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ORIGINAL ARTICLE

Milk powder induced lipid peroxidation reduction using Ku Ding tea (*Lactuca taiwaniana* Maxim) in rats

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Abstract The effects of Ku Ding tea (*Lactuca taiwaniana* Maxim) and milk powder on biochemical and immunological parameters of Sprague-Dawley rats were investigated and the possibility of use of Ku Ding tea to reduce physiological discomfort of drinking milk powder was assessed. Eighty rats were randomly assigned to four treatments: basal diet (control), basal diet plus whole milk powder (WM), basal diet plus Ku Ding tea (KD) and basal diet plus whole milk powder and Ku Ding tea (MK). Data was collected on animals' final body weight, hematological values, blood biochemical parameters, antioxidation parameters and immune organ weight index. Results showed that final body weight of male KD was significantly lower than that of WM. White blood cell count, monocyte count and granulocyte count of KD rats were significantly lower than those of WM. Compared to the control, single milk powder supplementation numerically increased plasma malondialdehyde. The malondialdehyde in the male KD and MK rats were lower than those in WM and control, although the differences were not significant. No significant differences were found in $Na^{+}K^{+}$-adenosine triphosphatase activity, spleen and thymus index in each group. Consumption of Ku Ding tea appeared to lower lipid peroxidation that was induced by milk powder in the rats.

Keywords Ku Ding tea · *Lactuca taiwaniana* · *llex latifolia* · Lipid peroxidation · Biochemical parameters · Hematological values · Antioxidation

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Introduction

Milk is one of the most nutritious foods, and the sole food for all young mammals during the early stage of life. Milk and dairy products contain an array of balanced nutrients and therefore play an important role in assisting both children and adults to meet their nutrient requirements (Huth et al 2006, MacDonald 2008, Michaelidou 2008). Although milk is a kind of proverbial nourishing food, the consumption of milk and milk products varies considerably among regions in the world, from about 180 kg yearly per capita in Island and Finland to less than 50 kg in Japan and China (Saxelin et al 2003). The regional difference in milk consumption may be linked with many factors. One is the different eating habit, and another is the discomfort symptom occasioned by consuming milk or milk products.

In Asia presently, cases of abstraction, conjunctivitis, inflammation of nasal, oral cavities and acne are common after drinking milk, especially milk powder. The occurrence of these symptoms may be as a result of allergy to milk by some people or an indication of lactose intolerance (MacDonald 2008) in others. In Chinese traditional medi-

cine, these symptoms are categorized as *Shang huo*, which literally means 'suffering from excessively internal heating'. This traditional medical system has also implicated milk and milk products as foods that could cause *Shang huo*, and thus are not recommended for consumption especially in summer (Zheng and Zhou 2005). Chinese traditional medicine basically formed a theoretical system to explain the reasons for *Shang huo*, but it is limited to the conceptual or 'systemic' level, lacking detailed concrete evidence (Ren et al 2003).

Ku Ding tea (*Lactuca taiwaniana* Maxim) is a herbal tea beverage consumed in China and is well known for its medicinal properties, including detoxification, diuretic, 'heat-clearance' and promoting disperse wind-heat (body imbalance) (Lau et al 2002). Although Ku Ding tea is derived from several species of plants similar in appearance (Tam et al 2006), 2 species are mainly used: the wax tree species *Ligustrum* and the holly species *Ilex* (Subhuti 2002). Currently, more than 90% of Ku Ding tea consumed in China is produced from *Ilex*.

Recent research has shown that Ku Ding tea promotes blood circulation, and lowers high blood pressure and lipids, including cholesterol (Pan et al 2004). Some studies have also demonstrated that Ku Ding tea has anti-oxidative function similar to ordinary tea, as well as anti-inflammatory property (Lau et al. 2002). We therefore, hypothesized that Ku Ding tea alleviates discomfort of *Shang huo* through its 'heat clearance' effect. This study was designed to test this hypothesis using rats fed various diets that contained milk products to mimic a process of *Shang huo*, supplemented with Ku Ding tea.

