

Effects of Cream Containing Ultralow Volume Radionuclides on Carrageenan-Induced Inflammatory Paw Edema in Mice

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Abstract

Low-dose irradiation activates antioxidative functions and inhibits oxidative damage. Although a wide range of health products have been developed using the activation of biological functions by low-dose irradiation, their effectiveness has not yet been confirmed. The purpose of this study was to determine whether cream containing ultralow volume radionuclides (UVR) protects against carrageenan-induced inflammatory paw edema in mice. Cream containing ultralow volume radionuclides (UVR-cream) or sham cream was applied to the right hind paw of mice and 50 μ L of carrageenan was subsequently injected to the right hind paw. Results showed that carrageenan administration induced paw edema; however, application of UVR-cream significantly decreased paw volume at 4 hours. Application of UVR-cream produced slight improvement in carrageenan-induced paw edema compared with sham cream. However, no significant changes were observed in paw edema between sham cream and UVR-cream. Carrageenan administration significantly decreased catalase activity and total glutathione content in paw. No significant changes were observed in the catalase activity or t-GSH content in paw among carrageenan-only, sham cream, and UVR-cream. In conclusion, the effects of UVR-cream differed from the beneficial effects induced by low-dose irradiation, since application of the cream did not activate antioxidative functions.

Keywords: Antioxidative function; Ultralow volume radionuclides; Cream; Paw edema

Introduction

Low-dose irradiation activates antioxidative functions as well as immune functions. For example, low-dose X-irradiation (0.5 Gy) increased antioxidative associated substances such as superoxide dismutase (SOD) and total glutathione (t-GSH) in the mouse brain and inhibited cold-induced brain edema [1]. Another study suggested that low-dose X-irradiation inhibited paw edema induced by ischemia-reperfusion in mice due to increased SOD activity [2]. Reactive oxygen species (ROS) induce this damage, and thus antioxidative functions play an important role in protection against oxidative damage.

Radon (^{222}Rn) is a radioactive element that mainly emits α -rays. Large numbers of patients undergo radon spa therapy in countries supporting this tradition, such as Japan [3], central Europe [4], and Russia [5]. Specifically, exposure to radon has a curative effect on several diseases [3,6]. We previously reported that radon inhalation activates antioxidative functions in the organs of mice and inhibits several oxidative damages [7,8]. The study indicates that antioxidative functions activated by radon inhalation likely play an important role in the beneficial effects of radon therapy. On the other hand, while low-dose irradiation has positive effects for human health [7,8], it is well known that radiation, especially high-dose irradiation, induces cancer.

A wide range of health products have been developed using the activation of biological functions by low-dose irradiation. These goods contain ore with naturally occurring radionuclides. Although health products containing ultralow volume radionuclides are available for sale, most goods are not tested for the effectiveness of the claimed effects. Therefore, it is reasonable to regulate goods containing

naturally occurring radioactive material (NORM) in order to reduce unnecessary exposure to radiation. However, a report evaluating cosmetics containing ultralow volume radionuclides revealed that the observed dose was lower than the effective dose limit [9].

The purpose of this study was to determine whether cream containing ultralow volume radionuclides (UVR-cream) activates antioxidative functions and protects against carrageenan-induced inflammatory paw edema in mice. We examined the following histological and biochemical parameters to assess the effects of cream treatment: paw histology, SOD activity, catalase activity, t-GSH content, and lipid peroxide level in mouse paw and NO level in serum.

Materials and Methods

Animals

Male ICR mice (age, 8 weeks; body weight, approximately 35 g) were obtained from Charles River (Yokohama, Japan). Ethical approval for all protocols and experiments was obtained from the animal experimentation committee of Okayama University. Mice were housed under a 12:12 h artificial light cycle (08:00 to 20:00) at a temperature of $23 \pm 2^\circ\text{C}$.

Treatment with cream

Table 1 shows the cream components (Sueam Co., Ltd. Tokyo, Japan). To clarify the effect of low-dose irradiation by ultralow volume radionuclides, sham cream was prepared that is identical to UVR-cream but without the ultralow volume radionuclides.

