CHAPTER 4

DISCUSSION AND CONCLUSION

The effects of 80% ethanol extract of lemon grass on antimutagenicity were examined in a Salmonella mutation assay. Lemon grass extract by itself had no mutagenic effect (Lomsri, 1993), but showed an inhibitory effect on the mutagenesis of some known mutagens in this study.

Lemon grass extract inhibited mutagenicity of AFB_1, a mutagen requiring activation by cytochrome P-450 dependent system, as well as some pyrolysate mutagens; such as Glu-P-1, Glu-P-2 and IQ, in a dose dependent manner. The results suggest that some active compounds in lemon grass may either deactivate these mutagens by directly trapping or deactivate involved enzymes in S9 mix. The antimutagenic effect of lemon grass on these mutagens may be due to prevention of the N-hydroxylation reaction. A major metabolic pathway in the presence of S9 mix of amino acid pyrolysates occured via N-hydroxylation (Hargraves, 1987; Hirayama, et al., 1989; Sato, 1986)

The enhancement of mutagenicity of Trp-P-1 and Trp-P-2 at a low concentration of lemon grass extract was observed. But at a high concentration, it could inhibit mutagenicity of these mutagens. These biphasic effects may be due to a decrease in the actual available amount of S9 by this extract. The same effect was also reported with the pine cone extract by Lee, et al (1993) and the active compound which may play a role was suggested to be lignin-related compound in pine cone extract (Oh-hara, et al, 1990; Sakagami, et al, 1990). A lignin-related compound might be one promising compound which may be responsible for

activity in lemon grass extract.

However, lemon grass extract cannot inhibit mutagensis induced by B(a)P. A promutagen form of B(a)P which is activated by cytochrome P-450 dependent mix function oxidase, namely P4501AI or P-448, which differs from the metabolic activation pathway of AFB_1. Therefore, it was suggested that the extract might not be able to influence this activation system.

Mutagenicity of direct acting mutagens, MNNG and AF-2, was inhibited by lemon grass extract. It was suggested that some active compounds in lemon grass may act directly on mutagens .

According to the terminology proposed by Kada and his colleages (Kada, et al,1986) lemon grass might contain desmutagens which inactivate mutagens before attacking DNA. Desmutagenicity of lemon grass may be due to the blocking or trapping of mutagens from DNA or may be involved in deactivated enzymes in cytochrome P-450 mixed function oxidase, but it could not deactivate enzymes in the cytochrome P-448 system.

The bioantimutagenicity of lemon grass was not investigate in this study, however, it has been found that the bioantimutagenicity of lemon grass was less active than desmutagenicity (Lomsri,1993).

The data obtained from Ames test showed higher variability than was expected from probabilistic arguments. Different variations occured from different sources (Edler,1992) such as genetically different tester strains, difference in: incubation time, culture time, culture conditions, number of spontaneous revertants, amount of histidine on plate, type of medium, amount of agar, solvents, S9 mix, cell densities, and unspecific laboratory effects.

In vitro cytotoxicity experiments indicated that lemon grass extract inhibited cancer cells growth to 50% of the control growth (ED_{50}) at a concentration of 401.52 µg/ml for murine lymphocytic leukemia cell (P388 cells) and at a concentration of 87.46 µg/ml for murine ascites mammary carcinoma cells (FM3A cells), whereas ED_{50} of 5-FU, a known anticancer drug, was only 0.06 and 0.008 µg/ml, respectively. Comparing 5-FU and lemon grass extract in terms of ED_{50}, 5-FU was 670 and 11,000 fold more active than lemon grass extract for P388 and FM3A cells, respectively. Cancer cells could be killed by using high dosages of lemon grass, thus, it was suggested that lemon grass extract had no cytotoxicity according to the modified criteria from National Cancer Institute protocol (Geran, et al,1972 ; Robert and Perdue, 1982).

In vivo testing for anti-tumor activity is time-consuming and costly. Mice model transplanted with fibrosarcoma needs a shorter time than other models such as, chemical induced carcinogenesis. The method of preparation is not complicated and is inexpensive. In vitro prescreening, such as cytotoxicity against cancer cells, was suggested and murine lymphocytic leukemia has been employed to facilitate the activity-directed separation process if the in vivo activity is coincidental with the in vitro cytotoxicity. However, this approach may miss antitumor compounds which are not cytotoxic. Thus, lemon grass extract was further studied in this model.

