Postprint: Antioxidant Effects of Green Tea Powder and Green Tea Polyphenols in Dogs

Authors: Li Yu, Huanqing Tao, Zhang Yudie, Xu Yizhong, Hao Ning, Zou Suping, Feng Shibin, Wang Xichun

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Abstract

The present experiment aimed to investigate the antioxidant effects of green tea powder and green tea polyphenols in dogs. Fifteen 5-month-old hybrid puppies were selected and randomly divided into three groups (n=5 per group). The control group was fed a basal diet, the green tea powder group was fed the basal diet supplemented with 1.0% green tea powder, and the green tea polyphenol group was fed the basal diet supplemented with 0.25% green tea polyphenols. The experimental period lasted 84 days. The results showed that, compared with the beginning of the experiment, body weight at the end of the experiment increased by 162% in the control group, 101% in the green tea powder group, and 132% in the green tea polyphenol group. Moreover, final body weight in the green tea powder and green tea polyphenol groups was significantly lower than that of the control group ($P<0.05$), being reduced by 61% and 30%, respectively. On day 84, serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in dogs from the green tea powder and green tea polyphenol groups were significantly or highly significantly lower than those of the control group ($P<0.05$ or $P<0.01$). Serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were highly significantly higher than those of the control group ($P<0.01$), serum malondialdehyde (MDA) content was highly significantly lower than that of the control group ($P<0.01$), and relative mRNA expression levels of hepatic antioxidant genes [glutamate-cysteine ligase catalytic subunit (GCLC), glutamate-cysteine ligase modifier subunit (GCLM), heme oxygenase-1 (HO-1), catalase (CAT)] and phase II detoxifying enzyme genes [glutathione S-transferase M1 (GSTM1) and quinone oxidoreductase 1 (NQO1)] were significantly higher than those of the control group ($P<0.05$). In conclusion, under the conditions of this experiment, dietary supplementation with 1.0% green tea powder or 0.25% green tea polyphenols exerted hepatoprotective, antioxidant, phase II detoxifying enzyme gene expression-promoting, and body weight-controlling effects in dogs.

Full Text

Antioxidant Effects of Green Tea Powder and Green Tea Polyphenols on Canines

LI Yu[1],[2], TAO Huanqing[2], *ZHANG Yudie[2], XU Yizhong[2], HAO Ning[2], ZOU Suping[2], FENG Shibin[2], WANG Xichun[2], ZHOU Yibin[1], WU Jinjie[2], WAN Xiaochun[1]*

[1]State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei 230036, China
[2]College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China

Abstract: This study investigated the antioxidant effects of green tea powder and green tea polyphenols (GTPs) on canines. Fifteen five-month-old hybrid puppies were randomly divided into three groups (n=5 per group). The control group received a basal diet, the green tea powder group received the basal diet supplemented with 1.0% green tea powder, and the GTPs group received the basal diet supplemented with 0.25% GTPs. The experimental period lasted 84 days. The results showed that compared with initial values, final body weight increased by 162% in the control group, 101% in the green tea powder group, and 132% in the GTPs group. Final body weight in both the green tea powder and GTPs groups was significantly lower than in the control group ($P<0.05$), representing reductions of 61% and 30%, respectively. On day 84, serum alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities in the green tea powder and GTPs groups were significantly or extremely significantly lower than in the control group ($P<0.05$ or $P<0.01$). Serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were extremely significantly higher ($P<0.01$), while serum malondialdehyde (MDA) content was extremely significantly lower ($P<0.01$) compared with the control group. The relative mRNA expression levels of hepatic antioxidant genes [glutamate cysteine ligase catalytic subunit (GCLC), glutamate cysteine ligase modifier subunit (GCLM), heme oxygenase-1 (HO-1), catalase (CAT)] and phase II detoxification enzyme genes [glutathione S-transferase M1 (GSTM1) and NAD(P)H quinone dehydrogenase 1 (NQO1)] were significantly higher than in the control group ($P<0.05$). In conclusion, under the conditions of this experiment, dietary supplementation with 1.0% green tea powder or 0.25% GTPs exerted hepatoprotective, antioxidant, and phase II detoxification gene-inducing effects while controlling body weight gain in canines.