The equivalent dose in case of application of 0.02 g of each cream to human skin was measured using a γ survey meter (Aloka TCS-161,

Tokyo, Japan). The equivalent dose of background, sham-cream, and UVR-cream was 0.08, 0.09, 0.10 μ Sv/hour, respectively.

	Sham cream	UVR-cream
Alcohol	Glycerin	Glycerin
	Cetyl alcohol	Cetyl alcohol
	Butylene glycol	Butylene glycol
Surfactant	PEG-40 castor oil	PEG-40 castor oil
	Glyceryl stearate	Glyceryl stearate
	Polysorbate 60	Polysorbate 60
Ester	Isopropyl palmitate	Isopropyl palmitate
	Cetyl palmitate	Cetyl palmitate
Oil	Squalane	Squalane
Silicon	Dimethicone	Dimethicone
Preservative	Methylparaben	Methylparaben
	Propylparaben	Propylparaben
Anti-inflammatory effect	Dipotassium glycyrrhizinate	Dipotassium glycyrrhizinate
Antioxidant effect	Asian ginseng fruit extract	Asian ginseng fruit extract
Radioactive substance		Zirconium oxide mineral

Table 1: Component differences between sham cream and cream containing ultralow volume radionuclides

The cream (0.02 g) was applied topically to the right hind paw. The paw was wrapped with cotton to prevent the removal of the cream by grooming. Two hours after application, the cream was removed from the paw by washing with a physiological saline solution.

Induction of carrageenan-induced paw edema

Carrageenan was dissolved in physiological saline (1% v/v). After the removal of cream, 50 μ L of carrageenan was injected to the right hind paw. Paw volume was measured before carrageenan administration and at 2 or 4 hours after carrageenan administration by water displacement using a circular plastic cylinder of radius 4.4 mm.

Behavioral testing: von Frey test

At 2 or 4 hours after carrageenan administration, the behavioral response of mice to mechanical stimuli was assessed using the von Frey test. Mice were individually placed in plastic cages with a wire-mesh floor (1 mm diameter wire placed 5 mm apart). The paw was touched with 1 of a series of 9 von Frey hairs (0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1, 1.4, and 2 g) (North Coast Medical Inc., CA, USA). A positive response was noted when the paw was sharply withdrawn. The 50% withdrawal threshold was determined using the updown method [10]. Briefly, the behavioral test was initiated with the 0.4 g von Frey hair, representing the middle of the series. A stronger stimulus was chosen if the paw showed a negative response. A weaker stimulus was chosen if the paw showed a withdrawal response. The critical 6 data points were noted after the response threshold was first crossed.

50% g threshold = $10^{X_f + k\delta}$ where X_f = value (in log units) of the final von Frey hair used; k = tabular value for the pattern of positive or negative responses [11]; and δ = mean difference (in log units) between stimuli. If continuous negative responses were observed to the maximum of the von Frey hair, values of 2.5 g were assigned.

Mice were sacrificed by an overdose of ether anesthesia and paws were quickly excised for determination of antioxidative associated substances and blood was drawn from the heart for determination of NO level. Serum was separated from the blood samples by centrifugation at $3,000 \times g$ for 5 min. These samples were stored at -80°C until use in the biochemical assays or fixed in 10% formalin for histological examination.

Biochemical assays

Paws were homogenized on ice in 10 mM phosphate buffer (PBS; pH 7.4). The homogenates were centrifuged at $12,000 \times g$ for 45 min at 4°C and the supernatants were used to assay SOD and catalase activities.

SOD activity in paws was measured by the Nitroblue Tetrazolium (NBT) reduction method [12] using the Wako-SOD test (Wako Pure Chemical Industries Ltd., Osaka, Japan). Briefly, the extent of inhibition of the reduction in NBT was measured spectrophotometrically at 560 nm. One unit of enzyme activity was defined as 50% inhibition of NBT reduction.

