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<원저>

Radioprotective Effects of Aronia on Radiation Irradiated Rats

- 방사선에 조사된 쥐에서 아로니아의 방사선 방호효과 -

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— Abstract —

The present study was intended to orally administer aronia to rats, irradiate radiation once to the whole bodies of the rats, and conduct blood tests to observe, compare, and analyze changes in blood cells, such as leukocytes, erythrocytes, and platelets, in order to examine the radioprotective effects of aronia.

As experimental animals, 15 male Sprague-Dawley (SD) rats aged six weeks weighing 200~250 g were taken and divided into the normal group (A) of five rats, the 5 Gy control group (B) of five rats, and the 5 Gy experimental group (C) of five rats. The normal group (A) was not~irradiated at all, the control group (B) was administered with general diets and irradiated, and the experimental group(C) was orally administered with 50 mg/kg/day of aronia two times per day to achieve a distilled water oral dose of 100 mg/kg/day and irradiated thereafter (5 Gy at 500 cGy/min) for 14 days. After the experiment, differences in leukocytes, erythrocytes, and platelets among the normal group (A), the control group (B), and the experimental group (C) were examined by comparing the counts of the blood cells and the results showed no statistically significant differences. However, on a detailed review, the normal group (A) showed statistically higher mean values for all of lymphocytes, hemoglobin, and mean corpuscular hemoglobin as compared to the control group (B) and the experimental group (C). Statistically significant differences in the counts of lymphocytes were shown between the normal group (A) and the control group (B), and between the normal group (A) and the experimental group (C); furthermore, statistically significant differences in mean corpuscular hemoglobin were shown between the normal group (A) and the experimental group (C).

Given the results of the present study, in irradiated rats, aronia was generally considered as having no radioprotective effect on leukocyte, erythrocyte, and platelet while having statistically significant radioprotective effects on lymphocytes, hemoglobin, and mean corpuscular hemoglobin. Based on the present experiment, diverse studies should be conducted hereafter.

Key Words: Aronia, Leukocyte, Erythrocyte, Platelet, Radioprotective

Ⅰ. INTRODUCTION

Following the rapid development of modern medicine, the use of radiation and radioactive isotopes can be easily found in even daily life due to the increases of their industrial use, medical use, and use in nuclear facilities[1]. When exposed to radiation, humans may develop gastrointestinal disorders and/or central nervous

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system disorders that, in worse cases, may even lead to death, mutation, and/or cancers. Therefore, as the use of radiation increases as such, the importance of the minimization of the exposure of living things and human bodies to radiation and protective effects has been increasing day by day. Disorders that may be caused by radiation include central nervous system disorders (100~300 Gy), gastrointestinal disorders (10~30 Gy), bone marrow lesions (4~8 Gy), and low-dose disorders (1~2 Gy or less) and each disorder shows acute, delayed, or genetic effects[2].

Signs or symptoms appearing when cell DNA had been cut due to the exposure to radiation and became extinct instead of being recovered so that proliferation was stopped are called radiation syndromes and, depending on the time of expression of the signs or symptoms, the resultant disorders are divided into acute disorders and chronic disorders depending[3].

Acute disorders refer to the disorders of which the signs or symptoms appear within a short time when exposed to relatively large doses in a short period of time; chronic disorders refer to those disorders of which the signs or symptoms are expressed when long periods of time have passed after exposure to radiation. Chronic disorders can induce malignant tumors including leukemia, shorten life spans, generate cancers, and show local impacts on tissues, such as sterility, and remain unrecoverable[3,4].

Agents that alleviate the actions of radiation are called radiation protective agents[5]. Radiation protective agents are thought to be utilizable in increasing survival rates and controlling carcinogenesis.

Aronia melanocarpa that is also called black choke-berry is the fruit of a plant that falls under berries that belong to Rosaceae and originally grows wild in the North America region[6,7]. Aronia contains the component anthocyanin up to 7.2~8 g per kg which is relatively more as compared to other berry plants [8]. In addition, the components anthocyanin and flavonoids contained in aronia show anti-oxidative activity that removes active oxygen formed in plant cells, act against allergic diseases, help blood circulation, suppress cardiovascular disorders, protect the gastric mucosa, and show anti-inflammatory effects, anti-diabetic effects, immune regulatory function, efficacy against hypertension, and effects to suppress eyesight deterioration and aging[8].

