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Radioprotective Effects of Grifola umbellata Extracts on γ-Irradiated Mice

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- 국문요약 -

저령(猪苓) 추출물이 감마선에 조사된 생쥐에 미치는 방사선 방호효과

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전신 γ-선 조사전 *Grifola umbellata*(*Gu*) 추출물의 단일 투여가 생쥐의 생존율, 체중, 기관 무게 및 혈액세포에 미치는 효과를 조사하였다. γ-선 조사 24시간 전에 *Gu* 추출물을 복강 투여하였을시, γ-선을 조사한 생쥐의 40-day 생존율을 65.5%에서 78.6%로 증가시켰다. *Gu* 추출물의 투여는 γ-선 조사에 의한 비장과 흉선의 무게 감소를 완전히 막아주었다(P<0.05, P<0.01). 또한 이와 유사하나 다소 적은 방사선 방호효과가 *Gu* 투여군의 고환에서도 관찰되었다(P<0.05). *Gu* 추출물의 투여는 γ-선 조사시 나타나는 조사후 7일째까지의 백혈구와 림프구수의 현저한 감소를 지연시켰고, 회복속도를 촉진시켰다. 또한 21일째후의 적혈구수의 회복을 촉진시켰다. 이와 같은 결과에서 *Gu* 추출물의 방사선 방호효과를 알 수 있다.

I. Introduction

Grifola umbellata, one of edible fungi belongs to *Polyporaceae* of *Basidiomycetes* and is termed officially as *Polyporus umbellatus* in the other scientific name[1, 2]. The fruiting bodies of *Grifola umbellata* have been used as Chinese medicines for curing human diseases such as inflammation of liver for a long time[2]. Nowadays, the substances of polysaccharide such as β-glucan which was isolated from fruiting bodies of *Grifola umbellata* have been reported to exhibit the outstanding anti-tumor effect for curing human diseases such as lung cancer, gastric cancer and cervical cancer[2, 4].

Ionizing radiation exerts the bulk of its toxic effects through the generation of oxygen-derived free radicals, in particular the hydroxyl radical[5, 6]. Such free radicals also occur during aerobic metabolism as a byproduct[7]. Therefore, the development of effective free radical scavenging compounds is important issue from the standpoint of radiobiology and gerontology.

A number of studies on the radioprotective compounds such as thiol complex, interleukin-1, tumor necrosis factor and granulocyte colony-stimulating factor[8~11]. However, they are not actually useful because of their additional toxic effect[10, 12]. Recently, study on the protective effects of natural products such as herbs against irradiation has become of general interest[13, 14].

Since no informations are available regarding the effect of *Grifola umbellata* extract on mice exposed to γ-irradiation we attempted to look at the effect of *Grifola umbellata* extract on the survival ratio, organ weight and blood cell counts of mice after whole-body γ-irradiation.

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Ⅱ. Materials and Methods

1. Chemicals

All chemicals used were of analytical grade and purchased from Sigma Chemical Co.(St. Louis, Mo., U.S.A.).

2. Extraction of sclerotium of *Grifola umbellata*

The sclerotium of *Grifola umbellata* was obtained from Kyongdong Chinese medicine market. This was cut into small pieces with blender and soaked in 80% aqueous methanol for 48 hrs at 4℃. The residues were extracted with water at 95℃ for 6 hrs. This procedure was repeated five times. The filtrate was concentrated and dialyzed. After centrifugation at 10,000 x g for 10 min precipitate was lyophilized.

3. Treatment of Animals

ICR mice, 2-weeks old male, were obtained from Green Cross Co. Ltd(Korea). They were housed in cages in a room at 25±2℃ and a humidity of 60~70%. They were maintained on a 12 hr-light/12 hr-dark cycle, with the light phase begining at 09:00 a.m. They had a free access to a commercial mouse diet and water until they reached approx. 28 g body weight. The mice were then divided into three groups: group 1 received 0.5 ml saline solution(0.9% NaCl) only; group 2 received 0.5 ml saline solution at 24 hr before whole-body irradiation with a ^{60}Co γ-ray source to 5.0 Gy in a homogeneous field of rays (dose rate : 2.5 Gy/min); group 3 received 0.5 ml saline solutions containing *Grifola umbellata* extract(100 mg/kg body weight) at 24 hr before irradiation under same condition to group 2. Animals were killed by decapitation and the blood was drawn into plastic tube and immediately analyzed.

4. Determination of Blood Cell Counts

The blood cell counts automatically analyzed at Jeil Hospital in Seoul.

