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Inhibition of Cyclophoshamide-Induced Mutagenicity by Microsized Powder of Selenium-Enriched Green Tea in Mice

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Previously, the antioxidant activity of Se-enriched green tea extracts has been studied in vitro. In the present study, an in vivo micronuclei test was employed to assess the antimutagenic effect of microsized Se-enriched green tea powder (MSTP) in mice bone marrow. Pretreatments of MSTP, micrometer-sized regular tea powder (MRTP), selenite, and MRTP + selenite were given by gavage for 29 consecutive days prior to cyclophoshamide (CP) treatment. Certain key antioxidant enzymes were also investigated to elucidate the mechanism of antimutagenic effect. Results indicated that MSTP and MRTP or selenite alone did not significantly induce micronuclei at either concentration, confirming its nonmutagenicity. In the CP-treated groups, significant suppressions in the micronuclei were recorded following pretreatment with MSTP, MRTP, and selenite administration. The antimutagenic effect of MSTP was evidently observed by significant reduction in the frequencies of micronuclei in bone marrow cells when compared to a positive control group. The administration of MSTP, selenite, and MRTP + selenite also increased the levels of selenium concentration, glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzymes in both blood and liver. However, no pronounced differences in activities of GPx and SOD were found among MSTP, selenite, and MRTP + selenite. The present findings demonstrate that the antimutagenic potential of MSTP could not be solely related to the enhancment of antioxidant enzymes of GPx and SOD.

KEYWORDS: Micrometer powder of Se-enriched green tea; micronucleus test; antimutagenicity; mutagenicity

INTRODUCTION

Increasing interests are raised on dietary compounds of plant origin that may prevent genetic effects of mutagens or carcinogens (*1*). Green tea, *Camellia sinensis*, is one of the most widely consumed beverages in the world, and its extract is therapeutically used by traditional Chinese medicine (TCM) for headaches, body aches and pains, digestion, depression, detoxification, and as an energizer. Green tea extracts contain a unique set of catechins that are responsible for its high biologic activity in antioxidant, antimutagenic, antiangiogenesis, and antiproliferative assays that are potentially relevant to the prevention and treatment of various forms of cancer. This health effect of green tea extract has been well-documented in the reviews of Cabrera et al. (*2*) and Cooper (*3, 4*).

Nevertheless, the chemical composition of green tea is quite complex with 15–20% of proteins and over 30% of phenolic compounds in dried leaves, while in tea infusion, only 4.5% of phenolic compounds and trace proteins or pigments is preserved (*2*). Considering the loss of active ingredients in tea infusion, such hypotheses were posed by food researchers that green tea leaves could be ground into microsized powders and added into food to increase the absorption of nutrients in green tea and to avoid the losses of nutrients during infusion. A microsized herbal medicine provided a higher biological utilization ratio than regular tea (*5*). Hasegawa et al. reported that powdered green tea (PGT) inhibited triglyceride (TG) accumulation in 3T3-L1 cells or caused degradation of intracellular lipid droplets (*6, 7*). However, such reports on the health effects of the consumption of PGT are very limited.

Selenium is viewed as a trace element needed for antioxidant defense or redox regulation mainly because of its essential role in glutathione peroxidase (GPx) and thioredoxin reductase (TR) (*8*). Epidemiological evidence and animal studies indicated that low Se levels in the diet are inversely correlated with the

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Table 1. Ingredients of Tea Powder[a]

sample tea	Se content (mg/kg)	total amino acid (g/kg)	tea polyphenols (g/kg)	vitamin C (g/kg)
regular tea powder	0.118 ± 0.006 c	33.3 ± 1.0 a	231.9 ± 19.2 a	1.65 ± 0.02 b
Se-enriched tea powder I	5.730 ± 0.245 b	35.20 ± 1.8 b	217.7 ± 18.9 a	2.22 ± 0.0.06 a
Se-enriched tea powder II	31.600 ± 0.09 a	37.8 ± 2.5 b	212.8 ± 20.0 a	1.16 ± 0.03 c

[a] Within the same column, means followed by a different letter are significantly different at $P < 0.05$.