According to the results of a clinical study in which 300 mg of aronia were administered every day to 26 adult males and females, aronia-derived anthocyanin had an effect of suppressing the oxidative stress caused by reactive oxygen species (ROS) that are a sort of free radicals[8].

The purpose of the present study was to orally administer aronia to rats, irradiate radiation once to the whole bodies of the rats, and conduct blood tests to observe, compare, and analyze changes in blood cells such as leukocytes, erythrocytes, and platelets in order to examine the radioprotective effects of aronia.

II. MATERIALS AND METHODS

1. Experimental animals

A total of 15 male Sprague-Dawley (SD) Rats aged 6 weeks weighing 200~250 g were bought from S Co Ltd (Osan, Gyeonggi-do). The temperature and humidity of the breeding room were maintained at 23±2°d and 50~60%, respectively. The stainless wire covered plastic cages were subjected to 12 hour light-darkness cycles shifting at 09:00 and 21:00. Each group of rats consisting of five rats was bred in each cage; feed and water were freely supplied. The experiment was conducted when the rats were healthy after passing an adaptation period of seven days immediately before the experiment and the animal laboratory of the Department of Food Engineering of Chonnam University was used as a breeding room.

2. Sample

Freeze-dried aronia powder (150g/pack) was purchased from Cheonjigayakcho Company Limited. The purchased powder was diluted in 1L of distilled water before use.

3. Experimental equipment

An automatic blood analyzer for animal only (Automatic hema Analyzer for multispecies Hemavet 950), a distilled water maker (MILLIPORE Milli-Q), blood collecting tubes (EDTA Tube), a Linac Accelerator 6 MegaVoltage (MV) X-ray therapeutic apparatus (CLINAC 21 IX model, VARIAN IX USA, 2007), and oral administration Zondes for rats were used.

4. Irradiation

Each of the rats was irradiated one time on its whole body using a Linac 6 MegaVoltage (MV) X-ray therapeutic apparatus (CLINAC 21 IX model, VARIAN IX USA, 2007). The radiation fields were equally set to 25 cm×25 cm, the Source-to-Surface Distance (SSD) was set to 100 cm, and the dose rate was set to 500 cGy/min so that 5 Gy was irradiated on the whole body.

5. Experimental method

The experimental animals were divided into the normal group (A) of five rats, the 5 Gy control group (B) of five rats, and the 5 Gy experimental group (C) of five rats. The normal group (A) was not irradiated at all, the control group (B) was administered with general diets and irradiated, and the experimental group (C) was orally administered with 50 mg/kg/day of aronia two times per day to achieve a distilled water oral dose of 100 mg/kg/day using zondes for rats for 14 days before irradiation.

The rats were ether-anesthetized for blood collection 24 hours after irradiation. The rats' abdomens were cut open to collect blood from the posterior vena cava vein and then the rats were sacrificed by cervical vertebral dislocation and used as analysis samples see Table 1.

6. Blood test

For accurate blood tests, blood was collected using blood collecting tubes (EDTA tube) and the blood samples were analyzed using an automatic blood analyzer for animals only (Automatic hema Analyzer for multispecies Hemavet 950).

7. Statistical processing

The data obtained through the above experimental methods were analyzed using SPSS version 23.0 program. The normality of the data was tested first as the number of the given data was small. Since the data did not satisfy the normality based on the test of normality, the data were analyzed using the Kruskal-Wallis test method which is a non-parametric method and Mann-Whitney tests were used as ex post facto tests (significance level α=0.05).

III. RESULTS

1. Comparison of leukocyte, erythrocyte, and platelet among the groups

The counts of leukocytes, erythrocytes, and platelets of the normal group, the control group, and the experimental group were compared with each other and the results are shown in Table 2. It can be seen that there was no statistically significant difference in leukocyte, erythrocyte, and platelets. On reviewing in detail, it can be seen that the mean count of leukocyte

〈Table 1〉 Experimental method

Division	Normal group (A)	Control group (B)	Experimental group (C)
Treatment	General diet	General diet+ Irradiation (5 Gy)	Aronia (100 mg/kg/day) + irradiation(5 Gy)
Rats (number)	5	5	5

Group A: Normal + General diet
Group B: Control + General diet + 5 Gy irradiation
Group C: Experimental + oral administration of aronia (100 mg/kg/day) for 14 day + 5 Gy irradiation

〈Table 2〉 Comparison of the counts of leukocytes, erythrocytes, and platelets among the groups

(Leukocyte, platelet Unit: K/㎕, erythrocyte Unit: M/㎕)

