Nutraceuticals & Food
Vol. 8, p. 96~104 (2003)

Nutraceuticals and Food

Antioxidant and Immunoenhancement Activities of Ginger (*Zingiber officinale* Roscoe) Extracts and Compounds in *In Vitro* and *In Vivo* Mouse and Human System

– Review –

F Zakaria-Rungkat†, Nurahman*, E Prangdimurt and Tejasari**

Department of Food Technology and Human Nutrition, Bogor Agricultural University, Indonesia
**Nutrition School, Semarang, Indonesia*
***Faculty of Agricultural Technology, University of Jember, Jember, Indonesia*

Abstract

Ginger (*Zingiber officinale* Roscoe) is traditionally used as appetite enhancer, improver of the digestive system, antithusive, anti-cold, antipyretic, analgesic, and antiinflammation. *In vitro* evaluation using human lymphocyte cultures showed almost similar indication with those in *in vivo* mouse study. NK cell lysing activity was improved significantly. Proliferation activity of B and T cells, and $CD3^+$ and $CD3^+CD4^+$ T cell subset were better observed using oleoresin or gingerol and shogaol fractions. Although there were higher activities in gingerol, the improvement was almost equal to that by oleoresin. Shogaol did not show better improvement except at higher concentration. It could be concluded that treatment with single bioactive compound, such as gingerol, did not show significant effects compared to oleoresin, the crude extract. In human study, involving healthy male adult, the improvement of NK cell lysing activity was again demonstrated and even more apparent. The mechanism involved in the protection seemed to be through the antioxidant activity of gingerol. However, other mechanism underlying the improvement of NK cell lysing activity must be involved since this improvement seemed to be specifically toward NK cell activity. Since NK cells are specific for the elimination of virus-infected cell and mutated cells, this positive effect on the immune system are very interesting. This work has also scientifically proved that the traditional beliefs that ginger had preventive effects on common cold appeared to be reasonable.

Key words: ginger extract, traditional drink, immunomodulator, lymphocytes, natural killer cell, antioxidant

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) has been used as a raw material in many traditional medicinal preparations since the ancient time. As a component of traditional health products, ginger is known to be effective as appetite enhancer, improver of the digestive system, antitussive, anti-cold, antipyretic, analgesic, and antiinflammation (1-3). Many chemical and health researches have focussed the attention on the chemical compounds of ginger and their relationship with the health effects produced. The analgesic, antipyretic and sleeping time prolongation properties have been associated with (6)-gingerol or (6)-shogaol, the major bioactive compounds in ginger (1). (6)-Shogaol was more effective as intestine decontractor than (6)-gingerol and was more powerful than the antitussive drug, dihydrocodein (2,4). The analgesic and antiinflammation effects of ginger was due to its ability in inhibiting prostaglandin biosynthesis (2). It was also shown that some of the bioactive compounds in ginger had stronger effect than the antiinflammation drug, endometasin. Both gingerol and shogaol function as antihepatotoxic against CCl_4 and galactosamine (5).

The present research results of the bioactive compounds found in ginger lead to the assumption that the bioactive compounds posses antioxidant activity *in vivo*. Most of these bioactive compounds are components found in the oleoresin fraction of ginger oil. The non-volatile fraction of the oleoresin contains 4 types of gingerol and 4 compounds of diarylheptanoid having strong antioxidant activity *in vitro*. All 8 compounds had stronger antioxidant activity than α-tocopherol, but weaker than that of the synthetic antioxidant BHT. The diarylheptanoid compounds had stronger antioxidant activity than gingerol compounds (6).

The evidence of bioactive compounds in ginger having various effects against many health disorders had led to the test of their effects on the immune system (7). Using mouse spleen lymphocytes, it was found that ethanol extracts, which contain oleoresin fraction of ginger, showed stimulation activity on the lymphocytes. It was also shown that the extracts reduced the percentage of cell death in cultures and concluded that the antioxidant activity of compounds in the oleoresin might be responsible for the pro-

†Corresponding author. E-mail: zakaria@bgr.centrin.net.id
Phone: +62-251-626725, Fax: +62-251-626725

rection against the cell death *in vitro*.

Proof of the immunostimulation activity of ginger extract is interesting considering the common traditional belief that ginger preparations exert protection against common cold, especially that occurs after a long hard work or extra physical activity. Influenza virus most probably causes the condition that produces the common cold at the time when the body immune system is low. In this research review, we provide more research results on the effects of ginger extract on the immune system using *in vitro* techniques, *in vivo* mouse experiments and human subjects.