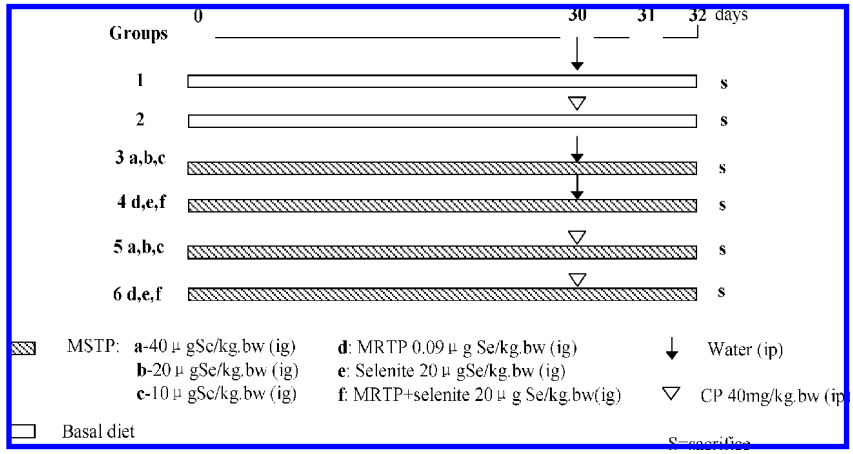

Figure 1. Experimental design to evaluate the effect of tea micropowder on the induction of micronuclei by CP. MSTP, micrometer Se-enriched tea powder; MRTP, micrometer regular tea powder; and MRTP + selenite, the combination of micrometer regular tea powder and selenite.

incidence of various forms of cancer (*9*). Se-enriched products of garlic, broccoli, and yeast were reported to be antimutagenic and anticarcinogenic (*10–12*). Our previous studies also proved that extracts of Se-enriched tea provide higher anticarcinogenic activities than regular tea (*13*), and Se-enriched rice is also able to suppress the mutation induced by cyclophosphamide (CP) or mitomycin C (MMC) by a micronucleus test and a chromosomal aberration test in mice (*14*). This protection effect suggests that enrichment of selenium in foods may be an effective strategy to reduce human cancer risk (*15*).

Considering the potential toxicity of Se compounds and tea prepared at high concentrations (*16, 17*), it is necessary to conduct toxicological tests to evaluate the safety of micrometer-ground Se-enriched tea powder (MSTP). In the present study, the mutagenicity and antimutagenicity potentials of MSTP were evaluated by a micronucleus test in mice bone marrow cells. The levels of selenium concentration and antioxidant enzymes including superoxide dismutase (SOD) and GPx were also investigated to explore the relevant mechanisms.

MATERIALS AND METHODS

Chemicals. CP was obtained from Sigma Chemical Co. (St. Louis, MO). The kits for Geimsa staining and measurement of protein, SOD, and GPx were purchased from Nanjing Jiancheng Bioengineering Institute. Other reagents were of analytical or high-performance liquid chromatography (HPLC) grade.

Preparation of Tea Powder and Se-Deficient Diets. The Se-enriched green teas I and II with various Se concentrations were produced by foliar application of selenite fertilizer in Zhe Luo Shan (Nanjing, China) tea plant as described previously (*15*). Se-enriched green tea and regular green tea were processed into micrometer powders [MSTP and micrometer-sized regular tea powder (MRTP)] by the machine (The Mikro-ACM air-classifier mill, Hosokawa Micron GmbH, Germany), and the mean diameters of the resulting tea particles were about 5 μm. Determination of ingredients of tea powder was performed according to national standards of the People's Republic of China (2003), and the results are shown in **Table 1**.

Basic diets were supplied in full compliance with recommendations of the AIN for mice diets (*18*), containing (percent by weight) casein (23.0), sucrose (30.0), cornstarch (32.0), cellulose fiber (5.0), corn oil (5.0), AIN-76 mineral mixture devoid of Se (3.5), AIN-76 vitamin mixture (1.0), choline dihydrogen citrate (0.2), L-methionine (0.3), and distilled H_2O. Ultimately, the Se content in diet was 4 μg/kg diets, which was negligible.

