A 90-DAY TOXICOLOGICAL ASSESSMENT OF KRG HARD CAPSULES CONTAINING A WATER EXTRACT OF KOREAN RED GINSENG IN *WISTAR* RATS

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The KRG hard capsule was produced by Daedong Korea Ginseng Co. Ltd., with Korean red ginseng (Panax ginseng C. A. Meyer) as its primary component. This study aimed to assess the toxicity of KRG following 90 days of repeated oral dosing. Male and female Wistar rats received the test capsule orally once daily at 120 and 360 mg/kg/day over 90 days. The Korean red ginseng extract administration did not lead to any significant toxicological effects regarding mortality, body weight, hematological parameters, serum biochemistry, gross pathological observations, or histopathological analysis. Consequently, based on this toxicological evaluation, the KRG hard capsules are considered safe and non-toxic. The findings may serve as adequate preclinical evidence to support the initiation of future clinical trials involving KRG.

Keywords: Korean red ginseng, toxicity, ginseng, rat.

I. INTRODUCTION

Traditional herbal substances have been widely utilized throughout history. Due to their natural origins, they are often perceived as effective with minimal adverse effects. In recent decades, the popularity of herbal substances has surged. Nevertheless, concerns about their safety have sparked considerable debate.[1]

For millennia, ginseng has been employed in traditional medicine as an adaptogen.[2] While it offers various pharmacological advantages for conditions like diabetes, immune system support, and mental health, it may also lead to adverse effects, including headaches, nausea, diarrhea, feelings of euphoria, breast pain, low blood pressure, and vaginal bleeding.[3]

The toxicological properties of red ginseng remain unclear, despite its extensive use and long-standing history, largely due to differences in the composition of various ginseng species and the methods used for sample preparation.[4,5] In our recent study, we administered Korean red ginseng extract, a dietary supplement

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presented in hard capsule form and primarily composed of ginsenosides Rb1, Rg1, and Rg3 derived from *Panax ginseng*, to *Wistar* rats over 90 days to evaluate its subchronic oral toxicity.

II. MATERIALS AND METHODS

1. Treatments and experimental animals

The KRG hard capsules were produced by Daedong Korea Ginseng Co. Ltd., with Korean red ginseng (*Panax ginseng* C. A. Meyer) as the primary ingredient. The manufacturing process involved several steps: fresh ginseng roots, aged six years, were thoroughly washed and then steamed at temperatures between 90 and 100°C for 80 to 100 minutes. Following this, the roots were dried using a hot air stream at temperatures ranging from 45 to 55°C, maintaining humidity below 15.5%. The dried red ginseng was then extracted with water, utilizing a raw material to solvent ratio of 1:10 (g: mL) at 85°C for 12 hours. Each 500-mg capsule contained 480 mg of KRG powder (the test product). The KRG contained three primary ginsenosides: Rg1, Rb1, and Rg3, with a total concentration of 5.27 mg/g. The specific amounts of Rg1, Rb1, and Rg3 were 0.1259 mg/g (2.93%), 1.7914 mg/g (29.85%), and 3.3542 mg/g (78.72%), respectively.

The KRG powder was suspended in distilled water. Adult *Wistar* rats, free from pathogens and of both genders, were obtained from the Dan Phuong Laboratory Animal Supply Center in Hanoi, Vietnam. A 90-day study was conducted involving thirty rats, equally divided into three groups of ten, selected randomly. The rats received Korean red ginseng extract at doses of 0 (vehicle), 120, and 360 mg/kg, with a dosing volume of 10 mL/kg. The test substance and vehicle were prepared fresh daily and administered orally once daily via a syringe equipped with an oral zoned needle.

2. Methods

Housing conditions

Rats used for this study were kept in standard metal cages under controlled environmental conditions, featuring a consistent 12-hour light/dark cycle, and had unrestricted access to food and water. Before the experiments, these animals underwent a 7-day acclimatization period. All experimental procedures adhered to the established guidelines for the care and use of laboratory animals.

Clinical signs and body weight

Clinical observations were conducted daily for all rats. These observations encompassed alterations in fur, mucous membranes, eyes, levels of physical activity, behavior, fecal output, and instances of mortality. The body weights of each rat were documented at the initiation of treatment and then recorded monthly until the day of necropsy.

