKF-525 A	750.0	$P < 0.001$	45.2*	$P < 0.01$
Phenobarbital	127.4	N.S.	35.2	$P < 0.02$
Simple coumarins				
Coumarin	108.3	N.S.	100.0	N.S.
Scopoletin	100.0	N.S.	111.4	N.S.
Scopolin	93.5	N.S.	104.0	N.S.
Aesculin	115.3	N.S.	122.7	N.S.
Osthol	125.8	N.S.	92.8	N.S.
Glabra-lactone	93.9	N.S.	126.3	N.S.
Furanocoumarins				
Imperatorin	1013.0	$P < 0.001$	63.4*	$P < 0.001$
Isoimperatorin	982.0	$P < 0.001$	56.8*	$P < 0.01$
Bergapten	898.0	$P < 0.001$	63.6*	$P < 0.05$
Oxypeucedanin	884.0	$P < 0.001$	83.0*	$P < 0.05$
Oxypeucedanin hydrate	516.0	$P < 0.001$	80.3*	$P < 0.05$
Prangolarin	352.0	$P < 0.001$	77.6*	$0.05 < P < 0.1$
Nodakenin	80.8	N.S.	81.6	N.S.
Nodakenetin	105.0	N.S.	92.8	N.S.
Pyranocoumarins				
Khellactone	88.5	N.S.	94.1	N.S.
Decursinol	119.3	N.S.	126.5	N.S.
Decursin	116.0	N.S.	123.8	N.S.
Related compounds				
p-Coumaric acid	88.0	N.S.	93.8	N.S.
Ferulic acid	117.0	N.S.	101.6	N.S.

* An asterisk indicates a single administration.

components.[8]

Effects of naturally occuring coumarins on drug metabolism: Coumarins are widely distributed plant products, and numerous pharmacological and physiological activities of medicinal plants such as anticoagulant activity, photosensitizing activity have been attributed to the coumarins.[9]

Some previous reports on drug metabolizing enzyme inducing effect of anticoagulant coumarins[10-12] and our finding led us to investigate the effect on activity of drug metabolizing enzymes of the naturally occurring coumarins and the related compounds.

Table II shows prolonging and shortening effects of some naturally occuring coumarins on hexobarbital induced sleeping time. Experimental conditions were the same as mentioned before. An asterisk indicates a single injection. In this experiment SKF-525A, one of potent enzyme inhibitor,[13] and phenobarbital, one of potent enzyme inducers,[6] were used as positive control substances.

As expected SKF-525A prolonged the action of hexobarbital during the first phase and shortened the action of the drug during the second phase. In contrast, phenobarbital did not affect the drug action during the first phase but markedly shortened the action of hexobarbital during the second phase.

