ORAL SINGLE- AND REPEATED-DOSE TOXICITY STUDIES ON GERANTI BIO-GE YEAST®, ORGANIC GERMANIUM FORTIFIED YEASTS, IN DOGS

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ABSTRACT — Single- and 13-week repeated-dose toxicities of Geranti Bio-Ge Yeast®, organic germanium fortified yeasts, were investigated in dogs. Both sexes of Beagle dogs were orally administered once at a dose of 2,000 mg/kg in single-dose toxicity or daily for 13 weeks at doses of 500, 1,000 or 2,000 mg/kg in repeated-dose toxicity tests. In single-dose toxicity test, no animal dead, moribund, or showing clinical signs or changes in body weight gain was found. In repeated-dose toxicity study, there were no considerable changes in ophthalmoscopy and urinalysis. Several alterations were observed in electrocardiography, hematology and blood biochemistry, including heart rate, R-R interval, QT correcting, reticulocytes, activated partial thromboplastin time and albumin/globulin ratio in only male dogs, but not in females, administered with Geranti Bio-Ge Yeast® in a dose-independent manner. In gross findings, several cases of abnormal findings were observed in both control and treatment groups, showing diffuse dark brown to black discoloration of liver, in a dose-independent manner. In microscopic examination, mild lesions, including cholestasis and inflammatory cell foci in liver, kidneys and prostate, were found sporadically in both control and treatment groups. In spite of some alterations in electrocardiography, hematology, blood biochemistry, gross and microscopic findings, such effects were not considered to include toxicopathological significance, based on the marginal changes within normal ranges and lack of dose-dependency, consistent time-course and gender relationship. Taken together, it is suggested that no observed adverse effect level (NOAEL) of Geranti Bio-Ge Yeast® is considered to be 2,000 mg/kg in dogs, and that long-term treatment in clinical trials might not exert adverse effects.

KEY WORDS: Geranti Bio-Ge Yeast®, Organic germanium fortified yeasts, Single and repeated-dose toxicities, Oral, Dogs

INTRODUCTION

Germanium (Ge), a grayish-white crystalline metal with atomic number of 32, is a naturally occurring trace element found in soil, water, plants and animal bodies, and daily intake in adults ranges from 0.367 to 1.5 mg (Tao and Bolger, 1997). It is well known that main pharmacological activities of germanium compounds include antimicrobial, antimutagenic, antitumor and immunomodulating effects (Kaars Sijpsteijn et al., 1964; Kada et al., 1984; Aso et al., 1985, 1989; Suzuki et al., 1985; Brutkiewicz and Suzuki, 1987; Goodman, 1988; Jao et al., 1990; Nakada et al., 1993; Olsen et al., 1995; Ikemoto et al., 1996; Gerber and Leonard, 1997; Kang et al., 2001), in addition to diverse therapeutic effects (Suzuki and...
Taguchi, 1983; Goodman, 1988; Hammett et al., 1992; Tao and Bolger, 1997), along with antagonistic action on heavy metal poisoning (Han et al., 1992; Lee et al., 1998). Accordingly, germanium-containing dietary supplements have become increasingly popular in recent years.

In spite of beneficial effects of the germanium compounds, long-term ingestion of the products containing a high level of germanium is not recommended, since at least 31 human cases of germanium intoxication were reported (Tao and Bolger, 1997), wherein the most common clinical symptoms were renal failure, anemia and muscle weakness. In fact, toxicity has been found to come from inorganic compounds such as germanium dioxide (GeO₂) and germanium tetrachloride. On the other hand, organic germaniums such as spirogermanium, germanium lactate citrate and carboxyethylgermanium sesquioxide (Ge-132) have been reported to exert negligible adverse effects, although the safety of organic germaniums is also in controversy (Tao and Bolger, 1997).

Organic germaniums can be chemically synthesized from germanium dioxide and organic acids through catalysis or extracted from medicinal plants containing high concentrations of germanium such as ginseng, aloes, garlic and Ganoderma lucidum Karst (Asai, 1980; Tao and Bolger, 1997). However, the extraction procedures cost too much, while purification procedures following chemical synthesis have not been proven to provide safe products, which limited its use as a food or drug (Tao and Bolger, 1997). It was suggested that the human cases of organic germanium intoxication were due to a high level of residual inorganic germanium (Krapf et al., 1992). Accordingly, residual level of inorganic germanium in organic products after synthesis and purification, and the possibility of release of atomic germanium from an organic form are on the focus of argument, in addition to the toxicity of the organic compound itself.

Recently, organic germaniums have been successfully obtained from microorganisms, especially from yeasts (Nobohiro et al., 1980; Klapcinska and Chmielewski, 1986; Vandyke et al., 1989; Slawson et al., 1992; Wei, 1992; Young and Tyk, 1997; Boder and Wittrup, 2000; Kushinirov, 2000). Geranti Bio-Ge Yeast®, a product of GerantiPharm Co., Ltd. (Seoul, Korea), is powdered yeast fortified with biosynthetic organic germanium. Geranti Bio-Ge Yeast® exerted remarkable immunostimulating and anti-arthritic effects (unpublished data). In previous studies, the dried yeast, before powdering, did not induce any adverse effects in rats and dogs (Ahn et al., 2001a, 2001b). Also, powdered Geranti Bio-Ge Yeast® was found to exert negligible toxicities in rats up to 2,000 mg/kg (unpublished data). In the present study, therefore, the overall adverse effects following acute or long-term exposure of dogs to Geranti Bio-Ge Yeast® were investigated.

MATERIALS AND METHODS

Test compound

Geranti Bio-Ge Yeast®, a dried yellowish powder of an edible yeast strain containing biosynthetic organic germanium, was stored at 4°C, dissolved in water, and administered in a volume of 8 ml/kg.

Test animals

Male and female Beagle dogs (7 months old), obtained from Gangwon Beagle Farm (Pyeongchang, Korea), were housed in an environmentally-controlled room with temperature of 23 ± 2°C, relative humidity of 50 ± 20%, air ventilation of 10 – 15 times/hr and a 12-hr light/dark cycle of 150 – 300 lux. Each dog was given 300 g/day of pellet feed (Purina Korea, Korea), and purified water was available ad libitum.

Single-dose toxicity study

The test was performed according to the “Guidelines for Toxicity Tests of Drugs and Related Materials” provided by the Korea Food and Drug Administration (KFDA) (Korea Food and Drug Administration, 1999). In brief, 2 dogs of both sexes in each group were weighed, and administered with 2,000 mg/kg of freshly-prepared Geranti Bio-Ge Yeast® or its vehicle (water) in a volume of 8 ml/kg. Clinical signs and mortality were examined every day for 14 days, and body weights were recorded on Days 0, 1, 3, 7 and 14 after administration.

