BIOMEDICAL AND ENVIRONMENTAL SCIENCES **16**, 276-282 (2003)

Lead Induced Oxidative Stress: Beneficial Effects of Kombucha Tea

P. DIPTI*, B. YOGESH, A. K. KAIN, T. PAULINE, B. ANJU, M. SAIRAM, B. SINGH, S.S. MONGIA, G. ILAVAZHAGAN DEVENDRA KUMAR, AND W. SELVAMURTHY

Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi-110054. India

Objective To evaluate the effect of oral administration of Kombucha tea (K-tea) on lead induced oxidative stress. **Methods** Sprague Dawley rats were administered 1 mL of 3.8% lead acetate solution daily alone or in combination with K-tea orally for 45 d, and the antioxidant status and lipid peroxidation were evaluated. **Results** Oral administration of lead acetate to rats enhanced lipid peroxidation and release of creatine phosphokinase and decreased levels of reduced glutathione (GSH) and antioxidant enzymes (superoxide dismutase, SOD and glutathione peroxidase, GPx). Lead treatment did not alter humoral immunity, but inhibited DTH response when compared to the control. Lead administration also increased DNA fragmentation in liver. Oral administration of Kombucha tea to rats exposed to lead decreased lipid peroxidation and DNA damage with a concomitant increase in the reduced glutathione level and GPx activity. Kombucha tea supplementation relieved the lead induced immunosuppression to appreciable levels. **Conclusion** The results suggest that K-tea has potent antioxidant and immunomodulating properties.

Key words: Lead; Oxidative stress; Kombucha tea; Antioxidant activity

INTRODUCTION

Kombucha tea, a popular health beverage, is made by incubating Kombucha mushroom in sweet black tea. The product is a carbonated acidic tea beverage with a slightly sweet taste. It is consumed worldwide, especially in China, Russia and Germany. It is suggested that Kombucha tea consumption can reduce blood pressure, relieve arthritis, increase immune response etc. Although these effects have not been proven scientifically, abundant knowledge and information are now surfacing as research is expanding in parallel to increased consumption of the fermented tea. The beneficial effects of K-tea are attributed to the presence of vitamins, amino acids, lactic acids, gluconic acid, antibiotic and variety of micronutrients[1, 2]. Recently Hartmann *et al.*[3] in their 3-year longitudinal study reported that K-tea ingestion led to the increased survival time in mice. K-tea has been reported to possess *in vitro* anti-microbial activity[2, 4] and anti-diabetic activity[5]. Recently, Sai Ram *et al.*[6] reported the anti-oxidant activity of Kombucha tea in rats on chromate (VI) induced oxidative stress. In view of these facts, the present study was designed to evaluate the beneficial effects of K-tea in terms of its anti-oxidant and immunomodulating activity in

*Correspondence should be addressed to P. DIPTI, E-mail: rnprasad32@hotmail.com

Biographical note of the first author: Prasad DIPTI, M. Sc. Ph.D, working as a scientist in the Experimental Biology Division of Defence Institute of Physiology and Allied Sciences.

0895-3988/2003
CN 11-2816

lead induced oxidative stress.

MATERIALS AND METHODS

Preparation of Kombucha Tea

Kombucha tea was prepared as per the method described by Sai Ram *et al.*[6]. Briefly, 10% sugar solution was prepared by boiling in a sterile conical flask and later 1 g/L black tea powder (Brooke bond, India) was added and allowed to cool at room temperature. The tea decoction was filtered, and cooled, and inoculum along with fungal mat (10%) and 100 mL of previous fermentation was added and incubated at 28±1℃ for 8-10 d under aseptic conditions.

Animals

Studies were conducted on male albino (Sprague-dawley) rats weighing 170-200 g. The animals were maintained at 22±1℃ and with a 12 h light and dark cycle, with food and water *ad libitum*. Rats were divided into 4 groups of 6 rats each. Group I served as control and was given 1 mL of 0.9% saline per rat. Group II was administered 1 mL of fermented tea (containing tea-fungus metabolites) per rat. Group III was administered 1 mL of 3.8% lead acetate solution per rat. Group IV was administered 1 mL fermented tea and 1 mL lead acetate solution. There was a gap of 5-6 h between administration of tea and lead and all the treatment was given for 45 d, orally with the help of gastric cannula.

