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Sesame Lignans Significantly Alleviate Liver Damage of Rats Caused by Carbon Tetrachloride in Combination with Kava

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ABSTRACT

The aim of this study was to examine if the liver damage caused by CCl_4 in combination with kava (*Piper methysticum*) could be alleviated via coadministration of sesame lignans. Six groups of male Sprague-Dawley rats were fed with corn oil as a control, kava only, kava plus sesame lignans, CCl_4 only, CCl_4 plus kava, and CCl_4 plus kava plus sesame lignans, respectively. Body weight, diet intake, animal behavior, and serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were recorded along with the course. Four weeks later, organ weights and histopathological data including liver steatosis and fibrosis were obtained after sacrifice. At day 28, both the diet intakes and body weights of rats decreased, and their relative liver weights increased considerably in all three CCl_4-treated groups compared to the control group. In accord with the levels of pathological steatosis and fibrosis, the liver damage caused by CCl_4 was somewhat worse when kava extract was coingested, but significantly attenuated when sesame lignans were coadministrated. No detectable liver damage was observed in the kava or kava plus sesame lignan group. The protective effect of sesame lignans against liver injury caused by CCl_4 with or without kava extract was also observed in the relative activities of plasma ALT, AST and ALP. It is concluded that sesame lignans significantly alleviate liver damage of rats caused by carbon tetrachloride with or without kava. The potential risk of kava's hepatotoxicity can be greatly circumvented and the applications of kava may be further extended by coadministration of sesame lignans.

Key words: hepatoprotection, kava (*Piper methysticum*), sesame lignan, sesamin, sesamolin

INTRODUCTION

Kava (*Piper methysticum*) has been used in Pacific islands as a medicinal and ceremonial plant for centuries. Conventionally, the rhizome of kava is used to prepare beverages, extracts, capsules, tablets, and topical solutions. In the past two decades, kava was introduced worldwide as an herbal tranquilizer and used as a dietary supplement for anxiety disorders. Effective dosages of kava extract showed relative minor side effects, such as drowsiness, dizziness and headaches that were commonly reported for patients taking benzodiazepine anxiolytics. Unlike brand medications currently used, no tolerance, physical dependence and withdrawal symptoms were observed for the long-term utilization of kava extract[1-8]. Extensive searches in databases, such as EMBASE, MEDLINE, AMED, CISCOM, Central/CCTR, and CCDANCTR, suggested a significant effect towards a reduction of the total score of the Hamilton Anxiety scale in patients receiving kava extract compared with those receiving placebo[9]. The adverse effects reported in a Cochrane review of kava as a treatment of anxiety were mild, transient and infrequent[10]. Nevertheless, allergic responses and hepatic toxicities have been noted.

More than 68 cases of suspicious kava hepatotoxicity have been documented since 1998. Some of

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the cases were severe and led to hepatic failure, such that the patients had to be rescued by liver transplantation. Consequently, kava based herbal products were either labeled with warning or temporarily banned in the United States and some European countries. Several possibilities have been proposed for the liver injury induced by kava products, including allergy to kava extract[11], insufficient activities of cytochrome P450-related gene products (such as CYP2E1) in patients[12,13], overdose and/or prolonged treatment[14], co-ingestion of other medications, and toxic alkaloids in leaf/stem (not found in root for making extracts).

Sesame seeds and sesame oil have long been used as health foods in Asia to increase energy and to prevent aging. It has been known that sesame oil possesses excellent antioxidant activities[15], which may be attributed to its abundant lignans, such as sesamin and sesamolin[16]. Sesame lignans were found to suppress lipid peroxidation in erythrocytes[17], to inhibit intestinal absorption of cholesterol and hepatic 3-hydroxy-3-methylglutaryl CoA reductase activity[18], to prevent chemically induced mammary cancer[19], to inhibit Δ5-desaturase and chain elongation of C18 fatty acids[20], to protect hypoxic neuronal and PC12 cells by suppressing ROS generation and MAPK activation[21], to enhance the antihypertensive effect[22], and to enhance liver detoxification of carbon tetrachloride and ethanol[23,24].