PROTECTIVE EFFECT OF GINGER EXTRACTS ON MICE IMMUNE SYSTEM AGAINST PARAQUAT

The evidence of antioxidant activity of ginger bioactive compounds (6) and their protective effect toward mouse spleen lymphocyte *in vitro* (7) lead to the hypothesis that the mechanism of the protective action was through the antioxidant activity of ginger ethanol extract exerted in the cell culture medium. To find out whether the antioxidant activity equally functions *in vivo*, four groups of mice aged 8 weeks (n = 6) were given standard laboratory diet and one group was given distilled water as drinking water ad libitum (control group). The second group (G group) received drinking water containing ginger water extract (0.2% total solid materials). The third group (GQ group) received the same drink as the second group and weekly intraperitoneal injection with 1 ml paraquat solution (10.4 mg/kg bw). The fourth group (Q group) received distilled water and intraperitoneal injection as for the third group. At the end of the 6 week experimental period, the mice were sacrificed, sterilized in 70% alcohol solution and the liver and spleen were taken out aseptically. The livers were weighted and kept in the freezer (-30°C) less than two weeks until used. Spleens were crushed and the cells were isolated by centrifugation at 600 g (8). The resulting splenocyte suspensions were allocated for the proliferation activity test, the percentage of $CD3^+$ subset T cell, the percentage of $CD3^+CD4^+$ subset T cells, NK cell lysing activity and malonaldehyde (MDA) content. All tests were performed immediately after the sacrifice. For the proliferation activity test, spleenocyte suspension of 10^6 cell/mL in standard RPMI-1640 solution containing glutamine and gentamicine were distributed 100 µL into microculture plate wells. Into the wells were added 20 µL of fetal calf serum (FCS). In addition, three wells of each mice group were added 80 µL of medium, or 80 µL of 100 mM paraquat solution, or 80 µL of ginger water extract (GWE) with total solid material (TSM) of 0.25% containing compounds with molecular weight less than 8 kD, or 80 µL concanavalin A solution (12.5 µg/mL), or 80 µL of *S. typhii* lipopolysaccharide (LPS) solution (12.5 µg/mL) (8). After 30 hr of incubation at 37°C at 5% CO_2, H^3-thymidine was added in the wells intended for the proliferation tests and incubation was continued up to 48 hr, then the cells were harvested and counted in β-counter for the radioactivity count. The percentage of $CD3^+$ and $CD3^+CD4^+$ T cell subset were analyzed using antimouse CD3 conjugated with R-phycoerythrin and antimouse CD4 conjugated with FITC. Both antibodies were from Sigma. Washed isolated cells were incubated with either antibody and measured with a flow cytometer using FACScan Research Software version 2.1 (9). Lysing activity of NK cell was measured by incubating the isolated cells with mouse leukemic cell line (YAC-1) as the target cells. The cells were mixed at the ratio of effector cells to target cells of 100 : 1 and 50 : 1. Before incubation, the target cells were tagged with H^3-thymidine overnight. After 4 hour incubation at 37°C and 5% CO_2, cells were harvested using Gelman cell-harvester membrane and the harvested cells were measured in β-counter to obtain the counts of the radioactive left. Results were expressed as the percentage of lyzed cells. Cell and liver MDA was measured according to a proposed method and calculated based on the cells protein content (10,11).

Table 1. Proliferation of mouse lymphocytes in cell cultures in the presence of ginger water extracts, paraquat, or mitogens concanavaline A (Con A) or *S. typhii* lipopolysaccharide (LPS) (5 µg/mL)

Condition of mouse lymphocyte culture	Mouse groups			
	Control	Receiving ginger (G)	Receiving paraquat (Q)	Receiving G and Q
Culture media only	364.8^{a}	464.1^{b}	324.6^{a}	442.2^{b}
Paraquat 40 µM	221.0^{ab}	271.3^{ab}	191.3^{a}	308.8^{b}
Ginger water extract	358.2^{ab}	523.4^{c}	312.2^{a}	454.3^{bc}
Paraquat 40 µM				
Concanavalin A	1133.0^{ab}	1426.2^{b}	877.3^{a}	1232.5^{ab}
S. typhii LPS	839.8	891.5	657.0	849.4

The mice had received ginger water extract for six weeks with or without intraperitoneal injection of paraquat (10.4 mg/kg bw). Data are presented as counts per a minute (cpm) of H^3-thymidine incorporated into the cells during the 96 hour culture. Superscript letters indicate significant different at $p<0.01$.

The results of the analysis in Table 1 showed that proliferation activities of cells from all groups of mice cultured in standard medium were significantly effected by ginger drink ($p < 0.05$). The effect of paraquat treatment, that was intended to cause damage of the immune cells, was not significant. This might be due to the low dose given to the mice and the short exposure time that were not sufficient to cause cellular damage to the immune system, although the dose used had reached 1/3 of the lethal dose. Doses higher than the dose given (12.5 mg/kg bw) caused death of 30 percent of the mice. When the cells of the treated mice were cultured in the presence of ginger water extract, paraquat or mitogens, there were indications of protective effect of ginger against paraquat and more responsive effect from mice drinking ginger. It could be assumed that drinking ginger by mice increased protection of immune cells against paraquat. These results show similarity with those obtained previously (5) demonstrating the ability of ginger extract in protecting hepatocytes against CCl_4.

Both paraquat and CCl_4 are known as xenobiotic compounds that are detoxified in the liver. Depending on the physiological condition encountered by the xenobiotic compounds during the exposure period, reactive metabolites could be produced and in turn could entail cellular damage (12). Paraquat is known to produce superoxide radical and had been shown to react with the flavoprotein from NADPH of cytochrome-P-450 reductase (13). In this research, we hypothesized that through intraperitoneal injection, the paraquat would be directly absorbed in the blood circulation without going through the intestinal digestion and that this absorbed paraquat would directly meet the blood cells, including the immune cells and hence might cause direct cellular damage that could be detected from spleen lymphocytes. This direct cellular damage had been shown in human blood lymphocytes and measurement of MDA (14). The authors stated that in mammalian cells, paraquat was transformed by the enzyme P-450 NADPH reductase. However, these *in vitro* phenomena might not be exactly the same as the *in vivo* system, where blood circulation might have reduced contact of paraquat and the cells, and hence reduced the possibility of paraquat absorption by the cells. It is also possible that the paraquat absorbed in intact form was not transformed to the radical superoxide in plasma or the damage of blood lymphocyte cells cannot be detected in the spleen cells. This phenomenon needs further investigation.