Oxidative Stress

It was induced by force-feeding of lead acetate solution daily (3.8%) for 45 d. The animals were anaesthetized and blood was collected by retro-orbital puncture and various biochemical parameters were assessed. Reduced glutathione was estimated in blood by the method of Kum-Talt and Tan[7]. Malondialdehyde (MDA) was determined by the method of Dousset *et al.*[8]. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) and CPK activity in blood were determined as per manufacturer's instruction using kits (RANDOX).

DTH Response

DTH response was induced as described by Atal *et al.*[9]. Separate groups of six rats each were immunized by injecting 25 μL of 5×10^9 sheep red blood cells (SRBC)/mL s.c into the right hind foot pad. Seven days later, the thickness of the left hind foot pad was measured using vernier calipers. Then the rats were challenged in the left hind foot pad with 25 μL of 5×10^9 SRBC intradermally. After 24 and 48 h of the challenge, the foot pad thickness was measured and the difference between pre- and post challenge foot pad thickness was taken as a measure of DTH response.

Antibody Titer

Separate groups of six rats each were injected 20 μL of 5×10^9 SRBC/mL sub-cutaneously into the right hind foot pad. After 7 d the rats were challenged with the same number of SRBC i.d. into the left foot pad. The blood samples were collected by retro-orbital puncture on the 14th day for determining antibody titer. Antibody levels were determined by haemaglutination as described by Sai Ram *et al.*[10]. Briefly, 25 μL of 0.1%

SRBC was added to 2 fold dilutions of serum samples made in saline containing 0.1% BSA in V-bottomed Takasty microtitration plates. After being mixed, the erythrocytes were allowed to settle down at 37℃ until the control wells showed a negative pattern (small button). The value of the highest serum dilution causing visible haemaglutination was taken as the Ab. titer.

DNA Fragmentation

DNA fragmentation was evaluated by the method of Rao *et al.*[11]. Briefly, the excised liver from control and treated animals were homogenized in ice cold lysis buffer pH 8.0 and centrifuged at 27 000 g for 30 min. Both pellet (intact chromatin) and supernatant (DNA fragments) fractions were assayed for DNA content using DAPI (4,6-diamidiono-2-phenylindole) reagent spectrofluorimetrically[12]. Briefly, 20 μL of sample was added to 2 mL of reagent, (100 ng/mL DAPI) and fluorescence intensity was measured at 450 nm with excitation at 362 nm. The level of DNA fragmentation was expressed as percentage value[13].

RESULTS

Oxidative Stress

The oxidative stress as measured by MDA and anti-oxidant level, induced by lead supplementation, are shown in Fig. 1. There is no change in MDA levels in control and K-tea alone fed groups. But lead administration significantly elevated the MDA levels ($P>0.01$) over control rats. K-tea feeding significantly inhibited lead induced lipid peroxidation.