Hematology

All animals underwent an overnight fasting period before blood collection. Blood samples were obtained from the femoral veins of the animals and transferred into vacutainer tubes containing EDTA as an anticoagulant. The following parameters were assessed using a hematological auto-analyzer (Horiba ABX Micros ES 60, Horiba Medical, France): white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), platelet count (PLT), neutrophil count (NEU), and lymphocyte count (LYM).

Serum biochemistry

A biochemical serum analysis was

conducted on all animals previously undergoing a hematological assessment. Blood samples were drawn from the femoral vein, transferred into non-heparinized tubes, and kept at room temperature. Serum was extracted following centrifugation at 3,000rpm for 10 minutes. The analysis was performed using a clinical chemistry semi-automated analyzer (Erba Chem 5x Clinical Chemistry Analyzer, Erba Diagnostics Mannheim GmbH, Germany) to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), creatinine (CREA), total cholesterol (TC), and total bilirubin (TBIL).

Gross findings

After the treatment period (on the 91st day), all surviving rats underwent external gross necropsy to assess changes in body condition, skin and fur, eyes, eyelids, and any abnormal discharges from natural openings. Blood samples were collected for the evaluation of hematological and biochemical parameters before the rats were euthanized via cervical dislocation. Subsequent dissection allowed for the examination of gross lesions in subcutaneous tissues (including edema, discoloration, and hematoma), superficial lymph nodes (noting any abnormalities in shape, size, color, and consistency), and the major body cavities (looking for abnormal positioning of viscera and excessive fluid or blood). The vital thoracic and peritoneal organs were then harvested, cleaned of any adhering tissues, and assessed for macroscopic changes in size, location, color, shape, consistency, surface appearance, cut surface appearance, and free margins.

Histopathological evaluation

Liver and kidney tissues were preserved in neutral buffered 10% formalin, followed by dehydration, embedding, sectioning, dewaxing, and staining with hematoxylin-eosin. Pathological changes were evaluated using an Olympus BX43 optical microscope, and photomicrographs were captured for documentation.

Statistical analysis

Quantitative data are expressed as mean ± standard deviation. The treatment groups' results were compared to those of the control group. Data analysis was conducted using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical methods were employed to evaluate body weight and hematological and biochemical parameters. One-way ANOVA was utilized to identify differences among the groups, while a paired sample T-test was applied to determine significant differences between days 0 and 90. A P-value of less than 0.05 was considered statistically significant.

III. RESULTS

1. Clinical signs and body weight

No sign of toxicity or fatality associated with KRG administration were noted at any dosage throughout the treatment period. All observational parameters, including general appearance, grooming, posture, gait, and behavior, remained within normal ranges during the study.

Chart 1 depicts the weight gain trajectories of the different rat groups. No notable difference in body weight was observed between the treated and control groups.

