Garlic Extract Inhibits the Enhanced Peroxidation and Production of Lipids in Carbon Tetrachloride-Induced Liver Injury

Kyoichi KAGAWA, Hisako MATSUTAKA, Yukari YAMAGUCHI and Chizuko FUKUHAMA
Pharmacological Laboratory, Hankyu-Kyoai Bussan Co., Ltd.,
180 Furue, Ikeda, Osaka 563, Japan
Accepted May 23, 1986

Abstract—Carbon tetrachloride (CCl₄) enhances lipid peroxidation, resulting in triglyceride accumulation in the liver. In this report, we studied the therapeutic, but not the preventive, effect of garlic extract on CCl₄-intoxicated liver, in comparison to the effect of vitamin E. Garlic extract was given orally to mice in the dose of 10, 100 or 500 mg/kg at 6 hr after CCl₄ administration. The increased conjugated-diene level was diminished significantly to 82% by the 100 mg/kg extract, and also thiobarbituric acid-reactivity was inhibited by all the doses of the extract. In addition to the above mentioned effects, the high doses of garlic extract lowered hepatic triglyceride and lipid contents. Highly significant and positive correlation was observed between hepatic triglyceride content and conjugated-diene level in the lipid fraction of the liver. Besides, vitamin E at the dose of 25 mg/kg inhibited only lipid peroxidation. We, therefore, conclude that not only is garlic extract effective on diminution of lipid peroxide and on alteration of peroxidative status to more reductive condition like the effect of vitamin E, but it also inhibits hepatic triglyceride accumulation in injured liver.

Garlic or its extract shows preventive effects on atherosclerosis in animal (1-3) and clinical experiments (4, 5). Although the mechanism is not clearly defined, its hypocholestermic (3-6) and hypolipidemic (4, 6, 7) actions have been observed. The addition of garlic to a cholesterol diet lowers plasma lipoprotein levels (3), and garlic also reduces the incorporation of ¹⁴C-U-acetate and ¹⁴C-U-sucrose into the lipid fraction of liver and plasma (8). These findings suggest that garlic inhibits lipogenesis.

In experimental liver injury, carbon tetrachloride (CCl₄) enhances lipid peroxidation, which is the primary alteration of liver function, resulting in other changes such as accumulation of triglyceride in the liver and eventually central necrosis. It was recently reported that garlic extract inhibits thromboxane synthesis (9) and aggregation of human platelets in vitro (10-12) and that garlic oil inhibits platelet fatty acid oxygenases in vitro (13).

In this report, we studied the effect of garlic extract on CCl₄-induced liver injury by measuring lipids and their peroxide contents and found a therapeutic, but not preventive, effect of garlic extract on liver injury.

Materials and Methods

Animals: Male mice (4-week old, ICR strain, specific pathogen-free) were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and maintained on standard pellet food (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad lib for 7-10 days. Their average weight was approx. 26 g.

After 16 to 18 hr-fasting, the mice were divided in two groups. Between 9:00 and 10:00 a.m., one group was given orally CCl₄ at a dose of 4 ml/kg body weight (14), and the other group received same volume of water (control). Six hours later, they were given orally 10, 100 or 500 mg/kg of garlic.
extract, 25 mg/kg of vitamin E, or water (CCI₄ alone). Control animals were given 500 mg/kg of the extract, 25 mg/kg of vitamin E, olive oil or water. After CCI₄ administration, all the animals had free access to food and water.

The garlic extract was diluted to 1 ml with water and given to the animals, and vitamin E (α-tocopherol acetate) was diluted with olive oil.

Preparation of samples: Twenty-four hours after CCI₄ administration, the blood was collected from the posterior vena cava under light ether anesthesia, and the liver was excised, washed free of blood with cold saline, blotted dry, weighed, and then homogenized in ice-cold 0.15 M KCl (1:9, w/v).

Lipid extraction: To 1 volume of 10% homogenate, 8 volumes of a 2:1 mixture of chloroform and methanol (v/v) were added and shaken for 10 min under N₂ gas. Then 4 volumes of water were added and the mixture centrifuged at 3000 rpm for 10 min. An aliquot of the lower chloroform phase was evaporated under reduced pressure. The extract was analyzed for total lipids, conjugated-diene, and thiobarbituric acid (TBA)-reactive substances.