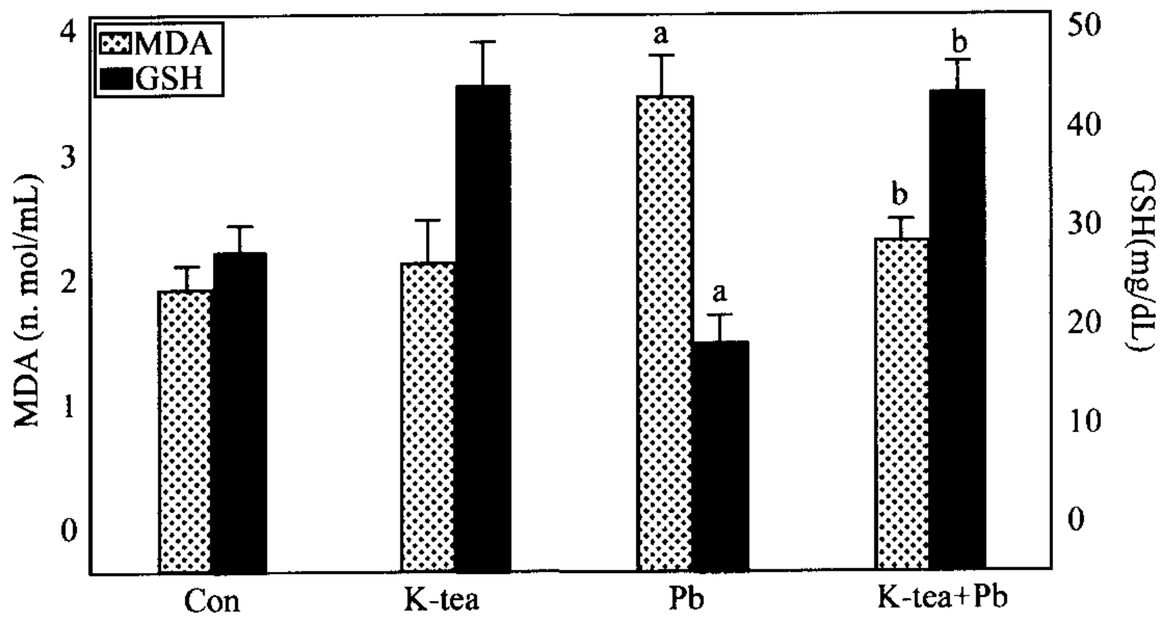

FIG. 1. Effect of K-tea feeding on lipid peroxidation (MDA) and reduced glutathione (GSH) levels in lead intoxicated rats. $P < 0.01$; [a] vs control; [b] vs Pb.

The blood glutathione levels decreased significantly in the lead administered group. However, the GSH level significantly increased in the K-tea fed group.

The activity of plasma CPK is depicted in Table 1. K-tea treatment did not alter the enzyme level compared to the control. However, CPK activity increased significantly in the lead treated group where as its level was restored to normal in the presence of K-tea.

Table 1 also depicts the antioxidant enzyme levels in different groups of rats. There was no significant change in SOD across the groups studied whereas GPx activity decreased

significantly in the lead treated group. Interestingly, the group receiving both K-tea and lead showed a significant elevation in GPx activity when compared with the group fed with lead.

TABLE 1

Effect of K-tea on Antioxidant Enzymes and CPK Levels in Lead Intoxicated Rats

	Control	K-tea	Pb	K-tea+Pb
GPx U/mL	43310±2077	44968±2492	36208[a]±2935	46371[b]±2908
SOD U/L	569±41	602±56	507±58	586±51
CPK U/L	127.82±6.57	101.96±19.66	307.76[a]±16.82	141.70[b]±17.66

Note. Values are $\bar{x} \pm s$; $P < 0.01$; [a]: vs control; [b]: vs Pb.

Immune Response

The effect of K-tea on immune response is depicted in Fig. 2. K-tea feeding enhanced the antibody titer marginally over the control. Lead treatment did not change the humoral response (Ab. titer), but inhibited the DTH response significantly. K-tea feeding reversed the immunosuppressive effect of lead on DTH response significantly.