Animals. Laboratory-bred Kun Ming Swiss albino mice, 6–8 weeks old, weighing 20 ± 2 g with quality certificated number SCXK 2003-0003, were procured from the Experimental Animal Centre of Chinese Academy of Science (Shanghai, China) and housed individually in polycarbonate cages with filter tops. An ambient temperature of 25 ± 2 °C, relative humidity of 50 ± 2%, and photoperiod of 12 h were maintained throughout the study. Mice had free access to food and deionized water. Body weights were recorded weekly during the 31 day experiments.

Micronucleus Assay. To investigate the protective effect of microtea powder against the mutagenicity induced by CP (40 mg/kg bw), the animals were divided into 14 groups of 10 mice each (**Figure 1**). Tea powder, selenite, and CP were suspended in 11 times the volume of distilled water. MSTP and MRTP were administered orally by intragastric route, whereas CP was by ip route. The dose volume for administration was calculated based on mice body weight as 0.2 mL/10 g body weight.

In group 1, mice received drinking water for 29 days and were intraperitoneally (ip) treated with water on days 29 and 30. Group 2 followed the same treatments with group 1 for 29 continuous days and then post-treated with CP (40 mg/kg bw, ip) on day 29. Group 3 received solutions of MSTP prepared at three different concentrations: 40 (a), 20 (b), and 10 μg Se/kg bw (c) for 29 continuous days before treatment with CP 40 mg/kg bw on days 29 and 30. Group 4 received solutions of MRTP, selenite, and MRTP + selenite with the Se concentrations of 20 μg Se/kg bw prior to the CP treatments, respectively. Groups 5 and 6 received only treatments of solutions of MSTP at three concentrations and MRTP, selenite, or MRTP + selenite for 30 days, to investigate a possible effect on spontaneous micronucleus frequencies.

On day 30, all animals were sacrificed at the sampling time of 6 h by cervical dislocation. A micronucleus assay from bone marrow was performed according to the protocol described by MacGregor et al. (*19*). The air-dried slides were fixed in methanol and subjected to Giemsa staining. A thousand polychromatic erythrocytes (PCEs) were analyzed per animal, including micronucleated polychromatic eryth-

Table 2. Body Weight Gain (Mean ± SD) after 30 Days of Treatment with Different Concentrations of MSTP, MRTP, and Selenite

treatments	Se dose (μg/kg bw)	no. of animals	body weight changes during the experiment (g)					body weight gain (g)
			0 weeks	1 weeks	2 weeks	3 weeks	4 weeks	
water	0	10	27.04 ± 1.78	31.97 ± 1.60	35.46 ± 1.92	35.88 ± 2.42	36.91 ± 2.23	9.87 ± 0.45
water + CP	0	10	27.17 ± 1.08	31.68 ± 1.30	34.78 ± 2.40	36.22 ± 3.10	37.45 ± 2.74	10.28 ± 1.66
MSTP(a) + CP	40	10	27.02 ± 1.04	29.60 ± 2.16	31.68 ± 1.80	32.84 ± 1.36	32.58 ± 2.86	5.56 ± 1.82[b]
MSTP(b) + CP	20	10	27.02 ± 1.02	29.45 ± 2.10	31.80 ± 2.20	31.86 ± 2.55	32.47 ± 1.36	5.45 ± 0.34[b]
MSTP(c) + CP	10	10	26.90 ± 0.96	28.98 ± 1.20	31.38 ± 1.00	32.72 ± 1.89	33.76 ± 2.10	6.86 ± 1.14[b]
MRTP + CP	0.09	10	26.67 ± 0.92	28.79 ± 1.51	31.85 ± 1.75	32.76 ± 1.75	32.53 ± 1.93	5.86 ± 1.01[b]
selenite + CP	20	10	27.09 ± 1.57	33.61 ± 2.25	37.00 ± 1.84	37.91 ± 2.52	39.17 ± 2.45	12.08 ± 0.88[a]
MRTP + selenite + CP	20	10	27.02 ± 2.00	28.59 ± 2.37	31.17 ± 3.83	32.48 ± 2.18	32.84 ± 2.14	5.82 ± 0.14[b]
MSTP(a)	40	10	26.93 ± 1.00	29.43 ± 2.22	31.63 ± 1.50	32.82 ± 2.47	32.32 ± 2.49	5.39 ± 1.48[b]
MSTP(b)	20	10	26.79 ± 1.25	29.01 ± 2.43	30.83 ± 3.19	31.39 ± 2.51	33.07 ± 1.99	6.28 ± 0.74[b]
MSTP(c)	10	10	27.19 ± 1.07	30.17 ± 2.39	31.81 ± 1.66	32.18 ± 2.15	32.28 ± 1.48	5.09 ± 0.41[b]
MRTP	0.09	10	26.91 ± 1.66	29.31 ± 2.81	31.59 ± 2.54	32.72 ± 2.91	32.93 ± 2.97	6.02 ± 1.31[b]
selenite	20	10	26.87 ± 1.32	32.86 ± 1.38	38.08 ± 1.98	37.10 ± 1.98	39.18 ± 2.06	12.31 ± 0.74[a]
MRTP + selenite	20	10	26.80 ± 1.44	29.68 ± 1.71	30.30 ± 2.60	31.10 ± 3.07	32.06 ± 3.22	5.26 ± 1.78[b]