Materials and methods

The use of animals and the experimental procedure were approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture of the Chinese Academy of Sciences.

Milk powder and Ku Ding tea: Whole milk powder was purchased from Hunan Avadairy Co., Ltd. (Changsha, Hunan., China) and it contained 185 g/kg protein, 200 g/kg fat, 600 g/kg carbohydrate, 200 g/kg sugar, 60 g/kg ash, 60 g/kg moisture, 2000 IU/kg vitamin A, 4000 μg/kg vitamin E, 30000 μg/kg vitamin C, 6000 mg/kg Ca, 4500 mg/kg P, 1000 μg/kg Fe, and 10000 μg/kg Zn.

Ku Ding tea extract was obtained by alcohol extraction from *llex latifolia* Thunb that was grown in Hainan Province, China, and was provided by the Hunan Engineering and Technology Center for Natural Products, Hunan Agricultural University (Changsha, Hunan., China).

Saponin compounds were analysed by spectrophometric method (Anon 1989). Briefly, with ursolic acid in *llex latifolia* Thunb as reference substance, and 8% vanillin in ethanol solution and 77% sulphuric acid solution as chromogenic reagents, after 10 min incubation at 60 °C, the content of total saponins in Ku Ding tea extract was determined at 534 nm using spectrophotometer (Lab Tech UV-2100, Columbia, Mo, USA). The amino acids content and total polyphenol were determined by ninhydrin and ferrous tartrate colorimetric method, respectively (Anon 1989). The Ku Ding tea extract had 18.2% saponin, 3.2% amino acids, and 11.0% total polyphenol.

Animals: Sprague-Dawley (SD) healthy rats, half male and half female, were purchased from the Dongchuang Lab Animal Science and Technology Inc. (Changsha, Hunan., China), and were kept in the Laboratory Animal Center, Hunan Agricultural University. The rats were allowed free access to fresh drinking water and to the basal diet (Dongchuang Lab Animal Science and Technology Inc. Changsha, Hunan., China), housed with sawdust as bedding which was replaced every 2 days. The basal diet pellet congained 83.2% dry matter, 24.2% crude protein, 4.57 crude fat, 4.2% fiber, 1.3% Ca and 0.8% Fe. The size of cage was 30 × 50 cm. The natural light/dark cycle was allowed. The room temperature was 26–28 °C during the 15 days adaptation period, and 22–24 °C during experimental period.

Experimental design: Eighty animals, each male weighing 86.5 ± 2.9 g and each female 85.2 ± 4.1 g were randomly divided into 4 groups, each consisting of 10 males and 10 females. They were housed in 4 cages per group, consisting of 5 male or female rats per cage and given 4 treatments (control basal diet, WM, 80% basal diet + 20% whole milk powder, KD, basal diet + drinking Ku Ding tea (1% w/w); MK, 80% basal diet + 20% whole milk powder + drinking Ku Ding tea). The trial began with an acclimatization period of 15 days, followed by a 45 days experimental period. The animals were fed *ad libitum* the basal diet during the initial period. During the experimental period, 20% milk powder of WM and MK was dissolved in water at a ratio of 1:4, filled into a feeding bottle, and offered to the animals twice (8–9 am and 5–6 pm) daily without any remnant. The drinking Ku Ding tea of KD and MK was formulated according to the standard 1% concentration (w/w) with water, and offered to the animals freely also in a feeding bottle. In order to ensure that the experimental rats drink Ku Ding tea, no drinking water was supplied to KD and MK treatments.

Rats were monitored daily for general physical condition. The body weights were recorded at intervals of 2 days. On 45th day all animals, after over-night fasting, were weighed. Blood samples were taken from the abdominal aorta and stored for further laboratory analysis. EDTA was used as an anti-coagulant for the blood samples. Serum was harvested by centrifuging blood samples at 1000 × g at 4 °C for 10 min, and stored at – 70 °C for further analysis. The collected blood

samples were used for hematological and serum biochemical studies. Animals were then killed by exsanguination from the abdominal aorta and then an autopsy was performed. The weights of the spleen and thymus gland were recorded for calculation of values relative to the body weights (thymus index and spleen index).