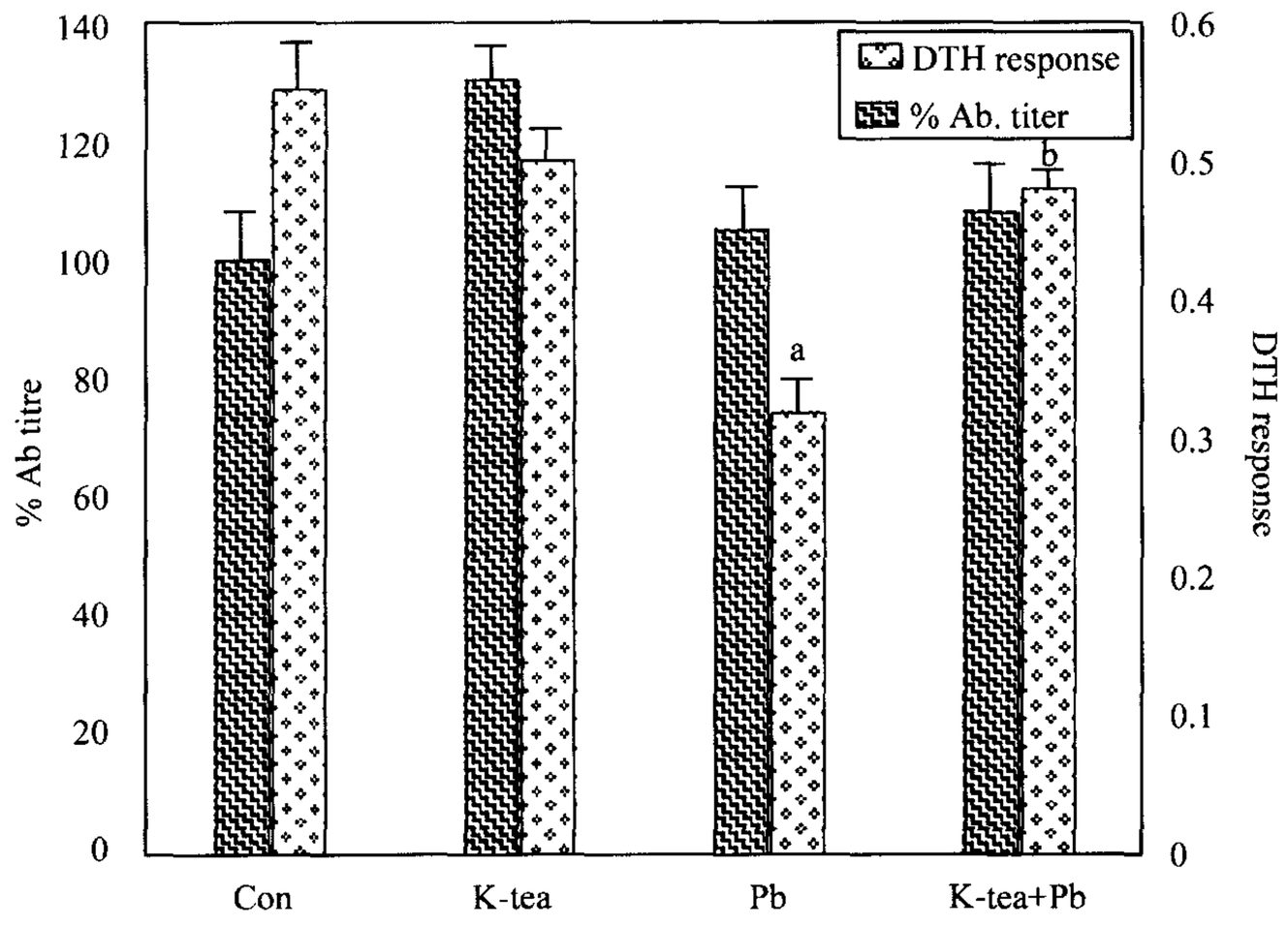

FIG. 2. Effect of K-tea feeding on antibody titer and DTH response in lead intoxicated rats. $P < 0.01$; [a] vs control; [b] vs Pb.

DNA Fragmentation

As seen in Fig. 3, there is a significant increase in DNA fragmentation in the group treated with lead whereas K-tea inhibited the level of lead considerably, thus helping to prevent DNA fragmentation.