[a] Significantly different from positive controls at $P < 0.05$. [b] Significantly different from positive controls at $P < 0.001$.

Table 3. Frequencies of MNPCEs in Mice Bone Marrow after Pretreatment with Various Concentrations of MSTP, MRTP, and Selenite and Post-treatment with CP

treatments	Se dose (μg/kg bw)	no. of analyzed cells	MNPCE		reduction (%)
			no.	%	
water	0	10000	62	0.62	
water + CP	0	10000	564	5.64	
MSTP(a) + CP	40	10000	104	1.04	91.6
MSTP(b) + CP	20	10000	107	1.07	91.0
MSTP(c) + CP	10	10000	123	1.23	87.8
MRTP + CP	0.09	10000	264	2.64	59.8
selenite + CP	20	10000	163	1.63	79.9
MRTP + selenite + CP	20	10000	136	1.36	85.3
MSTP(a)	40	10000	9	0.09	
MSTP(b)	20	10000	8	0.08	
MSTP(c)	10	10000	14	0.14	
MRTP	0.09	10000	43	0.43	
selenite	20	10000	22	0.22	
MRTP + selenite	20	10000	19	0.19	

rocytes (MNPCEs). Slides were scored blindly using a light microscope with a 100× immersion objective. The percentage of reduction in the frequency of MNPCEs was calculated using the following formula (*20*), reduction (%) = (frequency of MNs in A − frequency of MNs in B)/(frequency of MNs in A − frequency of MNs in C) × 100, where A is the group treated with CP (positive control); B is the group of cells with the MSTP, MRTP, selenite, and MRTP + selenite plus CP; and C is the negative control.

Considering the interaction between selenium and tea constituents, the expected theoretical combination effects were calculated according to the following equation: combined reduction effect (%) = (frequency of MNs in positive control − frequency of MNs in B)/(frequency of MNs in A − frequency of MNs in C) × 100.

Blood and Liver Samples Collection and Preparation. Blood samples were collected by extirpating the eyeballs in heparinized polypropylene tubes stored at −20 °C for measurement of Se content. The rest was centrifuged for 10 min at 1453*g*, 4 °C, to recover the plasma and stored at −20 °C for further measurement of enzyme activities. After the animals were sacrificed, the liver was removed and homogenized in 9 volumes of ice cold physiological saline solution and centrifuged at 3500*g* for 10 min at 4 °C, and then, the supernatant was decanted and stored at −20 °C until analysis.

Measurement of Antioxidant Enzymes and Selenium Contents. The SOD activity was determined by hydroxylamine assay developed from a xanthine oxidase assay described by McCord (*21*), which was presented as units per milligram of protein.

GPx activity measurements were conducted according to Tietze (*22*). Glutathione can react with DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] and form yellow final product at 412 nm by Colorimetric assays. One unit of GPx activity was defined as 1 μm/L GSH oxidized to GSSG (glutathione disulphide) per milligram of protein per minute after exclusion of nonenzymatic reactions.

The content of soluble tissue protein was measured by the Coomassie Brilliant Blue G-250 method (*23*). Coomassie Brilliant Blue G-250 has the potential to bind with protein. The binding of the dye to protein causes the maximum absorption at 595 nm.