If drinking ginger extract by mice did not show damage on the immune cells proliferation activity, the stimulation effect on the NK cells lysing activity was highly improved ($p < 0.01$). As shown in Table 2, the lysing activity of NK cells from mice given ginger extract significantly increased compared to that of control and mice receiving paraquat. When ginger drink was given to the group of mice receiving intraperitoneal injections of paraquat, the NK activity was brought back to the same level as that of the control group, although did not attain the level of the group receiving ginger drink alone.

NK cells are known to act on virus-infected and mutated cells. The mechanism of NK cells action in lysing their target cells includes enhancement of cytotoxic activity and increase of IFN-γ production, thereby promoting other immune cells activity, such as the cell response. In the case of immunopotentiating activity reports of various functional foods such as Japanese sake, the bioactive compound of nigerose in the drink was a disaccharide of α-(1-3)-linked D-glucose. When a mixture of this oligosaccharide, nigerosyl glucose and nigerosylmaltose was tested for its immunopotentiating activity *in vitro* and *in vivo* in mice, it was found that the oligosaccharide mixture activate NK and T cells by increased endogenous IL-2 and IL-12 that were locally produced by the activated resident macrophages (15). Macrophages and dendritic cells play a central role in promoting Th-1 response by producing IL-12. NK cells activity is stimulated by IL-12 (16). Another oligosaccharide, β-glucan, has been suggested to activate macrophages and NK cells through the β-glucan receptor. It would be interesting to investigate the mechanism of NK cell activation by ginger extract, especially through IL-12 stimulation by macrophage activation or by the pro-

Table 2. Liver and lymphocyte MDA, $CD3^+CD4^+$ T cell percentage and spleen natural killer activity of mice given water containing ginger water extract for six weeks with or without intraperitoneal (10.4 mg/kg bw)

	Control	Receiving ginger (G)	Receiving paraquat (Q)	Receiving G and Q
Liver weight (%)	4.4 ± 0.54	4.4 ± 0.40	4.8 ± 0.51	4.5 ± 0.34
$CD3^+$ Tcells (%)	61.07 ± 10.02	66.26 ± 14.55	54.51 ± 9.50	63.85 ± 13.40
$CD4^+$ Tcells (%)	27.68 ± 4.53	33.96 ± 4.96	28.67 ± 5.73	29.79 ± 8.63
Liver MDA (μmol)	2.73 ± 0.20	2.86 ± 0.16	3.04 ± 0.19	2.90 ± 0.14
Lymphocyte MDA (μM/g protein)	6.61 ± 2.12	6.16 ± 2.06	7.67 ± 1.59	6.69 ± 2.29
NK cell lysing activity (%)	41.00 ± 6.24^b	47.40 ± 4.24^c	24.50 ± 3.11^a	41.80 ± 4.48

Different letters indicate significant different at $p < 0.01$.

duction of γ -interferon. Chemoprevention from plants has been concluded as major areas of prevention research that promise to have a significant impact on cancer incidence, and in certain instances mortality (17). The finding in this research supports the traditional beliefs that ginger preparations increase body resistance to common cold, which is mostly caused by virus. In addition, its potential in increasing resistance to mutated cell proliferation, such as the case of tumor, should be considered.

Besides proliferation activity, cultured cells from all groups of mice did not show increased percentage of $CD3^+$ and $CD3^+CD4^+$ T cell subset. CD3 is a marker for all T cells while CD3CD4 is a marker for T cells bearing surface marker for Th-1 cells. This later surface marker is known to be vulnerable to virus HIV. It seems from this experiment that paraquat did not cause damaging effect to these T cell subsets and drinking ginger did not improve the proliferation activity of these cells.

MDA is a product of peroxidation reaction of unsaturated fatty acid caused by free radicals or other reactive compounds. Immune cells contain high level of unsaturated fatty acids on their membrane. These fatty acids play a central role in the integrity and function of the cells, which makes them sensitive to oxidation reactions (18). In this research, paraquat was assumed to be the source of superoxide radicals that could cause oxidation of membrane components, especially lipid fractions, upon the encountering of paraquat in the blood and the blood cells. MDA level in cells could indicate cell damage as results of membrane unsaturated lipid oxidation in the cells by paraquat or its derivatives. When the spleen cells and liver cells from all groups of mice were analyzed for MDA, there were no significant differences among the treated groups. Paraquat has been used as a source of oxidative stress *in vitro* tests. MDA production in human lymphocyte cells was induced with 20 mM of paraquat (14) while a dose of 30 mg/kg in mice, the LD50 dose, through intraperitoneal injection resulting in decrease in liver reduced glutathione, but there was no effect on lipid soluble antioxidant (19). This could indicate that the dose given to mice in this research might be insufficient to cause cellular oxidation or there is cell specificity of paraquat reactions.

EFFECTS OF NON-VOLATILE COMPOUNDS OF GINGER ON HUMAN LYMPHOCYTES *IN VITRO*

In mouse system, the effect of ginger water extract was more profound than that of ethanol extract in counteracting the effects of paraquat in exerting oxidative stress. In addition, the immune cell that was more significantly stimulated even in the presence of paraquat was the NK cell. It is then important to see if the effects would be the same in human immune cells. To investigate the effect of ginger extracts on human immune cells and to find out which compound among the known bioactive compounds in ginger that produce the most effect, sets of experiments were carried out using human lymphocyte obtained from one healthy adult male subject.