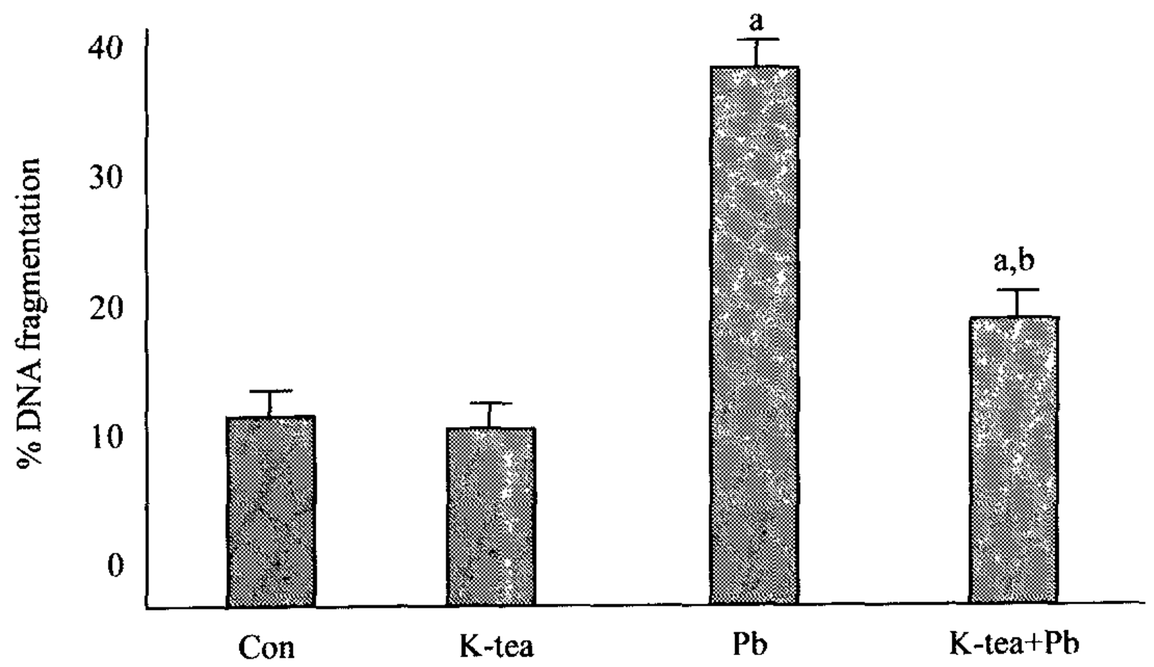

FIG. 3. Effect of K-tea on DNA fragmentation induced by Lead intoxication in liver. $P< 0.01$; [a] vs control; [b]vs Pb.

DISCUSSION

There has been wide interest in the possible benefits and toxicity of K-tea. Phan *et al.*[14] reported lead poisoning in two individuals who consumed K-tea fermented in a ceramic pot. Since ceramic pot contain lead while K-tea is highly acidic, it is evident that the lead has leached into the tea resulting in lead contamination. Sadjadi[15] reported the presence of anthrax bacillus in tea fermented in an unhygienic condition. Srinivasan *et.al.*[16] reported gastrointestinal toxicity of K-tea in four patients. However, all such reports refer to isolated cases and a few of persons (2-4 persons) are involved. Further, in all such cases, there is no scientific evidence that K-tea is directly responsible for toxicity. Many reports are also available which show therapeutic potentials of tea-fungus metabolites, but in most of such cases medication and meditation were also given to patients simultaneously with Tea-fungus metabolites. So there is no direct scientific proof of possible benefits or toxicity of K-tea.

A few laboratory tests were carried out by Kappa Laboratories in Miami, Florida (1995) and they reported that the tea was fit for human consumption. FDA (1995) also conducted microbiological tests and found no pathogenic microorganism in the tea, but warned the tea-fungus brewers of possible contamination when proper conditions were not maintained. Recently, Vijayaraghavan *et al.*[17] reported that oral administration of K-tea for 90 d to rats did not produce any toxic effects.

Lead is a ubiquitous environmental toxin that induces a wide range of physiological, biochemical and behavioral dysfunctions. Recent studies reported its potential for inducing oxidative stress[18]. It is also known to have some toxic effects on membrane structure and function[19]. Lead interacts with-SH groups of protein enzymes causing them to precipitate and results in the breakdown of several enzyme systems. It is also known to create an imbalance between TH1 (cell mediated) and TH2 activation (humoral) and result in immunodysregulation leading to impaired cell mediated immunity (CMI)[20]. But, despite the knowledge that lead can induce the oxidative stress, the usefulness of anti-oxidants in treating lead poisoning has not been thoroughly investigated.

In the present study, we found that lead treatment enhanced lipid peroxidation and decreased antioxidant enzyme level. In this regard, our studies fall in confirmation with earlier studies which also reported alterations in antioxidant enzyme activities such as SOD,

catalase and glutathione peroxidase and change in the concentration of some antioxidant molecules such as glutathione (GSH) in lead exposed animals[21,22] and workers[23-26]. Lead treatment inhibited DTH response significantly while humoral immunity remained unaffected. However, investigations of Koller and Kovacic[27] and Koller *et al.*[28] show that there is a reduction in humoral response in lead intoxicated mice.