5. Statistical Analysis

Analysis of variance was conducted according to Snedecor and Cochran[15] and treatment differences were subjected to the Student's Newman-Keuls multiple range test as outlined by Kirk[16].

Ⅲ. Results and Discussion

The effect of the single administration of *Grifola umbellata*(*Gu*) extract prior to γ-irradiation on body weight change in mice was shown in Fig. 1. The growth of 3 weeks old male mice in the irradiated group was slightly retarded as compared to those of control mice and mice treated with *Gu* extract prior to γ-irradiation.

The effect of *Gu* extract on 40-day survival ratio of mice after irradiation is shown in Table 1. The single pre-administration of *Gu* extract increased the 40-day survival ratio of irradiated mice from 65.5% to 78.6%.

The average spleen and thymus weights of the irradiated mice were lower than those of control mice ($P<0.05$). The decreases of both organ weights in irradiated mice may be due to the marked reduction of lymphocytes which are mainly produced in these organs[17]. In contrast the spleen and thymus weights of the *Gu* extract treated mice were almost the same as thoseof control mice. The result indicates that *Gu* extract may accelerate regeneration of both organs. The weight of testis in the irradiated mice was significantly reduced ($P<0.01$) as compared to that of control mice. However, the weight reduction of testis in the *Gu* extract treated mice was relatively slight as compared to that in the irradiated mice. This indicates that *Gu* extract exerted

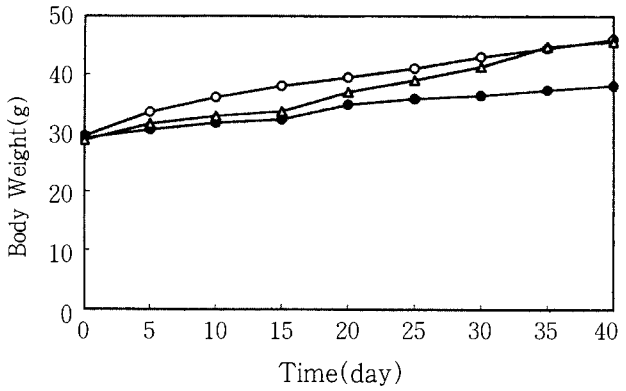

Fig. 1. Effect of the intraperitoneal administration of *Grifola umbellata* extract prior to γ-irradiation on body weight change in mice.
Administration of saline and *Grifola umbellata* extract and γ-irradiation were described in Materials and Methods.
○ : control group, ● : irradiated group, △ : *Grifola umbellata* extract treated group before γ-irradiation.

Table 1. Effect of *Grifola umbellata* extract on 40-day survival ratio of mice after irradiation.

Group	Number of survived mice	40-day survival ratio(%)	significance against control
Control	10 of 10	100	
Rad	19 of 29	65.5	P<0.001
Rad + *Gu*	22 of 28	78.6	P<0.01

Rad, irradiated group ; Rad + *Gu*, *Grifola umbellata* treated group before γ -irradiation.

Table 2. Effect of *Grifola umbellata* extract on organ weight of mice after γ-irradiation*.

Tissues	Control	Rad	Rad + *Gu*
		g	
Heart	0.18 ± 0.02^{a}	0.17 ± 0.02^{a}	0.17 ± 0.02^{a}
Kidney	0.70 ± 0.03^{a}	0.64 ± 0.04^{a}	0.61 ± 0.03^{a}
Liver	1.61 ± 0.16^{a}	1.55 ± 0.17^{a}	1.60 ± 0.18^{a}
Spleen	0.12 ± 0.01^{a}	0.09 ± 0.01^{b}	0.13 ± 0.01^{a}
Testis	0.35 ± 0.02^{A}	0.09 ± 0.01^{C}	0.15 ± 0.01^{B}
Thymus	0.06 ± 0.01^{a}	0.03 ± 0.00^{b}	0.05 ± 0.01^{a}

*Values are means ± S.D. for 10 animals sacrificed on day 40th.
Means not sharing a common superscript letter within a row are significantly different ($^{A,B,C}P<0.01$ or $^{a,b,c}P<0.05$).
Rad, irradiated group ; Rad + *Gu*, *Grifola umbellata* treated group before γ-irradiation.

relatively a little protective action on testis as compared that on the spleen and thymus. A similar result was also made with *Cordyceps sisnensis* extract[18]. Unlike these organs there were no significant differences in the weights of heart, kidney and liver among three groups(Table 2). Since the spleen, thymus and testis have been well known as radiosensitive organs[19], the protective action of *Gu* extract on irradiated mice may be responsible for its enhancing recovery of these organs.