Catalase activity was measured as the hydrogen peroxide (H_2O_2) reduction rate at 37°C and was assayed spectrophotometrically at 240 nm [13]. The assay mixture consisted of 50 μ L of 1 M Tris-HCl buffer containing 5 mM ethylenediaminetetraacetic acid (pH 7.4), 900 μ L of 10 mM H_2O_2 , 30 μ L deionized water, and 20 μ L paw supernatant. Activity was calculated using a molar extinction coefficient of $7.1 \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$. Catalase activity was measured by the amount of hydrogen peroxide split by catalase at 37°C . The reactions were initiated by the addition of supernatant.

T-GSH content was measured using the Bioxytech GSH-420™ assay kit (OXIS Health Products Inc., Portland, OR, USA). Briefly, paw tissue samples were homogenized in 10 mM PBS (pH 7.4), and mixed with ice-cold 7.5% trichloroacetic acid solution. The homogenates were centrifuged at $3,000 \times g$ for 10 min, and tissue supernatants were assayed. The assay is based on the formation of chromophoric thione, the absorbance of which can be measured at 420 nm, and is directly proportional to the t-GSH concentration.

Lipid peroxide levels were assayed using the Bioxytech LPO-586™ assay kit (OXIS Health Products Inc.). Briefly, paw samples were homogenized in 10 mM phosphate buffer (PBS; pH 7.4) on ice. Prior to homogenization, 10 μ L of 0.5 M butylated hydroxytoluene in acetonitrile was added per 1 mL of buffer-tissue mixture. After homogenization, the homogenate was centrifuged at $15,000 \times g$ for 10 min at 4°C , and the supernatant was used for the assay. The lipid peroxide level assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylidole, with malondialdehyde and 4-hydroxyalkenals at 45°C . The optical density of the colored products was read spectrophotometrically at 586 nm.

Protein content in each sample was measured by the Bradford method, using a Protein Quantification Kit-Rapid (Dojindo Molecular Technologies Inc., Kumamoto, Japan) [14].

Nitric oxide (NO) levels in serum were measured using the NO_2/NO_3 assay Kit C II (Dojindo Molecular Technologies, Inc.,

Kumamoto, Japan). This assay is based on the azo coupling reaction between diazonium salt compound and naphthyl ethylenediamine. The optical density of the colored products was read at 540 nm in a spectrophotometer.

Histological examination

Paws were fixed in 10% formalin and decalcified with Plank-Rychlo solution. Next, they were processed with a graded mixture of ethanol and xylene, and then embedded in paraffin. Tissue sections (6 microns) were prepared and stained with hematoxylin-eosin (HE). The number of inflammatory leukocytes was counted in a unit area (mm²).

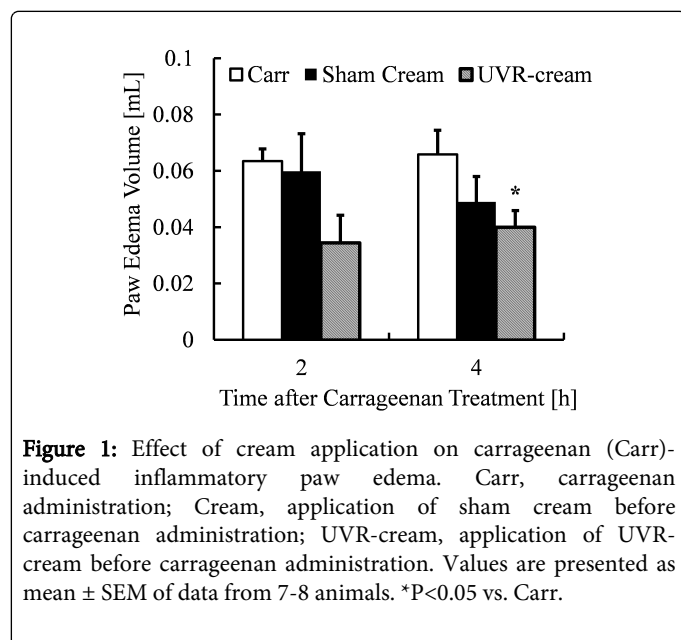
Statistical analyses

The data are presented as the mean \pm standard error of the mean (SEM). Each experimental group consisted of samples from 7-8 animals. Statistically significant differences were determined using Tukey's test for multiple comparisons where appropriate. P-values were considered significant at $P < 0.05$.