In the present study, we aimed to examine if the liver damage caused by ingesting kava of the highest recommended concentration (240 mg/kg) under a liver-injuring condition could be alleviated via coadministration of sesame lignans. A liver-injuring condition in rats was mimicked by treatment of CCl_4. Liver damage of rats fed with various combinations of kava extract, sesame lignans and CCl_4 was evaluated by morphological and pathological liver changes as well as serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

MATERIALS AND METHODS

I. *Reagents*

Kava root extract was a gift from Yushing Biotech Company (Taichung, Taiwan, R.O.C.). As revealed by HPLC analysis, compositions of the kava root extract (mainly kava ketones) used in this study was essentially identical both qualitatively and quantitatively to that of a commercial product available in the United States. Sesame lignans (mainly sesamin and sesamolin) were purified from sesame oil as described previously[25]. Purity of sesame lignans (sesamin and sesamolin) was also verified by HPLC chromatogram. Carbon tetrachloride, corn oil and chemicals were purchased from Sigma unless specified.

II. *Animals*

Male Sprague-Dawley rats initially weighing 220-260 g were obtained from the Animal Resource Center, National Science Council (Taipei, Taiwan, R.O.C.) and acclimated to the animal facility with free access to drinking water and standard rodent chow (Fwusow Ind. Corp., Taichung) at 22°C under a 12-h light/12-h dark cycle for 1 week before starting the experiment. The study protocols and care administered to the animals were approved by the institutional laboratory animal care and use committee.

III. *Experimental Design*

Six groups of animals, named control, Kava, K+S, CCl_4, C+K, and C+K+S, were included in this study, representing treatment with corn oil as a control, kava only, kava plus sesame lignans, CCl_4 only, CCl_4 plus kava, and CCl_4 plus kava plus sesame lignans, respectively. From day 1 to day 28, kava extract and sesame lignans (240 and 30 mg/kg body weight) were orally administrated to animals daily while CCl_4 (20% CCl_4/corn oil) was gavaged (0.5 mL/kg body weight) twice a week in a 2-day interval (Monday and Thursday) 5 h prior to administration of kava extract and/or sesame lignans if applicable. The control group was treated with corn oil and fed with rodent chow diet.

Body weight, diet intake and animal behavior were recorded daily. Blood samples were withdrawn from tail vein at 7, 14, 21, and 28 days after the first gavage, and serum samples were isolated for ALT, AST and ALP assessments. On day 28, rats were executed under anesthesia with diethyl ether and the order of execution was randomized among the groups. Liver and spleen tissues were immediately processed as described below in the Histology section.

IV. *Serum Analyses*

Serum samples were obtained from blood by centrifugation at 1000 ×*g* for 10 min at 4°C. Serum biochemical marker assay kits for liver, including ALT, AST and ALP, were purchased from Roche (Roche Diagnostics) and assayed according to International Federation of Clinical Chemistry (IFCC) reference procedures on 7600 Clinical Analyzer (Hitachi)[26].

V. *Histology*

The liver and other organs were dissected, weighed, and fixed in 10% buffered formalin. Tissue samples were embedded in paraffin and cut at 4 microns. Tissue sections were deparaffinized through xylene and graduated alcohol series to water and stained with hematoxylin and eosin (H & E) for evaluation using a standard light microscope. The consecutive sections were stained

with Masson's trichrome for assessment of fibrosis[27]. A semiquantitative evaluation for histological features was carried out randomizedly and blindly by a pathologist to the treatment groups and assessed the grades of tissue inflammation, fatty accumulation, and hepatotic fibrosis. Liver steatosis was graded on a three-point scale: 1+, hepatocytes in the area less than 33% of the lobules showed fatty accumulation, 2+, between 33 and 66% and 3+, for more than 66% hepatocytes. The criteria used for scoring fibrosis severity were as follows: 0, normal; 1+, fibrosis present (collagen fiber present that extends from portal triad or central vein to peripheral region); 2+, mild fibrosis (the collagen fiber present with extension without compartment formation); 3+, moderate fibrosis (the collagen fiber present with some pseudo lobe formation); and 4+, severe fibrosis (the collagen fiber present with thickening of the partial compartments and frequent pseudo lobe formation).