Assay: Conjugated-diene level in the hepatic lipid fraction was determined by spectral difference from the absorption of n-hexane at 233 nm (15). TBA-reactive substances in the liver homogenate and its lipid extract were measured by the method of Ohkawa et al. (16). Triglyceride levels of liver and plasma were measured using a triglyceride G-test Wako kit (Wako Pure Chemical Co., Ltd., Osaka, Japan) based on assay of the condensed product formed from 4-aminopyrine, p-chlorophenol, and H₂O₂ which was released from glycerol-3-phosphate after enzymatic hydrolysis of triglyceride (17). Protein and lipid contents were determined by the method of Lowry et al. (18) and by the gravimetric method, respectively. All the determinations were done in duplicate or triplicate. In some cases, two or three specimens which showed a similar lipid content in a group were mixed and used to determine hepatic triglyceride content.

Chemicals: The garlic extract was supplied by Hankyu-Kyoei Bussan Co., Ltd., Osaka, Japan. Extraction of garlic was done as follows: One kg of sliced garlic was extracted with one liter of 20% (v/v) ethanol for 3 months at room temperature, and then the filtrate was concentrated 7 times under reduced pressure below 40°C. The resulting solution was used as the garlic extract. The pH of the garlic extract was 4.0–4.5, and its moisture content was 31%. One g of the extract corresponds to the amount of extractive ingredients in approx. 5 g of raw garlic, so that dosage of the extract to mice is equivalent to 5 times the dose of raw garlic.

Statistics: The experimental data were expressed as the mean±S.E.M. in total amounts of liver, because there were no significant differences in liver weight among the CCl₄-treated groups. The weight, however, decreased to about 90% of the control group, due to poor food intake after CCI₄ treatment. The data of the control group were expressed as average values in all the control groups, i.e., water, olive oil, garlic extract or vitamin E alone-treated group, because there were no significant differences among them. Significant differences between CCl₄ alone and garlic extract or vitamin E were determined using Student’s t-test.

Results

Effect on conjugated-diene level: Recknagel and Ghoshal (15) demonstrated the presence of the conjugated-diene in liver, which resulted from lipid peroxidation in the membrane fraction at early intervals (2–4 hr) after CCl₄ poisoning.

As shown in Table 1, hepatic conjugated-diene level increased to 167% of that in the control (no CCl₄) even at 24 hr after CCl₄ administration. The increased level was significantly diminished to 82% and 86% by 100 mg/kg of garlic extract and 25 mg/kg of vitamin E, respectively. However, as mentioned in Statistics, they did not affect the conjugated-diene level in the normal liver (no CCl₄).

Effect on TBA level: As shown in Table 2, CCl₄ increased TBA values in liver homogenate and its lipid fraction to 740% and 380% of the control, respectively. The TBA value of the lipid fraction was significantly lowered.
Table 1. Inhibitory action of garlic extract and vitamin E on conjugated-diene formation in CCl₄-intoxicated liver

| Treatment          | Dose  | Conjugated-diene |
|--------------------|-------|------------------|
|                    | (mg/kg) | JE at 233 nm | % CCl₄ | N |
| No CCl₄            | —      | 6.27±0.31***    | —      | 38 |
| CCl₄ alone         | 4      | 10.47±0.52      | 100    | 17 |
| + Vitamin E        | 25     | 9.05±0.31*      | 88     | 20 |
| + Garlic extract   | 100    | 10.60±0.18      | 101    | 10 |
|                    | 500    | 8.57±0.41**     | 82     | 20 |

Garlic extract or vitamin E was given orally to mice at 6 hr after CCl₄ administration. The control group (no CCl₄) received 500 mg/kg of the extract, 25 mg/kg of vitamin E, or their vehicles without CCl₄ treatment. *P<0.05, **P<0.01 and ***P<0.001, significant different from CCl₄ alone.