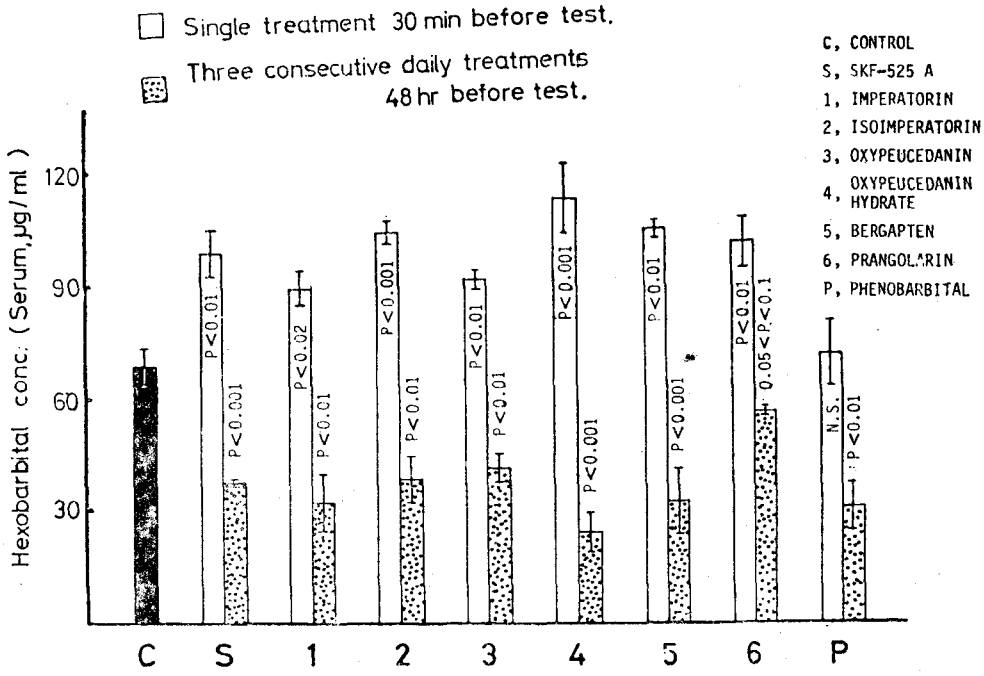

Fig. 1. Effects of SKF-525A, phenobarbital and furanocoumarins on metabolism of hexobarbital in mice

Serum hexobarbital concentration was measured 30 min after injection of 100mg/kg of hexobarbital sodium

Dose of sample pretreatment was 30 mg/kg (i.p.)

Among 19 compounds tested, only furanocoumarins such as imperatorin, isoimperatorin, oxypeucedanin. oxypeucedanin hydrate, bergapten and prangolarin altered the hexobarbital-induced sleeping time both during the first phase and during the second phase.

Other coumarins such as coumarins with acyclic substituents, pyranocoumarins, dihydrofuranocoumarins and related compounds such as p-coumaric acid, and ferulic acid were inactive at the dosage used.

In order to confirm these effects of SKF-525A, phenobarbital and the active six coumarins, the serum-level of hexobarbital in mice at given time were checked. The results are summarized in Fig. 1. In this experiment 100 mg/kg of hexobarbital was injected 30 minutes after a single pretreatment in the first phase test and 48 hours after a three consecutive daily pretreatment in the second phase test. 30 minutes after hexobarbital injection, animals were killed by a blow on the head and the serum level of hexobarbital was assayed according to the method described by Cooper and Brodie.[4]

It was demonstrated that SKF-525A caused a marked inhibition of hexobarbital metabolism during the first phase and an acceleration during the second phase. Phenobarbital, however, stimulated the barbiturate metabolism during the second phase only. The alterations of hexobarbital concentration in the animal groups treated with coumarins were very similar to those SKF-525A, indicating that these compounds may belong to an enzyme inhibitor rather than an enzyme inducer. From the results obtained, only furanocoumarins were demonstrated to be a strong drug metabolizing enzyme inhibitor and a double bond in furan ring seems to be essential for the manifestation of the activity, as the activity could not be observed in nodakenin and nodakenetin in which the furan ring is saturated.

Pharmacological properties of piperine, an active principle of *Piper* sp.: We observed in the initial screening test that extracts of two *Piper* sp. exhibited a prolongation in hexobarbital induced sleeping time but a marked reduction in strychnine mortality in the first phase test. This result strongly suggest that there should be some constituents in this plant which possess CNS-depressant property. Therefore, fractionation was carried out for active principles monitoring with prolongation of hexobarbital induced sleeping time and an active crystalline compound of mp 130° was isolated and identified as piperine by direct comparison with an authentic specimen.[14]

Piperine showed a strong potentiating effect on barbiturate-induced hypnosis (Table III) and

Table III. Influence of piperine on duration of action of hexobarbital in mice

Treatment	No. of mice	Dose (mg/kg)	Sleeping time (min) ±SE.	% increase
Control	6	—	27.0±3.1	
Piperine	6	100	181.3±3.4	571.5
Control	6	—	20.8±4.1	
Piperine	6	50	61.5±2.1	148.0
Control	6	—	18.2±1.2	
Piperine	6	30	34.8±3.1	91.7

Mice were administered with piperine 30 min prior to the administration of hexobarbital sodium (50mg/kg, i.p.).