Se sample preparation and measurement were performed by hydride generation atomic absorption spectrometry method as reported by Chen and co-workers (*24*). Briefly, samples were placed in flasks separately. Mixed acid of ultrapure nitric acid and perchloric acid (v/v, 3:1) was added into each flask and heated on an electric furnace until the solution became clear. Then, hydrochloric acid was added to reduce selenate to selenite, and the Se content was analyzed (*25*).

Statistical Analysis. Mean body weights and data from biochemical assays were analyzed by analysis of variance and posthoc compared by Tukey's HSD test in case of significance. The correlation between selenium concentration and antimutagenic effect was calculated by Kendall Tau. Multiple comparisons of means were done by a LSD (least significant difference) test. A probability value of $P < 0.05$ was considered significant. All computations were made by employing the statistical software (SAS, version 8.0).

RESULTS

Table 2 presents the means of body weight and body weight gains during the experiment. Animals in all groups showed increases in body weights, and no statistical differences were observed between CP-treated groups and their corresponding non-CP-treated groups. Groups receiving selenite have the higher body weight gains than negative control and other groups. Growth was not affected by dietary Se level. However, animals administered with MSTP, MRTP, and MRTP + selenite had a significantly lower body weight gain than groups with water or water + CP.

Table 3 shows frequencies of MNPCE in mice bone marrow after treatment with various concentrations of MSTP, MRTP, and selenite with or without CP post-treatment. No mutagenicity was found in the four kinds of administration or in three concentrations of MSTP without CP post-treatment. The percentages of micronuclei from the groups of MSTP, MRTP, and selenite were less than 0.43% when compared with the negative control. In addition, when mice received MSTP at any of concentrations used, the lowest percentages of MNPCE were produced with values under 0.14%. These results clearly indicated that MSTP, MRTP, and selenite had no effect on the induction of micronuclei in mice bone marrow cells.

In the antimutagenic assay, a significant reduction in the frequencies of MN on the animals treated with MSTP, MRTP, and selenite was observed followed by CP treatment, and for

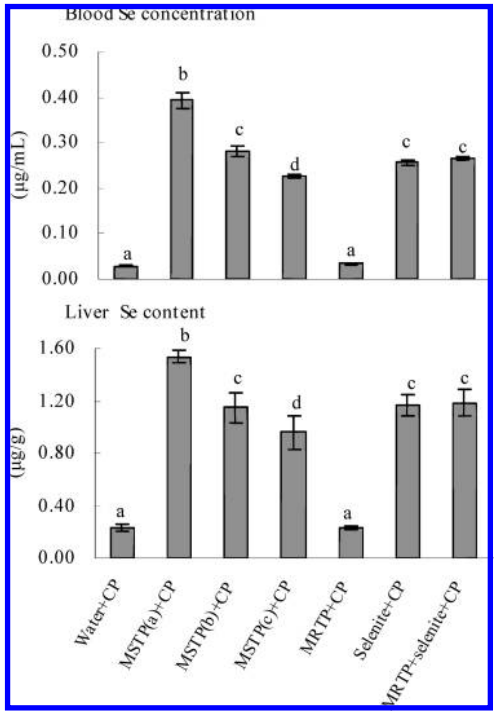

Figure 2. Effect of administration of MSTP, MRTP, and selenite on selenium levels in mice blood. Values are the means ± SD of 3−9 independent determinations. Different letters indicated significant differences from each other among testing groups with $P < 0.01$.

MSTP at the dose of 40 μg/kg bw, this inhibition effect reached the maximum of 91.6%. It is also noteworthy that MSTP at the dose of 20 μg/kg presented the higher inhibition activity of 91.0% than MRTP, selenite alone, or the combination of MRTP and selenite. This protective effect was significantly related to the Se concentrations administered.