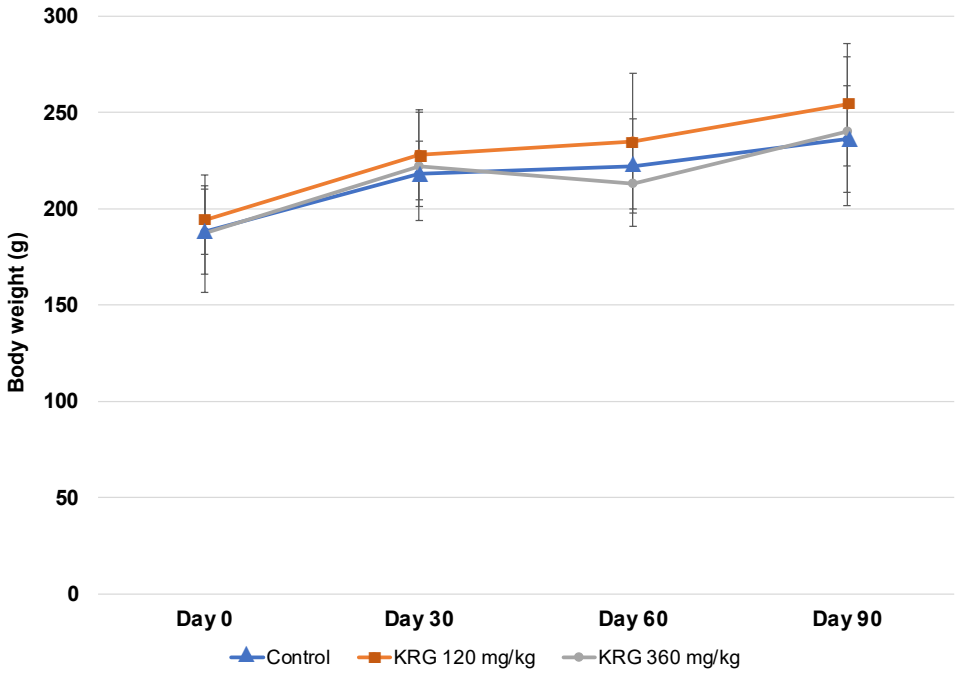

Chart 1. Body weight changes of the rats by dosage group and time in the 90-day subchronic toxicity study

2. Hematology

Table 1. Hematological parameters data by dosage group during the 90-day subchronic toxicity study

Parameters	Day 0			Day 90		
	Control	120 mg/kg	360 mg/kg	Control	120 mg/kg	360 mg/kg
RBC (10^{12}/L)	8.30 ± 1.56	8.25 ± 1.34	7.96 ± 1.59	7.96 ± 0.93	7.67 ± 1.03	7.89 ± 0.87
WBC (10^9/L)	8.50 ± 2.03	8.63 ± 2.32	8.07 ± 1.39	9.00 ± 1.46	8.58 ± 2.33	8.24 ± 1.86
HGB (g/dL)	11.89 ± 1.10	12.02 ± 1.54	11.13 ± 1.50	11.37 ± 1.67	11.05 ± 1.45	11.07 ± 1.69
HCT (%)	43.91 ± 7.26	43.33 ± 3.34	42.53 ± 5.93	42.03 ± 4.50	41.72 ± 6.77	42.42 ± 5.02
MCV (fL)	53.58 ± 3.23	52.60 ± 2.12	52.70 ± 3.30	52.78 ± 2.55	50.50 ± 2.72	51.30 ± 1.77
PLT (10^9/L)	534.30 ± 97.86	529.80 ± 107.12	535.30 ± 136.92	485.80 ± 53.25	461.40 ± 53.50	460.80 ± 111.28
NEU (%)	16.04 ± 4.05	15.28 ± 3.31	17.86 ± 3.62	14.05 ± 4.08	12.46 ± 3.70	15.05 ± 2.94

Parameters	Day 0			Day 90		
	Control	120 mg/kg	360 mg/kg	Control	120 mg/kg	360 mg/kg
LYM (%)	71.54 ± 6.50	71.08 ± 4.22	69.70 ± 5.06	74.41 ± 5.28	74.37 ± 3.40	72.93 ± 3.47

Values are presented as mean ± SD; One-way ANOVA followed by a Bonferroni post hoc test and paired sample T-test; p < 0.05 was considered statistically significant

Table 1 displays the hematologic indices across the different groups of rats. No significant alteration was noted in the KRG groups as compared to the control group. After the study period, no treatment-related variation was identified in these hematological parameters.

3. Serum biochemistry

Table 2 presents the clinical chemistry or biochemical parameters of rats that were administered KRG hard capsules orally over a 90-day period. The results indicated no significant difference between the dose and control groups in ALT, AST, ALB, TC, TBIL, and CRE levels.