Results

Effect of cream application on carrageenan-induced inflammatory paw edema

We assessed the effects of cream application on carrageenan-induced paw edema. Application of sham cream did not inhibit carrageenan-induced paw edema.



However, treatment with UVR-cream significantly decreased the paw volume at 4 hours. No significant differences were observed in paw edema between sham cream and UVR-cream (Figure 1).

Effect of cream application on 50% paw withdrawal threshold

Next, we assessed the effects of cream application on 50% paw withdrawal threshold. Carrageenan administration in the presence or absence of cream application significantly decreased the 50% paw withdrawal threshold compared with pre-treatment.

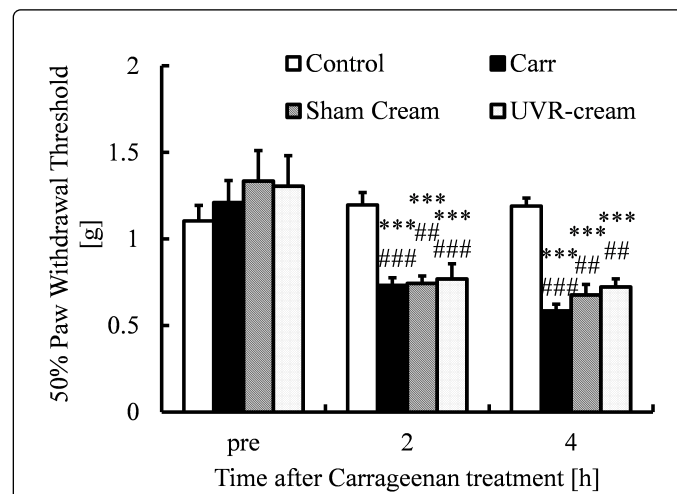


Figure 2: Effect of cream application on 50% paw withdrawal threshold. Carr, carrageenan administration; Cream, application of sham cream before carrageenan administration; UVR-cream, application of UVR-cream before carrageenan administration. Values are presented as the mean \pm SEM of data from 7-8 animals. *** $P < 0.001$ vs. Control, ## $P < 0.01$, ### $P < 0.001$ vs. Pre.

In addition, the 50% paw withdrawal threshold of mice treated with sham cream or UVR-cream was significantly lower than that of control. No significant changes were observed in the 50% paw withdrawal threshold between sham cream and UVR-cream (Figure 2).

Histological observation in paws following carrageenan administration

The migration of inflammatory leukocytes in paws was observed following carrageenan administration in the presence or absence of cream application.