Table 2. The level of thiobarbituric acid-reactive materials in CCl₄-intoxicated liver

| Treatment          | Dose  | Lipid fraction | Homogenate |
|--------------------|-------|----------------|------------|
|                    | (mg/kg) | μ moles MDA | % CCl₄ | μ moles MDA | % CCl₄ | N |
| No CCl₄            | —      | 0.59±0.04*** | —      | 0.70±0.05*** | —      | 38 |
| CCl₄ alone         | 4      | 2.23±0.24     | 100    | 5.15±0.30    | 100    | 17 |
| + Vitamin E        | 25     | 1.97±0.24     | 88     | 3.76±0.31**  | 73     | 20 |
| + Garlic extract   | 100    | 2.45±0.36     | 110    | 3.90±0.71    | 76     | 10 |
|                    | 500    | 1.53±0.17*    | 69     | 3.43±0.33*** | 67     | 20 |

MDA: malondialdehyde. See Table 1.

only by 100 mg/kg of garlic extract, where as the value in the homogenate was diminished by all the doses of the extract and vitamin E used in this experiment. Ten mg/kg of garlic extract showed an inhibitory effect on the increase of TBA value in the homogenate, but not in the lipid fraction.

In all fractions of the CCl₄-intoxicated liver, the TBA value in the homogenate was higher than that in the lipid fraction. Administration of garlic extract and vitamin E inhibited this amplification in the TBA value of the homogenate.

Correlation between TBA value and conjugated-diene level: It has been claimed that the TBA value does not accurately indicate the existing amounts of lipid peroxide in tissue, because the color development in the reaction with TBA is strongly affected by various cell components. However, in this experiment, a positive correlation was obtained between the conjugated-diene level and the TBA value in the lipid fraction (Fig. 1) or the homogenate (Fig. 2). Thus, the inhibitory action of garlic extract and vitamin E in vivo on lipid peroxidation was confirmed even in the assay of TBA value.

Effect on triglyceride level: Carbon tetrachloride caused the increase of triglyceride in the liver and its decrease in plasma (Table 3). The accumulation of triglyceride into liver was lowered by 100 and 500 mg/kg of garlic extract. Total lipid content in CCl₄-intoxicated liver was also decreased by them. In contrast, vitamin E, 25 mg/kg, could not lessen the increase in triglyceride and total lipid. On the other hand, plasma triglyceride level was slightly but not significantly increased by administration of 100 mg/kg of garlic extract.

The correlation between triglyceride and conjugated-diene levels in liver was examined with % changes of CCl₄ alone.
There was a good positive correlation between the lowering action of the extract on triglyceride accumulation and its inhibitory effect on lipid peroxidation in the CCl₄-intoxicated liver (Fig. 3). The values obtained in vitamin E-treated liver were not in agreement with this regression line.

**Discussion**

Garlic or its extract is reported to have hypocholesteremic (3–6) and hypolipidemic (4, 6, 7) effects and may have an inhibitory effect on the process of atherogenesis (1, 2). Practically all these effects are based on the preventive actions because garlic is administered together with a diet containing cholesterol or high-dose sucrose, which results in hypercholesteremia or hyperlipemia by feeding. In this report, we studied, in vivo, therapeutical application of garlic extract on the CCl₄ intoxicated liver as a model disease of liver injury.

**Table 3.** Effect of garlic extract and vitamin E on triglyceride and lipid contents in CCl₄-intoxicated liver

| Treatment       | Dose (mg/kg) | Triglyceride | Total lipids |
|-----------------|--------------|--------------|--------------|
|                 |              | Plasma (mg/dl) | Liver (mg) | Liver (mg) |
| No CCl₄         | —            | —            | —            | —          |
| CCl₄ alone      | 4            | 92.6± 4.7 (12) | 88.0±4.1 (19) | 139.7± 7.8 (19) |
| +Vitamin E      | 25           | 74.9± 3.9 (9)  | 86.7±4.2 (19) | 134.3± 7.3 (20) |
| +Garlic extract | 10           | 103.9±10.3 (9) | 89.1±8.8 (4)  | 122.6±14.1 (10) |
|                 | 100          | 110.1±13.8 (10)| 63.0±4.3 (15) | 109.6± 6.7 (20) |
|                 | 500          | 94.3±12.8 (11)| 69.3±4.1 (21) | 118.4± 7.3 (21) |

Figures in parentheses show numbers of experiments. See Table 1.
The hepatic lesion induced by \( \text{CCl}_4 \) is claimed to be as follows: The orally administered \( \text{CCl}_4 \) has been known to accumulate in hepatic parenchymal cells, to be metabolized and to yield free radicals. So, hepatic \( \text{CCl}_4 \) concentration reaches a maximum level within the first several hours and falls rapidly thereafter (19, 20). The free radicals in hepatic parenchymal cells attack the methylene bridge hydrogens of polyunsaturated fatty acids, especially in the microsomal fraction. The initial attack results in formation of a lipid radical and a concomitant shift of the double bond to the lower energy, conjugated-diene arrangement which can be detected by the intense absorption at 230–235 nm within 90 min after \( \text{CCl}_4 \) intoxication (15). The lipid radical reacts readily with molecular oxygen to produce a peroxyl radical.