Table IV. Effect of piperine on strychnine mortality in mice

Treatments	Dose (mg/kg, i.p.)	No. of died /No. of used
Exp. I[a]		
Control	—	6/10
Piperine	30	0/10
Exp. II[b]		
Control	—	8/10
Piperine	30	0/10

The dose of strychine nitrate was a) 1.2mg/kg and b) 1.3mg/kg.

a marked antagonism against strychnine mortality (Table IV).

Table V showed again that mice given 100 mg/kg of hexobarbital together with 30 mg/kg of piperine slept considerably longer than animals given the barbiturate alone.

However, there was no difference in the hexobarbital concentration in serum of mice 30 minutes after drug administration regardless of whether or not the animals had received piperine.

This indicates that the effect of piperine did not result from the inhibition of hexobarbital metabolism.

Table V. Effect of piperine on hexobarbital action and metabolism rate

Treatment	Dose (mg/kg, ip)	Hexobarbital (100mg/kg, ip)		
		Time post treatment	Sleeping time (min±S.E.)	Serum level[a] (μg/ml±S.E.)
Control	—	30 min	68.0±6.3(5)	64.1±4.5(10)
Piperine	30	〃	138.4±15.9(5)*	69.3±4.9 (8) (N.S.)
Control	—	48 hr	57.7±8.2(6)	66.9±5.4 (7)
Piperine	30×3 days	〃	54.3±5.8(6) (N.S.)	63.0±6.3 (8) (N.S.)

a) Hexobarbital concentration 30 min after the drug administration.
* $P<0.01$ vs. control, N.S.; Not significant

In the second phase experiment, piperine did not affect the hexobarbital induced sleeping time nor the rate of biotransformation of hexobarbital. Therefore the constituent in *Piper* sp. acting on drug metabolism is not piperine. The mode of potentiation induced by piperine is obviously different from that of SKF-525A and furanocoumarins. This difference was shown in another way. When SKF-525A was given intravenously to mice which had just recovered from hypnosis induced by hexobarbital, the animals were not visibly affected; but if piperine was given, the animals reverted almost immediately to a deep hypnosis.

This indicates that piperine caused subhypnotic amounts of hexobarbital to become hypnotic. Therefore piperine is a true potentiator, without activity altering drug metabolism. Hence, all evidences obtained suggest that other components may exist in the *Piper* sp. which are responsible for the enzyme inducing activity.

Neuropharmacological observations were performed on this compound for two hours by the screening procedures of Irwin[15] and Takagi, *et al.*[16] Table VI shows the results of general behavior evaluations in mice treated with piperine. In a dose of intraperitoneal 30 mg/kg of piperine, characteristic behavior of CNS depressant nature was observed throughout the period from 30 minutes to two hours after sample treatment: Strong decrease in motor activity; decrease in alertness, grooming, startle response, pinna and corneal reflex, rectal temperature (-1.3°C) and respiratory rate; increase in ptotic symptom.

Almost the same or rather stronger activity of above symptoms and besides increase in the symptom of passivity could be observed when piperine was administered orally in a dose of 100 mg/kg.

Contrary to our results, Singh, *et al.*[17] reported that piperine exhibited an analeptic activity, that is, increase in the ED_{50} (hypnotic activity response) and LD_{50} of pentobarbital sodium in mice.