VI. *Statistic Analysis*

Data were analyzed by one-way analysis of variance (ANOVA) using SAS general linear models program. Duncan's multiple tests were used to determine the differences among groups. Student's *t*-test was used in the two-group comparison. Values were expressed as mean ± SEM. Group means were considered to be significantly different at $p < 0.05$ as determined by the protective least significant difference technique when the ANOVA indicated an overall significant treatment effect.

RESULTS

The average daily food intakes were 36.2, 35.2, 37.7, 31.0, 29.7, and 31.9 g/day in the control, Kava, K+S, CCl_4, C+K, and C+K+S groups, respectively. Apparently, treatment of CCl_4 slightly affected the daily diet intakes of animals as observed in CCl_4, C+K and C+K+S groups. Body weight changes of animals in these six groups were shown in Table 1. There were no significant differences in body weights among groups before introducing the supplements (day 0). In contrast, average body weights of animals in CCl_4, C+K and C+K+S groups at day 28 were significantly lower than those in the control, Kava and K+S groups (Table 1). Among the three CCl_4-treated groups, food intake and body weight of animals in the C+K+S group (supplemented with sesame lignans) were evidently higher than those in the other two groups (supplemented without sesame lignans). Similar to animals in the control, Kava and K+S groups, animals in the C+K+S group looked active while those in the CCl_4 and C+K groups looked unhealthy with much less movement. The access of drinking water showed no differences among groups, except CCl_4-treated rats which increased water assess slightly on the days gavaged with CCl_4 (data not shown).

Mean relative liver weights of animals in CCl_4, C+K and C+K+S groups increased to 142, 149 and 146% over the relative liver weight in the control group and demonstrated statistic significances (Table 1). Though the relative liver weights of animals in Kava and K+S groups increased slightly compared to that in the control group, the statistics showed no differences. As compared to the CCl_4 group, the C+K and C+K+S groups showed significant reductions in relative liver weights. Treatment of CCl_4 resulted in the elevations of relative spleen weights of animals in CCl_4, C+K and C+K+S groups compared to those in the control, Kava and K+S groups that showed no significant differences among their relative spleen weights.

Animals fed with kava extract with or without sesame lignans did not possess obvious morphological and pathological liver changes in comparison with the control group (Figure 1A and 1B, a, b and c). As compared to the normal liver of animals in the control group, the injured liver of animals in the CCl_4 group showed concave light yellowish surface and swelling in size (data not shown), and the tissue section revealed lymphocytes infiltration in the central vein (Figure 1A, d). The hepatocytes were also necrotic and swelling. Fatty degeneration and cytoplasmic vascuolization were obvious in the central and mid-zone. Fibrotic injuries were observed in the CCl_4-treated liver by collagen accumulation and fiber extension in H&E (Figure 1A, d) and Masson's trichrome (Figure 1B, d) staining biopsies. The histopathological examination of liver morphological changes and damages induced by CCl_4 administration demonstrated evident liver steatosis (scored 2+~3+) and fibrosis (scored 2+~3+). Concave light yellowish surface and swelling in size were also observed in the liver of animals in the C+K group. The liver injuries observed in the C+K group was similar to those observed in the

Table 1. Body weight, relative liver and spleen weight of CCl_4-treated rats with or without the gavage of Kava extract and sesame lignans for 4 weeks

Group	Body weight (g)	Relative organ weight (g/100g of BW)	
		Liver	Spleen
Control	165 ± 13	3.5 ± 0.2	0.23 ± 0.02
Kava	176 ± 24	4.0 ± 0.2	0.22 ± 0.03
K+S	173 ± 25	4.1 ± 0.4	0.20 ± 0.03
CCl_4	107 ± 8**	5.7 ± 0.6**	0.27 ± 0.03
C+K	99 ± 33**	5.2 ± 0.5$^{**, \#}$	0.31 ± 0.09
C+K+S	126 ± 27$^{*, \#}$	5.1 ± 0.5$^{**, \#}$	0.30 ± 0.04

Values are means ± SEM, n = 4 for controls and n = 7 to 8 for others.
* Significantly different from the control group, $^{*}p < 0.05$, $^{**}p < 0.01$.
\# Significantly different from the CCl_4 group, $^{\#}p < 0.05$. The treatment protocols are described in details in Experimental design section.