Table 2. Biochemical parameters data by dosage group during the 90-day subchronic toxicity study

Parameters	Day 0			Day 90		
	Control	120 mg/kg	360 mg/kg	Control	120 mg/kg	360 mg/kg
AST (UI/L)	68.10 ± 16.64	70.90 ± 12.95	65.60 ± 12.97	60.80 ± 11.96	67.10 ± 14.04	67.30 ± 9.71
ALT (UI/L)	28.20 ± 6.46	29.20 ± 5.88	28.90 ± 4.75	29.50 ± 5.82	28.90 ± 6.57	32.60 ± 5.56
TBIL (mmol/L)	13.56 ± 0.49	13.34 ± 0.55	13.39 ± 0.74	13.13 ± 0.54	13.18 ± 0.70	13.17 ± 0.60
ALB (g/dL)	2.58 ± 0.45	2.67 ± 0.61	2.56 ± 0.45	2.57 ± 0.58	2.19 ± 0.44	2.44 ± 0.24
TC (mmol/L)	1.22 ± 0.27	1.22 ± 0.26	1.14 ± 0.19	1.23 ± 0.15	1.19 ± 0.13	1.23 ± 0.13
CREA (mg/dL)	0.95 ± 0.18	0.99 ± 0.11	0.98 ± 0.11	0.87 ± 0.18	0.94 ± 0.15	0.93 ± 0.17

Values are presented as mean ± SD; One-way ANOVA followed by a Bonferroni post hoc test and paired sample T-test; p < 0.05 was considered statistically significant