Chemical analysis: Hematological examination was performed using an XF9080 Semi-automatic Animal Hematology Analyzer (Perlong Corp., Nanjing, Jiangsu, China). The measurements included red blood cell count (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell volume distribution width (RDW), white blood cell count (WBC), lymphocyte count (LY#), monocyte count (MO#), granulocyte count (GR#), lymphocyte percentage (LY%), monocyte percentage (MO %) and granulocyte percentage (GR%).

Serum samples were analyzed for total protein (TP), albumin (ALB), blood urea nitrogen (BUN), total cholesterol (TC), glucose (GLU), aspartate aminotransferase activity (AST), alanine aminotransferase activity (ALT), and alkaline phosphatase activity (ALP), using a BS-200 Auto Chemical Analyzer (Mindray., Shenzhen, Guangdong, China), following the manufacturer's instructions.

Lipid peroxidation was estimated on the basis of serum malondialdehyde (MDA) concentration and antioxidant enzyme levels in serum were assessed by measuring superoxide dismutase (SOD, EC 1.15.1.1) activity. The analyses were performed using MDA Assay Kit and SOD Assay Kit, which were obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Serum MDA content was measured using the thiobarbituric acid (TBA) method (Tarladgis et al. 1960). MDA concentrations were calculated by the absorbance of TBA reactive substances at 532 nm and were expressed in nmol/ml. Serum SOD activity was measured according to the xanthine–xanthinoxidase method. This assay used the xanthine–xanthineoxidase system as the source of superoxide ions, and the absorbance at 550 nm was determined. The final results were expressed in U/ml. These analyses were performed in duplicates and absorbance was measured by a spectrophotometer (Lab Tech UV-2100 Columbia, MO., U.S.A).

The activity of red blood cell membrane Na^+K^+-adenosine triphosphatase (Na^+K^+-ATPase, EC 3.6.1.37) was determined to indicate cell energy metabolism and function. Na^+K^+-ATPase Kit were all provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu., China) and Na^+K^+-ATPase, activity was detected according to the theory that ATPase metabolizes ATP into inorganic phosphate. The final results were expressed in μmol Pi/10^7RBC/h.

Statistical analysis: All the results were presented as means ± SD. The effects of the diets on the various measured variables were subjected to analysis of variance (ANOVA), and the Duncan SSR—test was used for comparing the difference between groups using statistical software. Statistical significances were declared at 5% probability level.

Results and discussion

Body weight: There were no significant changes in general conditions of the rats in any group during the study. The body weight gains during the experimental period are shown in Fig 1. At the end of the experiment, the control, KD and MK groups showed slightly lower body weights compared with WM group in both male/female groups. However statistically significant difference ($p < 0.05$) in final body weight was only detected between male KD and male WM rats group.

The body weight of male KD rats (226 ± 34 g) at the end of the experiment was lower than that of CO (286 ± 19 g) rats, so also the body weight of male MK rats (323 ± 95 g) was lower than that of WM (369 ± 57 g) rats. In the female group, there was only slight decrease in mean body weight of MK rats, as compared with those of WM. This showed the ability of Ku Ding tea to reduce the rat body