Variable	Group	Mean	Standard deviation	*p*-value
Leukocyte	Normal group	2.3600	0.4492	0.289
	Control group	1.6000	0.6229	
	Experimental group	2.0160	1.7254	
Erythrocyte	Normal group	9.3020	0.4196	0.796
	Control group	9.1440	0.8251	
	Experimental group	9.5340	0.8725	
Platelet	Normal group	854.8000	118.4555	0.281
	Control group	961.0000	58.7878	
	Experimental group	872.6000	129.7027	

Group A: Normal + General diet
Group B: Control + General diet + 5 Gy irradiation
Group C: Experimental + oral administration of aronia(100 mg/kg/day) for 14 days + 5 Gy irradiation *(significance level α=0.05)

in the normal group was similar to that of the experimental group and that of the control group was lower compared to other groups. The mean count of erythrocyte in the experimental group was shown to be higher compared to other groups and the mean count of platelets in the control group was shown to be higher compared to other groups.

2. Comparison of the sub-elements of leukocyte, erythrocyte, and platelet among the groups

The sub-elements of leukocytes, erythrocytes, and platelets were compared among the normal group, the control group, and the experimental group to see the differences and the results are shown in Table 3. It can be seen that there were statistically significant differences in lymphocytes, hemoglobin, and mean corpuscular hemoglobin. On a detailed review, it could be seen that the normal group showed higher mean values for all of lymphocytes, hemoglobin, and mean corpuscular hemoglobin as compared to other groups. Statistically significant differences in the counts of lymphocytes were shown between the normal group and the control group, and between the normal group and the experimental group, while statistically significant differences in mean corpuscular hemoglobin were observed between the normal group and the experimental group. On reviewing other elements, it could be seen that the mean counts of neutrophilic leukocytes, monocytes among leukocytes, eosinophilic leukocytes, and basophilic leukocytes in the normal group were lower as compared to other groups.

3. Five elements that constitute leukocyte

Table 4 shows the means of ratios occupied by five elements (Neu, Lym, Mono, Eos, Baso) that constitute WBC (leukocyte). On a general review, it could be seen that lymphocytes accounted for the largest part in the normal group (A), while neutrophil leukocytes accounted for the largest part in the control group (B) and the experimental group (C). On a detailed review, it could be seen that in the normal group (A), lymphocytes accounted for the largest part of blood cells reaching 85.44%, followed by neutrophilic leukocytes reaching 10.76%. In the control group (B), neutrophilic leukocytes accounted for the largest part of blood cells reaching 69.90%, followed by lymphocytes reaching 20.25%. In the experimental group (C), neutrophilic leukocytes accounted for the largest part of blood cells reaching 49.27% followed by lymphocytes reaching 35.39%.

〈Table 3〉 Comparison of sub-elements of leukocytes, erythrocytes, and platelets among the groups

(Leukocyte, platelet Unit: K/㎕, erythrocyte Unit: M/㎕)

Variable		Group	Mean	Standard deviation	p-value
WBC	Neu	A	0.2500	0.1072	0.084
		B	1.1440	0.5593	
		C	1.1020	0.9689	
	Lym	A	2.1060	0.3681	0.007**
		B	0.3060	0.0780	
		C	0.5520	0.3834	
	Mono	A	0.0780	0.0217	0.165
		B	0.1380	0.0415	
		C	0.2340	0.2173	
	Eos	A	0.0140	0.0055	0.569
		B	0.0080	0.0084	
		C	0.0900	0.1233	
	Baso	A	0.0020	0.0045	0.266
		B	0.0000	0.0084	
		C	0.0380	0.1233	
RBC	HGB	A	17.9800	0.5404	0.007**
		B	15.9600	0.5941	
		C	16.2600	0.6693	
	HCT	A	46.9000	2.4980	0.564
		B	45.7200	3.9921	
		C	47.9600	3.2090	
	MCV	A	50.4200	1.5482	0.885
		B	50.0600	2.0501	
		C	50.4000	1.5083	
	MCH	A	19.3600	0.6950	0.044*
		B	17.5400	1.4571	
		C	17.1400	1.2462	
	MCHC	A	38.4000	1.9812	0.083
		B	98.6000	142.2368	
		C	34.0000	1.6763	
	RDW	A	22.3200	1.4184	0.588
		B	21.0200	2.0632	
		C	22.6200	2.3605	
PLT	MPV	A	6.2600	0.6066	0.167
		B	16.7000	22.5311	
		C	6.1600	0.3209	