4. Gross Pathology and Histopathology

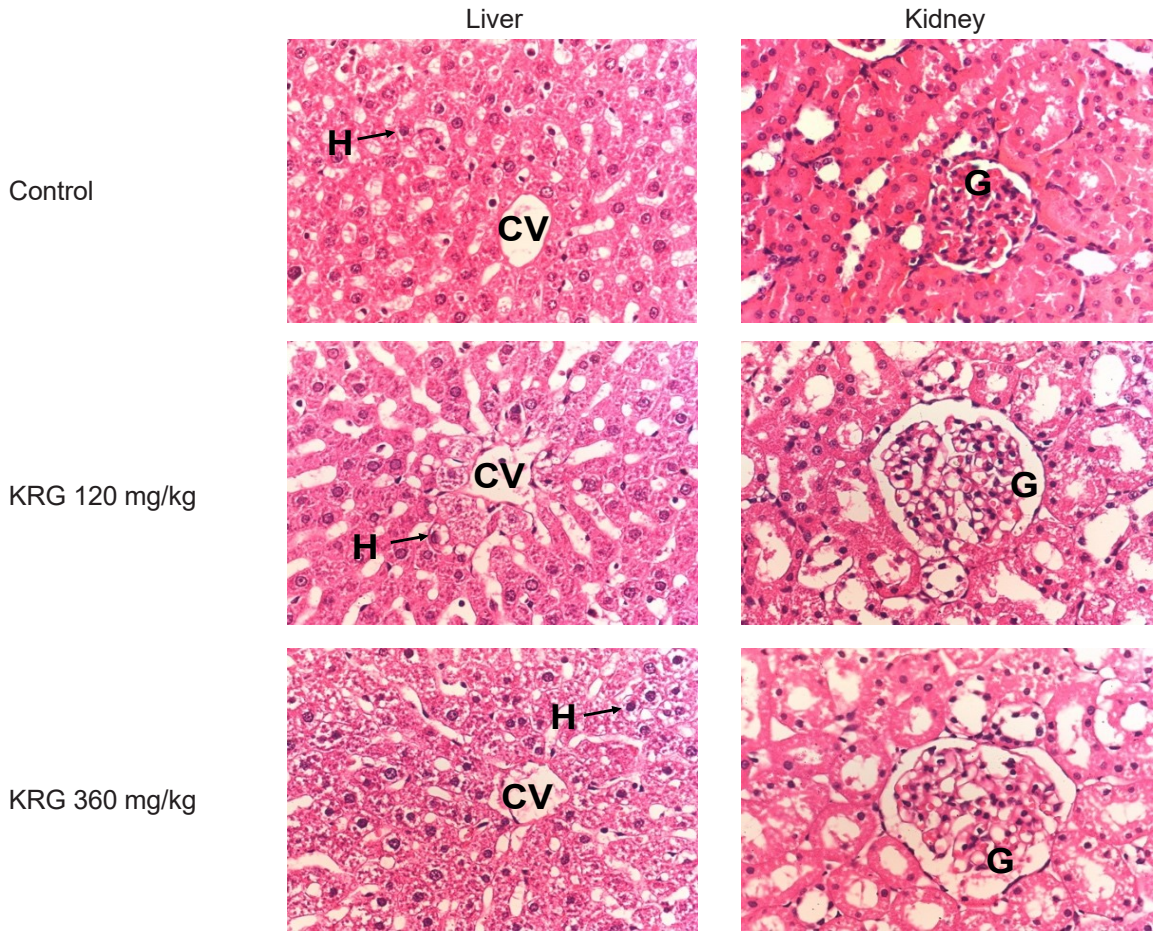

Figure 1. Photomicrographs of histological sections obtained from the liver and kidney of rats after the 90-day treatment period (400x magnification). Central veins (CV), hepatocyte (H), glomerulus (G)

The administration of KRG hard capsules did not lead to alterations in the external and internal organs of the animals in the gross pathological examination. A 90-day regimen of oral Korean red ginseng extract did not produce significant histopathological changes in either the kidney or liver (Figure 1). The findings suggest that the cellular structure of the tissues was preserved and resembled that of the control groups.

IV. DISCUSSION

Ginseng is a prominent herbal treatment in the traditional medicine practices of East Asia, with a history of use spanning more than two millennia across various nations to rejuvenate and boost vital energy. Extensive research indicates that the effectiveness of ginseng is primarily linked to ginsenosides, commonly referred to as ginseng saponins. These key constituents play a crucial role in the ginseng's biochemical and pharmacological properties.[2,3]

There are multiple varieties of ginseng, such as *Panax ginseng* (Korea), *Panax notoginseng* (China), *Panax japonicas* (Japan), and *Panax quinquefolius* (America). Among these, *Panax ginseng* is the most esteemed, noted for its

higher concentration of ginsenosides compared to the other species.[6] In Korea, traditional methods for preparing and processing *Panax ginseng* are prevalent. For medicinal use, ginseng is typically harvested at the age of six. The traditional method for producing red ginseng involves steaming and drying the roots without peeling, while white ginseng is prepared by simply drying and peeling the roots. Unlike white ginseng, red ginseng undergoes processing that results in the presence of ginsenosides Rg3, Rg5, and Rk1, which are known for their significant biological effects, including anticancer and anti-inflammatory properties. Furthermore, Korean red ginseng has demonstrated beneficial pharmacological effects on conditions such as high blood pressure, atherosclerosis, and hyperlipidemia by mitigating oxidative damage.[7,8]

Clinical trial data indicate that the occurrence of adverse events associated with ginseng mono preparations is comparable to that of a placebo. The most frequently reported adverse events include headaches, sleep disturbances, and gastrointestinal issues. While isolated case reports and data from spontaneous reporting systems suggest the potential for more severe adverse events, establishing a causal relationship based on the available evidence can be challenging.[8,9] Toxicities associated with ginseng preparations have been reported; however, the sample preparations differ significantly among various manufacturers. Furthermore, variations in content, quality, and preparation methods across different batches complicate the assessment of ginseng preparation toxicity in humans.[9]

Although ginseng has a long history of use and is widely consumed, its chronic effects remain poorly understood, and there is limited information regarding the toxicity of specific ginseng preparations. To gain a more thorough understanding of the toxicities associated with ginseng preparations, we evaluated the 90-day subchronic toxicity of Korean red ginseng extract, which contains three primary ginsenosides: Rg1, Rb1, and Rg3, in *Wistar* rats. The anticipated clinical dosage for KRG hard capsules is 19.2 mg/kg, assuming a 50 kg individual takes up to two capsules daily. Using a dose conversion factor of 6, we established the dose levels for the rats at 120 mg/kg (corresponding to the expected clinical dose) and 360 mg/kg (three times the expected clinical dose).

Clinical signs may indicate the adverse effects associated with the administration of KRG hard capsules. No noticeable change observed in general behavior, fur coloration, eye condition, mucous membranes, posture, mobility, or the processes of secretion and excretion.