Repeated-dose toxicity study

The test was also performed according to the “Guidelines for Toxicity Tests of Drugs and Related Materials” provided by KFDA (Korea Food and Drug Administration, 1999). Three animals of both sexes in each group were weighed, and administered with freshly-prepared Geranti Bio-Ge Yeast® at doses of 500, 1,000 or 2,000 mg/kg or its vehicle for 13 weeks. Clinical signs and mortality were examined every day throughout the experimental period, and body weights and feed and water consumptions were recorded every week. The animals were fasted after final administra-
tion for 24 hr, and subjected to ophthalmologic examination and electrocardiography (ECG). Heart rate, R-R interval, P-R interval, QRS duration, Q-T interval, QT correcting and mean electric axis (AXIS) were recorded using an ECG system (Cardiomax FX-3010, FUKUDA DENSHI, Japan). After urine and blood collection for urinalysis, hematology and blood biochemistry, all dogs were sacrificed under deep anesthesia with ketamine overdosage. Internal organs were removed, weighed, and gross findings were recorded. Also, the organs were examined under a microscope after formalin fixation, paraffin embedding, and hematoxylin-eosin staining.

Urine samples were analyzed by Combur® Test®M paper (Roche, Germany) for color, turbidity, specific gravity, pH, leukocytes, nitrite, protein, glucose, ketone, urobilinogen, bilirubin, occult blood and hemoglobin (Hb). For hematology, 1 ml of whole blood was collected into the CBC bottle (MEDI-LAND Co., Ltd., Korea) containing EDTA, and examined by a hematology autoanalyzer (CELL-DYN 3700, Abbott, USA) for total red blood cell (RBC) counts, Hb concentration, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, total white blood cell (WBC) counts and WBC differential counting (neutrophils, eosinophils, basophils, lymphocytes and monocytes). Reticulocytes were counted under a microscope after staining with methylene blue. Separately, prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured in plasma obtained from sodium citrate-treated blood using an automatic coagulation time meter (ACL 7000, Instrumentation Lab., USA). Serum biochemical analysis was performed using an automatic analyzer (INTEGRA 400, Roche, Germany) and an electrolyte analyzer (AVL9181, Roche, Germany) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GTP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, total bilirubin, creatine kinase (CK), total protein, albumin, albumin/globulin (A/G) ratio, total cholesterol, triglycerides (TG), glucose, phosphorus (P), calcium (Ca), potassium (K), sodium (Na) and chloride (Cl).

The experiments performed here were conducted according to Standard Operation Procedures (SOP) of Biotoxtech Co., Ltd. (Cheongwon, Korea) and the “Guide Principles in the Use of Animals in Toxicology” which had been adopted by the Society of Toxicology in 1989.

**Statistical analysis**

The data were expressed as the mean ± S.D., and analyzed for homogeneity of variance using Levene’s test (Montgomery, 1984). Tests of significance were performed using Dunnett’s t-test (Montgomery, 1984) after ANOVA analysis for homogeneous data or an appropriate t-test following rank or logarithmic transformation, with p < 0.05 as a criterion of difference. Separately, the categorical values in urinalysis were compared using X² test.

**RESULTS**

**Single-dose toxicity**

Throughout the experimental period, there were no animals dead, moribund, or showing clinical signs or changes in body weight gain in both control and treatment groups (Fig. 1), implying that the apparent toxic dose, following single oral dosage, of Geranti Bio-Ge Yeast® is over 2,000 mg/kg. Accordingly, the highest dose in repeated-dose toxicity study was set at 2,000 mg/kg.

**Repeated-dose toxicity**

Geranti Bio-Ge Yeast®, orally administered to dogs up to 2,000 mg/kg for 13 weeks, did not induce any remarkable clinical signs or affect the body weight in 2009.

**Fig. 1.** Changes in mean body weights of male (● and ▲) and female (▲ and ▼) dogs following single administration on Day 0 with 2,000 mg/kg of Geranti Bio-Ge Yeast® (■ and ▼) or its vehicle (● and ▲).
gain (Fig. 2). No significant changes in feed and water consumption were obtained during the treatment period (data not shown).

In addition, there were no considerable changes in ophthalmoscopy (data not shown). On the other hand, electrocardiogram exhibited a significant decrease in heart rate, increase in R-R interval and decrease in QTc in only male dogs administered with a high dose (2,000 mg/kg) of Geranti Bio-Ge Yeast® for 13 weeks (Table 1). However, such changes were not significantly different from pre-dosing values. Any significant alterations in parameters of urinalysis were not obtained following Geranti Bio-Ge Yeast® treatment (Table 2).

In male dogs, there was a significant decrease in the ratio of reticulocytes in the high-dose (2,000 mg/kg) group following 13-week treatment (Table 3B), which was not significant from pre-dosing value (Table 3A). There was an age-dependent decreasing trend in the ratio of reticulocytes, resulting in normalization of abnormally-high value in the medium-dose (1,000 mg/kg) group (Tables 3A). Also, APTT value in the high-dose (2,000 mg/kg) group exhibited a significant difference from control, but not from pre-dosing value. Although there were some variations in ALP and ALT before treatment, especially in medium- (1,000 mg/kg) and high-dose (2,000 mg/kg) groups of males and females, respectively, the values were recovered during Geranti Bio-Ge Yeast® treatment (Tables 4A and 4B). In addition, there was an increase in A/G ratio in low- (500 mg/kg) and medium-dose (1,000 mg/kg) groups, and a decrease in LDH in all (500 - 2,000 mg/kg) treatment groups, but not in females (Table 4B). However, the low values in LDH of male dogs were not significantly different from pre-dosing value (Table 4A). In comparison, the initial high LDH value of females treated with medium-dose (1,000 mg/kg) Geranti Bio-Ge Yeast® decreased to normal level.

Significant increases in the absolute weight of adrenals and relative weight of pituitary glands were observed in female dogs treated with the medium (1,000 mg/kg) and high (2,000 mg/kg) doses of Geranti Bio-Ge Yeast®, respectively (Tables 5 and 6). In gross findings, several cases of abnormal findings were observed in both control and treatment groups, showing diffuse dark brown to black discoloration of liver, in a dose-independent manner. There was 1 case each of unilateral aplasia of testis and compensatory renal hypertrophy following aplasia of the contralateral kidney. In microscopic examination, mild lesions, including cholestasis and inflammatory cell foci in liver, kidneys and prostate, were found sporadically in both control and treatment groups (Table 7).