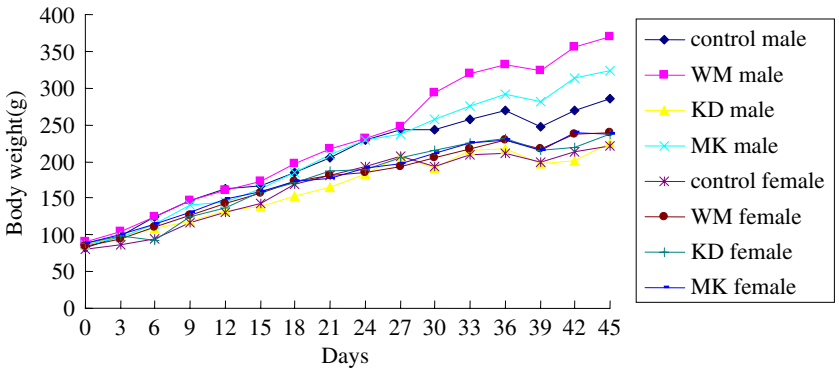

Fig 1 Cumulative body weight change of experimental rats

weight and this effect was more evident in the male than in female rats. *Ilex* species has been suggested for obesity management (Heck and Mejia 2007) and Ku Ding tea has also been believed to have weight loss properties in China. Zheng (2003) reported that 20 days administration of total terpene (TT) of Ku Ding tea to nutritional obese SD rats resulted in reduction of body weight. It has also been reported that triterpenes from Ku Ding tea exhibited acyl CoA cholesteryl acyl transferase (ACAT) inhibitory activity, thus potentially serving as new type of medicines to treat arteriosclerosis and obesity (Nishimura et al 1999a, b). The results of this study further supported the Ku Ding tea weight loss property.

Hematological parameters: In male rats, the RBC count and Hb of rats on MK diet were highest ($p < 0.05$) (Table 1). The MCH and MCHC of KD treatment were lower than the other ($p < 0.05$). So also the WBC, LY#, MO#, GR# and MO% of KD treatment were lower than those of other treatments, and the differences were all significant ($p < 0.05$) when compared with WM diet. In female rats however, there were no significant differences ($p > 0.05$) in RBC, Hb, MCH and MCHC among the four diets. As observed for male KD group rats, WBC, LY#, MO#, GR#, MO% and GR% of female rats fed KD diet were lower than those fed WM diet and the differences were significant ($p < 0.05$) only for WBC, MO#, GR# and MO%. There were no significant differences ($p > 0.05$) in HCT, MCV, RDW, LY% and GR% in either male or female groups.

In the present study, decrease of WBC counts in KD and MK rats was detected as compared with WM rats, and the reductions were also evident in different leukocyte counts in KD rats and female MK rats, and decreased MO# and GR# were observed in male MK rats. However, KD and MK rats had higher LY% and lower MO% and GR% as