Further, there is enhanced DNA damage level in lead intoxicated rats which could be attributed to increased oxidative stress. Ye[29] also reported a positive correlation between MDA and DNA damage in workers exposed to lead. Free radical mediated DNA damage is usually accompanied by depletion of intracellular reduced glutathione whose depletion lowers cell capacity to buffer against endogenous oxidants[30]. In the present study lead induced oxidative stress was associated with decreased activity of GPx. and GSH which in turn resulted in the decrease in immunity. The oxidant-antioxidant balance is an important determinant of immune cell function. Cells of immune system are particularly sensitive to change in oxidant-antioxidant balance because of the higher percentage of polyunsaturated fatty acids in the plasma membrane. Earlier studies also reported decreased immune response during oxidative stress[31,32]. However, K-tea treatment to lead exposed rats decreased lipid peroxidation as evident by decrease in MDA production and CPK release and a concomitant increase in reduced glutathione and GPx activity. The preservation of SOD and increase in GPx activity increased the antioxidant enzymatic defence which could be involved in ameliorative effect of K-tea on lead induced oxidative stress. Further, K-tea feeding reversed the immunosuppressive effect of lead on DTH response.

CONCLUSION

In conclusion, K-tea is able to decrease the oxidative stress induced by lead, and improve DTH response (cell mediated immunity) in rats. The ameliorative effect of K-tea could be due to decreased lipid peroxidation and DNA damage and increase in the concentration of cellular antioxidants, i.e. GSH and GPx.

REFERENCES

1. Mayser, P.S., Fromme, C., Leitzamann, and Gruender, K. (1995). The yeast spectrum of the tea fungus Kombucha. *Mycoses*. **38**(7-8), 289-295.
2. Greenwalt, C.J., Steinkraus, K.H., and Ledford, R.A. (2000). Kombucha, the fermented tea: microbiology, composition and claimed health effects. *Food Protection Org*. **63**(7), 976-981.
3. Hartmann, A.M., Burleson, L.E., Holmes, A.K., and Geist, C.R. (2000). Effects of chronic Kombucha ingestion on open-field behaviours, longevity, appetitive behaviours, and organs inc57-bl/6 mice: a pilot study. *Nutrition* **16**(9), 755-761.
4. Sreeramulu, G., Zhu, Y., and Knol, W. (2000). Kombucha fermentation and its antimicrobial activity. *J. Agric. Food Chem.* **48**(6), 2589-2594.
5. Chandralekha, S.K. (2000). Hypoglycemic activity of bio-tea in mice. *Ind. J. Exp. Biol.* **38**, 278-279.
6. Sairam, M., Anju, B., Pauline, T., Dipti Prasad, Kain, A.K., Mongia, S.S., Sharma, S.K., Singh, B., Singh, R., Ilavazhagan, G., Devendra Kumar, and Selvamurthy, W. (2000). Effect of Kombucha Tea on chromate (VI) induced oxidative tress in albino rats. *J. Ethanopharmacol.* **71**, 235-240.
7. Kum-Talt and Tan (1974). A new colorimetric method for determination of glutathione in erythrocytes. *Clin. Chem. Acta* **53**, 153-161.
8. Dousset, J.C., Trouih, M., and Yogi, K. (1983). Plasma MDA level during myocardial infarction. *Clin. Chim. Acta* **129**, 319-322.
9. Atal, C.K., Sharma, M.L., Kaul, A., and Khajuria, A. (1986). Immunomodulating agents of plant origin.I. Preliminary screening. *J. Ethnopharmacol.* **41**, 185-192.
10. Sairam, M., Sharma, S.K., Ilavazhagan, G., Devendra, K., and Selvamurthy, W. (1997). Immunomodulatory effects of neem oil, a volatile fraction from neem oil. *J. Ethnopharmocol.* **55**, 133-139.