CCl_4 group; no further severer damages existed (Figure 1A and 1B, e). In contrast, coadministration of sesame lignans in the C+K+S group rescued the liver fibrosis completely (scored 0-1+) and attenuated CCl_4-induced fatty accumulation almost completely (scored 0-1+) (Figure 1A and 1B, f).

Figure 2 shows time courses of activity changes in plasma ALT, AST and ALP after drug administrations. With the addition of CCl_4, plasma ALT and AST activities increased gradually while all other groups maintained at the background levels. ALP levels markedly declined with time in the control, Kava, K+S, and C+K+S groups, while were more reluctant to fall in the CCl_4 and C+K groups. On day 28, the plasma ALT, AST and ALP activities of the CCl_4 group increased to 270, 490 and 293% of those in the control group, respectively. Kava alone or in combination with sesame lignans had no effect on the serum levels of these three enzymes. In the presence of CCl_4, kava extract did not significantly enhance or reduce ALT, AST and ALP levels as compared with those in the CCl_4 group. Coadministration of sesame lignans in the C+K+S group greatly reduced ALT, AST and ALP activities to 57, 62 and 61% of those in the CCl_4 group, though still higher than those in the control group.

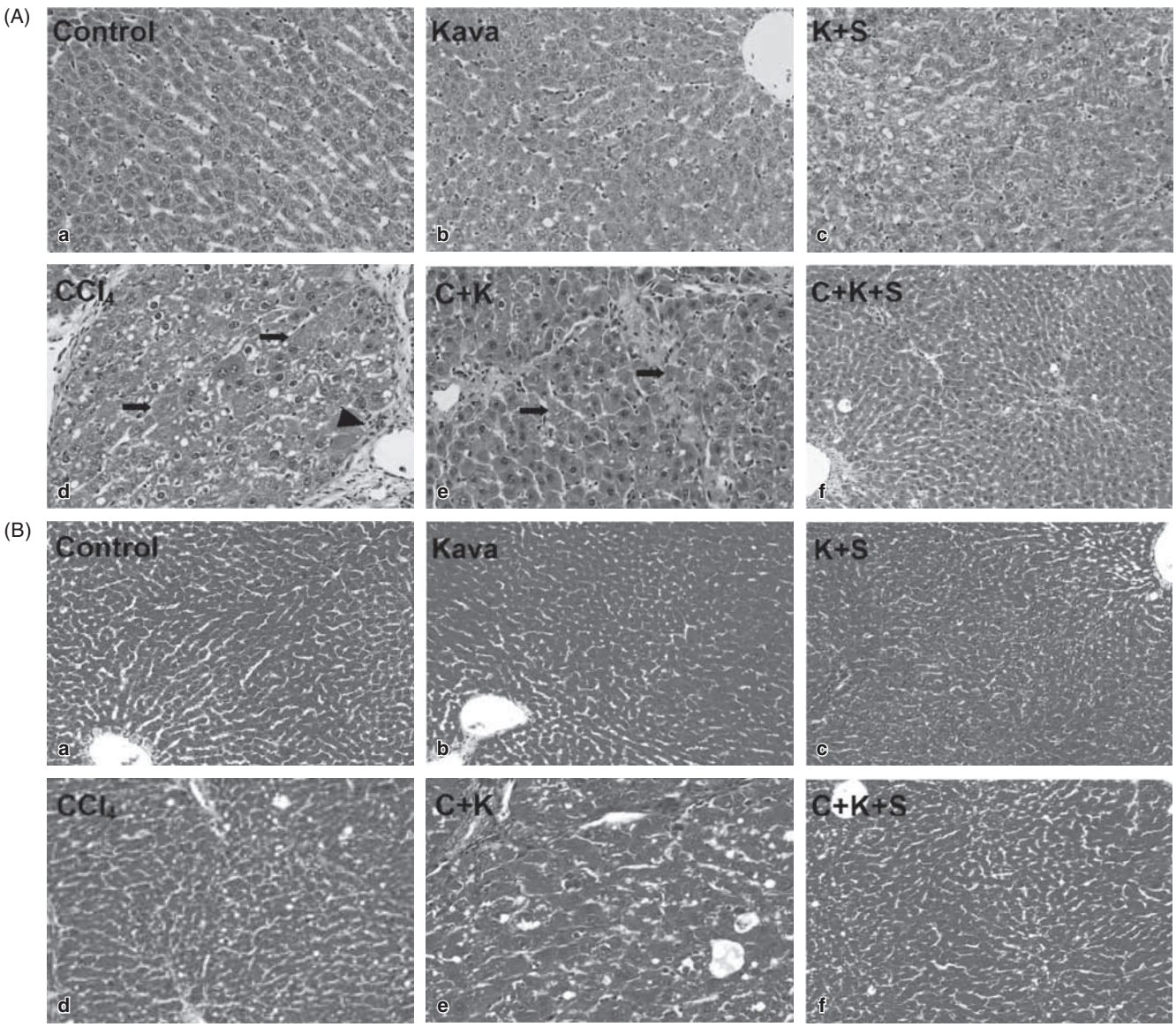

Figure 1. Effects of kava extract and sesame lignans on CCl_4-induced liver damage in SD rats: (a) control group treated with corn oil 0.5 mL/kg body weight, (b) animals treated with 240 mg/kg body weight kava extract, (c) animals treated with 240 mg/kg body weight kava extract and 30 mg/kg body weight sesame lignans, (d) animals treated with CCl_4, (e) animals treated with CCl_4 and 240 mg/kg body weight kava extract, and (f) animals treated with CCl_4, 240 mg/kg body weight kava extract and 30 mg/kg body weight sesame lignans. (A) Hematoxylin/eosin stain, (B) Masson stain; magnification 200×. The regions of lymphocyte infiltration and necrosis are labeled with arrowheads and arrows, respectively.

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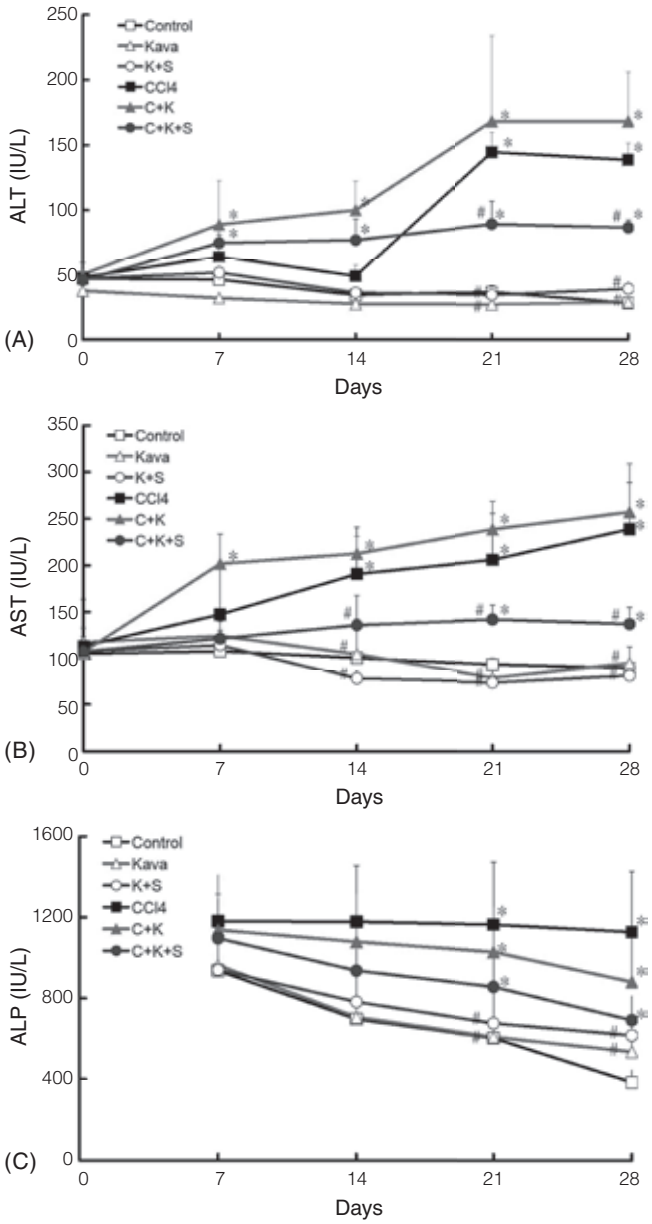

