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Anti-oxidant and natural killer cell activity of Korean red ginseng (*Panax ginseng*) and urushiol (*Rhus vernicifera* Stokes) on non-alcoholic fatty liver disease of rat

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ABSTRACT

Anti-oxidative and immunologic effects of the Korea red ginseng (KRG; *Panax ginseng*) and urushiol (*Rhus vernicifera* Stokes) on non-alcoholic fatty liver disease (NAFLD) were evaluated. Forty-five rats (five Long-Evans Tokushima Otsuka and 40 Otsuka Long-Evans Tokushima Fatty [OLETF] rats) received chew diets for 10 months; after this period. The OLETF rats were divided into the following four groups according to diet for 2 months: NAFLD (chew), KRG (chew + KRG [200 mg/kg/day]), urushiol (chew + urushiol [0.5 mg/kg/day]), and ursodeoxycholic acid (UDCA) (chew + UDCA [15 mg/kg/day]) groups. Liver function, lipid profiles and anti-oxidant activity of liver and serum, natural killer (NK) cell activity, and pathology were compared. In KRG and urushiol groups, the level of serum triglyceride ([302.0 ± 70.4 and 275.2 ± 63.8] vs. 527.7 ± 153.3 mg/dL) were lower compared with that of NAFLD group ($p < 0.05$). The levels of HDL-cholesterol (liver tissue: [4.8 ± 0.2 and 4.8 ± 0.5] vs. 4.2 ± 0.2 mg/g) and NK cell activity ([3485 ± 910 and 3559 ± 910] vs. 2486 ± 619 counts) were significantly higher than those of the NAFLD group ($p < 0.001$). Inflammation with neutrophil infiltration was observed in only two rats in the NAFLD group. These results suggest that 2 months of oral KRG or urushiol administration improves lipid profiles and stimulates NK cell activity, while inhibiting steatohepatitis in OLEFT rats.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is common in many regions; it is regarded as the most common cause of abnormality in liver function tests worldwide (Atabek, 2011; Lee et al., 2012). NAFLD is strongly associated with obesity, dyslipidemia, and metabolic syndrome, and can lead to cirrhosis and even hepatocellular carcinoma (Sanyal, 2011).

Understanding the pathogenesis of NAFLD is clinically critical for the prevention and treatment of patients with NAFLD. Oxidative stress increases lipid peroxidation and eventually causes hepatocyte injury associated with NAFLD (Pessayre, 2007). In addition, recent findings suggested that natural killer (NK) cells play an important role in the pathogenesis of NAFLD by inducing anti-fibrotic effects and hepatic satellite cell cycle arrest and apoptosis (Maher et al., 2008).

Ginseng is an oriental herb that has been consumed as both food and medicine for more than 2000 years. Among the several kinds of *Panax ginseng* products, Korean red ginseng (KRG) has been found to have the most potent pharmacological effects against immune deficiency, metabolic syndrome, and cancer (Kim et al., 2013; Park et al., 2012; Paul et al., 2012). Urushiol is

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an oil found in plants of the Family Anacardiaceae and constitutes a major organic component of the sap of the lacquer tree (*Rhus vernicifera* Stokes) (Suk et al., 2010). Urushiol has been found to have both antimicrobial and anti-oxidative effects (Kim et al., 1997a; Suk et al., 2010). More specifically, urushiol has been observed to have inhibitive effects against the growths of ovarian cancer, mouse leukemia, and human adenocarcinoma (Choi et al., 2001). All in all, these findings suggest that KRG and urushiol may inhibit the progression of NAFLD perhaps through the anti-fibrotic and anti-oxidative mechanisms associated with the NK cells. However, there are only few studies that directly study the effects of KRG and urushiol on NAFLD. Therefore, in this study, the anti-oxidative and immunological effects of KRG and urushiol on NAFLD were evaluated using the rat model.

2. Materials and methods

2.1. Chemicals

KRG extracts were provided by The Korean Society of Ginseng & Korea Ginseng Corp. (Seoul, Korea) as an undiluted solution. The KRG extracts contained the following seven glycosides, known as ginsenosides (mg/g): Rg_1 (2.481), Rb_1 (5.481), Rg_3(s) (0.197), Re (2.975), Rc (2.248), Rb_2 (2.175), and Rb (0.566). The extracts had a moisture content of 36.68%.

The preparation of urushiol involved the sap (40 mL) of lacquer tree being diluted to a volume of 1 L by the addition of distilled water, followed by extraction with 1 L of n-hexane which was performed twice. The hexane extract was concentrated under reduced pressure to yield brownish oil (26.9 g). The oil was then purified through silica gel column chromatography (Merck 7734) and eluted with 20% acetone/hexane. It was further purified by the same method (Merck 9385), followed by octadecyl silica gel column chromatography (YMC GEL ODS-A) using a specific gradient of methanol in water that best yields urushiols. The final concentration of extracted urushiol was 10 mg/mL (Suk et al., 2010).

Ursodeosycholic acid (UDCA) (URSA, Daewoong Corp., Seoul, Korea) was used as a control agent. To compare each group, KRG, urushiol, and UDCA were mixed evenly in a sterilized, normal chew diet for consumption (200 mg/kg body weight/day, 0.5 mg/kg body weight/day, and 15 mg/kg body weight/day, respectively).