**DISCUSSION**

It is well known that inorganic germaniums, such as germanium dioxide, exert diverse toxicities. In spite of general acceptance of negligible or minimal toxicity, the safety of the organic germanium compounds is in hot controversy, since conflicting results were achieved in long-term feeding studies of Ge-132 in animals (Kanda et al., 1990; Nakagawa et al., 1990; Taylor, 1991; Anger et al., 1992). Moreover, at least 4 reported
Table 1. Electrocardiogram of dogs (n = 3) administered with Geranti Bio-Ge Yeast® or its vehicle for 13 weeks.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sex</th>
<th>Dose (mg/kg)</th>
<th>Control</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
<th>control</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heart rate (bpm)</td>
<td>108.3±4.0</td>
<td>101.7±13.1</td>
<td>108.7±25.6</td>
<td>102.3±5.1</td>
<td>105.7±12.9</td>
<td>109.7±19.6</td>
<td>111.0±10.1</td>
<td>118.3±24.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R-R interval (sec)</td>
<td>0.552±0.020</td>
<td>0.594±0.080</td>
<td>0.570±0.143</td>
<td>0.585±0.031</td>
<td>0.570±0.072</td>
<td>0.556±0.099</td>
<td>0.542±0.052</td>
<td>0.520±0.101</td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td>P-R interval (sec)</td>
<td>0.092±0.003</td>
<td>0.098±0.016</td>
<td>0.096±0.009</td>
<td>0.085±0.004</td>
<td>0.092±0.006</td>
<td>0.100±0.014</td>
<td>0.090±0.004</td>
<td>0.093±0.005</td>
</tr>
<tr>
<td>treatment</td>
<td></td>
<td>QRS duration (sec)</td>
<td>0.059±0.002</td>
<td>0.054±0.004</td>
<td>0.063±0.005</td>
<td>0.059±0.004</td>
<td>0.055±0.010</td>
<td>0.056±0.008</td>
<td>0.054±0.005</td>
<td>0.048±0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QT interval (sec)</td>
<td>0.233±0.004</td>
<td>0.230±0.008</td>
<td>0.229±0.026</td>
<td>0.242±0.002</td>
<td>0.238±0.000</td>
<td>0.229±0.009</td>
<td>0.222±0.002*</td>
<td>0.234±0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QT correcting</td>
<td>0.314±0.003</td>
<td>0.300±0.010</td>
<td>0.306±0.019</td>
<td>0.317±0.009</td>
<td>0.318±0.018</td>
<td>0.310±0.016</td>
<td>0.302±0.013</td>
<td>0.328±0.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AXIS (degree)</td>
<td>61.2±20.6</td>
<td>72.7±11.6</td>
<td>65.3±18.2</td>
<td>67.7±20.0</td>
<td>79.0±20.6</td>
<td>72.7±11.6</td>
<td>65.3±18.2</td>
<td>79.0±5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heart rate (bpm)</td>
<td>114.7±8.0</td>
<td>99.0±15.7</td>
<td>109.7±12.1</td>
<td>81.3±8.5*</td>
<td>107.3±8.1</td>
<td>109.0±23.6</td>
<td>116.7±15.0</td>
<td>120.3±34.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R-R interval (sec)</td>
<td>0.523±0.037</td>
<td>0.615±0.094</td>
<td>0.550±0.066</td>
<td>0.741±0.073*</td>
<td>0.559±0.043</td>
<td>0.564±0.118</td>
<td>0.519±0.070</td>
<td>0.529±0.177</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td>P-R interval (sec)</td>
<td>0.093±0.006</td>
<td>0.098±0.004</td>
<td>0.094±0.010</td>
<td>0.089±0.002</td>
<td>0.085±0.009</td>
<td>0.097±0.012</td>
<td>0.089±0.008</td>
<td>0.091±0.005</td>
</tr>
<tr>
<td>treatment</td>
<td></td>
<td>QRS duration (sec)</td>
<td>0.058±0.008</td>
<td>0.061±0.006</td>
<td>0.065±0.003</td>
<td>0.053±0.008</td>
<td>0.055±0.003</td>
<td>0.062±0.005</td>
<td>0.057±0.002</td>
<td>0.063±0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QT interval (sec)</td>
<td>0.248±0.026</td>
<td>0.232±0.012</td>
<td>0.213±0.012</td>
<td>0.247±0.007</td>
<td>0.229±0.010</td>
<td>0.217±0.006</td>
<td>0.227±0.012</td>
<td>0.227±0.031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QT correcting</td>
<td>0.346±0.048</td>
<td>0.298±0.007</td>
<td>0.288±0.019</td>
<td>0.288±0.022*</td>
<td>0.307±0.019</td>
<td>0.304±0.022</td>
<td>0.315±0.006</td>
<td>0.316±0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AXIS (degree)</td>
<td>68.7±22.5</td>
<td>60.7±12.7</td>
<td>71.0±6.9</td>
<td>50.3±17.8</td>
<td>67.0±19.1</td>
<td>45.3±14.2</td>
<td>58.7±31.8</td>
<td>64.7±11.0</td>
</tr>
</tbody>
</table>