Table 1 Influence of diets on hematological data of rats

Parameters*	Dietary		Treatment		
	Control	WM	KD	MK	SEM
Male					
RBC, 10^9/l	8.3 ± 0.9^{b}	8.5 ± 0.7^{b}	8.2 ± 0.6^{b}	10.0 ± 0.5^{a}	0.345
Hb, g/l	144 ± 15.4^{b}	164 ± 16.9^{ab}	121 ± 14.4^{c}	175.2 ± 9.2^{a}	7.156
HCT, l/l	0.60 ± 0.12^{a}	0.63 ± 0.09^{a}	0.64 ± 0.04^{a}	0.72 ± 0.04^{a}	0.037
MCV, fl	71.7 ± 6.35^{a}	73.7 ± 8.22^{a}	78 ± 4.06^{a}	74.3± .69^{a}	2.874
MCH, pg	17.3 ± 1.45^{ab}	19.2 ± 1.44^{a}	14.9 ± 2.39^{b}	17.4 ± 0.82^{ab}	0.865
MCHC, g/l	242.3 ± 28.4^{a}	261.2 ± 14.8^{a}	190 ± 22.3^{b}	235.5 ± 20.2^{a}	10.778
RDW, %	17.7 ± 1.29^{a}	17.92 ± 1.48^{a}	18.5 ± 0.48^{a}	17.7 ± 0.85^{a}	0.524
WBC, 10^9/l	19.7 ± 3.34^{a}	24.22 ± 2.78^{a}	14.8 ± 2.09^{b}	23.3 ± 4.10^{a}	1.550
LY#, 10^9/l	14.1 ± 3.37^{ab}	16.15 ± 0.26^{a}	11.3 ± 2.03 ^{b}	16.8 ± 3.68 ^{a}	1.302
MO#, 10^9/l	2.8 ± 0.49^{a}	3.6 ± 0.94^{a}	1.5 ± 0.63^{b}	2.9 ± 0.66^{a}	0.356
GR#, 10^9/l	2.9 ± 1.05^{ab}	4.5 ± 1.99^{a}	2.0 ± 1.19^{b}	3.5 ± 1.32^{ab}	0.728
LY, %	71 ± 8.03^{a}	67.4 ± 7.89^{a}	76.6 ± 9.96^{a}	72.2 ± 7.64^{a}	4.335
MO, %	14.2 ± 2.08^{a}	14.6 ± 2.03^{a}	9.8 ± 3.47^{b}	12.6 ± 2.04^{ab}	1.315
GR, %	14.8 ± 5.93^{a}	18.0 ± 5.93^{a}	13.6 ± 6.57^{a}	15.2 ± 5.59^{a}	3.060
Female					
RBC, 10^9/l	7.6 ± 1.2^{a}	7.2 ± 0.7^{a}	7.8 ± 1.0^{a}	8.2 ± 2.3^{a}	0.750
Hb, g/l	140 ± 36.0^{a}	120.7 ± 20.5^{a}	123.3 ± 25.7^{a}	143.2 ± 46.7^{a}	17.825
HCT, l/l	0.592 ± 0.117^{a}	0.527 ± 0.021^{a}	0.58 ± 0.101^{a}	0.59 ± 0.169^{a}	0.060
MCV, fl	77.2 ± 5.38^{a}	74 ± 4.90^{a}	74.7 ± 4.16^{a}	72.8 ± 4.92^{a}	2.550
MCH, pg	18.2 ± 2.29^{a}	16.8 ± 1.51^{a}	15.8 ± 1.46^{a}	17.3 ± 1.02^{a}	0.856
MCHC, g/l	235 ± 27.0 ^{a}	228.2 ± 30.6^{a}	210.7 ± 19.7^{a}	238.5 ± 24.5^{a}	13.599
RDW, %	16.7 ± 0.99^{a}	17.4 ± 1.55^{a}	17.0 ± 0.99^{a}	17.6 ± 1.83^{a}	0.735
WBC, 10^9/l	16.4 ± 3.97^{ab}	17.7 ± 2.96^{a}	12.1 ± 2.15^{bc}	8.6 ± 1.95^{c}	1.517
LY#, 10^9/l	11.1 ± 3.52^{a}	12.2 ± 1.68^{a}	9.8 ± 3.06^{ab}	6.7 ± 1.75^{b}	1.339
MO#, 10^9/l	2.2 ± 0.42^{a}	2.4 ± 0.63^{a}	1.0 ± 0.42^{b}	0.87 ± 0.22^{b}	0.233
GR#, 10^9/l	3.0 ± 0.34^{a}	3.2 ± 1.2^{a}	1.3 ± 0.61^{b}	0.94 ± 0.39^{b}	0.382
LY, %	67.4 ± 6.14^{a}	69.1 ± 7.19^{a}	79.5 ± 10.53^{a}	78.3 ± 7.42^{a}	4.000
MO. %	13.8 ± 1.57^{a}	13.2 ± 1.90^{ab}	8.8 ± 4.64^{b}	10.3 ± 2.18^{ab}	1.358
GR, %	18.8 ± 4.6^{a}	17.6 ± 5.52^{a}	11.6 ± 6.45^{a}	11.3 ± 5.25^{a}	2.800

GR# granulocyte count, GR% granulocyte percentage, Hb haemoglobin HCT hematocrit, LY# lymphocyte count, LY% lymphocyte percentage, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, MCV mean corpuscular volume, MO# monocyte count, MO% monocyte percentage, RBC red blood cell count, RDW red blood cell volume distribution width, WBC white blood cell count

Means with the same superscripts within a row are not significantly different ($p > 0.05$) ($n = 10$ rats)

