Arch. Pharm. Res. 4(2), 123~127 (1981)

Effect of Six Chinese Drugs on Serum Transaminase Activity and Liver Tissue in Mice*

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(Received 10 October 1981)

Abstract□ The methanol extracts of *Patriniae* radix, *Dianthii* herba, *Melandrii* herba, *Echinopii* radix, *Siegesbeckiae* herba and *Magnoliae* cortex showed a significant elevation of serum transaminase activity accompanied by fatty degeneration and Kupffer cell activation in hepatic cells.

Keywords□ *Patrinia scabiosaefolia*, Valerianaceae-*Echinops latifolius*, *Siegesbeckia pubescens* Compositae-*Dianthus chinensis*, *Melandrium firmum*, Caryophyllaceae-*Magnolia abovata* Magnoliaceae-Hepatotoxicity.

In the course of the search for substances affecting mixed function oxidase activity in medicinal plants using a design, in which hexobarbital action was considered as an index of the enzyme activity, it was found that several plant extracts caused a significant prolongation of hexobarbital-induced sleeping time when tested 48 hr after the last dose of three consecutive daily administrations.[1,2] It was strongly suggested that this unexpected and interesting phenomenon was resulted from hepatic dysfunction provoked by some hepatotoxic substances in these plants. In order to prove it, serum transaminase activity, which is known to increase extremely in acute liver poisoning, was checked in mice given the plant extracts daily for 3 days and histopathological changes in the liver were examined by light microscopy.

*Part I in the series: Studies on Hepatotoxic Substances in Medicinal Plants.

MATERIALS AND METHODS

Preparation of Plant Samples

Six herbal drugs; roots of *Patrinia scabiosaefolia* (Valerianaceae) and *Echinops latifolius* (Compositae), whole plants of *Dianthus chinensis* (Caryophyllaceae), *Melandrium firmum* (Caryophllaceae) and *Siegesbeckia pubescens* (Compositae), and barks of *Magnolia obovata* (Magnoliaceae), used in this study were obtained commercially and botanically identified. Dried herb drugs were cut into small pieces and extracted three times with 90% methanol on a water bath and filtered.

The filtrate was evaporated to dryness under reduced pressure to obtain samples to be used for animal experiments.

Pretreatments

Male dd mice weighing 20±3g were allowed lab chows and water *ad lib.* maintaining in a fixed temperature environment throughout the experiment. A definite amounts of the

test samples suspended in 0.5% CMC were administered intraperitoneally daily for three consecutive days to each group of 10 mice. The control mice were given vehicle only. Twenty four hr after the last treatment of the extracts, mice were weighed, killed by cutting carotid artery and blood was collected carefully for the determination of sGOT and sGPT activities. After collection of blood, the liver was removed for the preparation of specimen for histological observation and protein determination.

Serum Transaminase Activity Measurement

Blood from two mice was pooled and allowed to clot. The serum was separated from the cells by centrifugation at 1,000 rpm for 15 min. The sGOT and sGPT activities were estimated according to the procedures described by Kessler *et al.*[3] using Technicon MT II Autoanalyzer.

Histopathological Observation

For the histopathological observation of the liver, an aliquot of the liver was sliced and fixed in 10% buffered formalin.

Subsequently, the liver was imbedded in paraffin, sectioned in 4 μm in thickness and stained with hematoxylin and eosin.

Primary microscopic lesions in the livers were observed pathologically.

Protein Determination

Another aliquot of the liver was homogenized in ice-cold KCl-phosphate buffer (pH, 7.4) in a glass homogenizer with a motor driven tepflon pestle. The homogenate was centrifuged for 30 min at 10,000g. The contents of the total protein in the homogenate and soluble protein in 10,000g supernatant were estimated by the method described by Lowry, *et al.*[4]

RESULTS

Table I indicates changes in body weight, liver weight and contents of total protein and soluble protein in the liver of mice which were sacrificed 24 hr after 3 day pretreatment of the plant extracts. No apparent body weight changes in the animal groups treated with 4 plant extracts were observed, however treatment of extracts of *Dianthii Herba* and *Patriniae Radix* caused a significant body weight decrease during the three day periods. A significant decrease in the liver weight was also observed in the group treated with *Dianthii Herba*, however, the other groups exhibited no measurable liver weight changes. The soluble protein content of the liver treated with *Dianthii Herba*, *Melandrii Herba*, *Magnoliae Cortex*, *Echinopii Radix* and *Patriniae Radix* was significantly reduced, whereas *Siegesbeckiae Herba* caused a significant increase in protein content.

In Fig. 1, GOT and GPT activity in serum are shown of mice which were pretreated with vehicle and six herbal drug extracts. It is seen that all of the experimental groups showed significant elevation of GOT activity by pretreatment of herbal drug extracts. The group treated with *Dianthii Herba* exhibited the highest value of the enzyme activity.

A simiar tendancy in significant elevation of GPT activity was observed in mice pretreated with plant extracts except in those pretreated with *Echinopii Radix*.