Administration of MSTP, selenite, and MRTP + selenite significantly elevated the levels of hepatic selenium concentrations, GPx, and serum Se, SOD, and GPx enzymes when compared to the positive groups. **Figure 2** shows the increase in Se level in both blood and liver after oral administration of MSTP, selenite, and MRTP + selenite, which was highly correlated to administered selenium level with $R^2 = 0.902$ and $P < 0.001$ for blood and $R^2 = 0.0.8643$ and $P < 0.001$ for liver (**Figure 3**.) Treatment with MSTP resulted in more than 7-fold increase over the positive control while at a Se dose of 40 μg/kg bw blood Se concentration reached the highest of 0.394 ± 0.02 μg/mL and hepatic Se 1.537 ± 0.04 μg/g.

Table 4 shows the effect of supplementation with MSTP, selenite, and MRTP on the status of antioxidant enzymes in mice. CP treatment alone led to significant reductions in the activities of antioxidant enzymes like SOD and GPx in both liver and blood as compared with the negative control, which indicated the existing cellular oxidative damage and was in agreement with the reports from others that CP treatment caused decreases in the activities of catalase (CAT), GPx, and glutathione reductase (GR) (*26*). After administration with MSTP, selenite, and MRTP or MRTP + Se and post-treatment with CP, the levels of SOD and GPx in CP-treated mice were partly restored, and this enhancing effect was quite specifically unique for MSTP. GPx activities in mice blood were increased with the rising administered dose ranging from 710.3 ± 74.7 to 1061.2 ± 93.6 μg/mL, while no pronounced difference was found between the dose of 20 and 40 μg/kg bw. However, we could not find the dose–response effect of selenium on liver,

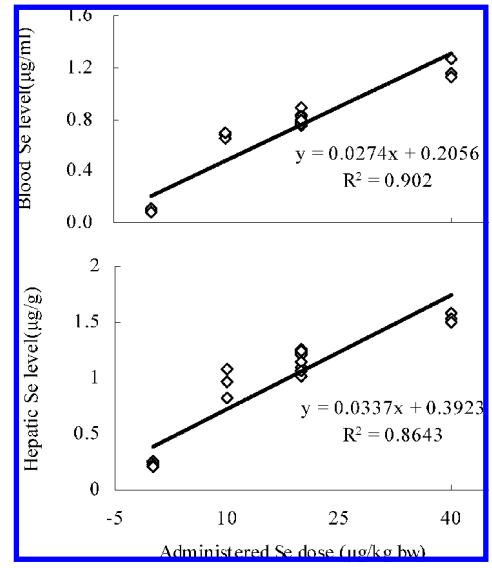

Figure 3. Linear regression to link-administered Se dose to selenium level in blood or liver.

and varying the administered dose caused slight alterations of liver GPx activities or SOD.

DISCUSSION

Most of the results of the *Salmonella* assay clearly showed that all green tea extracts have a strong antimutagenic potential, and this higher protection has been assumed to be due to the powerful scavenging and antioxidative properties of high concentrations of unpolymerized catechins and their gallates (*27, 28*). Selenium also appears to function as an antimutagenic agent (*29*), preventing the malignant transformation of normal cells and the activation of oncogenes, and all of these are closely associated with its presence in the GPxs (*30*) and TR (*31*). Green tea enriched with selenium also showed a higher antimutagenic activity than regular tea or selenite alone (*32*), which indicated a possible synergetic effect of selenium and active components in tea. However, the mechanisms are not yet completely understood.

Because of the increasing consumption of microtea powder in many food industries, the present study evaluated its protective effect on mice bone marrow PCEs. Oral treatment was chosen because it is the main route in the human body. In the present study, we found that administered MSTP or MRTP resulted in a lower body weight gain, which was in accordance with the reports from Sayama (*33*). Explanations for this could be that lipid metabolism in mice was suppressed by the administration of green tea powder (*34*) and that hypocholesterolemic activity of PGT might be due to the inhibition of the synthesis of cholesterol in the liver (*35*).

The mutagenicity potential of MSTP evaluated in mice bone marrow cells by micronucleus test demonstrated that MSTP at various administered doses did not cause any micronuclei as compared to negative control (**Table 3**). Biochemical assay also shows that mice receiving either form of MSTP, selenite, or selenite + MRTP had enhanced GPx activities and Se levels in blood and liver as compared to mice receiving only water with lower GPx activites (48.7 ± 9.5 U/mg in liver and 854.2 ± 92.2 U/mL in blood) and decreased Se levels (data not shown). These results indicated that consumption of MSTP is safe and enhance the in vivo antioxidant systems.

