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Radio-protective Effects of the Extracts of Various Medicinal Plants against Human Normal Lung Cells

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Abstract – *Rhapontica uniflora, Platycodon grandiflorum* and *Solanum tuberosum* extracts affected the recovery of radiation-induced damage at all tested doses (15.6, 31.3, 62.5 and 125 μg/ml). The survival rate of pre-treatment with extracts was increased by 2 times more than its compared with untreated cells. All tested extracts protected the growh-delay of normal cells and haematological parameters from their radiation-induced fall. In tested extracts, *Solanum tuberosum* was significantly superior to *Platycodon grandiflorum* and *Rhapontica uniflora* in radio-protection activity.
Keywords – *Rhapontica uniflora, Platycodon grandiflorum, Solanum tuberosum*, radio-protection

Introduction

Of the several chemicals considered for radio-protection, WR-2721 [S-2-(3-aminopropylamino)phosphorothioic acid], a derivative of cysteamine, has been found to be the most effective in protecting mammals (Ganasoundari, *et al.*, 1997; Uma Devi, *et al.*, 1980; Yuhas, *et al.*, 1980). Other synthetic compounds were MPG (2-mercaptopropionyglycine), zinc aspartate, cysteamine and aminothiol. Unfortunately, these compounds showed several side effects (Floersheim and Bieri, 1990). Among these compounds, difficulties were encountered when administering WR-2721 to humans, who experienced adverse toxic effects such as hypotension, nausea and allergy when exposed to the drug (Cairnie, 1983; Turrisi, *et al.*, 1983).

We carried out the screening for radio-protector that have low toxicity form plant materials. In this paper, we report the reduction of the damage against normal human lung fibroblast, and the haematological change in mice.

Materials and Methods

Sample extraction and treatment – *Rhapontica uniflora, Platycodon grandiflorum*, and *Solanum tuberosum* were used as radio-protection materials. Aqueous extract was prepared by refluxing the dried material in 70% methanol and lyophilized.

In SRB assay, All compounds were prepared wih the final dose of 15.6, 31.3, 62.5, and 125 μg/ml. For blood count, all compounds were prepared with the final dose of 150 mg/kg, and injected intraperitoneally. Groups of mice were irradiated by 6 Gy.

Isolation of fibroblast cells from human lung – Normal lung tissue specimens was isolated from patients with advanced lung cancer treated in the Korea Cancer Center Hospital. After resection, a representative portion of the normal specimen was obtained and transported in PBS containing penicillin-streptomycin (200 U/ml).

Enzymatic disaggregation of the biopsied lung tissue was occupied with collagenase type II. When tissue was disaggregated, removed collagenase by centrifugation, and then seeded cells at a high concentration. After incubation at 37°C for 48 hours, attachment fibroblast cells were isolated by trypsin-EDTA. Collected fibroblast cells were maintained by RPMI 1640 containing 10% FBS.

***In vitro* radio-protection assay** – For radio-protection, SRB assay was used (Skehan, *et al.*, 1990). Cells were harvested by trypsinization, counted and plated in 4 well plates. After treatment of each plant extract for 48 hours, plates was irradiated by 6Gy (Cs137), and media were immediately removed and washed 3 times with PBS, and replaced with fresh medium. Culture medium was replaced by fresh medium every 3 days. Recovery time was 8 days. Results were expressed as relative percentages of absorbance

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as compared with untreated controls.

Blood counts – These were performed with non-tumor bearing Balb/c mice (male) (Floersheim, *et al.*, 1988). Blood samples were taken from the retro-orbital plexus and counted with a coulter counter. The blood count response was expressed as percentage of the normal count determined 1 day before irradiation. The absolute values for the blood counts in normal mice were as follows: thrombocytes, 800,000-1,100,000/m^3; erythrocytes, (7.0-7.4)×10^6/mm^3, leukocyte, 9,000-10,000/mm^3, haematocrit, 80-88%. The LD_{50} for γ-irradiated Balb/c mice was 8 Gy. The effect of radioprotectors on blood counts was assessed with a radiation dose of 6 Gy.

Results and Discussion

Radio-protection of human lung fibroblast cells by plant extracts – *In vitro* radio-protection effect was exhibited by survival rate. The radiation-induced damage was exhibited varying extent on established primary lung cells. The range of survival rate was 28-47% on primary lung cells. All tested extracts affected the recovery of radiation-induced damage at all tested doses. The effect was slightly increased dose-dependently. The survival rate of pre-treatment with extracts was increased by 2 times more than its compared with radiation alone.

All tested extracts alone had no effect on the growth as compared with untreated cells.

Haematological change – As can be seen in Fig. 1 (a), the three tested extracts reduced the radiation-induced fall of peripheral blood thrombocyte count, but to varying extent. On day 10, when the thrombocyte of the irradiated controls had fallen to a low of 15% compared with the initial values, the respective counts in the treated groups were 35% with *Solanum tuberosum*, 32% with *Platycodon grandiflorum*, and 25% with *Rhapontica uniflora*. Substantial differences from the irradiated controls were also at day 20, namely 69% with *Solanum tuberosum* compared with the control value of 32%, 58% with *Platycodon grandiflorum*, and 68% with *Rhapontica uniflora*.

Fig. 1(b) illustrates that haematocrit fell in the irradiated controls reaching a minimum value at day 10. In each group pre-treated with tested extracts, the complete protection in the haematocrit was exhibited.

In untreated irradiated group, erythrocyte counts at day 10 and day 25 had fallen to 62% and 68% of pre-irradiation values, while in the group treated with *Solanum tuberosum*, the respective values were 79% and 87%. The group of pre-treated with *Platycodon grandiflorum* and *Rhapontica uniflora* were not exhibited significant protection (Fig. 1(c)).