* Significantly different from control (p < 0.05).
<table>
<thead>
<tr>
<th>Time</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>control 500 1,000 2,000</td>
<td>control 500 1,000 2,000</td>
</tr>
<tr>
<td>Color</td>
<td>light yellow</td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>clear turbid</td>
<td></td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.000 1.005 1.010</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7 8 9</td>
<td>7 1 1 1 1</td>
</tr>
<tr>
<td>Leukocytes (cells/µl)</td>
<td>− 10-25 75</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
<tr>
<td>Nitrite</td>
<td>− +</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
<tr>
<td>Protein (mg/dl)</td>
<td>− 30 100</td>
<td>2 3 3 2 3 3 3 3</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>normal 50 100</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
<tr>
<td>Ketone</td>
<td>− + ++</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
<tr>
<td>Urobilinogen (mg/dl)</td>
<td>normal 1 4</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>− + ++</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
<tr>
<td>Occult blood (cells/µl)</td>
<td>− 5-10 25</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
<tr>
<td>Hemoglobin (cells/µl)</td>
<td>− 10 25</td>
<td>3 3 3 3 3 3 3 3</td>
</tr>
</tbody>
</table>
Table 3A. Hematology of dogs (n = 3) before administration with Geranti Bio-Ge Yeast® or its vehicle.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Control</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
<th>Control</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10⁶/µl)</td>
<td>5.82 ± 0.64</td>
<td>6.10 ± 0.60</td>
<td>5.90 ± 0.27</td>
<td>6.11 ± 0.51</td>
<td>6.66 ± 0.18</td>
<td>6.61 ± 0.53</td>
<td>5.99 ± 0.32</td>
<td>6.70 ± 0.41</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.2 ± 1.4</td>
<td>13.6 ± 1.5</td>
<td>13.0 ± 0.3</td>
<td>13.6 ± 1.0</td>
<td>14.5 ± 0.3</td>
<td>14.3 ± 1.4</td>
<td>13.4 ± 0.6</td>
<td>14.9 ± 1.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.7 ± 3.8</td>
<td>41.0 ± 3.2</td>
<td>39.3 ± 0.6</td>
<td>40.3 ± 2.9</td>
<td>43.9 ± 1.2</td>
<td>43.3 ± 4.1</td>
<td>41.3 ± 2.1</td>
<td>44.8 ± 2.2</td>
</tr>
<tr>
<td>RBC indices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>70.1 ± 1.5</td>
<td>67.3 ± 1.6</td>
<td>66.7 ± 1.9</td>
<td>66.0 ± 1.4</td>
<td>65.9 ± 1.9</td>
<td>65.5 ± 1.2</td>
<td>68.9 ± 0.9*</td>
<td>66.8 ± 0.9</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>22.8 ± 0.3</td>
<td>22.4 ± 0.8</td>
<td>22.0 ± 0.7</td>
<td>22.2 ± 0.8</td>
<td>21.8 ± 0.8</td>
<td>21.6 ± 0.4</td>
<td>22.4 ± 0.2</td>
<td>22.2 ± 0.4</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>32.5 ± 0.5</td>
<td>33.2 ± 1.0</td>
<td>33.0 ± 0.4</td>
<td>33.6 ± 0.4</td>
<td>33.2 ± 0.3</td>
<td>32.9 ± 0.6</td>
<td>32.5 ± 0.5</td>
<td>33.3 ± 0.9</td>
</tr>
<tr>
<td>Platelets (×10³/µl)</td>
<td>419 ± 94</td>
<td>649 ± 116</td>
<td>502 ± 121</td>
<td>446 ± 122</td>
<td>487 ± 89</td>
<td>550 ± 79</td>
<td>491 ± 63</td>
<td>385 ± 92</td>
</tr>
<tr>
<td>WBC (×10³/µl)</td>
<td>12.08 ± 4.74</td>
<td>14.20 ± 2.21</td>
<td>11.46 ± 3.44</td>
<td>11.28 ± 2.88</td>
<td>9.13 ± 1.59</td>
<td>8.95 ± 1.09</td>
<td>9.31 ± 2.10</td>
<td>10.64 ± 0.92</td>
</tr>
<tr>
<td>Neutrophils</td>
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<td>63.1 ± 5.2</td>
<td>53.2 ± 9.7</td>
<td>51.7 ± 2.7</td>
<td>60.3 ± 3.6</td>
<td>59.1 ± 2.8</td>
<td>57.4 ± 11.9</td>
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<tr>
<td>Lymphocytes</td>
<td>36.2 ± 16.1</td>
<td>23.3 ± 16.9</td>
<td>28.8 ± 9.1</td>
<td>39.4 ± 9.2</td>
<td>38.3 ± 3.2</td>
<td>25.2 ± 2.8</td>
<td>30.8 ± 10.4</td>
<td>33.9 ± 7.7</td>
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<td>Monocytes</td>
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<td>4.3 ± 4.0</td>
<td>3.7 ± 3.9</td>
<td>8.0 ± 0.8</td>
<td>10.5 ± 1.2</td>
<td>7.3 ± 6.1</td>
<td>6.4 ± 5.5</td>
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<tr>
<td>Eosinophils</td>
<td>1.6 ± 0.5</td>
<td>1.8 ± 2.0</td>
<td>1.1 ± 0.8</td>
<td>1.7 ± 1.6</td>
<td>0.1 ± 0.1</td>
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<td>0.6 ± 0.9</td>
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<td>3.5 ± 0.8</td>
<td>1.4 ± 1.7</td>
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<td>Coagulation</td>
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<td>PT (sec)</td>
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<td>5.95 ± 0.09</td>
<td>6.05 ± 0.23</td>
<td>5.95 ± 0.09</td>
<td>6.00 ± 0.00</td>
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<td>APTT (sec)</td>
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<td>20.8 ± 2.4</td>
<td>19.2 ± 4.2</td>
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* Significantly different from control (p < 0.05).
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<th></th>
<th></th>
<th>Female</th>
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<td>2,000</td>
<td>Control</td>
<td>500</td>
<td>1,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>6.88 ± 0.65</td>
<td>6.91 ± 0.61</td>
<td>6.85 ± 0.21</td>
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<td>7.46 ± 0.64</td>
<td>7.25 ± 0.13</td>
<td>7.29 ± 1.00</td>
<td>7.50 ± 0.10</td>
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<td>RBC (×10^6/µL)</td>
<td>15.2 ± 1.2</td>
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<td>15.1 ± 0.7</td>
<td>16.1 ± 1.6</td>
<td>16.2 ± 1.0</td>
<td>15.9 ± 1.1</td>
<td>16.8 ± 2.5</td>
<td>16.7 ± 0.5</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>47.1 ± 2.8</td>
<td>46.9 ± 3.7</td>
<td>46.3 ± 1.9</td>
<td>48.4 ± 4.7</td>
<td>48.7 ± 3.6</td>
<td>48.0 ± 2.9</td>
<td>50.0 ± 6.3</td>
<td>50.1 ± 0.6</td>
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<td>Hematocrit (%)</td>
<td>1.45 ± 0.12</td>
<td>1.07 ± 0.09</td>
<td>1.36 ± 0.08</td>
<td>1.04 ± 0.27</td>
<td>0.71 ± 0.29</td>
<td>1.39 ± 0.32</td>
<td>1.40 ± 0.18</td>
<td>1.26 ± 0.62</td>
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<td>RBC indices</td>
<td>68.6 ± 2.3</td>
<td>68.0 ± 1.2</td>
<td>67.7 ± 0.9</td>
<td>65.8 ± 1.1</td>
<td>65.4 ± 1.0</td>
<td>66.2 ± 3.9</td>
<td>68.7 ± 1.2</td>
<td>66.7 ± 0.2</td>
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<td>MCV (fl)</td>
<td>22.1 ± 0.3</td>
<td>22.3 ± 0.5</td>
<td>22.0 ± 0.3</td>
<td>21.9 ± 0.3</td>
<td>21.7 ± 0.6</td>
<td>22.0 ± 1.2</td>
<td>23.1 ± 0.3</td>
<td>22.3 ± 0.6</td>
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<td>MCHC (g/dl)</td>
<td>32.3 ± 0.9</td>
<td>32.8 ± 0.2</td>
<td>32.5 ± 0.2</td>
<td>33.3 ± 0.5</td>
<td>33.2 ± 0.4</td>
<td>33.2 ± 1.4</td>
<td>33.6 ± 1.0</td>
<td>33.4 ± 0.9</td>
</tr>
<tr>
<td>Platelets (×10^3/µL)</td>
<td>340 ± 53</td>
<td>399 ± 97</td>
<td>400 ± 78</td>
<td>380 ± 48</td>
<td>381 ± 50</td>
<td>337 ± 285</td>
<td>460 ± 40</td>
<td>442 ± 58</td>
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<tr>
<td>WBC (×10^3/µL)</td>
<td>9.07 ± 2.75</td>
<td>9.78 ± 2.38</td>
<td>9.73 ± 1.35</td>
<td>9.92 ± 1.39</td>
<td>5.92 ± 0.67</td>
<td>8.63 ± 2.45</td>
<td>9.59 ± 4.01</td>
<td>9.72 ± 2.12</td>
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<tr>
<td>Neutrophils</td>
<td>62.1 ± 20.6</td>
<td>67.1 ± 13.4</td>
<td>69.6 ± 15.0</td>
<td>61.6 ± 3.1</td>
<td>82.9 ± 1.4</td>
<td>72.4 ± 19.4</td>
<td>64.7 ± 18.9</td>
<td>59.8 ± 20.4</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>36.4 ± 18.9</td>
<td>28.1 ± 17.0</td>
<td>26.8 ± 12.5</td>
<td>34.9 ± 6.2</td>
<td>13.0 ± 2.8</td>
<td>24.7 ± 19.3</td>
<td>33.5 ± 17.0</td>
<td>38.5 ± 22.0</td>
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<tr>
<td>Macrophages</td>
<td>0.1 ± 0.2</td>
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<td>0.9 ± 1.5</td>
<td>0.5 ± 0.3</td>
<td>0.1 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.4 ± 2.3</td>
<td>4.2 ± 2.8</td>
<td>3.4 ± 2.9</td>
<td>1.9 ± 1.8</td>
<td>3.6 ± 3.3</td>
<td>2.7 ± 0.4</td>
<td>1.2 ± 1.7</td>
<td>1.5 ± 1.8</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.7</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>6.25 ± 0.09</td>
<td>6.15 ± 0.15</td>
<td>6.30 ± 0.30</td>
<td>6.05 ± 0.09</td>
<td>6.25 ± 0.09</td>
<td>6.60 ± 0.60</td>
<td>6.35 ± 0.23</td>
<td>6.20 ± 0.09</td>
</tr>
<tr>
<td>Basophils</td>
<td>24.3 ± 0.4</td>
<td>22.1 ± 1.4</td>
<td>22.8 ± 1.1</td>
<td>21.6 ± 0.7*</td>
<td>24.4 ± 1.0</td>
<td>22.3 ± 1.7</td>
<td>21.9 ± 1.0</td>
<td>21.3 ± 2.3</td>
</tr>
</tbody>
</table>