* $p<0.05$, ** $p<0.01$

Group A: Normal + General diet

Group B: Control + General diet + 5 Gy irradiation

Group C: Experimental + oral administration of aronia (100 mg/kg/day) for 14 days + 5 Gy irradiation

*Mann-Whitney

〈Table 4〉 The ratios of individual elements that constitute leukocyte in individual groups

(Units:%)

List	A	B	C
Neu	10.76	69.90	49.27
Lym	85.44	20.25	35.39
Mono	3.19	9.39	11.62
Eos	0.55	0.42	2.57
Baso	0.06	0.03	1.16
Total	100.00	100.00	100.00

IV. DISCUSSION

To examine the radioprotective effects of aronia in irradiated rats, a total of 15 rats irradiated with 5 Gy radiation on the whole bodies using a Linac 6 MegaVoltage (MV) X-ray therapeutic apparatus (CLINAC 21 IX model) were divided into the normal group (A), the control group (B), and the experimental group (C) consisting of five rats each and blood tests were conducted. To review cell susceptibility according to Bergonie-Tribondeau's law, susceptibility to radiation was higher among cells with larger neogenesis abilities, those with longer processes of cell division, those with lower degrees of morphological and functional differentiation, and those that are younger and weaker. Accordingly, reproductive tissues and hematocrit tissues can be regarded to be the most susceptible to radiation [9]. Although leukocyte values were close to normal values in a previous study [10], in the present experiment, lymphocyte values could be identified as being similar to normal values through comparison. In addition, unlike the present experiment, in a previous experiment that examined the radioprotective effects of red ginseng in mice [11], remarkable decreases in leukocyte and blood cell values could be observed.

The present experiment was conducted to examine the radioprotective effects of aronia. No previous study was available in South Korea. Aronia is also called back chokeberry and the experiment was conducted to verify if aronia can be used as a radioprotective substance. Radioprotective substances are substances that reduce radiation sensitivity and protective synthesis includes the sulfhydryl (SH) group, such as cysteine and cysteamine[12].

The paper entitled "Protective effects of chitosanoligosaccharides against gamma irradiation in mice" reported that blood cells were quickly recovered in the pretreatment group, which was supplied with chitosan before gamma irradiation[13].

These results are consistent with those the results of the present study. The paper entitled "The biological effects of low dose X-ray irradiation on the sperms of rats" reported that the results of blood tests showed no significant difference[14].

This study conducted an experiment to see "whether red ginseng extracts can be used as a radioprotective substance" and reported that red ginseng extracts had components with antioxidative activity[15].

We expected that aronia would be usable as a radioprotective substance. The results of the present experiment Table 2 did not show any statistically significant differences in leukocytes, erythrocytes, or platelets. However, these results are not considered sufficient for the conclusion that aronia has no radioprotective effect, because the normal group might have included some rats with poor health conditions.

When the results of the present experiment were examined in detail to compare and analyze the leukocytes in the control group and the experimental group. Based on the results, the mean values of the control group were lower than those of other groups. Therefore, a recovery of the number of leukocytes in the experimental group administered with aronia could be identified. Given this result, aronia is considered to have radioprotective effects. The mean corpuscular hemoglobin concentration (MCHC) of the

control group was 98.6 g/dL and that of the experimental group was much different at 34.0 g/dL. The control group was irradiated and its MCHC value was more than double of the value of the normal group, which was 38.0 g/dL. Given the present experiment, the group administered with aronia showed protective effects in terms of prevention. In the results, the control group showed a higher count of platelets compared to the experimental group. This can be attributed to irradiation.

The leukocyte experimental results shown in Table 3 indicate that the leukocyte count of the control group was higher than that of the experimental group thereby demonstrating that leukocytes increased in the control group, which was irradiated. Therefore, the irradiation at the dose of 5 Gy to rats is considered to have led to the increase in leukocytes. The mean of platelets of the control group was shown to be higher as compared to other groups. To review in detail, the mean of lymphocyte among leukocytes decreased in the control group. However, the mean of lymphocyte of the experimental group was lower compared to the normal group and the difference was statistically significant ($P<0.007$).

The fact that the lymphocyte count of the experimental group was not high is considered to indicate the radioprotective effects of aronia.

The content of hemoglobin in erythrocytes in the experimental group was higher as compared to the control group and the difference was statistically significant ($p<0.007$). Hemoglobin plays the role of transporting oxygen in vivo. The fact that the content of hemoglobin in the experimental group was higher than that in the control group is considered to indicate the radioprotective effects of aronia.