Table VI. Central and autonomic nervous system activity of piperine in mice

Profiles	30mg/kg i.p.			100mg/kg p.o.		
	30	60	120	60	90	120 (min)
Ⅰ. Awereness						
1. Alertness	−2	−2	−2	−2	−2	−2
2. Visual placing						
3. Passivity				+4	+4	+4
4. Stereotypy						
5. Traction test						
Ⅱ. Mood						
1. Grooming	−2	−2	−4	−4	−4	−4
2. Vocalization						
3. Irritability						
Ⅲ. Motor activity						
1. Spontaneous movement	−1	−3	−3	−3	−3	−3
2. Touch response	−2	−2	−2	−4	−2	−2
3. Pain response (tail clip)	−2	−2	−2	−2	−2	−2
Ⅳ. CNS excitation						
1. Startle response	−2	−2	−2	−4	−4	−4
2. Straub's tail response						
3. Tremors						
4. Twitches						
5. Convulsions						
Ⅴ. Body posture						
Ⅵ. Motor incoordination						
1. Abnormal gait						
2. Righting reflex						
3. Paralysis (hind paw)						
Ⅶ. Muscle tone						
1. Grip tone						
2. Body tone						
Ⅷ. Reflex						
1. Pinna reflex	−4	−4	−2	−2	−2	−2
2. Corneal reflex				−2	−2	−2
Ⅸ. Autonomic profile						
1. Piloerection						
2. Body temperature (rectal)	−2	−2	−2	−2	−2	−2
3. Pupil size						
4. Palpebral opening	−2	−2	−2	−4	−4	−4
5. Exophthalmos						
6. Lacrimation						
7. Salivation						
8. Urination						
9. Fecal excretion						
10. Diarrhea						
11. Writhing						
12. Vomitting						
13. Respiratory rate	−2	−2	0			
14. Skin color						

In order to clarify such a discrepancy, further pharmacological study of piperine was carried out by the subcutaneous pentetrazole seizure threshold test, the maximal electroshock seizure test and rotating rod test (Table VII). In this experiment, male mice were used and all the samples suspended in 0.5% CMC solution were administered orally. The numerical values indicated are ED_{50}'s determined at the time when peak effect of test compounds spepeared.

Phenytoin, one of antiepileptics, and chlormezanone, one of muscle relaxant tranquilizers, were used as the reference drugs for comparison. Piperine showed a slight antielectroshock activity but a marked antichemoshock activity and caused muscular incoordination in mice. Phenytoin showed much more potent antielectroshock activity, whereas showed no antichemoshock. Chlormezanone showed more potent antipentetrazole activity compared with antielectroshock.

From this result, it is considered that piperine is not appropriate as an anticonvulsant, but expected to be used as a muscle relaxant. A detailed evaluation for its muscle relaxant activity is in progress.

Table VII. Protective evaluation of compounds in convulsion and rotarod test in mice

Compounds	Time post p.o. treatment	scPT ED_{50}* (mg/kg)	MES ED_{50}** (mg/kg)	PR ED_{50}*** (mg/kg)
Piperine	60 min	211.5(82.7—402.5)	>1300	89.1 (63.2—125.7)
Phenytoin	120 min	Inactive	16.8 (9.8— 31.6)	489.8(360.1—666.1)
Chlormezanone	60 min	14.9 (6.6— 39.3)	197.3(169.5—220.7)	129.7 (90.0—185.4)

* Subcutaneous pentetrazole seizure threshold test, median effective dose.
** Maximal electroshock seizure test, median effective dose.
*** Rotarod test, median effective dose.
Figures in parentheses indicate the 95% confidence limits.

Plants possessing hepatotoxicity: Initial screening result showed that seven plant materials prolonged hexobarbital induced sleeping time during the second phase though they did not affect during the first phase except *Patriniae Radix*. This result was suggested to be due to liver damage by the plant materials.

Table VIII. Effect of plant extracts on serum GOT activity in mice

Treatment	Daily dose (mg/kg, i.p.) for 3 days	Body wt. (g±S.E.) initial	Body wt. (g±S.E.) final	Liver wt. (g±S.E.)	Total protein[a] mg/g of liver±S.E.	Soluble protein[a] mg/g of liver±S.E.	Serum GOT activity[b] (U/l±S.E.)
Control	—	20.8±0.3	21.3±0.5	1.37±0.05	85.5±4.5	62.7±0.5	187.2±9.6
Dianthi herba	250	19.7±0.5	16.7±0.7†	0.95±0.07*	87.4±2.8	43.6±1.4*	634.5±113.6**
Melandrii herba	62.5	20.3±0.7	19.2±1.4	1.19±0.11	81.0±1.4	48.2±1.2*	317.3±41.8***
Echinopii radix	500	20.4±0.3	20.5±0.8	1.35±0.07	85.7±2.2	57.9±1.7	276.0±19.7**
Siegesbeckiae herba	125	21.5±0.3	21.2±0.5	1.35±0.07	85.2±2.0	67.2±0.8**	367.2±28.0*
Magnoliae cortex	250	21.0±0.5	20.0±0.5	1.36±0.06	80.1±2.5	54.6±1.2*	449.3±91.6***
Patriniae radix	125	21.0±0.4	17.9±0.6†	1.26±0.05	68.6±0.6**	45.2±0.5**	392.6±46.3*