Table I: Effect of herbal drug extracts on liver weight and liver protein contents 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 (mg/g of liver ±S.E.)	Soluble protein (mg/g of liver ±S.E.)
Control (0.5% CMC)		20.8±0.3	21.3±0.5	1.37±0.05	85.5±4.5	62.7±0.5
Dianthii Herba	250	19.7±0.5	16.7±0.7†	0.95±0.07*	87.4±2.8	43.6±1.4*
Melandrii Herba	62.5	20.3±0.7	19.2±1.4	1.19±0.11	81.0±1.4	48.2±1.2*
Echinopii Radix	500	20.4±0.3	20.5±0.8	1.35±0.07	85.7±2.2	57.9±1.7***
Siegesbeckiae Herba	125	21.5±0.3	21.2±0.5	1.35±0.07	85.2±2.0	67.2±0.8**
Magnoliae Cortex	250	21.0±0.5	20.0±0.5	1.35±0.06	80.1±2.5	54.6±1.2*
Patriniae Radix	125	21.0±0.4	17.9±0.6†	1.26±0.05	68.6±0.6**	45.2±0.5*

Singnificance of difference;
* $P<0.001$, ** $p<0.01$, *** $p<0.05$ vs. control, † $p<0.01$ vs. the initial body weight.

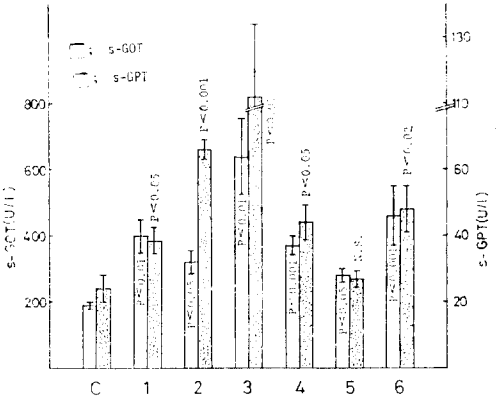

Fig: 1. Effects of herbal drug extracts on serum GOT and GPT activities in mice.
Bars represent mean±S.E. of 3 determinations.
Significance of differences was determined by student t-test.
N.S. =not significant
C. control; 1; *Patriniae Radix*, 2; *Melandrii Herba*, 3; *Dianthii Herba* 4; *Siegesbeckiae Herba*, 5; *Echinpii Radix*, 6; *Magnoliae Cortex*

Representative sections of the liver tissues of the control and herbal drug extract-pretreated animals were shown in plate 1-7.

Almost no histopathological changes but only a slight Kupffer cell activation could be observed in the control animals. In all groups treated with plant extracts, however, distinct fatty degeneration accompanying Kupffer cell activation in hepatic cells were seen; fine fatty degeneration especially in the centrilobular area was observed in the groups treated with *Patriniae Radix, Melandrii Herba* and *Dianthii Herba*, and in the panlobular area in the groups treated with *Magnoliae Cortex* and *Echinopii Radix*. The appearance of giant cells could be observed in the groups treated with *Siegesbeckiae Herba* and *Magnoliae Cortex*.

DISCUSSION

As a result of the successive disclosures on toxicity of modern synthetic drugs and remarkable effects of chinese drugs in healing problems that have proved often recalcitrant to standard western methods, traditional chinese medicine has received increasing popularity, in spite of disadvantage that the mechanism of the action is difficult to be explained scientifically. Chinese drugs are mostly natural herbal preparations and

about two thousand different plants were listed in old oriental medicinal books.

Nowadays Korean herbal doctors use, however, only about five hundred plant materials and among them two hundred and eighty plant materials are commonly used[5].

Our experiments showed that the methanol extracts of *Patriniae Radix, Dianthii Herba Melandrii Herba, Echinopii Radix, Siegesbeckiae Herba* and *Magnoliae Cortex* elevated the activity of serum transaminases and prolonged barbiturate-induced hypnosis.[2] These findings were reflected in the hepatocellular damages induced by the herbal drug extracts.

Morphological examination revealed the fatty degeneration and appearance of giant cells as well as Kupffer cell activation in the liver tissues of the animals which are regarded as an indication of the early morphologic changes due to the liver poisoning. Severe hepatic lesions such as cellular necrosis were not observed under the present experimental conditions. This result seems to be in part due to insufficiency in exposure to toxic substances as the duration of the sample treatment was relatively short. Systematic fractionations of these plant materials are being undertaken in order to find out toxic principles.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to the Institute of Reproductive Medicine and Population, Seoul National University for measurement of transaminase activity and to Dr. Y. I. Kim, Seoul National University Hospital for his help with histopathological observation.

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Explanation of the Plates

1. A liver section of mice treated with vehicle. *magnif.* 100×
2. A liver section of mice treated with *Patriniiae Radix* *magnif.* 100×
3. A liver section of mice treated with *Dianthii Herba.* *magnif.* 100×
4. A liver section of mice treated with *Melandrii Herba.* *magnif.* 200×
5. A liver section of mice treated with *Siegesbeckiae Herba* *magnif.* 200×
6. A liver section of mice treated with *Magnoliae Cortex.* *magnif.* 200×
7. A liver section of mice treated with *Echinopii Radix.* *magnif.* 200×