Table 4. Effect of Supplementation with MSTP, Selenite, and MRTP on Levels of Antioxidant Enzymes in Mice[a]

		SOD enzyme		GPX	
treatments	Se dose (μg/kg w)	liver (U/mg)	blood (U/mL)	liver (U/mg)	blood (U/mL)
water	0	353.2 ± 36.1	232.3 ± 19.3	48.7 ± 9.5	854.2 ± 92.2
water + CP	0	174.4 ± 19.0[c]	80.5 ± 29.9[c]	24.4 ± 3.4[b]	507.8 ± 67.5[b]
MSTP(a) + CP	40	202.0 ± 35.9[c]	220.4 ± 21.9[c]	88.3 ± 7.7[c]	1061.2 ± 93.6[b]
MSTP(b) + CP	20	205.0 ± 34.3[c]	194.5 ± 31.8[c]	86.5 ± 11.1[c]	965.2 ± 103.4
MSTP(c) + CP	10	237.8 ± 37.3[c]	155.9 ± 33.2[c]	81.5 ± 14.6[c]	710.3 ± 74.7
MRTP + CP	0.09	216.6 ± 25.1[c]	125.5 ± 37.3[c]	30.6 ± 11.2	732.6 ± 122.4
selenite + CP	20	206.9 ± 23.8[b]	109.5 ± 16.1[c]	83.2 ± 11.1[c]	931.6 ± 126.6
MRTP + selenite + CP	20	226.3 ± 32.1[c]	172.9 ± 34.9[c]	79.9 ± 13.9[c]	960.7 ± 89.9
MSTP(a)	40	343.4 ± 20.0	315.4 ± 14.6	119.2 ± 16.7[c]	1143.2 ± 68.9[b]
MSTP(b)	20	357.7 ± 32.7	311.3 ± 26.6	109.2 ± 11.1[c]	1172.8 ± 110.8[b]
MSTP(c)	10	339.0 ± 40.5	292.8 ± 25.1	100.5 ± 20.6[c]	1236.36 ± 97.5[b]
MRTP	0.09	346.8 ± 25.3	272.1 ± 20.9	59.8 ± 14.3	960.3 ± 88.7
selenite	20	333.1 ± 37.38	259.0 ± 18.9	104.2 ± 11.2[c]	1195.4 ± 81.9[b]
MRTP + selenite	20	337.0 ± 22.1	294.3 ± 15.6	104.6 ± 8.7[c]	1181.5 ± 85.9[b]

[a] Values are the means ± SD of nine independent determinations. [b] Significantly different from positive controls at $P < 0.05$. [c] Significantly different from positive controls at $P < 0.001$.

As no deleterious effect on bone marrow cells was detected, we evaluated the protective action of MSTP against CP-induced micronuclei in bone marrow cells. Three dosages of MSTP were administered and exhibited antimutagenic activities by decreasing the frequencies of MNPCE; the dose–response effect was observed, which agreed with our previous study on Se-enriched rice, where a 30 day oral administration with ground Se-enriched rice dose dependently reduced the frequencies of mouse bone marrow MNPCEs induced by CP (*14*). MRTPs are adopted as references for they have almost similar compositions of amino acid, total polyphenol, and vitamin C (**Table 1**). In this case, we found 264 micronuclei in the group of MRTP plus CP treatment and 163 in selenite + CP. The sum of micronuclei of MRTP and selenite will be 427 in the observed 20000 cells, and on the basis of the equation, the theoretical sum reduction effect was calculated as 69.8%, which is quite lower than the measured reduction effect of 85.27%. Noteworthy, this result indicated the presence of synergistic potential interaction between selenite and tea constituents, which is consistent with the data from Amantana and its co-workers (*32*). It is rather more interesting that at the same administered Se dose of 20 μg/kg bw, microsized Se-enriched tea powder presented the even much higher antimutagenic capacity of 91.0% than selenite of 79.9% or MRTP + selenite of 85.3%.