Figure 2. Time courses of ALT (A), AST (B) and ALP (C) activities in plasma of SD rats of the six groups (control, Kava, K+S, CCl_4, C+K, and C+K+S) as described in Figure 1. Values are means ± SEM, n = 4 for control and n = 7 to 8 for others. *Significantly different from the control group ($p < 0.05$). #Significantly different from the CCl_4 group ($p < 0.05$). The treatment protocols are described in details in Experimental design section.

DISCUSSION

The adverse effects of kava were often unapparent and rare, though prolonged and/or overdose kava usage was a reportedly potential rare but life-threatening risk factor for hepatotoxicity[28]. Causal relationship between kava and tissue damage has not been directly established, and few literature reports have ever identified kava components which may affect liver function. *In vitro* studies have shown that kava lactones, the active principles of kava extracts, inhibited a variety of human CYP isoforms, while 28 days of kava supplementation only affected CYP2E1 phenotype in a clinical trial, and caused no elevations in serum liver enzymes[12]. It seems that kava does not directly cause detectable liver injury, but may possibly raise metabolic burden of liver and enhance liver injury in the coexistence of other liver-damaging factors. If the unfavorable factors could be counteracted by coadministration of liver-protecting agents, consumption of kava may not lead to the suspicious risk of liver injury. In the present study, *in vivo* compensatory or protective effect of sesame lignans was evaluated under the stress generated by carbon tetrachloride with or without kava extract. Administration of the highest recommended dosage (240 mg/kg body weight) or a relatively low dosage (60 mg/kg body weight; data not shown) of kava extract for 4 weeks did not result in observable liver damages, though the liver weight showed slight increase (not statistically significant) which may indicated an increased burden of liver to metabolize the kava extract.

Carbon tetrachloride is a potent inducer of hepatic injury and serves in a model protocol to investigate the possible medication for liver damage. While kava extract alone did not elicit noticeable liver injury, it did enhance CCl_4-induced liver damage when coadministrated at a high dosage. This is concordant with previous findings showing that patients suffering liver disease or taking drug products that affect the liver are more vulnerable to liver injuries when coingested a high dosage of kava[29]. Carbon tetrachloride is known to induce oxidative stress by inducing ROS formation, depleting GSH of phase II enzyme, and reducing antioxidant enzyme and substrates. Thereafter, free radicals and lipid peroxidation resulted from oxidative stress caused hepatic damages[30,31]. Sesame lignans have been found to possess superior antioxidative activities and to alleviate CCl_4- and ethanol-mediated liver injuries[18,23,32]. In this study, detrimental effects of CCl_4 with or without kava extract were significantly alleviated by sesame lignans, as demonstrated by profound reduction in serological AST, ALT and ALP activities and greatly improved histopathology status in liver steatosis and fibrosis.

CONCLUSIONS

Our results demonstrate the *in vivo* hepatoprotective effects of sesame lignans on the liver damage induced by carbon tetrachloride in combination with a high dosage of kava extract. We suggest that the potential risk of kava's hepatotoxicity can be greatly circumvented and the applications of kava may be further extended by coadministration of sesame lignans. Of course, highlighted precautions in kava applications are indispensable for avoiding overdose or combined usage with other

drugs that may affect liver severely, and for alerting patients of liver disease or genetic deficiency.