For dietary formulations control, WM, KD, MK: See text

Table 2 Influence of diets on Plasma biochemical of rats

Parameter*	Dietary		Treatment		
	Control	WM	KD	MK	SEM
Male					
TP, g/dl	83.9 ± 7.87[a]	81.3 ± 2.19[a]	76.1 ± 4.9[a]	83.5 ± 6.4[a]	2.747
ALB, g/dl	43.7 ± 0.23[ab]	42.2 ± 1.57[ab]	41.2 ± 1.38[b]	44.9 ± 3.25[a]	0.996
BUN, mmol/l	8.3 ± 1.70[a]	7.6 ± 0.60[a]	8.6 ± 0.81[a]	7.4 ± 0.72[a]	0.483
GLU, mg/dl	5.2 ± 0.36[a]	5.4 ± 0.20[a]	5.7 ± 0.95[a]	6.1 ± 1.08[a]	0.399
TC, mg/dl	1.96 ± 0.34[a]	1.63 ± 0.19[a]	2.1 ± 0.43[a]	1.9 ± 0.17[a]	0.156
AST, IU/l	208.7 ± 122.0 [a]	137.8 ± 17.3[a]	180.5 ± 6.1[a]	237.9 ± 106.5[a]	37.044
ALT, IU/l	61.5 ± 8.24[a]	51.5 ± 12.71[a]	69.2 ± 14.75[a]	79.0 ± 34.04[a]	10.255
ALP,IU/l	396.9 ± 126.97[ab]	343.4 ± 79.9[b]	472.2 ± 147.6 [ab]	587.7 ± 97.9[a]	59.522
Female					
TP, g/dl	81 ± 2.75[a]	82.6 ± 1.65[a]	82.3 ± 1.63[a]	82.38 ± 5.12[a]	1.616
ALB, g/dl	41.2 ± 4.63[a]	42.8 ± 1.78[a]	45.1 ± 0.59[a]	44.0 ± 2.43[a]	1.339
BUN, mmol/l	7.8 ± 1.12[a]	7.7 ± 0.90[a]	9.0 ± 1.156[a]	8.0 ± 1.11[a]	0.526
GLU, mg/dl	5.2 ± 0.46[b]	5.2 ± 0.72[b]	6.3 ± 1.14[ab]	7.2 ± 0.86[a]	0.406
TC, mg/dl	1.98 ± 0.29[a]	1.78 ± 0.30[a]	1.81 ± 0.16[a]	2.1 ± 0.28[a]	0.130
AST, IU/l	186.5 ± 76.4[a]	147.0 ± 27.1[a]	177.1 ± 25.9[a]	202.6 ± 77.8[a]	28.915
ALT, IU/l	54.3 ± 20.47[a]	55.2 ± 12.62[a]	46.8 ± 6.19[a]	63.4 ± 18.13[a]	7.586
ALP, IU/l	417.1 ± 198.2 [a]	294.3 ± 164.0 [a]	340.8 ± 110.0 [a]	316.0 ± 105.6 [a]	71.290

ALB Albumin, *ALP* alkaline phosphatase, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *BUN* blood urea nitrogen, *GLU* glucose, *TC* total cholesterol, *TP* total protein
Means with the same supercripts within a row are not significantly different ($p > 0.05$), ($n = 10$ rats)

compared with WM rats. The reason remains unknown regarding the sensitivity of WBC subpopulations.

Plasma biochemical parameters: In males, ALB of KD group was lower ($p < 0.05$) than that of MK group (Table 2). ALP in MK group was the highest among all the groups and increased to 171.1% compared to the male WM group ($p < 0.05$). There were no significant differences in TP, BUN, GLU, TC, AST and ALT among male groups. In females, KD and MK treatments showed increased GLU level as compared with control and WM groups, but the increase was statistically significant ($p < 0.05$) only in rats fed MK diets. No significant differences of other serum parameters were found in female groups. According to the serum biochemical data, the indices in the plasma to liver (ALT and AST) and kidney functions (BUN) of the rats were not significantly different among all the groups, which might imply that the liver and kidney functions were normal and were not affected by drinking milk powder and Ku Ding tea.