* Significantly different from control (p < 0.05). #Significantly different from pre-dosing value (p < 0.05).
Table 4A. Blood biochemistry of dogs (n = 3) before administration with Gerani Bio-Ge Yeast® or its vehicle.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Control</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
<th>Control</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>29.0 ± 19.9</td>
<td>37.5 ± 16.4</td>
<td>112.4 ± 114.8</td>
<td>26.6 ± 12.4</td>
<td>61.4 ± 30.7</td>
<td>48.0 ± 31.6</td>
<td>105.1 ± 117.9</td>
<td>198.9 ± 152.0</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>34.6 ± 7.7</td>
<td>40.5 ± 13.7</td>
<td>41.8 ± 11.2</td>
<td>35.8 ± 3.6</td>
<td>41.6 ± 15.1</td>
<td>44.2 ± 6.8</td>
<td>44.7 ± 11.8</td>
<td>54.1 ± 16.5</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>135.5 ± 36.5</td>
<td>154.9 ± 37.8</td>
<td>233.6 ± 32.1*</td>
<td>106.1 ± 8.4</td>
<td>157.6 ± 34.8</td>
<td>141.8 ± 28.0</td>
<td>204.4 ± 87.9</td>
<td>316.8 ± 249.5</td>
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<tr>
<td>CK (U/l)</td>
<td>289.7 ± 85.3</td>
<td>303.0 ± 93.6</td>
<td>251.3 ± 32.0</td>
<td>244.7 ± 34.8</td>
<td>383.7 ± 177.5</td>
<td>395.0 ± 93.5</td>
<td>306.0 ± 18.2</td>
<td>317.3 ± 74.4</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>62.5 ± 11.7</td>
<td>46.4 ± 7.9</td>
<td>65.2 ± 10.5</td>
<td>56.9 ± 4.0</td>
<td>65.9 ± 10.6</td>
<td>61.1 ± 2.9</td>
<td>59.9 ± 9.4</td>
<td>54.0 ± 7.9</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>8.49 ± 1.04</td>
<td>10.29 ± 3.19</td>
<td>9.08 ± 1.10</td>
<td>10.20 ± 1.95</td>
<td>8.91 ± 1.60</td>
<td>10.38 ± 2.22</td>
<td>8.36 ± 2.43</td>
<td>9.19 ± 1.81</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.47 ± 0.09</td>
<td>0.39 ± 0.04</td>
<td>0.38 ± 0.05</td>
<td>0.46 ± 0.05</td>
<td>0.42 ± 0.04</td>
<td>0.41 ± 0.04</td>
<td>0.51 ± 0.08</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>T-bilirubin (mg/dl)</td>
<td>0.13 ± 0.03</td>
<td>0.19 ± 0.07</td>
<td>0.17 ± 0.03</td>
<td>0.18 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.27 ± 0.12</td>
<td>0.18 ± 0.03</td>
<td>0.19 ± 0.05</td>
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<tr>
<td>T-cholesterol (mg/dl)</td>
<td>190.43 ± 49.03</td>
<td>121.85 ± 26.81</td>
<td>167.68 ± 51.64</td>
<td>160.72 ± 24.30</td>
<td>179.37 ± 17.04</td>
<td>143.94 ± 29.64</td>
<td>150.68 ± 26.94</td>
<td>146.47 ± 6.38</td>
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<tr>
<td>TG (mg/dl)</td>
<td>30.6 ± 8.0</td>
<td>25.9 ± 7.0</td>
<td>26.3 ± 3.7</td>
<td>31.0 ± 4.2</td>
<td>26.3 ± 2.8</td>
<td>25.9 ± 6.1</td>
<td>38.9 ± 10.4</td>
<td>30.3 ± 4.0</td>
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<tr>
<td>T-protein (g/dl)</td>
<td>5.0 ± 0.4</td>
<td>5.2 ± 0.5</td>
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<td>5.3 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.5</td>
<td>5.2 ± 0.2</td>
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<tr>
<td>Albumin (g/dl)</td>
<td>2.5 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>2.7 ± 0.2</td>
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<tr>
<td>A/G ratio</td>
<td>1.02 ± 0.14</td>
<td>0.90 ± 0.13</td>
<td>1.05 ± 0.06</td>
<td>1.05 ± 0.06</td>
<td>1.15 ± 0.05</td>
<td>1.13 ± 0.12</td>
<td>1.10 ± 0.10</td>
<td>1.10 ± 0.11</td>
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<td>γ-GTP (U/l)</td>
<td>4.17 ± 1.48</td>
<td>3.42 ± 1.41</td>
<td>5.24 ± 3.21</td>
<td>3.03 ± 0.65</td>
<td>4.02 ± 0.57</td>
<td>3.01 ± 0.88</td>
<td>3.76 ± 0.79</td>
<td>6.04 ± 2.47</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>255.0 ± 68.6</td>
<td>231.3 ± 128.0</td>
<td>163.7 ± 6.4</td>
<td>182.7 ± 69.0</td>
<td>116.0 ± 54.1</td>
<td>230.7 ± 94.5</td>
<td>256.3 ± 42.2*</td>
<td>107.3 ± 21.1</td>
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<td>P (mg/dl)</td>
<td>7.18 ± 1.53</td>
<td>6.64 ± 0.26</td>
<td>6.45 ± 0.79</td>
<td>6.33 ± 0.75</td>
<td>6.65 ± 0.44</td>
<td>7.16 ± 0.41</td>
<td>6.88 ± 0.54</td>
<td>7.34 ± 0.49</td>
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<tr>
<td>Ca (mmol/l)</td>
<td>2.28 ± 0.22</td>
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<td>2.31 ± 0.06</td>
<td>2.34 ± 0.17</td>
<td>2.52 ± 0.03</td>
<td>2.37 ± 0.27</td>
<td>2.43 ± 0.09</td>
<td>2.38 ± 0.06</td>
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<tr>
<td>Na (mmol/l)</td>
<td>143.7 ± 0.6</td>
<td>143.0 ± 1.0</td>
<td>143.7 ± 2.1</td>
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<td>143.0 ± 1.0</td>
<td>142.0 ± 2.0</td>
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<td>143.7 ± 1.5</td>
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<td>K (mmol/l)</td>
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<td>Cl (mmol/l)</td>
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<td>111.3 ± 1.2</td>
<td>112.0 ± 1.0</td>
<td>110.3 ± 1.2</td>
<td>111.3 ± 0.6</td>
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* Significantly different from control (p < 0.05).
Table 4B. Blood biochemistry of dogs (n = 3) after administration with Geranti Bio-Ge Yeast® or its vehicle for 13 weeks.