Thus, lipid peroxidation, once initiated, advances autocatalytically and eventually hydroperoxide and/or endoperoxide are formed. Even at 24 hr later, the higher level of the lipid peroxidative process has been observed (21–23). The rapid accumulation of hepatic triglyceride has also been noticed at an early time (24–28) and the elevation observed to continue for 20 to 24 hr (26, 28).

In order to investigate therapeutic effects, namely, the recuperative effects of garlic on the liver already injured by \( \text{CCl}_4 \), we decided to administer the extract to mice at 6 hr later after \( \text{CCl}_4 \) poisoning and to study the biochemical changes, namely lipid peroxidation and triglyceride accumulation in the liver in the subsequent 18 hr.

Hepatic lipid peroxidation has been evaluated with various assay methods including direct methods that detect the absorbance at approx. 233 nm due to the conjugated-diene function by titration of the peroxide groups with iodine and indirect methods that measure the breakdown products of lipid peroxides, such as malondialdehyde from endoperoxides, ethane and pentane from hydroperoxides, and chemiluminescences produced by the further reaction of malondialdehyde with nitrogenous materials (29). In the present study, two methods, measurements of conjugated-diene and malondialdehyde, were employed to determine lipid peroxidation levels.

The high doses of garlic extract, 100 and 500 mg/kg, and 25 mg/kg of vitamin E inhibited therapeutically the increase in the conjugated-diene level (Table 1) and the TBA value (Table 2) in both the lipid fraction and tissue homogenate in \( \text{CCl}_4 \)-intoxicated liver, whereas they did not influence the normal levels. On the other hand, 10 mg/kg of garlic extract could lower only the TBA value in the homogenate.

It has been claimed that the TBA assay using tissue homogenate is not suitable for assessing lipid peroxidation in vivo. According to Mihara and Uchiyama (22), the TBA value is considered to indicate the existing amount of lipid peroxides and complex status of tissue peroxidation including several antioxidative and prooxidative factors. Hitherto, ascorbic acid, glutathione, selenium, or pyridine nucleotides, etc. have been reported as a water-soluble factor affecting the hepatic TBA value (30–33). In the present experiment, a water-soluble factor(s) caused amplification of the TBA value in the homogenate, and both garlic extract and vitamin E in vivo reduced this amplification (Table 2). The inhibition was observed even in a dose of 10 mg/kg of garlic extract. These results and findings suggest that garlic extract and vitamin E act, indirectly or directly, on such factor(s) and affect the peroxidative status in tissue.

In this experiment, we, however, found that the TBA value was positively correlated with the conjugated-diene level (Figs. 1 and 2). The latter is accepted as a direct parameter of lipid peroxide level in vivo. Thus, the inhibitory action of garlic extract and vitamin E in vivo on the enhanced lipid peroxidation was confirmed not only in the conjugated-diene level but also in the TBA value. We, therefore, consider that both garlic extract and vitamin E alter peroxidative status to a more reductive condition and also inhibit lipid peroxidation itself in liver, and they seem to act more prominently on amplification of TBA value than on lipid peroxide formation. In addition, the TBA assay appears to be useful and convenient as a parameter of lipid peroxidation in tissues.
to evaluate pharmacological efficacy of drugs, although this assay method using tissue homogenate may or may not reflect the lipid peroxidative status.