Mean body weights were analyzed to evaluate the effects of KRG on animal growth. The relationship between nutrition from food and water intake and body weight is direct. Additionally, various stressors can impact body weight. A notable and sensitive indicator of adverse effects following drug exposure is a reduction in body weight gain.[10] No obvious influence on food and water consumption was noted. One-way ANOVA statistical analysis indicated no significant difference among the groups ($p > 0.05$), implying that KRG treatment did not influence the weight gain of the rats.

Hematological analyses serve as critical indicators for evaluating the substances' toxicity in humans and animals. Examination of hematological parameters indicates that administering a test substance influences blood composition.[10,11] There were no notable variation between the dose and control groups,

suggesting that the product did not affect hematological parameters.

Biochemical parameters serve as essential diagnostic criteria in clinical settings.[10] They can reveal the negative effects induced by various substances. Biochemical analyses play a critical role in identifying, detecting, and characterizing the toxic impacts of harmful compounds. These analyses are vital for assessing the specific target organs affected by these substances and offer important insights into disease mechanisms.[12] The creatinine (CREA) level is utilized to evaluate kidney damage, while liver function is assessed through markers such as ALT, AST, TC, ALB, and TBIL.[11] Both the kidneys and liver, which are responsible for eliminating xenobiotics, are sensitive organs that can be adversely affected by chemicals, including pharmaceuticals and botanical substances.[12] The results of biochemical analyses were not affected by KRG compared with the control group, which indicated that KRG did not alter kidney and liver functions.

The histopathological study is intended to observe any abnormalities in gross pathology and organ histopathology.[10] The histopathological evaluation of the vital organs, including the kidney and liver, showed that the structures were normal, and no atypical microscopic changes were identified in the kidney and liver.

Siegel RK reported negative effects resulting from excessive exposure to ginseng and introduced the term “ginseng abuse syndrome”.[13] The clinical presentation of ginseng abuse syndrome encompasses hypertension, gastrointestinal disturbances, insomnia, nervousness, confusion, and depression.[14] In the prevailing work, we did not detect any clinical symptoms associated with ginseng abuse syndrome in male and female rats administered with Korean red ginseng extract. Our current observations align with subchronic toxicity investigations, where male and female SD rats were administered diets supplemented with ginseng extract at doses of 0, 1.5, 5, and 15 mg/kg/day over 13 weeks, showing no histopathological alteration.[15] Furthermore, another study found that rats did not exhibit any toxic effects after consuming ginseng extract at dosage levels ranging from 105 to 210 mg/kg/day for 25 weeks.[9] The dose levels utilized in these studies were deemed low; however, we examined higher doses (120 and 360 mg/kg/day) of ginseng extract and found no sign of toxicity. In a long-term study involving mice, the intake of *Panax ginseng* enhanced behavioral reactions to mild stress; nevertheless, there were no notable variation in average weights or survival rates.[16] Additionally, a three-month administration of ginseng extract to both male and female beagle dogs revealed no sign of toxicity.[17] Ginsenosides, recognized as steroid-like saponins, are regarded as ginseng’s primary active pharmacological components.[2,3] The safety profile of ginsenosides has garnered significant interest among researchers. Investigations into the chronic toxicity of ginsenoside Re indicated that there were no fatality among the test rats, and histopathological evaluations revealed no abnormality.[18] The highest safe dosage of ginsenoside Rg3 administered via oral gavage in Sprague-Dawley rats was determined to be 180 mg/kg.[18] Furthermore, ginsenosides Rb1 and Rg1 are regarded as key ginsenosides in ginseng, exhibiting low toxicity and a range of potential therapeutic benefits for neurological, neurodegenerative, metabolic, and cardiovascular conditions.[19,20] These aforementioned research findings consistently indicate that ginseng use is safe, corresponding

with the results of the current study.

V. CONCLUSION

In this investigation, the administration of KRG hard capsules via oral route during the 90-day subchronic toxicity assessment in Wistar rats did not result in any fatality or observable adverse effects in terms of clinical signs, body weight changes, or hematological and biochemical parameters. Additionally, necropsy and histological evaluations revealed no treatment-related finding. Consequently, the results obtained at the administered doses (120, 360 mg/kg BW) indicate that KRG is unlikely to exhibit toxicological effects and is considered safe for use in herbal medicine.

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