嘉南藥理科技大學專題研究計畫成果報告

計畫名稱:食用豆類之保肝作用

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摘要

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It estimated the effects of various hot water extracts concentration (100, 500 and 1000 mg/kg) and silymarin (25 mg/kg) on acetaminophen (APAP)-induced liver injury by measuring serum glutamate-oxalate-transaminase (sGOT) and serum glutamate-pyruvate-transaminase (sGPT) activities in rats. The sGOT and the sGPT activities, increased by APAP, were significantly decreased ($P<0.05$) when treating with increasing amounts up to 1000 mg/kg of the extracts. Especially, the mung bean agueous extract indicated the best hepatoprotective effect on APAP-induced hepatotoxicity. The pathological changes of injurious liver caused by APAP, were improved by the treatment with all of HWEL, which were compared to silymarin as a standardized drug. Above these results, the extract of mung bean acted as a potential hepatoprotective agent in dietary supply.

□□關鍵字: Legumes; GOT; GPT; hepatoprotective

INTRODUCTION

Recently, investigations showed that acetaminophen (APAP)-induced liver damage could be inhibited by the crude extracts of *Terminalia catappa* (Lin et al. 2000). Liver injury is caused by different agents, such as chemicals, alcohol, viruses, and auto-immune diseases (Sugiyama et al. 1999). It was reported that large doses of acetaminophen could produce serious liver necrosis in humans and in Experimental animals (Prescott et al. 1971; Mitchell 1988). Both acetaminophen

induced necrosis and covalent binding of the acetaminophen metabolite to liver protein were increased by inducers of cytochrome P-450 enzymes in liver microsomes. (Potter et al. 1973). The peroxidation has been attributed to the superoxide ion generated by the biotransformation of the acetaminophen or the active metabolites (N-acetyl-*p*- benzoquinoneimine, NAPQI) (Farber and Gerson 1984). The extent of liver injury caused by the APAP could be estimated by measuring the activities of serum enzymes, such as glutamate-oxalate-transaminase (sGOT)and glutamate-pyruvate-transaminase (sGPT). It is necessary therefore to assay the hepatoprotective activity of HWEL.

This study was undrtaken to evaluate the antioxidant and hepatoprotective activity of the HWEL, and histopathological changes of rat hepatic tissues.

MATERIAL and METHODS

Samples of mung bean (*Phaseolus radiatus*), adzuki bean (*Phaseolus aureus*), and black bean (*Glycine max*) were obtained from Tainan District Agriculture Improvement Station, Taiwan, while that of rice bean (*Phaseolus calcaratus*) was purchased from a chinese herb store in Tainan.

2.1. Chemical

Acetaminophen (APAP) and carboxymethyl cellulose (CMC) were purchased from Sigma Chemical Co.(St. Louis, MO).

2.2. Preparation of Extracts

Each sample (100g) was extracted with 1 *L* of boiling water for 1 hour. The extracts were filtered; the residue was re-extracted under the same conditions, and the combined filtrated were evaporated to dryness under vacuum and the yield of soluble constituents (mung bean, adzuki bean, black bean and rice bean) were 18.76, 21.80, 15.64 and 24.14%.

2.3. Animals

6-7 weeks old were obtained from the animal center, National Cheng Kung University, Tainan. They were housed in an air- conditioned room at 22± 3℃, 55 ± 5% humidity, and fed with a standard laboratory diet and tap water throughout the investigation.

2.4. APAP-induced hepatotoxicity in rats

The acute hepatotoxicity inducement was determined by the method of Lin and Shieh et al. (1997). Animals were fasted for 16 h, then divided into fifteen groups of ten rats each. Group A was given normal saline (10 ml/kg, i.p.) as the normal control, and group B was injected with APAP (500 mg/kg, in 25% PEG 400) (APAP, Sigma, USA). The other thirteen groups were treated with HWEL (100, 500 and 1000 mg/kg

in saline, i.p.), or with the standard reference silymarin (25 mg/kg in 1% CMC, i.p.),with a 2 h lag behind APAP administration.

2.5.Estimation of serum sGOT and sGPT activity

On the APAP-induced model system, the rats were anesthetized with ether, 24 h after APAP administration. Blood was taken from the carotid artery and then centrifuged at 3000 rpm at 4℃ for 10 min to separate the serum. The activities of serum glutamate-oxalate-transaminase (sGOT) and glutamate-pyruvate-transaminase (sGPT) were measured by the method of Reitaman and Frankel (1957).

2.6.Statistical analysis

Statistical analysis involved use of the Statistical Analysis System (SAS) software package.

The hepatoprotective data were indicated as the mean± S.E.M and statistically performed by ANOVA procedures. Significant differences between the means of all samples were determined by the Duncan's multiple range test (Duncan, 1957), $p<0.05$ was regarded as significant.