Carbon tetrachloride-induced fatty liver has been demonstrated to be associated with a decrease in hepatic triglyceride secretion (26, 27, 34–36) resulting from the inhibition of low-density lipoprotein synthesis (27, 36) and with enhancement of lipid synthesis (35). Eventually, lipids such as triglyceride accumulate in the liver, and, on the contrary, triglyceride decreases in plasma. In our present report, these findings and a slight decrease in hepatic protein content (a 5–8% reduction from the control) were confirmed after CC14 administration. Garlic extract in the doses of 100 and 500 mg/kg, but not 10 mg/kg, inhibited the increase of hepatic triglyceride content caused by CC14. Conversely, plasma level tended to increase after administration of garlic extract, and especially, after the 100 mg/kg dose, the level became not significantly different from the normal level (no CC14). From the balance of triglyceride concentration between the liver and plasma, it is thought that garlic extract affects triglyceride secretion from the liver to the plasma and its synthesis in the liver. Chang and Johnson (8) observed that dietary garlic reduced hepatic lipogenesis by inhibiting the incorporation of carbon moiety into lipids. In comparison with garlic extract, vitamin E had no action on triglyceride accumulation in liver, but yet furthered its decrease in plasma.

There was a highly significant correlation between conjugated-diene level and triglyceride accumulation in all the groups, with the exception of the vitamin E group (Fig. 3). It is clear that 10 mg/kg of the garlic extract is ineffective on both triglyceride accumulation and lipid peroxide formation, whereas either 100 or 500 mg/kg of garlic extract has a therapeutic effect on both of them. Moreover, the result shown in Fig. 3 suggests that it is possible that garlic extract may act on other site(s) besides that of vitamin E. The recovering mechanism of garlic and vitamin E remains to be ascertained when they are used therapeutically. In the case of administration prior to injury, however, vitamin E is generally acknowledged to function as a singlet oxygen quencher and radical scavenger in the lipid peroxidative process.

The characteristic components of garlic were reported to be as follows: In the aqueous fraction, alliin and sulfur-containing amino acid derivatives such as S-allyl cysteine, S-allyl mercapto cysteine, S-propyl cysteine and S(1-propylenyl)-cysteine were reported. Diallyl disulfide and diallyl trisulfide were found in the water-insoluble fraction. Allicin was observed as an enzymatic fission product of alliin.

However, only a few relationships between garlic constituents and their pharmacological effects have been investigated. It was recognized that volatile unsaturated oils of garlic, diallyl disulfide and allyl propyl disulfide, have hypolipidemic and hypocholesteremic effects in both serum and liver (6). Ariga et al. (11) noticed that methyl allyl trisulfide, which has been isolated from garlic oil as a minor component, showed strong inhibitory effect on platelet aggregation in vitro, and the main constituents of garlic oil, diallyl disulfide and diallyl trisulfide, had less effect than methyl allyl trisulfide. This effect has been claimed to be through interfering with thromboxane synthesis (9) and thromboxane oxygenases (13). Naito et al. (37) reported that disulfides, which were separated from the ethanolic extract of boiled garlic by ion exchange column chromatography, showed antioxidative activity against peroxidation of linoleic acid, and this effect was additive to that of d-δ-tocopherol. Allicin has been known to show antibacterial action, whereas there are very few accounts in the literature of the aqueous ingredients. Although enhancement of vitamin B1 availability by allicin was observed, it seems that more detailed examinations are required to verify this effect. It is possible that sulfur-containing amino acid derivatives may mimic the action of methionine since this amino acid has been reported to function as a free radical scavenger in injured liver (38–40).

However, at the present time, the causal relationship between constituents of garlic and the therapeutical effects pointed out in
this report remains obscure. We would like to study in more detail the relationships between the pharmacological effects and components of garlic in not only the oily fraction but also the aqueous fraction because garlic was extracted with a relatively low concentration of ethanol.

In summary, we conclude that although vitamin E has only an inhibitory action on lipid peroxidation, the high dose of garlic extract is effective not only on suppression of lipid peroxide formation and reduction of peroxidative status but also on inhibition of triacylglyceride accumulation in the injured liver. However, these effects in the case of the 500 mg/kg dose would seem to be influenced by toxicity of the extract, because they were lesser than those of the 100 mg/kg dose. Furthermore, since the latter action for lipid peroxidation is detectable even at the 10 mg/kg dose, these two actions of garlic extract appear to be caused separately by at least two components in the extract used in this experiment. So, we conclude that the 10 mg/kg dose may be the critical level to show therapeutic effects in injured liver, and the safety range seems to be some ten times the critical dose.