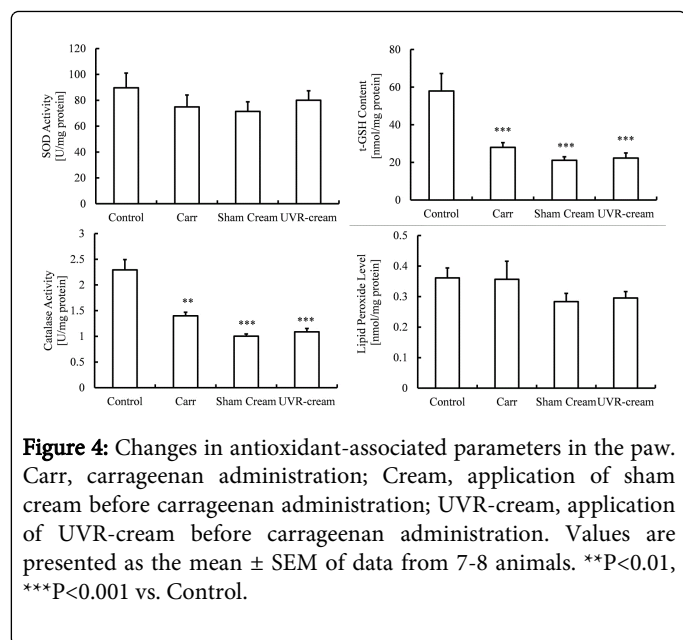
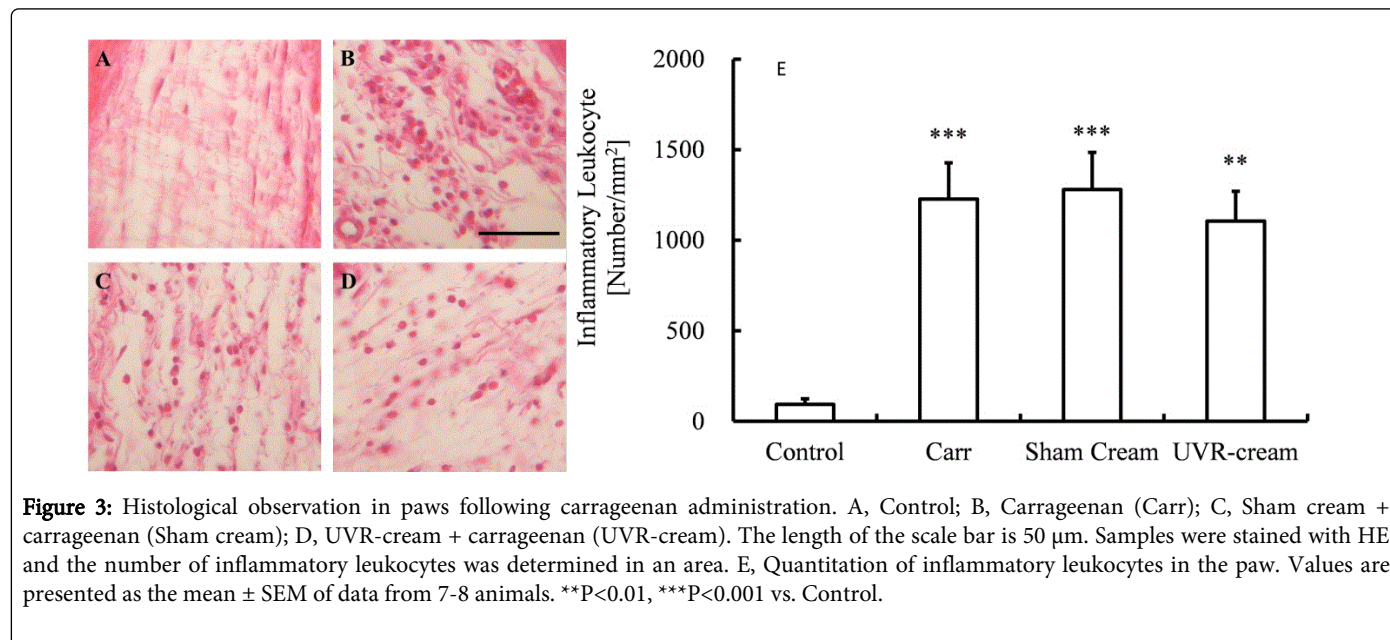
There were significantly more inflammatory leukocytes per unit area in mice administered carrageenan compared to control. No significant changes were observed in leukocyte migration in paw among carrageenan-only, sham cream, and UVR-cream (Figure 3).

Effect on antioxidative functions in paw following carrageenan administration

We examined the effect of cream treatment on the antioxidative-associated substances in paw. The results shown in Figure 4 indicate that carrageenan administration in the presence or absence of cream application significantly decreased catalase activity and t-GSH content in paws.