<table>
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<tr>
<th>Sex</th>
<th>Dose (mg/kg)</th>
<th>Control</th>
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<th>1,000</th>
<th>2,000</th>
<th>Control</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
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<td>ALT (U/l)</td>
<td>32.8 ± 15.5</td>
<td>48.2 ± 21.5</td>
<td>116.4 ± 137.8</td>
<td>26.4 ± 8.7</td>
<td>85.2 ± 86.1</td>
<td>49.4 ± 29.0</td>
<td>91.2 ± 99.1</td>
<td>212.6 ± 164.4</td>
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<td>AST (U/l)</td>
<td>35.1 ± 8.6</td>
<td>34.8 ± 4.9</td>
<td>42.1 ± 13.6</td>
<td>28.1 ± 2.1</td>
<td>30.5 ± 10.2</td>
<td>32.0 ± 8.8</td>
<td>39.2 ± 15.4</td>
<td>50.1 ± 15.6</td>
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<tr>
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<td>ALP (U/l)</td>
<td>108.4 ± 29.2</td>
<td>105.8 ± 11.4</td>
<td>178.4 ± 83.7</td>
<td>84.1 ± 9.4</td>
<td>110.8 ± 38.6</td>
<td>164.8 ± 8.8</td>
<td>171.6 ± 84.2</td>
<td>211.8 ± 125.3</td>
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<td>CK (U/l)</td>
<td>211.7 ± 11.0</td>
<td>191.7 ± 40.9</td>
<td>171.3 ± 31.0</td>
<td>177.0 ± 13.7</td>
<td>137.3 ± 6.5</td>
<td>192.3 ± 74.3</td>
<td>195.3 ± 98.2</td>
<td>174.0 ± 44.4</td>
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<tr>
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<td>Glucose (mg/dl)</td>
<td>86.6 ± 2.8</td>
<td>115.6 ± 36.5</td>
<td>99.0 ± 8.9</td>
<td>93.7 ± 1.9</td>
<td>91.1 ± 11.9</td>
<td>83.5 ± 5.6</td>
<td>90.8 ± 9.6</td>
<td>87.9 ± 6.8</td>
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<tr>
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<td>BUN (mg/dl)</td>
<td>9.41 ± 2.15</td>
<td>10.69 ± 2.03</td>
<td>9.22 ± 0.08</td>
<td>11.35 ± 2.87</td>
<td>12.63 ± 0.42</td>
<td>10.78 ± 1.33</td>
<td>11.03 ± 2.65</td>
<td>10.82 ± 0.59</td>
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<tr>
<td></td>
<td>Creatinine (mg/dl)</td>
<td>0.78 ± 0.06</td>
<td>0.78 ± 0.03</td>
<td>0.69 ± 0.09</td>
<td>0.80 ± 0.07</td>
<td>0.72 ± 0.03</td>
<td>0.75 ± 0.09</td>
<td>0.84 ± 0.16</td>
<td>0.81 ± 0.01</td>
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<tr>
<td></td>
<td>T-bilirubin (mg/dl)</td>
<td>0.21 ± 0.08</td>
<td>0.17 ± 0.04</td>
<td>0.19 ± 0.04</td>
<td>0.17 ± 0.02</td>
<td>0.15 ± 0.03</td>
<td>0.22 ± 0.06</td>
<td>0.22 ± 0.01</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>T-cholesterol (mg/dl)</td>
<td>215.14 ± 50.04</td>
<td>201.20 ± 63.32</td>
<td>180.66 ± 45.06</td>
<td>189.59 ± 23.64</td>
<td>201.05 ± 15.09</td>
<td>161.68 ± 48.07</td>
<td>163.15 ± 18.55</td>
<td>163.86 ± 10.80</td>
</tr>
<tr>
<td></td>
<td>TG (mg/dl)</td>
<td>40.95 ± 5.17</td>
<td>34.32 ± 15.72</td>
<td>32.00 ± 5.65</td>
<td>38.17 ± 8.31</td>
<td>28.60 ± 4.62</td>
<td>34.46 ± 8.06</td>
<td>44.23 ± 6.86</td>
<td>36.96 ± 2.99</td>
</tr>
<tr>
<td></td>
<td>T-protein (g/dl)</td>
<td>5.9 ± 0.4</td>
<td>5.5 ± 0.3</td>
<td>5.5 ± 0.2</td>
<td>5.9 ± 0.2</td>
<td>5.7 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.9 ± 0.1</td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Albumin (g/dl)</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>3.2 ± 0.0</td>
<td>3.0 ± 0.2</td>
<td>3.2 ± 0.3</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>A/G ratio</td>
<td>0.98 ± 0.05</td>
<td>1.21 ± 0.23*</td>
<td>1.21 ± 0.07*</td>
<td>1.11 ± 0.10</td>
<td>1.29 ± 0.10</td>
<td>1.28 ± 0.05</td>
<td>1.17 ± 0.2</td>
<td>1.14 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>γ-GTP (U/l)</td>
<td>4.38 ± 2.56</td>
<td>3.17 ± 0.53</td>
<td>6.16 ± 4.62</td>
<td>3.36 ± 0.49</td>
<td>4.01 ± 1.15</td>
<td>2.92 ± 1.71</td>
<td>3.90 ± 1.06</td>
<td>6.62 ± 3.09</td>
</tr>
<tr>
<td></td>
<td>LDH (U/l)</td>
<td>284.0 ± 59.9</td>
<td>135.7 ± 31.2*</td>
<td>146.7 ± 73.9*</td>
<td>125.3 ± 47.3*</td>
<td>68.0 ± 7.2</td>
<td>226.0 ± 176.7</td>
<td>117.0 ± 34.8*</td>
<td>109.7 ± 61.3</td>
</tr>
<tr>
<td></td>
<td>P (mg/dl)</td>
<td>6.63 ± 1.30</td>
<td>5.89 ± 0.53</td>
<td>6.75 ± 0.65</td>
<td>6.21 ± 0.50</td>
<td>6.04 ± 0.50</td>
<td>6.21 ± 0.61</td>
<td>6.57 ± 0.10</td>
<td>6.12 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Ca(mmol/l)</td>
<td>2.73 ± 0.19</td>
<td>2.60 ± 0.02</td>
<td>2.63 ± 0.13</td>
<td>2.65 ± 0.07</td>
<td>2.73 ± 0.05</td>
<td>2.73 ± 0.04</td>
<td>2.75 ± 0.09</td>
<td>2.72 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Na (mmol/l)</td>
<td>145.0 ± 2.0</td>
<td>146.3 ± 2.1</td>
<td>146.3 ± 0.6</td>
<td>147.0 ± 1.7</td>
<td>144.3 ± 1.5</td>
<td>145.3 ± 1.5</td>
<td>145.7 ± 0.6</td>
<td>146.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>K (mmol/l)</td>
<td>5.3 ± 0.4</td>
<td>5.0 ± 0.3</td>
<td>4.8 ± 0.4</td>
<td>4.9 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>5.1 ± 0.4</td>
<td>5.0 ± 0.1</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Cl (mmol/l)</td>
<td>110.0 ± 0.0</td>
<td>110.0 ± 1.7</td>
<td>111.3 ± 0.6</td>
<td>110.7 ± 0.6</td>
<td>110.3 ± 1.2</td>
<td>110.0 ± 1.2</td>
<td>110.0 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from control (p < 0.05). # Significantly different from pre-dosing value (p < 0.05).
Table 5. Absolute organ weights of dogs (n = 3) administered with Geranti Bio-Ge Yeast® or its vehicle for 13 weeks.