Lipid peroxidation and SOD activity in plasma: The lipid peroxidation is indicated as MDA concentration. In male rats, at the end of the experimental period, the MDA concentration in male control group was 4.49 nmol/ml (Table 3). Compared with the control group, single milk powder supplement increased MDA concentration in plasma to 5.00 nmol/ml. The MDA levels in the male KD and MK rats were lower than those in the WM and control rats, although the difference was not significant ($p > 0.05$). In females, the MDA level for KD rats was 3.37 nmol/ml and was significantly lower ($p < 0.05$) than those in the MK treatment. The activity of SOD in the KD and MK groups was significantly ($p < 0.05$) lower than that in the WM group in both sexes.

Table 3 Effects of Ku Ding tea and milk powder on MDA concentration and SOD activity in plasma of Sprague-Dawley rats

	Dietary		Treatment		
	Control	WM	KD	MK	SEM
Male					
MDA, nmol/ml	4.5 ± 0.83[a]	5.0 ± 0.80[a]	3.4 ± 0.86[a]	4.1 ± 1.29[a]	0.489
SOD, U/ml	277 ± 103.0 [ab]	348 ± 16.6 [a]	231 ± 42.4[b]	210 ± 31.9[b]	26.654
Female					
MDA, nmol/ml	3.6 ± 0.72[ab]	4.0 ± 0.36[ab]	3.7 ± 0.55[b]	4.9 ± 1.48[a]	0.459
SOD, U/ml	365 ± 19.9[a]	308 ± 17.6[b]	193 ± 18.1[c]	215 ± 28.3[c]	10.718

MDA: Malondialdehyde, SOD: Superoxide dismutase
Means with the same superscripts within a row are not significantly different ($p > 0.05$), ($n = 10$ rats)

Table 4 Effects of Ku Ding tea and drinking milk powder on Na^+K^+-ATPase activity in red blood cell membrane of 'Sprague-Dawley' rats

	Dietary		Treatment		
	Control	WM	KD	MK	SEM
Male					
Na^+K^+-ATPase, μmol Pi/10^7RBC/h	17.8 ± 3.86[a]	14.7 ± 1.74[a]	17.8 ± 5.49[a]	13.2 ± 3.66[a]	2.056
Female					
Na^+K^+-ATPase, μmol Pi/10^7RBC/h	14.0 ± 3.31[a]	13.9 ± 1.53[a]	12.9 ± 5.07[a]	10.8 ± 2.10[a]	1.566

Na^+K^+-ATPase, Na^+K^+-adenosine triphosphatase

Means with the same superscript within a row are not significantly different ($p > 0.05$), ($n = 10$ rats)

Lipid peroxidation is an indicator of oxidative stress in human beings and other organisms (McCord 2000) and it has been proposed as a general mechanism for cell injury and death (Miki et al 1987). MDA is an end product of lipid peroxidation whose formation is accelerated by oxidative stress (Horie et al 1997) and thus detection of MDA can reflect the level of oxygen free radicals and the extent of lipid peroxidation.

Bay et al (1999) investigated effects of skim milk and cultured milk supplementation on peroxidative stress in brains of weanling rats and reported a reduction in brain TBA reactive substances concentration in milk-supplemented animals as compared with controls. However Bricarello et al. (2004) observed low fat cow milk to cause a slight increase of TBA reactive substances in comparison with baseline, in patients with primary hypercholesterolemia.

Our results showed that MDA in plasma was increased in the WM rats as compared with control group. The MDA concentration for KD rats was the lowest among both male and female groups. And MDA of male MK group rats was lower than that in the WM group. This indicates that Ku Ding tea could inhibit the formation of MDA and reduce lipid peroxidation.