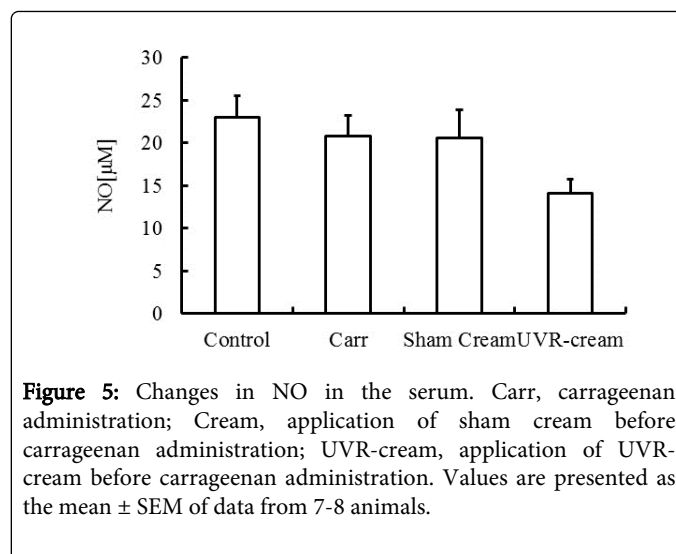
No significant changes were observed in the catalase activity or t-GSH content in paws among carrageenan-only, sham cream, and

UVR-cream. Lipid peroxide level in the paws of mice administered sham cream or UVR-cream was slightly lower than that of carrageenan-administered mice (Figure 4).



Effect on NO level in serum following carrageenan administration

To assess the anti-inflammatory effect following carrageenan administration, NO, which is a mediator of inflammation, was examined. However, no significant changes were observed in NO level in serum among all groups (Figure 5).



Discussion

Carrageenan administration produces ROS such as superoxide, hydrogen peroxide, and hydroxyl radical, and the production of ROS contributes to the development of oxidative damage [15]. We previously reported that radon inhalation activated antioxidative functions in mice and inhibited carrageenan-induced paw edema. In this study, we investigated the effect of a UVR-cream on carrageenan-induced paw injury. The cream contains an antioxidant, Asian ginseng, which inhibits the production of hydroxyl radicals [16]. It is therefore assumed proposed to inhibit carrageenan-induced paw injury. Results of this study showed that application of UVR-cream significantly inhibited paw edema induced by carrageenan administration. While sham cream also inhibited paw edema, these

differences were not statistically significant. In addition, no significant changes were observed in paw edema between sham and UVR-creams. These findings suggested that the substances in the cream mainly contributed to the inhibition of paw edema.

Pain is often accompanied by inflammation. Carrageenan administration induced mechanical allodynia and significantly decreased the 50% paw withdrawal threshold. A report indicated that non-invasive pulsed magnetic field ameliorated carrageenan-induced inflammatory pain due to activation of antioxidative functions [17]. We previously reported that radon inhalation inhibited formalin-induced inflammatory pain [18]. This study also suggested that activation of antioxidative functions plays an important role in the inhibition of inflammatory pain. While carrageenan administration significantly decreased the 50% paw withdrawal threshold, application of the UVR-cream did show ameliorating effects.

Carrageenan-induced inflammatory response is linked to neutrophil infiltration and the production of neutrophil-derived ROS [15]. Our results showed that carrageenan administration significantly increased inflammatory leukocytes in paws; however, no significant changes were observed in leukocyte migration among carrageenan-only, sham cream, and UVR-cream groups. These findings indicated that the UVR-cream did not inhibit the inflammatory response, such as leukocyte migration, and neutrophil-derived ROS production.

NO is a mediator of carrageenan induced inflammatory edema and contributes the production of ROS [15]. However, our results indicated that no significant changes were observed in NO level in serum among all groups. These results suggested that the main inflammatory response under the conditions was leukocyte migration.

To examine the mechanism of the effects, antioxidative functions in paws were analyzed. The results showed that carrageenan administration significantly decreased catalase activity and t-GSH content in paw, suggesting oxidative stress. However, no significant differences were observed in the catalase activity or t-GSH content in paw among carrageenan-only, sham cream, and UVR-cream, suggesting that the UVR-cream did not contribute to the activation of antioxidative functions. These findings suggested that the antioxidative or anti-inflammatory substances in cream mainly contributed to the inhibition of paw edema.