A paper reported that when "the degree of recovery of blood cell components after administration of ethanol extract of wilfordii roots following irradiation" was observed, it could be seen that the number of leukocytes decreased. The paper also reported that when one day passed after irradiation, platelets were recovered in the group irradiated at a dose of 7 Gy after the administration of wilfordi root extract. When 1, 4, 7 days passed after irradiation, no significant differences in the reduction of leukocyte counts among the groups were observed. However, when 21 days passed after irradiation, the speed of recovery of leukocytes was shown to be higher in the group irradiated at a dose of 7 Gy after the administration of wilfordii root extract compared to the control group just irradiated at a dose of 7 Gy[16]. These results were the same as the results of the present experiment.

A previous study entitled "Radiation defense mechanism of platelets after administration of alliin" reported that when the platelets of rats whole-body irradiated at a dose of 5 Gy after alliin injection were observed, the shapes of the platelets were found to be the same as those of the normal control group[17]. In the present experiment too, the content of platelets of the group administered with aronia was shown to be lower as compared to the control group. This is considered to indicate the effects of aronia as a radioprotective substance.

In the present experiment, several limitations were derived. First, since the number of rats per the experimental group was too small, accurate and reliable data could not be easily produced. Second, the rats were under severe stress before being irradiated and this seemed to have resulted in poor results and the same stress had to be imposed on individual rats. Third, the experimental period had to be divided into three and the rats had to be irradiated on day 7, 14, and 30 instead of setting the experimental period as 14 days. In future studies, the number of rats per experimental group should be increased, the same stress should be imposed on the animals before irradiation, and the experimental period should be longer.

V. CONCLUSION

Given the results of the present study, in irradiated rats, aronia was generally considered as having no radioprotective effect on leukocytes, erythrocytes, and platelets while having statistically significant

radioprotective effects on lymphocytes, hemoglobin, and mean corpuscular hemoglobin. Based on this experiment, diverse studies should be conducted hereafter.

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•국문초록

방사선에 조사된 쥐에서 아로니아의 방사선 방호효과

문환식·이준행

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본 연구에서는 아로니아를 Rat에 경구 투여 후 방사선을 1회 전신 조사한 다음 혈액검사를 실시해 백혈구, 적혈구, 혈소판 등의 혈구세포의 변화 상태를 관찰하고 비교 분석하여 아로니아의 방사선 방어효과를 알아보고자 하였다.

실험동물은 SD계 Rat 6주령 200~250g 수컷 15마리를 이용하여 5마리를 하나의 군으로 정상군 (A), 5 Gy 대조군(B), 5 Gy 실험군(C)으로 설정하였고, 정상군(A)은 방사선 조사를 실시하지 않고, 대조군 (B)은 일반식이를 투여하여 방사선조사를 하였고, 실험군(C)은 방사선조사 전 14일 동안 아로니아, 증류수 경구투여량(100 mg/kg/day)로 1일 2회씩 50 mg/kg/day로 각각 쥐에 14일 동안 경구 투여 후 방사선 조사(500 cGy/min로 5 Gy)를 하였다. 그 결과 정상군(A), 대조군(B), 실험군(C)에 따른 백혈구, 적혈구, 혈소판 차이를 비교해 본 결과 통계적으로 유의한 차이가 없었지만, 세부적으로 살펴보면, 림프구, 혈색소, 평균적혈구색소량에서 모두 정상군(A)이 대조군(B), 실험군(C)에 비해서 평균수치가 높게 나타나 통계적으로 유의한 차이가 있음을 알 수 있었다. 림프구에서는 정상군(A)과 대조군(B), 정상군(A)과 실험군(C)간에 통계적으로 유의한 차이가 보였으며, 평균적혈구색소량에서는 정상군(A)과 실험군(C)에서 통계적으로 유의한 차이를 보였다.

본 연구의 결과로 보아 방사선에 조사된 쥐에서 아로니아의 방사선방호효과가 전체적으로 백혈구, 적혈구, 혈소판 등은 없는 것으로 사료되었고, 부분적으로는 림프구, 혈색소, 평균적혈구혈색소량은 통계적 유의성이 있는 것으로 알 수 있었다. 이 실험을 바탕으로 향후 다양한 연구가 선행되어야 할 것으로 사료된다.

중심 단어: 아로니아, 백혈구, 적혈구, 혈소판, 방사선방호