2.2. Animals

The Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Otsuka Long Evans Tokushima Fatty, Otsuka, Inc., Tokushima, Japan) were selected to investigate the effect of KRG, urushiol, and UDCA on NAFLD; these rats spontaneously develop obesity, insulin resistance, and NAFLD, which progressively worsens with advancing age (Rector et al., 2010). Long Evans Tokushima Otsuka (LETO) rats (Long Evans Tokushima Otsuka, Otsuka, Inc., Tokushima, Japan) were used control.

A total of 40 male OLETF rats and five male LETO rats with an age of 6 weeks were housed individually in steel micro-isolator cages maintained at 22 °C with a 12-h light and 12-h dark cycle. The control group of five LETO rats received normal chow diets (Purina LabDiet, St. Louis, MO, USA) for 12 months (Rector et al., 2010). On the other hand, OLETF rats received a normal chow diets for 10 months, followed by randomized assignment into four dietary groups for 2 months (10 rats each: NAFLD (normal chow diet), KRG (normal chow and KRG diet [200 mg/kg body weight/day]), urushiol (normal chow and urushiol diet [0.5 mg/kg body weight/day] (Suk et al., 2011), and UDCA (normal chow and UDCA diet [15 mg/kg body weight/day]) (Fig. 1). After 12 months of feeding, the rats were sacrificed after having fasted for 8 h. The rats were euthanized with ether anesthesia. A midline abdominal incision was performed and blood was collected at the heart. Livers were rapidly resected for the pathology and stored at −80 °C for the evaluation of lipid profiles. Whole blood (10 mL) was collected for the evaluation of natural killer (NK) cell activity. Other blood samples were collected, and centrifuged at 3000 rpm for 15 min to collect serum.

Animals received humane care and all procedures were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and were approved by the Hallym University College of Medical Institutional Animal Care and Use Committee.

2.3. Liver function test and lipid profiles

From the serum, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), total cholesterol, high density lipoprotein (HDL)-cholesterol, and triglyceride (TG) were analyzed by biochemical analyzer of blood (KoneLab 20, Thermo Fisher Scientific, Waltham, Finland).

For the calculation of lipid profiles of liver tissue, 1 g of liver tissue was mixed with 3 ml of saline solution; chloroform and methanol solution (chloroform:methanol = 2:1) was then added to homogenize the mixture. The homogenate was centrifuged at 3000 rpm for 10 min, and the lower level was collected. Isoprophanol was added and lipid profiles were evaluated by using quantitative kit (LQ DIA, Asanpharm, Hwasung, Korea). Total cholesterol, HDL-cholesterol, and TG of the liver tissue were analyzed.

2.4. Antioxidant activity

Antioxidant activity was evaluated in the liver tissue and serum. For the calculation of antioxidant activity in liver tissue, 1 g of liver tissue homogenate was mixed with 0.25 M sucrose (pH 7.0). After the homogenates was centrifuged at

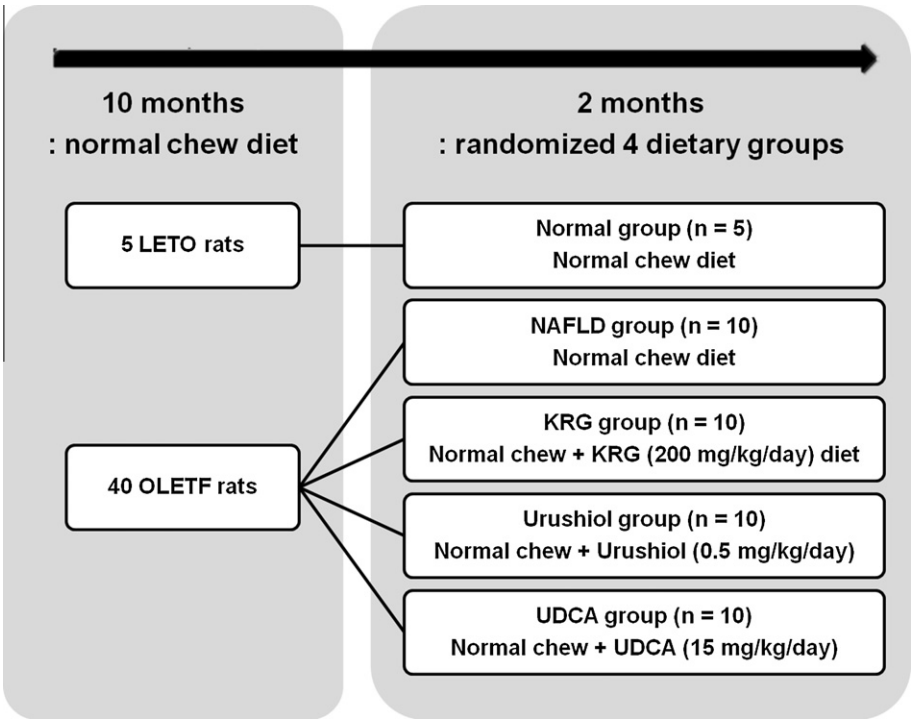

Fig. 1. Flow chart of study design. LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; *n*, number; KRG, Korea Red Ginseng; UDCA, ursodeoxycholic acid.