As shown in Fig. 2 the leukocyte counts in the irradiated group were dramatically decreased during the first 7 days and gradually recovered thereafter whereas those in the *Gu* extract treated group were not decreased as rapidly as those in irradiated group(P <0.01). The recovery tendency of leukocyte counts in extract treated group was similar to that in the *irradiated* group. Fig. 3 shows similar reduction tendency of

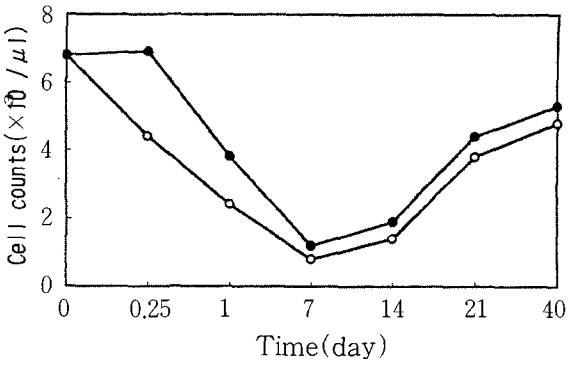

Fig. 2. Effect of the intraperitoneal administration of *Grifola umbellata* extract prior to γ-irradiation on the leukocyte counts of mice.
○ ; irradiated group, ● ; *Grifola umbellata* extract treated group before γ-irradiation.

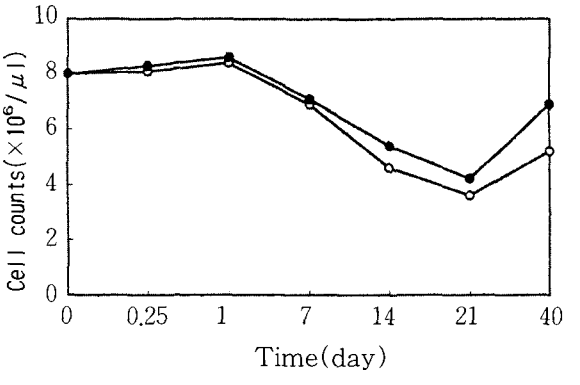

Fig. 3. Effect of the intraperitoneal administration of *Grifola umbellata* extract prior to γ-irradiation on the erythrocytes counts of mice.
○ ; irradiated group, ● ; *Grifola umbellata* extract treated group before γ-irradiation.

erythrocytes counts in both groups but more rapid recovery of cell counts in extract treated group. As shown in Fig. 4 the lymphocyte counts in the irradiated group were significantly decreased during the first 7 days and gradually recovered thereafter. However, those in the extract treated group did not fall as low as those in the irradiated group and recovered more rapidly. The results indicate that the recovery of both erythrocyte and lymphocyte counts after irradiation was clearly accelerated by the extract. The similar report was also made with the ginseng extract[20]. Overall results indicate that *Gu* extract by unknown manner accelerates the regeneration of the hemopoietic tissues but does not prevent the cellualr destruction.

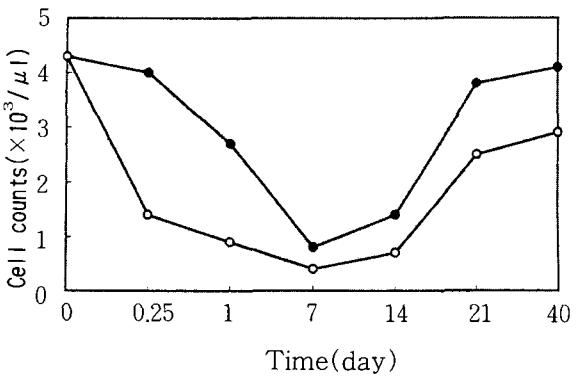

Fig. 4. Effect of the intraperitoneal administration of *Grifola umbellata* extract prior to γ-irradiation the lymphocyte counts of mice.
○ : irradiated group, ● : *Grifola umbellata* extract treated group before γ-irradiation.

Although the exact mechanism in protective effect of *Gu* extract on irradiated mice is not clear yet, the present study is the first report regarding the *Gu* which was tested and found to be a potential radioprotective agent. Further investigation with fractionation and subsequent examination of *Gu* extract is required for elucidating major active component of *Gu* in its radioprotective action.

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