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REFERENCES

1. Singh, Y. N. 1992. Kava: an overview. J. Ethnopharmacol. 37: 13-45.
2. Lindenberg, D. and Pitule-Schodel, H. 1990. D,L-kavain in comparison with oxazepam in anxiety disorders. A double-blind study of clinical effectiveness. Fortschr. Med. 108: 49-50.
3. Kinzler, E., Kromer, J. and Lehmann, E. 1991. Effect of a special kava extract in patients with anxiety-, tension-, and excitation states of non-psychotic genesis. Double blind study with placebos over 4 weeks. Arzneimittelforschung 41: 584-588.
4. Volz, H. P. and Kieser, M. 1997. Kava-kava extract WS 1490 *versus* placebo in anxiety disorders--a randomized placebo-controlled 25-week outpatient trial. Pharmacopsychiatry 30: 1-5.
5. Scherer, J. 1998. Kava-kava extract in anxiety disorders: an outpatient observational study. Adv. Ther. 15: 261-269.
6. Pittler, M. H. and Ernst, E. 2000. Efficacy of kava extract for treating anxiety: systematic review and meta-analysis. J. Clin. Psychopharmacol. 20: 84-89.
7. Cagnacci, A., Arangino, S., Renzi, A., Zanni, A. L., Malmusi, S. and Volpe, A. 2003. Kava-Kava administration reduces anxiety in perimenopausal women. Maturitas. 44: 103-109.
8. Feltenstein, M. W., Lambdin, L. C., Ganzera, M., Ranjith, H., Dharmaratne, W., Nanayakkara, N. P., Khan, I. A. and Sufka, K. J. 2003. Anxiolytic properties of Piper methysticum extract samples and fractions in the chick social-separation-stress procedure. Phytother. Res. 17: 210-216.
9. Pittler, M. H. and Ernst, E. 2003. Kava extract for treating anxiety. Cochrane Database Syst. Rev. CD003383.
10. Ernst, E. 2006. Herbal remedies for anxiety - a systematic review of controlled clinical trials. Phytomedicine. 13: 205-208.
11. Suss, R. and Lehmann, P. 1996. Hematogenous contact eczema cause by phytogenic drugs exemplified by kava root extract. Hautarzt 47: 459-461.
12. Gurley, B. J., Gardner, S. F., Hubbard, M. A., Williams, D. K., Gentry, W. B., Khan, I. A. and Shah, A. 2005. *In vivo* effects of goldenseal, kava kava, black cohosh, and valerian on human cytochrome P450 1A2, 2D6, 2E1, and 3A4/5 phenotypes. Clin. Pharmacol. Ther. 77: 415-426.
13. Gurley, B. J., Swain, A., Hubbard, M. A., Williams, D. K., Barone, G., Hartsfield, F., Tong, Y., Carrier, D. J., Cheboyina, S. and Battu, S. K. 2008. Clinical assessment of CYP2D6-mediated herb-drug interactions in humans: effects of milk thistle, black cohosh, goldenseal, kava kava, St. John's wort, and Echinacea. Mol. Nutr. Food Res. 52: 755-763.
14. Teschke, R., Schwarzenboeck, A. and Hennermann, K. H. 2008. Kava hepatotoxicity: a clinical survey and critical analysis of 26 suspected cases. Eur. J. Gastroenterol. Hepatol. 20: 1182-1193.
15. Budowski, P. M. K. 1951. The Chemical and physiological properties of sesame oil. Chem. Rev. 48: 125-151.
16. Fukuda, Y., Nagata, M., Osawa, T. and Namiki, M. 1986. Contribution of lignan analogues to antioxidative activity of refined unroasted sesame seed oil. J. Am. Oil Chem. Soc. 63: 1027-1031.
17. Osawa, T., Namiki, M. and Kawakishi, S. 1990. Role of dietary antioxidants in protection against oxidative damage. Basic Life Sci. 52: 139-153.
18. Hirose, N., Inoue, T., Nishihara, K., Sugano, M., Akimoto, K., Shimizu, S. and Yamada, H. 1991. Inhibition of cholesterol absorption and synthesis in rats by sesamin. J. Lipid Res. 32: 629-638.
19. Hirose, N., Doi, F., Ueki, T., Akazawa, K., Chijiiwa, K., Sugano, M., Akimoto, K., Shimizu, S. and Yamada, H. 1992. Suppressive effect of sesamin against 7,12-dimethylbenz[a]-anthracene induced rat mammary carcinogenesis. Anticancer Res. 12: 1259-1265.
20. Fujiyama-Fujiwara, Y., Umeda-Sawada, R., Kuzuyama, M. and Igarashi, O. 1995. Effects of sesamin on the fatty acid composition of the liver of rats fed N-6 and N-3 fatty acid-rich diet. J. Nutr. Sci. Vitaminol. 41: 217-225.
21. Hou, R. C., Huang, H. M., Tzen, J. T. and Jeng, K. C. 2003. Protective effects of sesamin and sesamolin on hypoxic neuronal and PC12 cells. J. Neurosci. Res. 74: 123-133.
22. Nakano, D., Kwak, C. J., Fujii, K., Ikemura, K., Satake, A., Ohkita, M., Takaoka, M., Ono, Y., Nakai, M., Tomimori, N., Kiso, Y. and Matsumura, Y. 2006. Sesamin metabolites induce an endothelial nitric oxide-dependent vasorelaxation through their antioxidative property-independent mechanisms: possible involvement of the metabolites in the antihypertensive effect of sesamin. J. Pharmacol. Exp. Ther. 318: 328-335.
23. Akimoto, K., Kitagawa, Y., Akamatsu, T., Hirose, N., Sugano, M., Shimizu, S. and Yamada, H. 1993. Protective effects of sesamin against liver damage caused by alcohol or carbon tetrachloride in rodents. Ann. Nutr. Metab. 37: 218-224.
24. Chang, C. Y., Chen, Y. L., Yang, S. C., Huang, G. C.,