Antioxidant enzymes, such as SOD, are widely distributed in all cells (McCord 2000). SOD protects the cell against O_2^- by dismutation of the highly reactive superoxide anion to O_2 (Hseu et al 2008). Therefore SOD can clear the superoxide anion radical to protect the cells from being injured, and plays an important role in maintaining the balance between oxidation and anti-oxidation of the organism. Observation from this study showed that SOD activity in KD and MK groups were lower than in other groups. Although KD and MK groups had lower SOD activity, their MDA concentration was also lower. The possible reason may be that Ku Ding tea was able to scavenge superoxide and free radicals and inhibit lipid peroxidation, so that the concentration of MDA was at a relatively low level, and SOD enzyme activity at the lower level could maintain the normal antioxidant status of the body. Some researchers have also demonstrated that Ku Ding tea or its extracts markedly exhibited antioxidant potency in scavenging free radicals and prevented RBC from hemolysis in *in vitro* or *in vivo* tests (Lau et al 2002, Liu et al 2009, Thuong et al 2009). The results of this study further supported that Ku Ding tea had antioxidation effect.

Na^+K^+-ATPase activity in red blood cell membrane of rats: There were no significant differences ($p > 0.05$) in this parameter among the treatments in both sexes (Table 4). In animal cells, Na^+K^+-ATPase is the major ion motive ATPase and it is a membrane-bound enzyme that couples the free energy contained within the ATP molecule to the translocation of Na^+ and K^+ across the membrane (Horisberger et al 1991). Therefore ATPase activity is a useful index to energy metabolism in various cells. Our results showed that the Na^+K^+-ATPase activity of KD group was relatively high (17.8 and 12.9 μmol Pi/107RBC/h) in 4 groups and the possible reason was that Ku Ding tea could activate the Na^+K^+-ATPase. However, in MK group the

Table 5 The spleen index and thymus index for 'Sprague-Dawley' rats

	Dietary		Treatment		
	Control	WM	KD	MK	SEM
Male					
Spleen index, mg/g	3.5 ± 0.42[a]	3.5 ± 0.7[a]	3.0 ± 1.04[a]	3.7 ± 0.83[a]	0.414
Thymus index, mg/g	1.8 ± 0.88[a]	1.8 ± 0.15[a]	1.6 ± 0.49[a]	1.79 ± 0.26[a]	0.244
Female					
Spleen index, mg/g	4.0 ± 1.27[a]	3.74 ± 0.98[a]	3.1 ± 0.81[a]	2.9 ± 0.60[a]	0.450
Thymus index, mg/g	2.2 ± 0.21[a]	1.89 ± 0.51[a]	2.2 ± 0.39[a]	2.3 ± 0.24[a]	0.171

Means with the same subscript within a row are not significantly different ($p > 0.05$), ($n = 10$ rats)

ATPase activity was the lowest (13.2 and 10.8 μmol Pi/107RBC/h) both in the male and female groups. The difference in the ATPase activity between KD and MK groups could be attributed to the existence of interaction between Ku Ding tea and milk powder.

Immune organ weight index: The spleen index and thymus index, as shown in Table 5, were not significantly influenced ($p > 0.05$) by experimental diets in both sexes. Therefore, drinking Ku Ding tea and/or milk powder did not affect the size of spleen and thymus in male and female rats.

Thymus and spleen are important immunological organs and their organ indexes may to some extent reflect the strength of the immune function. Few studies have reported the effect of Ku Ding tea on the immune tissue. The current study showed no difference in term of thymus weight index and the spleen weight index among all the groups, indicating that taking Ku Ding tea and drinking milk powder had no significant impact on the immune tissues. Further research is necessary to elucidate the impact of Ku Ding tea on cell and humoral immunity in animals.

Conclusion

Ku Ding tea could inhibit lipid peroxidation, through its antioxidant function. Furthermore, supplementation of Ku Ding tea appeared to reduce milk powder-induced lipid peroxidation. Therefore, our findings suggest that lipid peroxidation may be one aspect of milk-induced *Shang huo* mechanisms and Ku Ding tea may have a function to alleviate milk-induced *Shang huo* symptom through its antioxidation effects.

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