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th></th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dose (mg/kg)</td>
<td></td>
<td>Control</td>
<td>500</td>
<td>1,000</td>
<td>2,000</td>
<td>Control</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
<td>9.75 ± 0.97</td>
<td>9.31 ± 0.26</td>
<td>9.85 ± 2.06</td>
<td>9.63 ± 0.30</td>
<td>8.37 ± 0.92</td>
<td>8.37 ± 0.73</td>
<td>8.57 ± 0.62</td>
</tr>
<tr>
<td>Liver (g)</td>
<td></td>
<td>265.5 ± 17.4</td>
<td>282.9 ± 49.8</td>
<td>304.3 ± 44.0</td>
<td>312.8 ± 30.7</td>
<td>229.3 ± 10.4</td>
<td>265.9 ± 17.6</td>
<td>222.8 ± 8.6</td>
</tr>
<tr>
<td>Kidneys (g)</td>
<td>left</td>
<td>23.1 ± 1.4</td>
<td>23.1 ± 4.3</td>
<td>22.9 ± 2.4</td>
<td>22.6 ± 3.6</td>
<td>17.3 ± 1.0</td>
<td>17.3 ± 1.4</td>
<td>22.4 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>right</td>
<td>23.0 ± 0.6</td>
<td>20.0 ± 1.0</td>
<td>22.7 ± 2.2</td>
<td>21.3 ± 3.5</td>
<td>17.7 ± 1.6</td>
<td>16.8 ± 0.6</td>
<td>18.0 ± 3.4</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td></td>
<td>23.8 ± 2.4</td>
<td>20.4 ± 3.3</td>
<td>20.3 ± 3.8</td>
<td>20.5 ± 1.8</td>
<td>22.7 ± 4.3</td>
<td>21.0 ± 4.8</td>
<td>20.1 ± 1.8</td>
</tr>
<tr>
<td>Adrenals (mg)</td>
<td>left</td>
<td>464.9 ± 40.9</td>
<td>435.7 ± 45.5</td>
<td>413.0 ± 78.3</td>
<td>375.1 ± 92.0</td>
<td>375.1 ± 12.7</td>
<td>488.4 ± 13.2</td>
<td>605.1 ± 161.0*</td>
</tr>
<tr>
<td></td>
<td>right</td>
<td>457.9 ± 49.7</td>
<td>453.7 ± 79.0</td>
<td>445.6 ± 71.1</td>
<td>382.3 ± 30.3</td>
<td>376.6 ± 17.1</td>
<td>514.0 ± 106.7</td>
<td>500.3 ± 26.8*</td>
</tr>
<tr>
<td>Brain (g)</td>
<td></td>
<td>81.5 ± 4.0</td>
<td>77.2 ± 1.5</td>
<td>72.7 ± 6.8</td>
<td>72.2 ± 4.1</td>
<td>69.1 ± 5.3</td>
<td>72.4 ± 2.8</td>
<td>71.2 ± 2.7</td>
</tr>
<tr>
<td>Pituitary (mg)</td>
<td></td>
<td>72.4 ± 7.2</td>
<td>57.9 ± 4.0</td>
<td>69.5 ± 3.3</td>
<td>58.0 ± 8.7</td>
<td>49.9 ± 5.0</td>
<td>53.1 ± 13.8</td>
<td>60.7 ± 7.0</td>
</tr>
<tr>
<td>Lung (g)</td>
<td></td>
<td>94.1 ± 16.1</td>
<td>83.8 ± 6.1</td>
<td>88.1 ± 11.1</td>
<td>81.0 ± 0.9</td>
<td>79.5 ± 15.0</td>
<td>81.1 ± 4.4</td>
<td>88.4 ± 8.8</td>
</tr>
<tr>
<td>Heart (g)</td>
<td></td>
<td>76.2 ± 7.0</td>
<td>76.9 ± 2.1</td>
<td>82.1 ± 14.5</td>
<td>74.4 ± 7.0</td>
<td>72.2 ± 8.1</td>
<td>70.4 ± 6.9</td>
<td>71.2 ± 5.7</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td></td>
<td>14.5 ± 3.7</td>
<td>10.8 ± 3.5</td>
<td>10.0 ± 2.3</td>
<td>10.5 ± 2.3</td>
<td>11.0 ± 3.6</td>
<td>12.3 ± 3.2</td>
<td>11.0 ± 2.9</td>
</tr>
<tr>
<td>Thyroid glands (mg)</td>
<td>left</td>
<td>385.4 ± 91.5</td>
<td>347.5 ± 48.7</td>
<td>603.4 ± 257.1</td>
<td>342.5 ± 124.7</td>
<td>391.9 ± 100.5</td>
<td>415.5 ± 97.5</td>
<td>358.0 ± 52.2</td>
</tr>
<tr>
<td></td>
<td>right</td>
<td>384.0 ± 61.7</td>
<td>359.9 ± 17.7</td>
<td>558.6 ± 224.5</td>
<td>360.1 ± 140.2</td>
<td>443.0 ± 98.9</td>
<td>347.7 ± 29.2</td>
<td>354.3 ± 93.3</td>
</tr>
<tr>
<td>Testes (g)</td>
<td>left</td>
<td>6.69 ± 0.81</td>
<td>6.85 ± 0.30</td>
<td>6.47 ± 0.03</td>
<td>6.41 ± 0.89</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>right</td>
<td>6.70 ± 0.91</td>
<td>8.11 ± 1.75</td>
<td>6.12 ± 0.53</td>
<td>6.86 ± 0.69</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Epididymides (g)</td>
<td>left</td>
<td>1.08 ± 0.06</td>
<td>1.28 ± 0.14</td>
<td>1.08 ± 0.24</td>
<td>1.21 ± 0.05</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>right</td>
<td>1.04 ± 0.14</td>
<td>1.28 ± 0.43</td>
<td>1.12 ± 0.22</td>
<td>1.29 ± 0.11</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Prostate (g)</td>
<td></td>
<td>3.48 ± 2.88</td>
<td>3.90 ± 2.05</td>
<td>3.73 ± 2.34</td>
<td>5.48 ± 1.22</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ovaries (mg)</td>
<td>left</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>381.3 ± 147.2</td>
<td>401.3 ± 306.7</td>
<td>259.1 ± 46.9</td>
</tr>
<tr>
<td></td>
<td>right</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>486.6 ± 367.7</td>
<td>461.6 ± 362.8</td>
<td>279.6 ± 11.0</td>
</tr>
<tr>
<td>Uterus (g)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6.3 ± 6.1</td>
<td>10.6 ± 13.6</td>
<td>3.2 ± 1.0</td>
</tr>
</tbody>
</table>