Peripheral blood was withdrawn in the morning at 8 : 00 using a venoject containing heparin and was immediately centrifuged against ficoll. Blood lymphocytes were withdrawn from the blood and ficoll interface, washed and allocated for the proliferation activity test, percentage of $CD3^+CD4^+$ subset T cells and NK cell lysing activity. All tests were performed immediately after cell isolation. Ginger extracts were prepared according to the method developed in our laboratory (20). Ginger bioactive compounds including oleoresin, gingerol, and shogaol were extracted using ethanol, isolated with thin layer chromatography and verified by high-pressure chromatography. The three compounds were used in the test with ginger water extract as control. The proliferation activity test, the percentage of $CD3^+CD4^+$ and the NK cell lysing activity methods were basically similar to the test used in mouse experiments, except for the antibody for CD3 and CD4 that were antihuman, and the target cells for human NK cell were human leukemic cell line, K562. To create oxidative stress *in vitro*, cells for all tests were treated with paraquat at the concentration of 3 mM prior to incubation. Oleoresin, gingerol, shogaol and ginger water extract were added to the culture at various concentrations for the proliferation test and cultured for 4 days. To measure cell proliferation activity, 18 hr before harvesting, H-3thymidine were added to the cultures. Cells were harvested and counted in β-counter as mention previously in the mouse experiments. For the analysis of $CD3^+CD4^+$ T cells, after cell incubation of 48, cells were harvested by centrifugation and incubated with double antibody including antihuman CD3 conjugated with phycoerythrin and with antihuman CD4 conjugated with fluorescein isothiocyanate. For NK cell analysis, isolated human lymphocytes were treated with paraquat and the test bioactive compounds then incubated with the target cells at the ratio of effector cells to target cells equal to 100 : 1 (21).

Table 3 shows the results of the proliferation test, the percentage of $CD3^+CD4^+$ T cell subset and the lysing activity of NK cells (21). In the presence of paraquat as the source of oxidative stress in the cultures, oleoresin, gingerol and shogaol appeared capable of improving B and T cell proliferation as demonstrated by the significant proliferation stimulation indices, especially at lower dose for oleoresin and gingerol (50 μg/mL), and higher doses for shogaol (100 and 200 μg/mL). Protection for T cells appeared to occur at wider concentration compared to that for B cells. This was supported by the increases of the

Table 3. The effects of ginger bioactive compounds on *in vitro* proliferation of human T cells, percentge of $CD3^+$ and $CD3^+CD4^+$ T cells, and cytolytic activity of natural killer (NK) cells

Compounds added to cell culture	Proliferation activity (cpm)		$CD3^+$ T cell (%)	$CD3^+CD4$ T cell (%)	NK cell activity (%)
	B cells	T cells			
Media only	3046.2^{abc}	2347.4^{cdc}	44.39^{f}	21.29^{b}	55.26^{cf}
Oleoresin (μg/mL)					
50	4667.7^{a}	4420.7^{b}	41.69^{g}	27.61^{g}	70.05^{cf}
100	3801.4^{ab}	3020.8^{bcde}	39.40^{b}	30.18^{f}	67.28^{ab}
150	4101.6^{a}	2917.0^{bcde}	47.82^{c}	35.04^{c}	64.35^{bc}
200	3758.4^{ab}	3419.2^{bcd}	34.01^{f}	31.40^{f}	63.42^{bc}
Gingerol (μg/mL)					
50	4559.4^{a}	7241.6^{a}	52.91^{d}	41.19^{d}	67.05^{ab}
100	2002.1^{bcd}	6307.5^{b}	60.51^{b}	50.47^{b}	65.64^{b}
150	1421^{cd}	3777.1^{bc}	56.76^{c}	39.44^{d}	63.72^{bc}
200	807.1^{d}	2133.6^{cde}	57.06^{c}	39.16^{d}	61.17^{cd}
Shogaol (μg/mL)					
50	2892.0^{abc}	2740.5^{bcde}	49.65^{a}	40.50^{d}	52.91^{f}
100	2027.4^{bcd}	2687.9^{cde}	55.10^{c}	43.96^{c}	49.04^{g}
150	3228.9^{abc}	3105^{bcde}	45.41^{f}	32.43^{f}	47.25^{g}
200	3128.7^{abc}	3641.9^{bcd}	47.97^{c}	41.19^{d}	46.36^{g}
Ginger extracts (μg/mL)					
Water	2875.5^{abc}	2039.3^{de}	69.57^{a}	59.16^{a}	54.68^{f}
Ethanol	2071^{bcd}	1394.2^{e}	59.33^{b}	45.78^{c}	58.59^{de}

The cells were isolated from peripheral blood of one healthy subject. For proliferation activity, cells were cultured in RPMI-1640 medium containing 10% fetal calf serum with or without the addition of ginger compounds.