RESULTS

Effect on APAP-induced hepatotoxicity

Results indicated that administration of APAP (500 mg/kg, in 25% PEG 400, i.p.) had increased in liver transaminases activity and was significantly different from control (saline) and HWEL-treated groups (Table 1 ,2 and 3). The sGOT and sGPT activities of HWEL-treated groups were evaluated after APAP injection. HWEL (100, 500 and 1000 mg/kg) and silymarin (25 mg/kg) significantly suppressed enzyme activities by APAP-injection ($p<0.05$).The hepatoprotective effect of HWEL in serum transaminases activity reduced with increasing doses up to 1000 mg/kg. The mung bean (*P. radiatus*) showed the best effect in serum enzyme activity than others. Thereforce, the effects of the extracts were dose-dependent.The hepatoprotective effect of legumes increased with concentration. Dietary supplementation with HWEL significantly prevented the toxicity effects of acetaminophen.

DISCUSSION

This study showed that injection with APAP displayed the highest levels of sGOT and sGPT. Compared with the APAP group, it is found that treatment with HWEL and silymarin appeared to enhance the repair from the APAP-induced hepatotoxicity as judged from the levels of sGOT and sGPT ($P<0.05$). The water extracts of mung bean represented the best hepatoprotective effect in serum enzyme test. The crude extracts of mung bean at 1000 mg/kg showed greater repaired effect than did 25 mg/kg of silymarin, which is a hepatic protective agent.

In conclusion, the mechanism by which the APAP produced liver injury is different.

However, the APAP-induced model must rely on the cytochrome P-450 system to produce reactive metabolites and NAPQI. Therefore, the possible hepatoprotective mechanisms of water extracts of legumes on the chemical-induced liver injuries may be due to the following factors: (1) inhibition of the cytochrome P-450 activity; (2) prevention of the process of lipid peroxidation; (3) stabilization of the hepatocellular membrane; and (4) enhancement of the protein synthesis.

Table 1. Effects of HWEL (100mg/kg) and silymarin on sGOT and sGPT activities in APAP-induced rats

Group	**Dose** (mg/kg)	**sGOT** (IU/l)	**sGPT** (IU/l)
APAP/PEG 400	500	5634.2± 579.7	1420.5± 239.1
Control (saline)	_	83.2± 15.6*^{c}	23.0± 5.2 *^{d}
Silymarin	25	159.4± 30.8*^{b}	50.6± 6.8 *^{c}
Mung bean	100	244.7± 28.2*^{ab}	73.7± 14.5*^{bc}
Adzuki bean	100	267.9± 60.0*^{a}	101.3± 23.5*^{ab}
Black bean	100	336.2± 49.2*^{a}	132.1± 30.4*^{a}
Rice bean	100	276.3± 58.5*^{a}	95.6± 25.6*^{ab}

Data were presented as the means± S.E.M for six rats.* P<0.05 significantly different from APAP group. Values in a column with no common letters were significantly at P<0.05.

Table 2. Effects of HWEL (500 mg/kg)and silymarin on sGOT and sGPT activities in APAP-induced rats

Group	**Dose** (mg/kg)	**sGOT** (IU/l)	**sGPT** (IU/l)
APAP/PEG 400	500	5634.2± 579.7	1420.5± 239.1
Control (saline)	_	83.2± 15.6*^{c}	23.0± 5.2 *^{d}
Silymarin	25	159.4± 30.8*^{b}	50.6± 6.8 *^{c}
Mung bean	500	167.2± 25.9*^{ab}	76.8± 20.3*^{b}
Adzuki bean	500	167.6± 42.7*^{ab}	62.6± 13.9*^{bc}
Black bean	500	298.5± 35.6*^{a}	103.8± 24.6*^{a}
Rice bean	500	222.3± 55.0*^{ab}	80.5± 14.7*^{b}

Data were presented as the means± S.E.M for six rats.* P<0.05 significantly different from APAP group. Values in a column with no common letters were significantly at P<0.05.

Table 3. Effects of HWEL (1000 mg/kg) and silymarin on sGOT and sGPT activities in APAP-induced rats

Group	Dose (mg/kg)	**sGOT** (IU/l)	sGPT (IU/l)
APAP/PEG 400	500	5634.2± 579.7	1420.5± 239.1
Control (saline)	_	83.2± 15.6*^{c}	23.0± 5.2 *^{d}
Silymarin	25	159.4± 30.8*^{b}	50.6± 6.8 *^{c}
Mung bean	1000	157.5± 37.0*^{b}	32.6± 8.1*^{b}
Adzuki bean	1000	178.6± 35.7*^{ab}	58.5± 12.1*^{ab}
Black bean	1000	202.5± 50.6*^{a}	76.8± 19.7*^{a}
Rice bean	1000	173.6± 35.5*^{ab}	55.9± 17.3*^{ab}

Data were presented as the means± S.E.M for six rats.* $P<0.05$ significantly different from APAP group. Values in a column with no common letters were significantly at $P<0.05$.