* Significantly different from control (p < 0.05).
Table 6. Relative organ weights of dogs (n = 3) administered with Geranti Bio-Ge Yeast® or its vehicle for 13 weeks.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>Control</td>
<td>500</td>
<td>1000</td>
<td>2000</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>9.75 ± 0.97</td>
<td>9.31 ± 0.26</td>
<td>9.85 ± 2.06</td>
<td>9.63 ± 0.30</td>
</tr>
<tr>
<td>Liver (%)</td>
<td>2.75 ± 0.46</td>
<td>3.05 ± 0.61</td>
<td>3.12 ± 0.24</td>
<td>3.24 ± 0.24</td>
</tr>
<tr>
<td>Kidneys (%)</td>
<td>0.24 ± 0.04</td>
<td>0.25 ± 0.05</td>
<td>0.24 ± 0.03</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Spleen (%)</td>
<td>0.24 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Adrenals (%)</td>
<td>4.77 ± 0.16</td>
<td>4.67 ± 0.40</td>
<td>4.22 ± 0.46</td>
<td>3.88 ± 0.83</td>
</tr>
<tr>
<td>Pituitary (%)</td>
<td>0.84 ± 0.06</td>
<td>0.83 ± 0.04</td>
<td>0.75 ± 0.11</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>Thymus (%)</td>
<td>0.15 ± 0.06</td>
<td>0.12 ± 0.04</td>
<td>0.10 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Thyroid (%)</td>
<td>4.04 ± 1.37</td>
<td>3.73 ± 0.53</td>
<td>5.97 ± 1.50</td>
<td>3.53 ± 1.17</td>
</tr>
<tr>
<td>Testes (%)</td>
<td>68.64 ± 6.18</td>
<td>73.52 ± 0.36</td>
<td>67.45 ± 12.48</td>
<td>66.48 ± 8.13</td>
</tr>
<tr>
<td>Epididymides (%)</td>
<td>11.12 ± 0.79</td>
<td>13.79 ± 2.06</td>
<td>11.00 ± 0.83</td>
<td>12.62 ± 0.82</td>
</tr>
<tr>
<td>Prostate (%)</td>
<td>35.27 ± 28.20</td>
<td>42.01 ± 22.20</td>
<td>35.83 ± 14.86</td>
<td>57.20 ± 14.14</td>
</tr>
<tr>
<td>Ovaries (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Uterus (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Significantly different from control (p < 0.05).
Repeated-dose toxicity of Geranti Bio-Ge Yeast®.

Human cases in European countries were caused by the ingestion of organic germanium-lactate-citrate (Van der Spoel et al., 1990; Krapf et al., 1992; Hess et al., 1993). Interestingly, some preparations of organic germanium were confirmed to include high concentrations of inorganic germanium dioxide, which was claimed to be the organic Ge-132 or germanium-lactate-citrate of unknown purity (Tao and Bolger, 1997). Since germanium dioxide is a starting material for these organic forms, it is not excluded that the final products could contain residual germanium dioxide, depending on the thoroughness of good manufacturing practice. Otherwise, it is possible that atomic germanium released from the organic forms during absorption, metabolism and excretion is responsible for the toxicity.

The most common signs and symptoms in patients include weight loss, fatigue, gastrointestinal disturbances including nausea, emesis and anorexia, anemia, muscle weakness and renal failure (Tao and Bolger, 1997). The target organs of germaniums are kidneys (Sanai et al., 1991a, 1991b; Schauss, 1991; Raisin et al., 1992; Hess et al., 1993), muscles (Yim et al., 1999), nerves (Matsumuro et al., 1993) and liver (Raisin et al., 1992). It is believed that functional disorder of mitochondria is one of the toxic mechanisms, inferred from the characteristic features of swollen mitochondria in renal tubules and muscles (Matsumuro et al., 1993; Yim et al., 1999).

Acute oral toxicity of germanium compounds was found to be low. It was reported that the LD50 values in rats were 3,700 mg/kg and 11,000 - 11,700 mg/kg for germanium dioxide and Ge-132, respectively (Asai, 1984; Vouk, 1986). In comparison, repeated exposure to germanium dioxide was highly toxic, resulting in 50% mortality following 4-week treatment at 100 - 1,000 ppm in drinking water or diet (Rosenfeld and Wallace, 1953). In a set of studies using rats, however, Geranti Bio-Ge Yeast® did not induce any adverse effects following 13-week administration (unpublished data). Although there is no report on the toxicity of germanium compounds in dogs, in the present single-dose toxicity study, no death, clinical signs and pathological findings related to the treatment were observed. In addition, 13-week long-term treatment to dogs with Geranti Bio-Ge Yeast® did not induce mortality, only minimal physiological changes.

It is important to note that there were significant changes in heart rate, R-R interval and QTc. However, the changes in electrocardiogram were observed in only male dogs administered with a high dose (2,000 mg/kg) of Geranti Bio-Ge Yeast® for 13 weeks, and not significantly different from their initial values. In addition, the alterations in cardiac function were not accompanied by histopathological findings and LDH level which was rather much lower than that of the control group. In spite of several cases of alteration in electrocardiography, hematology, blood biochemistry, organ weights, gross and microscopic findings, therefore, such effects were not considered to include toxicopathological significance, based on the lack of dose-dependency, consistent time-course and gender relationship. Furthermore, such alterations were within normal ranges, observed at extremely-high doses, and also found in control group according to maturing and aging of animals.

In previous genotoxicity tests, Geranti Bio-Ge Yeast® did not cause bacterial mutagenesis, chromosomal aberration, and micronucleus formation (Min et al., 2004). Furthermore, dried yeasts, before powdering, did not exert any adverse effects following 10-month long-term oral treatment in rats and dogs (Ahn et al., 2001a, 2001b). In the previous and present studies on the both dried and powdered yeasts, respectively, the possible target organs of germanium, such as kidneys, muscles, nerves and liver, were not affected, except negligible inflammatory cell foci in a dose-independent manner. In particular, mitochondrial injuries were not seen in all tissues observed. As inferred from the results, the release of inorganic germanium from Geranti Bio-Ge Yeast® during the long-term treatment to rats and dogs with high doses (up to 2,000 mg/kg).

<table>
<thead>
<tr>
<th>Table 7. Histopathological findings of rats (n = 3) administered with Geranti Bio-Ge Yeast® or its vehicle for 13 weeks.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Table 7. Histopathological findings of rats (n = 3) administered with Geranti Bio-Ge Yeast® or its vehicle for 13 weeks.</td>
</tr>
</tbody>
</table>
kg) might be negligible. Thus, such results imply that Geranti Bio-Ge Yeast® does not include harmful inorganic germanium, and that the organic features of germanium atom in Geranti Bio-Ge Yeast® are devoid of acidic or enzymatic degradation in gastrointestinal tracts, liver, kidneys or muscles.

Taken together, it is suggested that no observed adverse effect level (NOAEL) of Geranti Bio-Ge Yeast® is considered to be 2,000 mg/kg in dogs, and that long-term oral treatment at an estimated clinical dose might not exert remarkable adverse effects.

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Materials, Documentation #1999-61, Korea Food and Drug Administration, Seoul.

Young, M.R. and Tyk, B.K. (1997) : Mcm2 and Mcm3 are constitutive nuclear proteins that exhibit distinct isoforms and bind chromatin during specific cell cycle stages of Saccharomyces cerevisiae. Mol. Biol. Cells, 8, 1587-1601.