11. Rao, P.V.L., Bhattacharya, R., Parida, M.M., Jana, A.M., and Bhaskar, A.S.B. (1998). Fresh water cyanobacterium microcystis aeruginosa (UTEX 2385) induced DNA damage *in vivo* and *in vitro*. *Environ. Toxicol. Pharmacol.* **5**, 1-6.
12. Brunk, C.F.K, Jones, K.C., and James, T.W. (1979). Assay of nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* **92**, 497.
13. Bhattacharya, R. and Lakshmana, Rao, PV. (1997). Pharmacological cytotoxicity and DNA damage in isolated rat thymocytes and their protective efficacy *in vivo*. *Toxicol. Lett.* **119**, 59-70.
14. Phan, T.G., Estell, J., Duggin, G., Beer, I., Smith, D., and Ferson, M.J. (1998). Lead poisoning from drinking Kombucha tea brewed in a ceramic pot. *Med. J.* **169** (11-12), 644-646.
15. Sadjadi, J. (1998). Cutaneous anthrax associated with the Kombucha mushroom in Iran. *J. Am. Med. Assoc.* **280**(8), 1567-1568.
16. Srinivasan, R., Smolinske, S., and Greenbaum, D. (1997). Probable gastrointestinal toxicity of Kombucha tea; is this breverage healthy or harmful? *J. Gen. Int. Med.* **12**(10), 643-644.
17. Vijayaraghavan, R., Singh, M., Rao P.V.L., Bhattacharya, R., Kumar, P., Sugendran, K., Kumar, O., Pant, S.C., and Singh, R. (2000). Sub acute (90 days) oral toxicity studies of Kumbucha Tea. *Biomed. Environ. Sci.* **13**, 293-299.
18. Gurer, H. and Ercal, N. (2000). Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radical Biol. and Med.* **29**, 927-945.
19. Donaldson.W.E. and Knowles, S.O.(1993). Is lead toxicosis a reflection of altered fatty acid composition of membranes? *Comp. Biochem. Physiol.* C**104**, 377-379.
20. Hoe, Y., Pasons, P.J., and Lawrence, D.A. (1993). Lead differentially, modifies cytokine production *in vitro* and *in vivo*. *Toxicol. Appl. Pharmacol.* **138**(1), 149-157.
21. Hsu, J.M. (1981). Lead toxicity related to glutathione metabolism. *J. Nutr.* **111**, 26-33.
22. McGowan, C. and Donaldson, W.E. (1986). Changes in organ non-protein sulfhydrl and glutathione concentrations during acute and chronic administration of inorganic lead to chicks. *Biol. Trace Element Res.* **10**, 37-47.
23. Ito, Y., Niiya, Y., Kurita, H., Shima, S., and Sarai, S. (1985). Serum lipid peroxide level and blood superoxide dismutase activity in workers with occupational exposure to lead. *Br. J. Ind. Med.* **48**, 239-242.
24. Monteiro, H.P., Abdalla, D.S.P., Arcuri, A.S., and Bechara, E.J.H. (1985). Oxygen toxicity related to exposure to lead. *Clinical Chemistry* **31**, 1673-1676.
25. Solliway, B.M., Schaffer, A., Pratt, H., and Yanni, S. (1996). Effects of exposure to lead on selected biochemical and haematological variables. *Pharmacol. Toxicol.* **78**, 18-22.
26. Sugawara, E., Nakamura, K., Miyake, T., Fukumura, A., and Seki, Y. (1991). Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. *Br. J. Ind. Med.* **48**, 239-242.
27. Koller, L.D. and Kovacic, S. (1974). Decreased antibody formation in mice exposed to lead. *Nature (Lond)* **250**, 148.
28. Koller, L.D, Exon, J.H., and Roan, J.G. (1976). Humoral antibody response in mice after single dose exposure to lead or cadmium. *Proc. Soc. Exp. Biol. (NY)* **151**, 399.
29. Ye, X.B., Fu, H., Zhu, J.L, Ni, W., Lu, Y.W., Kuang, X.Y., Yang, S.L., and Shu, B.X. (1999). A study on oxidative stress in lead exposed workers. *J. Toxicol. Environ Health* **57**(3), 161-172.
30. Slater, A.F.G., Stefan, C., Nobel, I., van den, Dobbelsteen, D.J., and Orrenius, S. (1995). Signalling mechanisms and oxidative stress in apoptosis. *Toxicol. Lett.* **82/83**, 149-153.
31. Miller, R.A. (1994). Aging and immune function: Cellular and biochemical analyses. *Exp. Gerontol.* **29**, 21-25.
32. Meydani, S.N., Wu, D., Santos, M.S., and Hayek, M.G. (1995). Antioxidants and immune status in aged persons: overview of present evidence. *Am. J. Clin. Nutr.* **62**, 1462S-1476S.

(*Received September* 6, 2001 *Accepted February* 18, 2003)