Keywords: canines; green tea powder; green tea polyphenols; liver function; antioxidant genes; phase II detoxification genes

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In recent years, advances in medical and veterinary care have deepened our understanding of free radicals, revealing that chronic diseases in dogs are as-

sociated with oxidative damage caused by free radical accumulation. Puppies (generally defined as small breeds from weaning to approximately 8 months and large breeds from weaning to 12 months) have immature immune systems, low disease resistance, and the highest morbidity and mortality rates. During this stage, puppies require specialized diets to meet their nutritional needs for growth. Adult dogs (generally over 8–12 months) have mature immune systems and stronger disease resistance, and are fed adult maintenance diets. Puppy management requires not only appropriate nutrition based on developmental characteristics but also functional additives to support their immature digestive systems and enhance immunity.

China possesses abundant tea resources, and developing new applications for tea is of significant importance. Tea functionality is determined by its various components, with polyphenols—polyhydroxy phenolic compounds—being the primary bioactive constituents. Since Japanese scientists discovered the antioxidant properties of tea polyphenols, research on their extraction and application has attracted widespread attention. While green tea powder and GTPs have demonstrated beneficial effects in livestock production and the feed industry, their application as natural antioxidants in companion animals such as dogs remains poorly documented.

Therefore, this study aimed to investigate the antioxidant effects of green tea powder and GTPs in canines, explore their application potential, and provide theoretical guidance for the development of tea-containing pet foods.

1.1 Experimental Materials

Green tea powder was provided by the College of Tea and Food Science at Anhui Agricultural University, while GTPs were purchased from Wuhu Tianyuan Technology Co., Ltd. The major component contents of both materials are presented in Table 1 .

Basal diets for different canine developmental stages were Hentoo complete dog food, a co-development of Shanghai Taoleisi Technology Co., Ltd. and the U.S. Taoleisi Experimental Center. This extruded pellet feed had nutrient levels shown in Table 2 .

1.2 Experimental Animals and Management

Fifteen five-month-old hybrid puppies were purchased from a local kennel in Hefei. All dogs were housed in kennels at the Animal Hospital of Anhui Agricultural University and received routine vaccinations. Dogs were fed twice daily at 09:00 and 17:00, with feed amounts adjusted according to body weight changes. Fresh water was available ad libitum. Kennels were cleaned daily to maintain hygiene and were regularly disinfected.

1.3 Experimental Design

The fifteen puppies were randomly divided into three groups (n=5): control, green tea powder, and GTPs groups. The basal diet was ground and mixed thoroughly with additives. The control group received the basal diet only, the green tea powder group received basal diet plus 1.00% green tea powder, and the GTPs group received basal diet plus 0.25% GTPs.

1.4 Sample Collection

Blood samples: Body weight was measured and 4 mL of blood was collected from the forelimb vein after 12-hour fasting on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84. Blood was centrifuged at 3,000 rpm for 10 minutes, and serum was separated and stored at -20°C for analysis.

Liver tissue samples: At the end of the experiment, six dogs from each group were anesthetized with Sumianxin (0.1 mL/kg body weight, intramuscular). Liver tissue was collected via laparotomy, snap-frozen in liquid nitrogen, and stored at -80°C for determination of antioxidant and phase II detoxification gene mRNA expression levels.

1.5 Serum Biochemical Assays

Serum ALT, AST, and LDH activities were measured using commercial kits (Changchun Huili Biotechnology Co., Ltd.). Serum SOD and GSH-Px activities and MDA content were determined using ELISA kits (Nanjing Senbeijia Biotechnology Co., Ltd.). All assays were performed according to manufacturer instructions.

1.6 Determination of Hepatic Antioxidant and Phase II Detoxification Gene mRNA Expression

Total RNA was extracted from liver tissue using Trizol Reagent (TaKaRa, Japan). Complementary DNA was synthesized using a reverse transcription kit (TaKaRa, Japan) and stored at -20°C. Primers were designed using Primer 5 software based on target sequences from the NCBI database (Table 3). Relative mRNA expression levels of antioxidant genes (GCLC, GCLM, HO-1, CAT) and phase II detoxification enzyme genes (GSTM1, NQO1) were quantified by real-time PCR using SYBR Green I (TaKaRa, Japan) on an ABI-7500 system (ABI, USA). β-actin served as the internal reference gene, and results were expressed as $2^{\wedge}(-\Delta\Delta Ct)$.