percentage $CD3^+$ and $CD3^+CD4^+$ T cell subset. In the proliferation activity test, T cell proliferation was measured by using Con A mitogen, which is a more specific stimulator for T cells. All T cells bear CD3 molecule in their plasma membrane, while Th-1 bears CD4 molecule in addition to CD3. The measurement of the percentage of these subset cells represents equally the proliferation activity of these subset cells. Gingerol and shogaol are constituents of oleoresin, but their antioxidant activity is not the same. Gingerol is known to have higher antioxidant activity than shogaol (2,6). It is probable that the two compounds in oleoresin exert synergic effect on the T cell proliferation. The doses used in these experiments were adjusted from the amount of ginger used in normal household drinks (equal to 50 μg/mL oleoresin). Therefore, the dose effects at low doses are closer to the normal drinks. It appeared from this study that doubling the doses of either oleoresin or gingerol would be better than single dose, and triple dose did not entailed better improvement. Particularly for $CD3^+CD4^+$ T cell subset proliferation, gingerol demonstrated better results. Since $CD3^+CD4^+$ T cell subset is the Th-1 cell that is vulnerable to HIV virus, this finding needs more elaboration and confirmation in human study.

Of greatest concern, NK cell lysing activity in the presence of paraquat was highly significantly improved by both oleoresin and gingerol ($p < 0.01$; Table 3). On the contrary, shogaol reduced the activity of these cells, while ginger water and ethanol extracts did not demonstrate improvement in cell activity. This finding is in accordance with that in mouse *in vivo* study previously done where mice given ginger water extract drink demonstrated improvement in NK cell activity, both with and without paraquat.

In this experiment using human lymphocyte cultured *in vitro*, the effects of ginger water extract were not significant. It is probable that when the ginger drink consumed by the mice, most of the compounds absorbed was gingerol. This should be verified by analyzing gingerol content in plasma. The most probable mechanism of gingerol protection against paraquat was through its antioxidant activity. In this research, it was also found that all ginger extracts and their derivatives reduced MDA level in human lymphocytes cultured in the presence of paraquat (21). This reduction in MDA was inversely correlated with the level of glutathion in the cells. Since both gingerol and shogaol are constituents of oleoresin and ginger water and ethanol extracts, both ginger water extract and oleoresin could be more efficiently and practically used in everyday health drinks or other types of health preparations to improve NK cell activity. This cell activity is important in body mechanism of eliminating infected and mutated cells, which might lead to tumor development.

THE EFFECT OF DRINKING GINGER DRINK ON NK CELL ACTIVITY AND ANTIOXIDANT STATUS OF ADULT MALE SUBJECTS

In both *in vivo* mouse and *in vitro* human lymphocyte studies, we constantly observed improvement in the im-

mune system as results of drinking ginger in mice and ginger bioactive compounds in human lymphocytes (9,21-23). The proliferation activity was improved even after exposing human lymphocytes to paraquat as the source of oxidative stress. In *in vivo* mouse experiment, paraquat through intraperitoneal injection did not cause impairment of the immune system when mice were given ginger in their drinking water. In both mouse and human lymphocyte experiments, NK cells were found to be more active in lysing leukemic target cells in the presence of ginger extract *in vivo* in mice or ginger bioactive compounds *in vitro*. Oleoresin, the ethanol extract of ginger oil, and gingerol, one of the constituents of oleoresin, showed the same capacity in NK cell activity improvement in human *in vitro* lymphocyte test. The mechanism of this improvement in the immune system might be due to the antioxidant activity of ginger bioactive compounds. Nevertheless, other mechanism might involve such the stimulation of Th-I cell, cytokine and interferon. In this study, we observed the effect of ginger drink given daily to healthy adult male subjects on NK activity and the antioxidant status, including vitamin E and MDA in plasma. Gingerol is a semipolar compound, so it is more probable that this compound will have positive interaction with vitamin E. MDA in plasma has been considered as one of the parameter to evaluate the oxidative stress in the body (24). High MDA level signals high lipid peroxidation in the body that could be prevented by either vitamin E or gingerol, directly or indirectly.

In this study, healthy male students aged between 19~27, living in a local religious dormitory were recruited to volunteer. The students were grouped in two groups of 12, and explained the objectives and mechanism of the study. The students were organized to meet in the dormitory every day for one month except Sunday to drink the freshly prepared ginger drink, which was prepared according to the traditional dose, but extraction method was slightly modified to obtaine standard procedure of preparation. Before participation, all students had physical examinations by a medical doctor and peripheral blood was withdrawn aseptically in a local clinic for analysis to ensure health of the participants. Ginger drink was provided in the afternoon at 17 : 00 to the treated group accompanied by light biscuits. The second group, which was the control group received biscuits with water. Intervention was done with discussion about nutrition and food safety when necessary and was constantly supervised to ensure intake of the drink. After 30 days of intervention with ginger drink, all subjects were subjected to physical examination and blood was withdrawn for analysis. The intervention was approved by the Department of Higher Education, Ministry of Education. Ginger drink is considered as normal traditional drink that has been used traditionally by the population, therefore, there should not be side effects of this drink intervention. The question lied on the health effects the drink could bring out. Blood analysis includes: NK cell lysing activity, MDA and vitamin E in plasma. Peripheral blood obtained by using disposable syringe containing EDTA as anticoagulant, was immediately centrifuged on ficoll to obtain the lymphocytes and plasma. Lymphocytes were incubated with target cells (K562) that have been previously incubated with H^3-thymidine as mention in the previous studies. MDA was analyzed (10) and vitamin E was measured using spectrofluorometer (22,23).