The protocols for studying the antitumor effects of lemon grass extract in mice transplanted with fibrosarcoma were divided into 2 protocols. Protocol 1 was designed for administration of the extract orally 10 days daily after tumor transplantation. In protocol 2, the extract was administered daily for 7 days before

and 10 days after transplantation. The mice in each group were weighed every day during the experiment. It was found that there was no significant difference in the weight of the two groups of mice during the experiment. Twenty days after tumor transplantation, their weight decreased. The decrease in weight may be duo to cachexia from cancer (Balducci and Hardy, 1985). Fibrosarcoma cells which were injected into mice muscularly or subcutaneously were prepared from a strain of mouse in which fibrosarcoma cells had originated spontaneously. Because of the high susceptibility to the induction of fibrosarcoma, tumor was always observed in C3H mice when injected with tumor cells into target tissue such as muscle. All the mice in the experiment developed tumors in their right hind legs about 7-10 days after tumor transplantation. One parameter for evaluating in an animal antitumor model is the survival time or number of survival days of the animal after it was transplanted with cancer cells. It was found that, the untreated control animals survived 25.87 days in the case of protocol 1 and 25.7 days in protocol 2. The treated groups survived 24.62 days in protocol 1 and 25.7 days in protocol 2. The T/C value of both protocols was 95 and 100, respectively. Extract of lemon grass did not increase the life span of fibrosarcoma bearing mice, although it was administrated before tumor transplantation. However, murine fibrosarcoma may be an aggressive tumor model, and the survival time of mice short. Further study should be done with more diluted fibrosarcoma cell concentration.

Fibrosarcoma most frequently developed within subcutaneous and muscular tissue (Stewart, 1974). It was white, grey or pink, firm , and shaped somewhat spherically. Leg tumors were assessed for necrotic tissue and classified into

4 degrees (See Table 7). The disappearance of tumor necrosis in leg tumors of the mice receiving lemon grass extract after tumor transplantation (protocol 1) was more than in the mice fed only control diets. The result was similar to mice in protocol 2. Furthermore, the tumors of mice fed lemon grass extract in both protocols tended to be less severe than the mice fed only control diets. Also the tumors were less in diameter than in the control groups. It seems that lemon grass affords some retardation of tumor growth.

Only lung metastases from primary leg tumors have been observed in fibrosarcoma bearing mice (Ando, et al,1983) and also in this study. Mice receiving lemon grass extract developed fewer severe metastases than the control mice of both protocols. The effect of giving lemon grass after tumor transplantation was compared with that of giving before and after, it was found that lung metastases in the latter group was reduced more than in the former group. The frequency of metastases varied depending on the rapidity of growth and the length of survival of animals following the appearance of tumors (Stewart, 1974). In this study, the survival time between the treated groups and control groups did not differ, but the correlation between tumor necrosis and lung metastases was observed. The reason for the reduction of lung metastases by lemon grass may be due to the reduction in the growth rate of established primary tumors in the legs of mice receiving lemon grass extract, reducing the pool of tumor cells free to disseminate (Ando, et al, 1983). Although lemon grass extract did not prolong the life span of mice, it seemed to delayed tumor growth and reduce lung metastases. Furthermore, administration of lemon grass before transplantation gave a better result than administration after. To understand exact mechanism of antitumor

compounds in lemon grass, the stimulation of the immunological system should be investigated, such as killer suppressor T cells, tumor necrosis factors, interleukins, etc. Further examination of the chemoprotective activity of lemon grass in other chemical carcinogenesis models could provide more reasonable explanations for the development and assessment of chemoprotective investigations.