Determined 24hr after the last dose of the extracts.
a) Lowry, *et al.*: *J. Biol. Chem.* **193**, 265 (1951)
b) Kessler, *et al.*: *Clinical Chemistry* **16**, 530 (1970)
* $P<0.001$ ** $P<0.01$ *** $P<0.05$, vs. control. † $P<0.01$, vs. the initial body wt.

Therefore, serum GOT activity which is known to increase extremely in acute liver poisoning was checked in mice, which were given the plant extracts daily for three days (Table Ⅷ). At the same time, weights of body and the liver and protein contents in the liver were measured.

All plants extracts tested increased serum GOT activity and decreased soluble protein content in the liver.

Dianthii Herba and *Patriniae Radix* decreased body weights, liver weights and total protein content in the liver. These results suggested that some plants of medicinal plants used frequently would cause the damage to the liver.

Conclusion

Crude plant materials have been prescribed in combination as drugs without knowledge of their effects on the drug metabolizing enzymes in the liver. These experimental results showed that at least 29% of medicinal plants tested affected hexobarbital induced sleeping time. It is, therefore, eagerly required that more thorough investigations on the relationship between the active constituents of the medicinal plants and the drug metabolizing enzymes should be undertaken which will contribute to the modernization of ancient therapies under scientific control and moreover, to develop new and safe drugs from natural resources.

References

1) Conney, A.H.: *Pharmacot. Rev.* **19**, 317 (1967)
2) Woo, W.S., Shin, K.H., Kim, I.C. and Lee, C.K.: *Arch. Pharm. Res.*, **1**, 13 (1978) and papers cited therein.
3) Woo, W.S. and Shin, K.H.: *Arch. Pharm. Res.*, **2**, 115 (1979)
4) Cooper, J.R. and Brodie, B.B.: *J. Pharm. Exp. Ther.*, **114**, 409 (1955)
5) Baxter, R.M., Dandiya, P.C., Kandel, S.I., Okany, A. and Walker, G.C.: *Nature*, **185**, 446 (1960)
6) Kato, R., Chiera, E. and Vassanelli, P.: *Biochem. Pharmacol.*, **13**, 69 (1964)
7) Kato, R., Chiera, E. and Vassanelli, P.: *Experientia*, **18**, 453 (1962)
8) Ryu, K.S. and Yook, C.S.: *J. Pharm. Soc. Korea*, **12**, 59 (1968)
9) Shin, K.H. and Chi, H.J.: *Kor. J. Pharmacog.*, **10**, 1 (1979)
10) Feuer, G.: *Can. J. Physiol. Pharmacol.*, **48**, 232 (1970)
11) Deckert, F.W. and Remmer, H.K.: *Chem-Biol. Interactions*, **5**, 255 (1972)
12) Ritschel, W.A. and Lee, L.H.: *Drug Dev. Ind. Pharm.*, **3**, 401 (1977)
13) Cook, L., Toner, J.J. and Fellows, E.J.: *J. Pharm. Exp. Ther.*, **111**, 131 (1954)
14) Shin, K.H., Yun (Choi), H.S., Woo, W.S. and Lee, C.K.: *Kor. J. Pharmacog.*, **10**, 69 (1979)
15) Irwin, S.: *Science*, **136**, 123 (1962)
16) Takagi, K., Saito, H.. Higuchi, Y. and Yamaguchi, A.: *Pharmacometrics*, **5**, 5 (1971)
17) Singh, N., Kulshrestha, V.K., Srivastava, R.K. and Kohli, P.P.: *J. Res. Ind. Med.*, **8**, 1 (1973)