1.7 Statistical Analysis

Data are expressed as mean $\pm$ standard deviation (SD). One-way ANOVA and Duncan' s multiple comparison tests were performed using SPSS 17.0 software. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

2.1 Effects of Green Tea Powder and GTPs on Canine Body Weight

As shown in Table 4 , initial body weights were similar across the three groups (P>0.05). During weeks 0–2, all groups showed rapid weight gain without significant differences between treatment and control groups (P>0.05). As the experiment progressed, body weight increased in all groups. From week 3 onward, the green tea powder and GTPs groups showed lower body weights than the control group, though the difference was not significant at week 3 (P>0.05). From week 4 through week 12, both treatment groups had significantly lower body weights than the control group (P<0.05). By week 12, body weight had increased by 162% in the control group, 101% in the green tea powder group, and 132% in the GTPs group compared with initial values. Final body weights in the green tea powder and GTPs groups were significantly lower than in the control group (P<0.05), representing reductions of 61% and 30%, respectively. These results indicate that dietary supplementation with 1.00% green tea powder or 0.25% GTPs effectively controlled weight gain in canines.

2.2.1 Effects on Serum ALT Activity

As shown in Table 5 , serum ALT activity was significantly lower in the green tea powder group (P<0.05) and extremely significantly lower in the GTPs group (P<0.01) compared with the control group on day 28. Both treatment groups showed significantly lower ALT activity on days 35, 42, 56, and 63 (P<0.05). On days 49 and 70, ALT activity was significantly lower in the green tea powder group (P<0.05) and extremely significantly lower in the GTPs group (P<0.01). On days 77 and 84, both treatment groups showed extremely significantly lower ALT activity than the control group (P<0.01).

2.2.2 Effects on Serum AST Activity

As shown in Table 6 , serum AST activity was significantly lower in both treatment groups compared with the control group on day 56 (P<0.05). The GTPs group showed significantly lower AST activity on days 63 and 70 (P<0.05). Both treatment groups had significantly lower AST activity on day 77 (P<0.05) and extremely significantly lower activity on day 84 (P<0.01).

2.2.3 Effects on Serum LDH Activity

As shown in Table 7 , serum LDH activity was significantly lower in both treatment groups compared with the control group on day 28 (P<0.05). The GTPs group showed significantly lower LDH activity on day 35 (P<0.05). On day 49, the green tea powder group showed significantly lower LDH activity (P<0.05) while the GTPs group showed extremely significantly lower activity (P<0.01). Both treatment groups exhibited extremely significantly lower LDH activity on days 56, 63, 70, and 77 (P<0.01), and significantly lower activity on day 84 (P<0.05).

2.3.1 Effects on Serum GSH-Px Activity

As shown in Table 8 , serum GSH-Px activity was significantly higher in the GTPs group compared with the control group on day 42 ($P<0.05$). Both treatment groups showed significantly higher GSH-Px activity on days 49 and 56 ($P<0.05$). On day 63, the green tea powder group showed extremely significantly higher activity ($P<0.01$) while the GTPs group showed significantly higher activity ($P<0.05$). Both treatment groups had significantly higher GSH-Px activity on day 70 ($P<0.05$). Serum GSH-Px activity increased gradually over time in all three groups, with the treatment groups showing higher activity than the control group from day 14 onward.

2.3.2 Effects on Serum SOD Activity

As shown in Table 9 , serum SOD activity was significantly higher in the GTPs group compared with the control group on day 42 ($P<0.05$). Both treatment groups showed significantly higher SOD activity on days 49 and 56 ($P<0.05$). On days 63 and 70, the green tea powder group showed significantly higher activity ($P<0.05$) while the GTPs group showed extremely significantly higher activity ($P<0.01$). Both treatment groups had significantly higher SOD activity on day 77 ($P<0.05$) and extremely significantly higher activity on day 84 ($P<0.01$). Overall, serum SOD activity increased gradually over time in all groups, with treatment groups showing higher activity than the control group from day 14 onward.

2.3.3 Effects on Serum MDA Content

As shown in Table 10 , serum MDA content was significantly lower in the GTPs group compared with the control group on day 49 ($P<0.05$). Both treatment groups showed significantly lower MDA content on days 56 and 63 ($P<0.05$) and extremely significantly lower content on days 70, 77, and 84 ($P<0.01$). Overall, serum MDA content decreased gradually over time in all groups, with treatment groups showing lower content than the control group from day 7 onward.

2.4 Effects on Hepatic Antioxidant and Phase II Detoxification Gene Expression

As shown in Table 11 , the relative mRNA expression levels of GSTM1, GCLC, GCLM, NQO1, HO-1, and CAT in liver tissue were significantly higher in both treatment groups compared with the control group ($P<0.05$).

3.1 Effects on Serum Biochemical Indicators

ALT, AST, and LDH are primarily located in hepatocyte mitochondria and normally exhibit low activity in peripheral blood. When free radicals damage hepatocyte membrane lipids, lipid peroxidation compromises membrane integrity, causing massive release of ALT, AST, and LDH into circulation. Consequently,

elevated serum activities of these enzymes serve as sensitive indicators of hepatocellular damage.