Radiation induced skin damage is a deterministic effect of radiation. Therefore, only when the exposure dose exceeds a certain threshold dose, radiation results in damage. Measurement of equivalent dose showed that the equivalent dose from UVR-cream was slightly higher than that of the background level. Therefore, radiation from UVR-cream has an insignificant effect on skin damage.

In conclusion, the application of UVR-cream produced slight improvement of carrageenan-induced paw edema compared with sham cream. However, the observed effects differed from the beneficial effects induced by low-dose irradiation, in that the UVR-cream did not activate antioxidative functions. These findings indicated that the components of the cream such as Asian ginseng fruit extract mainly contribute to the inhibition of carrageenan-induced paw edema, because dipotassium glycyrrhizinate, which has anti-inflammatory effects, did not inhibit anti-inflammatory responses.

References

1. Yoshimoto M, Kataoka T, Toyota T, Taguchi T, Yamaoka K (2012) Inhibitory effects of prior low-dose X-irradiation on cold-induced brain injury in mouse. *Inflammation* 35: 89-97.
2. Kataoka T, Mizuguchi Y, Yoshimoto M, Taguchi T, Yamaoka K (2007) Inhibitory effects of prior low-dose x-irradiation on ischemia-reperfusion injury in mouse paw. *J Radiat Res* 48: 505-513.
3. Mitsunobu F, Yamaoka K, Hanamoto K, Kojima S, Hosaki Y, et al. (2003) Elevation of antioxidant enzymes in the clinical effects of radon and thermal therapy for bronchial asthma. *J Radiat Res* 44: 95-99.
4. Becker K (2004) One century of radon therapy. *Int J Low Radiation* 1: 334-357.
5. Iashina LM, Shatrova LE, Zhdanova KS, Kuznetsova TA (2011) [The influence of radon baths on the lipid profile of patients with cardiovascular diseases and dyslipidemia]. *Vopr Kurortol Fizioter Lech Fiz Kult* : 3-4.
6. Yamaoka K, Mitsunobu F, Hanamoto K, Mori S, Tanizaki Y, et al. (2004) Study on biologic effects of radon and thermal therapy on osteoarthritis. *J Pain* 5: 20-25.
7. Kataoka T (2013) Study of antioxidative effects and anti-inflammatory effects in mice due to low-dose X-irradiation or radon inhalation. *J Radiat Res* 54: 587-596.
8. Yamaoka K (2006) Activation of Antioxidant System by Low Dose Radiation and Its Applicable Possibility for Treatment of Reactive Oxygen Species-Related Diseases. *J Clin Biochem Nutri* 39: 114-133.
9. Furuta E, Nakahara H (2008) Dose Estimation of Radiation Exposure from Hormesis Cosmetics. *hoken buturi* 43: 341-348.
10. Dixon WJ (1980) Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol* 20: 441-462.
11. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1994) Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 53: 55-63.
12. Baehner RL, Murrmann SK, Davis J, Johnston RB Jr (1975) The role of superoxide anion and hydrogen peroxide in phagocytosis-associated oxidative metabolic reactions. *J Clin Invest* 56: 571-576.
13. Aebi H, Wyss SR, Scherz B, Gross J (1976) Properties of erythrocyte catalase from homozygotes and heterozygotes for Swiss-type acatalasemia. *Biochem Genet* 14: 791-807.
14. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
15. Cuzzocrea S, Zingarelli B, Hake P, Salzman AL, Szabó C (1998) Antiinflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. *Free Radic Biol Med* 24: 450-459.
16. Zhang D, Yasuda T, Yu Y, Zheng P, Kawabata T, et al. (1996) Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. *Free Radic Biol Med* 20: 145-150.
17. Mert T, Ocal I, Cinar E, Yalcin MS, Gunay I (2014) Pain-relieving effects of pulsed magnetic fields in a rat model of carrageenan-induced hindpaw inflammation. *Int J Radiat Biol* 90: 95-103.
18. Yamato K, Kataoka T, Nishiyama Y, Taguchi T, Yamaoka K (2013) Antinociceptive effects of radon inhalation on formalin-induced inflammatory pain in mice. *Inflammation* 36: 355-363.