Acknowledgments: We should like to express our gratitude to President Toshihiro Yamanaka for his constant encouragement and Mr. Shin-ichi Hasegawa for his technical assistance.

References
1 Jain, R.C.: Effect of alcoholic extract of garlic in atherosclerosis. Am. J. Clin. Nutr. 31, 1982–1983 (1978)
2 Sainani, G.S., Desai, D.B., Natu, M.N., Katrodia, K.M., Valame, V.P. and Sainani, P.G.: Onion, garlic, and experimental atherosclerosis. Japan. Heart J. 20, 351–357 (1979)
3 Kamanna, V.S. and Chandrasekhar, N.: Effect of garlic (Allium sativum Linn) on serum lipoproteins and lipoprotein cholesterol levels in albino rats rendered hypercholesteremic by feeding cholesterol. Lipids 17, 483–488 (1982)
4 Jain, R.C.: Effect of garlic on serum lipids, coagulability and fibrinolytic activity of blood. Am. J. Clin. Nutr. 30, 1380–1381 (1977)
5 Sainani, G.S., Desai, D.B., Gorhe, N.H., Natu, S.M., Pise, D.V. and Sainani, P.G.: Effect of dietary garlic and onion on serum lipid profile in Jain community. Indian J. Med. Res. 69, 776–780 (1979)
6 Adamu, I., Joseph, P.K. and Augusti, K.T.: Hypolipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two-month period. Experientia 38, 899–901 (1982)
7 Chi, M.S.: Effects of garlic products on lipid metabolism in cholesterol-fed rats. Proc. Soc. Exp. Biol. Med. 171, 174–178 (1982)
8 Chang, M.L.W. and Johnson, M.A.: Effect of garlic on carbohydrate metabolism and lipid synthesis in rats. J. Nutr. 110, 931–936 (1980)
9 Makheja, A.N., Vanderhoek, J.Y. and Bailey, J.M.: inhibition of platelet aggregation and thromboxane synthesis by onion and garlic. Lancet 1, 781 (1979)
10 Bordia, A.: Effect of garlic on human platelet aggregation in vitro. Atherosclerosis 30, 355–360 (1978)
11 Ariga, T., Oshiba, S. and Tamada, T.: Platelet aggregation inhibitor in garlic. Lancet 1, 150 (1981)
12 Samson, R.R.: Effects of dietary garlic and temporal drift on platelet aggregation. Atherosclerosis 44, 119–120 (1982)
13 Vanderhoek, J.Y., Makheja, A.N. and Bailey, J.M.: Inhibition of fatty acid oxygenases by onion and garlic oils: Evidence for the mechanism by which these oils inhibit platelet aggregation. Biochem. Pharmacol. 29, 3169–3173 (1980)
14 Di Luzio, N.R. and Costales, F.: Inhibition of the ethanol and carbon tetrachloride induced fatty liver by antioxidants. Exp. Mol. Pathol. 4, 141–154 (1965)
15 Recknagel, R.O. and Ghoshal, A.K.: Quantitative estimation of peroxidative degeneration of rat liver microsomal and mitochondrial lipids after carbon tetrachloride poisoning. Exp. Mol. Pathol. 5, 413–426 (1966)
16 Ohkawa, H., Ohishi, N. and Yagi, K.: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358 (1979)
17 Spayd, R.W., Bruschi, B., Burdick, B.A., Dappen, G.M., Eikenberry, J.N., Esders, T.W., Figueras, J., Goodhue, C.T., LaRossa, D.D., Nelson, R.W., Rand, R.N. and Wu, T.-W.: Multilayer film elements for clinical analysis: Applications to representative chemical determinations. Clin. Chem. 24, 1343–1350 (1978)
18 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
19 Baccino, F.M., Satta, M. and Mameli, L.: Therapeutic Effect of Garlic Extract
Distribution of CCl₄ among liver cell fractions. Biochim. Biophys. Acta 90, 606–608 (1964)

20 Recknagel, R.O. and Litteria, M.: Biochemical changes in carbon tetrachloride fatty liver: Concentration of carbon tetrachloride in liver and blood. Am. J. Pathol. 36, 521–531 (1960)

21 Comporti, M., Saccocci, C. and Dianzani, M.U.: Effect of CCl₄ in vitro and in vivo on lipid peroxidation of rat liver homogenates and subcellular fractions. Enzymologia 29, 185–204 (1965)