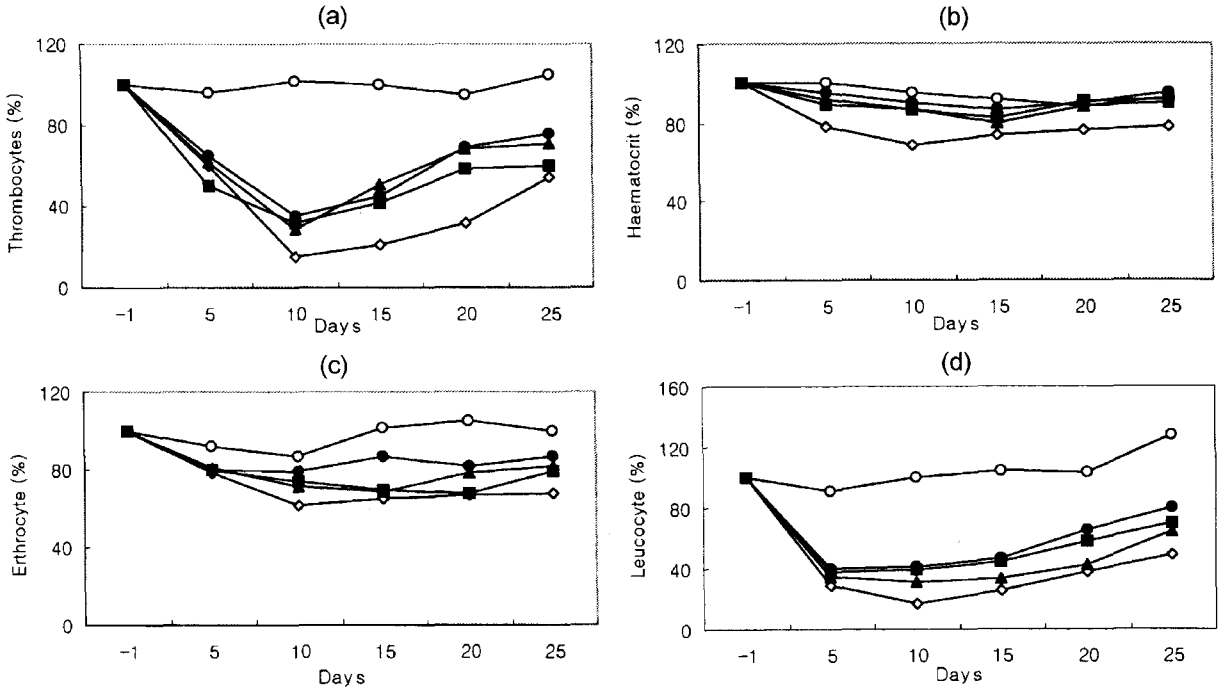

Fig. 1. Response of (a) the thrombocyte count, (b) the haematocrit, (c) the erythrocyte count and (d) the leucocyte count to irradiation and treatment with various plant extracts. The pre-irradiation values were taken as 100%. ○ normal mice; ◇ irradiated only; ▲ 150 mg/kg of *Rhapontica uniflora* and irradiated; ■ 150 mg/kg of *Platycodon grandiflorum* and irradiated; ● 150 mg/kg of *Solanum tuberosum* and irradiated.

Table 1. Radio-protective effects of various plant extracts on human normal lung fibroblast cells

Isolated lung cell No.	Extract	Radiation dose (Gy)	Dose (μg/ml)	Survival rate (%)
Fibroblast 309*	*Rhapontica uniflora*	6	0	38
		6	15.6	62
		6	31.3	58
		6	62.5	53
		6	125	55
	Platycodon grandiflorum	6	0	38
		6	15.6	54
		6	31.3	56
		6	62.5	59
		6	125	59
	Solanum tuberosum	6	0	38
		6	15.6	58
		6	31.3	60
		6	62.5	62
		6	125	65
Fibroblast 211	*Rhapontica uniflora*	6	0	28
		6	15.6	28
		6	31.3	32
		6	62.5	46
		6	125	55
	Platycodon grandiflorum	6	0	28
		6	15.6	29
		6	31.3	29
		6	62.5	36
		6	125	46
	Solanum tuberosum	6	0	28
		6	15.6	47
		6	31.3	55
		6	62.5	57
		6	125	59
Fibroblast 208	*Rhapontica uniflora*	6	0	47
		6	15.6	56
		6	31.3	57
		6	62.5	62
		6	125	63
	Platycodon grandiflorum	6	0	47
		6	15.6	45
		6	31.3	47
		6	62.5	51
		6	125	55
	Solanm tuberosum	6	0	47
		6	15.6	54
		6	31.3	57
		6	62.5	51
		6	125	55

*The number of established primary cells from patient's lung tissue.

A similar protection as for thrombocytes was seen for leucocyte (Fig. 1(d)). All tested extracts were exhibited radio-protection effect, but the leucocyte count was protected less markedly.

All tested extracts alone had no significant effect on blood counts as compared with normal group.

All tested extracts protected the growth-delay of normal cells and haematological parameters from their radiation-induced fall. In tested extracts, *Solanum tuberosum* was significantly superior to *Platycodon grandiflorum* and *Rhapontica uniflora* in radio-protection activity. The present finding that *Solanum tuberosum* is good radio-protector *in vitro* suggests that this extract may be promising agent for human radiation protection in radiotherapy. The further study will be carried the purification of active fraction.

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