Numerous studies have investigated the hepatoprotective effects of green tea powder and tea polyphenols. He et al. demonstrated that tea polyphenols significantly reduced elevated serum ALT activity in mice with experimental liver injury, showing protective effects against both carbon tetrachloride (CCl_4) and ethanol-induced hepatic damage. Zhang et al. reported that tea polyphenols significantly reduced serum aminotransferase activity in rats with alcohol-induced liver injury. Li et al. found that catechin compounds at medium (100 mg/kg) and high (200 mg/kg) doses significantly decreased serum ALT activity and MDA content in rats with CCl_4-induced chronic liver injury. Zhou et al. observed that various concentrations of tea polyphenols and catechins significantly reduced serum ALT activity in rats with alcoholic liver disease. Li et al. reported that dietary supplementation with 2,000 mg/kg green tea powder significantly improved serum antioxidant capacity and reduced ALT and AST activities in geriatric dogs. Chen et al. demonstrated that high (2.0 mg/kg) and medium (1.0 mg/kg) doses of tea polyphenols significantly reduced serum ALT and AST activities in lead-exposed rats, indicating hepatoprotective effects. LDH is an intracellular marker enzyme whose release reflects cell membrane permeability and cellular damage. Wang et al. showed that tea polyphenols significantly reduced LDH activity in oxidatively stressed bovine mammary epithelial cells in a concentration-dependent manner. Yu et al. reported that tea polyphenols improved survival rates and reduced LDH release and apoptosis in mouse testicular Sertoli cells exposed to herbicide-induced damage. These findings collectively demonstrate that appropriate concentrations of tea polyphenols can significantly reduce serum ALT, AST, and LDH activities. In the present study, supplementation with 1.00% green tea powder or 0.25% GTPs significantly or extremely significantly reduced serum ALT, AST, and LDH activities by the end of the experiment, indicating hepatoprotective effects in canines. Although some fluctuations were observed across the three groups, likely due to physiological variations during the feeding period, the overall trends remained stable.

3.2 Effects on Antioxidant Capacity

Normal metabolic processes generate free radicals, and excessive accumulation can cause pathological damage associated with cancer and cardiovascular diseases. GSH-Px, SOD, and CAT are endogenous antioxidant enzymes that function independently or synergistically to combat oxidative stress. MDA, a stable end-product of lipid peroxidation, serves as an indirect marker of cellular free radical damage.

Cai et al. reported that dietary supplementation with 0.3% and 0.4% tea polyphenols improved pork quality and significantly increased serum GSH-Px activity in pigs. Wang et al. demonstrated that tea polyphenols at 200 mg/kg significantly enhanced GSH-Px activity in various tissues of young roosters. Hong et al. observed significantly increased serum GSH-Px activity in Boer

goats receiving 80 mg of tea polyphenols daily. Hu et al. also reported elevated serum GSH-Px activity in chickens fed tea polyphenols. These findings align with our results, showing that 1.00% green tea powder or 0.25% GTPs extremely significantly increased serum GSH-Px activity in canines by the end of the experiment.

Li et al. reported that dietary supplementation with 1.0, 2.0, and 3.0 g/kg green tea powder significantly increased serum SOD activity in geriatric dogs. Xu and Wang demonstrated that 0.3% tea polyphenols enhanced antioxidant capacity and significantly increased serum SOD activity in puppies. Cheng showed that 0.5% GTPs significantly increased serum SOD activity, confirming the antioxidant benefits of GTPs supplementation in canine diets. Marnewick et al. reported that rats fed diets containing green and black tea showed significantly reduced hepatic oxygen radical concentrations and increased SOD activity. While low levels of free radicals are beneficial, excessive free radicals attack polyunsaturated fatty acids in biological membranes, initiating lipid peroxidation and forming lipid peroxides (LPO). Cao and Shao demonstrated that tea polyphenols significantly inhibited lipid peroxidation and reduced intracellular LPO content, exhibiting anti-aging effects. Li et al. reported that tea polyphenols at $ $300 mg/kg significantly reduced MDA content in liver tissue and improved meat quality in broilers. Li showed that ultrafine green tea powder significantly reduced serum lipid peroxidation levels in broilers, with more pronounced effects over extended feeding periods. Yin reported that tea polyphenols extremely significantly reduced LPO content in liver, plasma, and egg yolk of laying hens. Li and Xie demonstrated that tea pigments significantly increased SOD activity and reduced serum MDA content in aged mice in a dose-dependent manner. In the present study, 1.00% green tea powder or 0.25% GTPs extremely significantly increased serum GSH-Px and SOD activities while extremely significantly reducing serum MDA content. These effects likely relate to the direct free radical scavenging properties of tea polyphenols and catechins present in our test materials, thereby enhancing canine antioxidant capacity.