22 Mihara, M. and Uchiyama, M.: Evaluation of thiobarbituric acid (TBA) value as an index of lipid peroxidation in CCl₄-intoxicated rat liver. Yakugaku Zasshi 101, 221–226 (1981) (in Japanese)

23 Yoshikawa, T., Furukawa, Y., Murakami, M., Takemura, S. and Kondo, M.: Effects of vitamin E on D-galactosamine-induced or carbon tetrachloride-induced hepatotoxicity. Digestion 25, 222–229 (1982)

24 Schotz, M.C. and Recknagel, R.O.: Rapid increase of rat liver triglycerides following carbon tetrachloride poisoning. Biochim. Biophys. Acta 41, 151–152 (1960)

25 Rubinstein, B. and Rubinstein, D.: The effect of carbon tetrachloride on hepatic lipid metabolism. Can. J. Biochem. Physiol. 42, 1263–1273 (1964)

26 Schotz, M.C., Baker, N. and Chavez, M.N.: Effect of carbon tetrachloride ingestion on liver and plasma triglyceride turnover rates. J. Lipid Res. 5, 569–577 (1964)

27 Lombardi, B. and Ugazio, G.: Serum lipoproteins in rats with carbon tetrachloride–induced fatty liver. J. Lipid Res. 6, 498–505 (1965)

28 Stern, P.H., Furukawa, T. and Brody, T.M.: Rat liver and plasma lipids after carbon tetrachloride administration. J. Lipid Res. 6, 278–286 (1965)

29 Corongiu, F.P., Lai, M. and Milia, A.: Carbon tetrachloride, bromotrichloromethane and ethanol acute intoxication: New chemical evidence for lipid peroxidation in rat tissue microsomes. Biochem. J. 212, 625–631 (1983)

30 Bernheim, M.L.C.: The effect of carbon tetrachloride, ethionine and chloretone on the ascorbic acid content of rat liver. Biochem. Pharmacol. 7, 59–64 (1961)

31 Mihara, M., Uchiyama, M. and Yamane, Y.: Effect of the addition of biological antioxidants on the thiobarbituric acid (TBA) reaction. Yakugaku Zasshi 103, 889–894 (1983) (in Japanese)

32 McCay, P.B., Gibson, D.D. and Hornbrook, K.R.: Glutathione-dependent inhibition of lipid peroxidation by a soluble, heat-labile factor not gultathione peroxidase. Fed. Proc. 40, 199–205 (1981)

33 Tam, B.K. and McCay, P.B.: Reduced triphosphopyridine nucleotide oxidase-catalyzed alterations of membrane phospholipids. III. Transient formation of phospholipid peroxides. J. Biol. Chem. 245, 2295–2300 (1970)

34 Recknagel, R.O., Lombardi, B. and Schotz, M.C.: A new insight into pathogenesis of carbon tetrachloride fat infiltration. Proc. Soc. Exp. Biol. Med. 104, 608–610 (1960)

35 Maling, H.M., Frank, A. and Horning, M.G.: Effect of carbon tetrachloride on hepatic synthesis and release of triglycerides. Biochim. Biophys. Acta 64, 540–545 (1962)

36 Seakins, A. and Robinson, D.S.: The effect of the administration of carbon tetrachloride on the formation of plasma lipoproteins in the rat. Biochem. J. 86, 401–407 (1963)

37 Naito, S., Yamaguchi, N. and Yokoo, Y.: Fractionation of antioxidant extracted from garlic. Nippon Shokuhin Kogyo Gakkaishi 28, 465–470 (1981) (in Japanese)

38 Miller, L.L., Rogers, C.A., Lamson, B.G. and Lambie, M.W.: Protein depletion and hepatic function in the dog: Rationale of methionine in therapy. Am. J. Med. Sci. 214, 84–88 (1947)

39 Miller, L.L., Ross, J.F. and Whipple, G.H.: Methionine and cystine, specific protein factors preventing chloroform liver injury in protein-depleted dogs. Am. J. Med. Sci. 200, 739–756 (1940)

40 Miller, L.L. and Whipple, G.H.: Liver injury, liver protection, and sulfur metabolism: Methionine protects against liver injury even when given after anesthesia. J. Exp. Med. 76, 421–435 (1942)