The results of MDA analysis from plasma of the treated subject before intervention was 2.36 μmol/L (Fig. 1). This value was much higher than the normal value suggested (25) that was 1.01 μmol/L, and could be the results of several causes such as internal health status and oxidative stress in the subjects. After receiving ginger drink intervention for 30 days, this level was reduced to 1.94 μmol/L, which was 17.79% lower than before the intervention

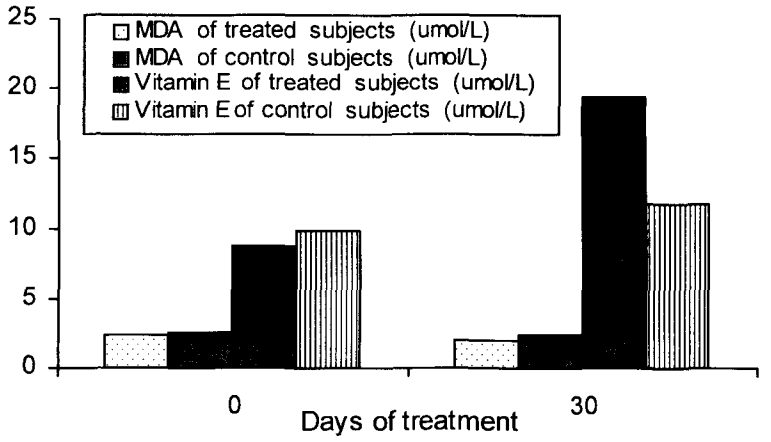

Fig. 1. MDA and vitamin E content in plasma of subjects before (0 day) and after receiving ginger drink for 30 days (n = 12). The subjects were healthy male students living in the same dormitory.

and statistically significant ($p < 0.01$). On the contrary, the control subject did not show significant changes in MDA level at day 0 and 30 (2.49 μmol/L to 2.39 μmol/L). Fig. 1 also shows the vitamin E level in the treated subjects and the control. Before intervention, the average vitamin E level in the treated subjects was 8.74 mg/L. After intervention, there was marked increase in vitamin E level of this treated group to 19.4 mg/L, which was 121.97% higher ($p < 0.01$). For the control group, vitamin E level showed insignificant increase from 9.79 mg/L to 11.84 mg/L ($p > 0.05$). It seems from this study that there was positive interaction among bioactive compound in ginger and vitamin E. The major bioactive compound in ginger water extract was gingerol (data not shown), and this semi-polar compound is known to have the most antioxidant activity (6). When ginger drink was consumed, the compounds must have been absorbed and there was possibility that the antioxidant effect was already effective in the blood circulation as well as in the cell membrane. In addition, like carotenoids from food plants that are absorbed and accumulated in the liver (26). The same fate might be experienced by the semi polar ginger compounds absorbed. Vitamin E has been reported to protect LDL in the blood and lipid fraction in the cell membrane (27). The introduction of gingerol in the blood and cells might substitute vitamin E in the oxidation reactions; therefore vitamin E was used up during the intervention period. In the same experiment, it was also found that the peak resembled that of gingerol in the HPLC analysis of the subjects plasma (data not shown).

Before intervention, control group had NK cell lysing activity of 29.96% at 100 : 1 ratio of effector cells to target cells, and 15.86% at 50 : 1 ratio (Fig. 2A). Treated group had, respectively, 30.18% and 13.18% NK cell lysing activity. Reducing of effector cells to half, reduce equally the activity of the cells in lysing their target cells. After intervention, the treated group that had consumed ginger drink for 30 days showed highly significant increase in NK cell lysing activity to 51.11% at the ratio of 100 : 1, and 52.37% at 50 : 1 (Fig. 2B). The reduction of effector cells to a half did not change the lysing activity of the NK cells. In the control group, after 30 days, there was no change in NK cell lysing activity (9). The percent lysis of target cells remains low (24.83% and 19.50% for ratio of effector cells to target cells of 100 : 1 and 50 : 1 respectively). It was clearly demonstrated in this human study that drinking ginger extract improved antioxidant capacity by substituting and/or protecting vitamin E in plasma and probably in the cells. NK cells lysing activity was significantly improved even at the low ratio (50 : 1) (22,23).

This antioxidant activity might be directly or indirectly related to the NK cell activity. Protection of ginger bioactive compounds, which was most probably gingerol, when this compound was in the blood circulation, might entail better performance of the immune cells in the blood. However, in the vitro system, even though gingerol had shown antioxidant activity, not all immune cells were equally protected as shown by the only NK cells activity that was highly and constantly improved. Perhaps, in addition to the antioxidant activity, which had substituted or protected vitamin E in the blood and might be in the