3.3 Effects on Hepatic Antioxidant Gene Expression

Glutathione (GSH) is a crucial intracellular reducing agent that protects cells from oxidative stress, maintains redox balance, and participates in macromolecular biosynthesis. Intracellular GSH is synthesized through the coordinated action of glutamate cysteine ligase (GCL) and glutathione synthetase (GSS). The rate-limiting step involves GCL-catalyzed formation of a dipeptide from cysteine and glutamate, which is then converted to GSH by GSS. GCL comprises two subunits: the catalytic subunit (GCLC) containing the active site, and the modifier subunit (GCLM) that regulates GCL activity without catalytic function. HO-1 is an antioxidant protein with anti-inflammatory and anti-apoptotic properties that catalyzes heme degradation to produce bilirubin, carbon monoxide (CO), and ferrous iron (Fe^{2+}), all of which possess antioxidant functions.

Understanding HO-1' s protective mechanisms against oxidative damage could aid in treating related diseases. CAT is a heme-containing enzyme sensitive to peroxides and UV radiation that catalyzes hydrogen peroxide decomposition into water and oxygen, preventing free radical-mediated cellular damage. Together with GSH-Px and SOD, CAT forms an enzymatic defense system that eliminates superoxide anions and hydrogen peroxide, protecting cells, nucleic acids, and proteins from free radical attack.

GCLC, GCLM, HO-1, and CAT are hepatic antioxidant genes that are upregulated in response to environmental stressors, enabling antioxidant defense. Hepatocyte-specific GCLC deletion causes steatosis and liver failure, while low-dose proteasome inhibitors upregulate GSS and GCLC expression to treat alcoholic liver disease in rats, highlighting GCLC' s critical role in alleviating oxidative liver injury from various causes. Na et al. reported that catechins upregulated GCLC and HO-1 mRNA expression in human mammary epithelial cells. Yu et al. demonstrated that pu-erh black tea extract upregulated hepatic HO-1 mRNA expression in quinocetone-treated SD rats. Chen showed that various tea types upregulated hepatic SOD and CAT mRNA expression, with green tea exhibiting superior capacity compared to other teas. Our findings indicate that green tea powder and GTPs enhanced expression of these antioxidant genes, likely through direct free radical scavenging components that influence downstream antioxidant protein expression, consistent with the observed increases in antioxidant enzyme activities, though the precise mechanisms require further investigation.

3.4 Effects on Hepatic Phase II Detoxification Gene Expression

GSTM1 and NQO1 are phase II detoxification genes expressed in the liver. GSTM1 is a glutathione S-transferase (GST) isoform; GSTs are phase II metabolic enzymes primarily expressed in the liver with lower levels in kidney, small intestine, and testis. GSTs participate in redox reactions, catalyzing the conjugation of various metabolites and toxins generated during oxidative stress. Environmental toxins undergo phase I metabolism followed by phase II detoxification, forming water-soluble compounds for excretion, thereby protecting cells from oxidative damage. NQO1 is expressed in most eukaryotic cells and confers resistance to numerous natural and synthetic compounds while reducing oxidative damage to organelles and genetic material. NQO1 is a highly inducible reductase that uses NADH or NADPH as electron donors to catalyze quinone reduction, reducing quinone activity and decreasing reactive oxygen intermediate production from redox cycling, thus protecting normal cells from quinone-induced damage.

Li reported that antioxidant-rich foods significantly increased hepatic GSTM1 and NQO1 mRNA expression and enhanced antioxidant capacity in mice. Espinosa et al. demonstrated that white tea significantly increased hepatic GST and NQO1 expression, improving resistance to oxidative damage. Our results showed that 1.00% green tea powder or 0.25% GTPs significantly increased

hepatic GSTM1 and NQO1 mRNA expression in canines, indicating that these concentrations confer detoxification capacity and hepatoprotective effects.

Under the conditions of this experiment, dietary supplementation with 1.00% green tea powder or 0.25% GTPs improved liver function, reduced oxidative stress, enhanced expression of hepatic antioxidant and phase II detoxification genes, and controlled body weight gain in canines.

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Note: Figure translations are in progress. See original paper for figures.