Similar results were observed in the level of selenium, SOD, and GPx in blood and liver. MSTP administered increasingly led to a rise in these parameters. Pretreatment with MSTP, selenite, and MRTP + selenite with the same selenium dose did not cause any significant difference among these groups followed by CP treatment. It is interesting to find that no difference in Se level, SOD, or GPx after the administration of MSTP, selenite, and MRTP + Se at 20 μg/kg bw was observed. Considering selenium bioavailability in mice, these results suggested that this antimutagenic activity is independent of the selenium level in blood and liver.

In this study, enzymatic levels of GPx and SOD were investigated because they were enzymes involved in attenuating free radical-induced oxidative damage, while free radicals are closely related to DNA damage and carcinogenesis. Because selenium was able to activate SOD and GPx, it could be hypothesized that selenium compounds possess general antioxidant effects involved in different phases of free radical detoxification (*36*). Our results have also shown that, after 1 month of Se-deficient diet, Se levels decreased in either blood or liver in mice, which is coupled with decreased hepatic and plasma GPx activities in mice. Concerning the effects of Se deficiency on GPx activity, our results were consistent with those of Muller et al. (*37, 38*) that Se deficiency decreased GPx activity in plasma and organs in rats. Our data also confirmed that the decrease of GPx activity was related to the decrease of SOD activity in plasma in Se-depleted animals. It could be supposed that this decrease in SOD activity occurred in response to GPx inhibition. The same observation was made by Matsumo and Kim et al. (*39, 40*), which showed that Se content and GPx activities were significantly lower with declined activity of SOD. On the other hand, Se supplementation especially in the form of MSTP could prevent the decline of these two antioxidant enzymes activities induced by mutagens. Thus, it is possible that the synergic effect of Se and the ingredients of tea powder, which lead to increased SOD activity, may explain the prevention of the antioxidant detoxification system.

The mechanism of selenium anticarcinogenicity is not yet fully well-established. Possible ways were proposed in the review of Combs that selenium at low concentrations is mainly purposed for restoring the activities of selenoproteins (GPx and TrxR) while supernutritional doses of Se may suppress transformed cells through a combination of effects involving Se−protein activities as well as anticancer activities of Se metabolites (*41, 42*). Thereby, our choice of selenium dosage requires mention. Basically, at dietary levels no greater than 0.5 μg/kg bw, the known selenoproteins appear to be expressed maximally in animal tissues and the GPx activities seemed to reach a plateau in all tissues at 0.06−0.1 μg/g diet (*26*). At least 10-fold Se intakes will be required to observe that antimutagenic or antitumorigenic effect in animals. Here, our selection of selenium dosage is 10, 20, and 40 μg/kg bw, which is higher than the dose of 0.5 μg/kg bw for maintaining the function of the selenoproteins. This dosage was also adopted by other researchers to observe the least anticarcinogenic effect. Hereby, we need to note out that animals after 7 days of acclimatation and 29 days of experiment before CP treatment can be categorized into two groups: Se-deficient groups receiving water or MRTP with or without CP and Se supernutritonal groups for MSTP, selenite, or MGTP + Se treatment. Our results indicated that Se-deficient mice have lower Se levels and GPx or SOD activities, and this status was even worsened after CP treatment, indicated by a higher percentage of micronuclei and reduction in the activities of GPx and SOD. On the contrary, mice received greater than 10 μg/kg had a significantly higher inhibition activity against micronuclei, which was coupled with the enhancement of GPx or SOD activities. However, no pronounced difference was found in these parameters among

Se supranutrional groups. There, the mechanisms for this could be that supranutritional Se is beneficial to alter phase I and/or phase II enzymes in a manner leading to inhibition of CP−DNA adduct formation in mice erythrocytes (*43*).

Taken together, our results showed that MSTP was able to suppress the mutation induced by CP in a dose-dependent manner. This effect was higher than MRTP, selenite alone, and MRTP + selenite. Data from our studies also indicated a possible synergetic interaction between selenium and tea ingredients. When MSTP is utilized as a Se supplementation of low Se diets or as an assistant chemotherapeutic agent, it is able to reduce the toxicity and side effects of chemotherapeutic drugs. Further studies need to be conducted to explore its synergistic antimutagenic effects of chemotherapeutic drugs and components.

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