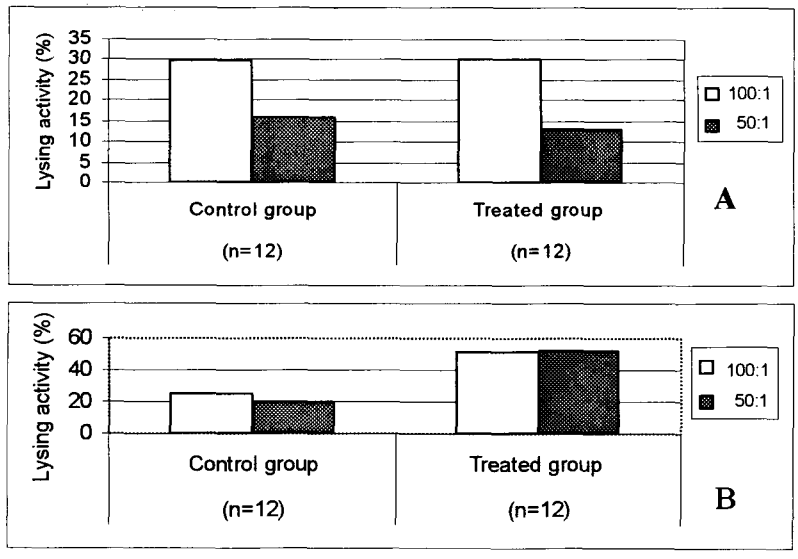

Fig. 2. NK cell lysing activity of 12 healthy male subjects given ginger drink and the control group. Ginger drink was given for 30 days every afternoon and peripheral blood was drawn aseptically for lymphocyte isolation. The cells were incubated with target cells which had been treated with H^3-thymidine. Data was expressed as count per minute of the unlysed target cells.

cell as well, there were other immunomodulation activities of ginger compounds, especially gingerol in stimulating NK cell lysing activity. This stimulation could be more specific to those related with the mechanism of NK cell activation such as Th-1 cell roles, cytokines and interferon production. In the immune system, more direct observation of oxidative response could be better observed through macrophage cells (28-30). The application of the mouse experiment in human subjects is important considering that genistein from soybean was shown to inhibit cancer cell growth *in vitro*. However, it was concluded that the plasma concentration required to inhibit cancer cell growth *in vivo* was unlikely to be achieved from a dietary dosage of genistein (31).

Based on the fact that drinking ginger by both mice and the student subjects did not lower the activity of NK cells, further observations on other immune functions might be of interest, since immune-lowering effects of soybean antioxidants had been indicated (32). The authors reported that genistein, a flavonoid estrogen, injections in mice decreased thymic weight and thymic and splenic $CD4^+$ $CD8^-$ T cell numbers and resulted in lymphocytopenia, thymic atrophy and immune suppression. WCRF & AICR (33) has proposed the prevention of cancer through food and vegetables, however, the importance of spices and herbals could also be considered (34,35). In addition, supplementation with local vegetables and fruits carrying vitamin A, C and E to workers exposed to food chemical contaminations had shown improvement in the immune cell functions (36).

REFERENCES

1. Tang W, Eisenbrand G. 1992. *Chinese Drugs of Plant Origin: Chemistry, Pharmacology and Use in Traditional and Modern Medicine*. Springer-Verlag, New York. p 20.
2. McCaleb R. 1991. Herbal Gram. *Res Rev* 25: 45-56.
3. Keys JD. 1981. *Chinese Herbs: Their Botany, Chemistry and Pharmacodynamics*. Charles E. Tuttle Co, Tokyo. p 65.
4. Suekawa MA, Ishige A, Yuasa K, Sudo K, Aburada M, Hosoya E. 1984. Pharmacological studies on ginger I. Pharmacological actions of pungent constituents, (6)-gingerol and (6)-shogaol. *J Pharmacobiodyn* 7: 836-848.
5. Hikino H, Kiso Y, Kato N, Hamada Y, Shioiri T, Aiyama R, Itokawa H, Kiuchi F, Sankawa U. 1985. Antihepatotoxic actions of gingerols and diarylheptanoids. *J Ethnopharmacol* 14: 31-39.
6. Nakatani N. 1997. Natural antioxidants from spices. In *Phenolic Compounds in Food and Their Effects on health II*. Huang M, Ho C, Lee CY, eds. Am Chem Soc., Washington DC.
7. Zakaria-RF, Darsana L, Wijaya H. 1996. Immunity enhancement and cell protection activity of ginger buds and fresh ginger on mouse spleen lymphocytes. Paper presented at the Symp Non-Nutritive Health Factors for Future Foods. Korean Soc Food Sci & Technol, Korea.
8. Zakaria FR, Belleville F, Nabet P, Linden G. 1992. Allergenicity of bovine casein I. Specific lymphocyte proliferation and histamine accumulation in the mastocyte as results of casein feeding in mice. *Food Agric Immunol* 4: 41-51.
9. Zakaria FR, Wiguna Y, Hartoyo A. 1999. Consumption of ginger (*Zingiber officinale* Roscoe) drink increased natural killer cell activity in students from Ulil Alkaab in Bogor. *Food Technol Industry Bull* 9: 23-29.
10. Conti M, Morand PC, Levillain P, Lemonnier A. 1991. Improved fluorometric determination of malonaldehyde. *J Clin Chem* 37: 1273-1275.
11. Kobayashi KK, Yoshida A, 1986. Effect dietary ascorbic acid and vitamin E on metabolic changes in rats and guinea pigs exposed to PCB. *J Nutr* 116: 98-106.
12. Zakaria FR. 1996. Synthesis of radical and electrophile compounds in and by food compounds. In *Radical Compounds and Food System: Biomolecular reaction, Effects on Health and Prevention*. Zakaria FR, Dewanti R, Yasni S, eds. CFNS, IPB, Bogor, Indonesia. p 31.
13. Fritz KL, Nelson TL, Ruiz-Velasco V, Mercurio SD. 1994. Acute intramuscular injection of oils or oleic acid component protects mice against paraquat lethality. *J Nutr* 194: 425-429.
14. Clausen J. 1995. An *in vitro* system for evaluation of oxidative stress and effects of antioxidants. Abstract presented at the International Symposium on Natural Antioxidants. Peking, China.
15. Murosaki S, Muroyama K, Yamamoto Y, Kusaka H, Liu T, Yoshika Y. 1999. Immunopotentiating activity of nigero-oligosaccharides for the T helper 1-like immune response in mice. *Biosci Biotechnol Biochem* 63: 373-378.
16. Ross GD, Vetvicka V. 1993. CR3 (CD11b, CD18): a phagocyte and NK cell membrane receptor with multiple ligand specificity and functions. *Clin Exp Immunol* 92: 181-184.
17. Singletary K. 2000. Diet, natural products and cancer chemoprevention. *J Nutr* 130: 465S-466S.
18. Meydani SN, Wu D, Santos MS, Hayek MG. 1995. Antioxidants and immune response in aged persons: Overview of present evidence. *Am J Clin Nutr* 62: 146S-147S.
19. Bus JS, Cagen SZ, Olgaard M, Gibson JE. 1976. A mechanism of paraquat toxicity in mice and rats. *Toxicol Appl Pharmacol* 35: 501-513.
20. Wikandari P. 1995. Development of extraction methods for the analysis gingerol in fresh and processed ginger. *Thesis*. Food Science Graduate School, Bogor Agricultural University, Indonesia.
21. Tejasari, Zakaria-R F. 2000. Functional properties of ginger: fractions 1 dan 2 of ginger (*Zingiber officinale* Roscoe) bioactive oleoresin compounds decreased lymphocyte membrane lipid peroxidation *in vitro*. In *Proceeding of Food Industry Nat Seminar*. Budiyanto S, Muchtadi M, eds. PATPI, Surabaya. Vol II.
22. Zakaria F, Susanto H, Hartoyo H. 2000. The effects of ginger (*Zingiber officinale* Roscoe) consumption on malonaldehide and vitamin E levels in students from Ulil Alkaab Kedung Badak Bogor. *Food Technol Industry Bull* 10: 36-40.
23. Nurahman, Zakaria FR, Sanjaya, Sayuthi D. 1999. The effect of ginger consumption on the protective effect of lymphocytes from oxidative stress in students from Ulil Alkaab, Kedung Badak, Bogor. In *Proceeding of National Seminar on Food Technology*. Zakaria-Rungkat F, Suhartono M, Astawan M, eds. PATPI & MENPANGHOR, Jakarta.
24. Aruoma OI. 1997. Assessment of potential prooxidant and antioxidant actions. *Am Oil Chemist Soc* 73: 1617-1625.
25. Wasowicz W, Neve J, Peretz A. 1993. Optimized steps in fluorometric determination of thiobarbituraric acid-reactive