Tsi, D., Huang, C. C., Chen, J. R. and Li, J. S. 2009. Effect of schisandrin B and sesamin mixture on CCl_4-induced hepatic oxidative stress in rats. Phytother. Res. 23: 251-256.

25. Cheng, F. C., Jinn, T. R., Hou, R. C. and Tzen, J. T. 2006. Neuroprotective effects of sesamin and sesamolin on gerbil brain in cerebral ischemia. Int. J. Biomed. Sci. 2: 284-288.
26. Schumann, G. and Klauke, R. 2003. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin. Chim. Acta 327: 69-79.
27. Karaki, Y., Munakata, S., Saeki, T., Hirota, S. and Fujimaki, M. 1991. Appearance of a carcinoid-like pattern in rat hepatic tumors induced by 3'-methyl-4-dimethylaminoazobenzene. Jpn. J. Cancer Res. 82: 397-402.
28. Stickel, F., Baumuller, H. M., Seitz, K., Vasilakis, D., Seitz, G., Seitz, H. K. and Schuppan, D. 2003. Hepatitis induced by Kava (Piper methysticum rhizoma). J. Hepatol. 39: 62-67.
29. Currie, B. J. and Clough, A. R. 2003. Kava hepatotoxicity with Western herbal products: does it occur with traditional kava use? Med. J. Aust. 178: 421-422.
30. Weber, L. W., Boll, M. and Stampfl, A. 2003. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit. Rev. Toxicol. 33: 105-136.
31. Kodai, S., Takemura, S., Minamiyama, Y., Hai, S., Yamamoto, S., Kubo, S., Yoshida, Y., Niki, E., Okada, S., Hirohashi, K. and Suehiro, S. 2007. S-allyl cysteine prevents CCl_4-induced acute liver injury in rats. Free Radic. Res. 41: 489-497.
32. Matsumura, Y., Kita, S., Tanida, Y., Taguchi, Y., Morimoto, S., Akimoto, K. and Tanaka, T. 1998. Antihypertensive effect of sesamin. III. Protection against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats. Biol. Pharm. Bull. 21: 469-473.