Partial purification of antimutagenic substances in lemon grass extract was performed by Sephadex LH-20 chromatography. All active antimutagenic principles still decreased mutagenesis of AFB_1, Glu-P-1, Glu-P-2, IQ and MNNG. However, they could not inhibit mutagenesis by B(a)P and AF-2. The mechanism of partially purified antimutagens may act as desmutagen, which is the same as crude extract. The fractions have no cytotoxic effects on both cell lines, as well as their crude extract. Interestingly, only fraction No 4 inhibited the mutagenicity of Trp-P-1 and Trp-P-2. Compared with the crude extract in the same concentration, crude extract did not inhibit mutagenesis of both mutagens whereas fraction No 4 did. Thus, compounds in fraction No 4 may be a major antimutagen inhibited mutagenesis of Trp-P-1 , Trp-P-2 and other mutagens in this study. Further isolation of fraction No 4 was investigated by HPLC, the active compound was the last eluate. After characterizing its structure by GC-MS, it was shown that its molecular weight was about 355. The common compounds present in lemon grass usually have a molecular weight of not more than 300 such as citral (mw = 152), myrcene (mw = 136), limonene (mw = 136). Investigating according to NAPRALERT(SM), one compound in lemon grass, chlorogenic acid, has a molecular weight almost equal to unidentified compound in this study. Lignin related compounds may be another promising active principle in lemon grass

extract. Some studies have reported that many substances in lignin such as phenylpropanoid, caffeic acid, and *p*-coumaric acid inhibit the mutagenicity of AFB_1 (San and Chan, 1987) and B(a)P (Sakai, et al, 1990) in the Ames test. Therefore, fraction 4.V of lemon grass extract might be or have a structure like chlorogenic acid (phenylpropanoid). However, the exact structure needs further identification.

The optimal way for dealing with virtually all disease is prevention, and this certainly is the case for cancer. If the disease cannot be prevented, then the second goal is effective therapy. In reality, all cancer reseach is directly part of these two goals.

Chemoprevention can be defined as the use of one or several chemical agents to prevent the occurrence of cancer. It is a preventive strategy. Chemopreventive agents can be categorized into two major groups, blocking agents and suppressing agents. Blocking agents prevent cancer producing compounds from reaching or reacting with critical target sites. They exert a barrier function. Suppressing agents prevent the evolution of the neoplastic process in cells which would otherwise become malignant (Wattenberg, 1985; Wattenberg, 1993).

In experiments the two can generally be separated by the fact that blocking agents are effective when administered prior to or simultaneously with cancer producing compounds. In some instances, a compound will have both blocking and suppressing action. Apart from cancer, mutagenesis causes many degenerative diseases such as heart disease, aging (Ames and Gold, 1991), thus lemon grass extract might also be used to prevent these conditions. Furthermore, studies suggest that the predicted cancer chemoprotective activity of lemon grass is

found in the Salmonella mutation test, decreasing tumor development and metastases before and after carcinogenesis. Lemon grass might act both as a blocking and suppressing agent.

Beside the induction of mixed function oxidase activities, the enhancement of several phase II enzymes such as glutathione-S-transferase (GST), UDP-glucuronyl transferase and DT-diaphorase due to dietary exposure to cruciferous vegetables has been described (Aspry and Bjeldanes, 1983; Bogaerds, et al,1990; Salbe and Bjeldanes, 1986). Lemon grass has also enhanced GST activity in mouse intestine (Luke and Boling, 1991). The enhancement of these mainly detoxifying enzyme systems is associated with a decrease in tumorigenesis similar to that reported for the antioxidants butylated hydroxyanisole and butylated hydroxytoluene (Kensler, et al, 1985)

Lemon grass seems to have several advantages as a chemoprotective agent. First, lemon grass extract completly inhibits mutagenesis of many mutagens in the Salmonella mutation system. A second advantage of lemon grass is that it inhibits chromosomal abberration in human lymphocytes exposed to mitomycin C (Meevatee, et al, 1993) and inhibits micronucleus formation in rats exposed to cyclophosphamide (Pinseang, 1993). A third advantage of lemon grass may be its retardation of tumor growth and lessening the degree of tumor metastases in this investigation. A forth advantage of lemon grass may be its safety. Pharmacological studies have revealed of lemon grass is be nontoxic (Carlini, et al, 1986; Souza, et al, 1986). A fifth advantage of lemon grass is that it contains a large group of chemical substances, some of which appear to be effective in antimutagenesis, such as caffeic acid and *p*-coumaric acid (San and Chan, 1987;

Sakai, et al,1990). Thus the prospect that development and assessment of chemoprotective agents from lemon grass seems reasonable. Therefore, chemoprotective agents from lemon grass could be administered to high-risk, but otherwise healthy individuals improve to their quality of health.