substance in serum: importance of extraction pH and influence of sample preservation and storage. *Clin Chem* 39: 2522-2526.
26. Zakaria-Rungkat F, Djaelani M, Setiana, Rumondang E, Nurrochma. 2000. Retinol accumulation in rat livers as a measure of carotenoid bioavailability of green vegetables and carbohydrate containing foods. *J Food Comp Anal* 13: 297-310.
27. Halliwell B, Gutteridge JMC, Cross CE. 1992. Free radicals, antioxidant and human diseases: where are we now? *J Lab Clin Med* 119: 508-620.
28. Hwang JK, Lim TS, Na K, Yun JM, Choi EM. 2002. Abstract no 13 presented at 2nd Intl Symp on Antioxidants in Nutr & Therapy: Mechanism in Physiology-Pathology-Pharmacology. Kuta, Bali, Indonesia.
29. Furhman B, Rosenblat M, Hayek T, Colerman R, Aviram M. 2000. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprottein E-deficient mice. *J Nutr* 130: 1124-1131.
30. Zakaria-Rungkat F, Septiana AT, Sulistiyani. 2001. Ginger (*Zingiber officinale* Roescoe) extracts increase human LDL resistance to oxidation and prevent cholesterol accumulation in macrophage. Abstract presented at The Second Intl Symp on Natural Antioxidant: Molecular Mechanism and Health Affects, Beijing, China.
31. Santell RC, Kieu N, Helferich WG. 2000. Genistein inhibits growth of estrogen-independent human breast cancer cells in culture but not in athymic mice. *J Nutr* 130: 1665-1669.
32. Yellayi S, Naaz A, Szewczykowski MA, Sato S, Woods JA, Chang J, la Segre M, Allred CD, Helferich WG, Cooke PS. 2002. The phytoestrogen genistein induces thymic and immune changes: A human health concern? *Proc Natl Acad Sci* USA 99 (Issue 11): 7616-7621.
33. WCRF & AICR. 1997. Food, Nutrition and the Prevention of Cancer: a Global Perspective. WCRF & AICR, London.
34. Murakami A, Ohigashi H, Koshimizu K. 1999. Chemoprevention: insights into biological mechanisms and promising food factors. *Food Rev Int* 15: 335-395.
35. Craig WJ. 1999. Health promoting properties of common herbs. *Am J Clin Nutr* 70: 491S-499S.
36. Zakaria-Rungkat F, Irawan Bus, Pramudya SM, Sanjaya. 2000. Fruits and vegetables as the sources of vitamin C dan E intervention increased the immune system in industry workers in Bogor. *Food Technol Industry Bull* 11 (2): 21-27.

(Received December 10, 2002; Accepted February 20, 2003)