

Antitumor Effects and Acute Oral Toxicity Studies of a Plant Extract Mixture Containing *Rhus verniciflua* and Some Other Herbs

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Abstract

A novel antitumor agent was developed from six kinds of herbs containing *Rhus verniciflua* (Rv-PEM01). The components were traditionally established for each formula for traditional medicine. The formula was designed to affect antitumor effect as well as maintain host immune functions. First, we investigated the antiproliferative activities of Rv-PEM01 on human and canine tumor cell lines *in vitro*, and on antitumor effects using BALB/cAJcl-nu/nu mice *in vivo*. Acute oral toxicity of Rv-PEM01 was also investigated *in vivo* in ddY mice. Rv-PEM01 exhibited antiproliferative activities against PC-3 (IC₅₀: 0.328 ± 0.081 mg/ml), A549 (IC₅₀: 0.520 ± 0.070 mg/ml), D-17 (IC₅₀: 0.124 ± 0.037 mg/ml) and MRC-5 (IC₅₀: 0.505 ± 0.058 mg/ml) cells. Luteolin 7-β-D-glucopyranoside and apigenin 7-β-D-glucopyranoside were identified as the main active compounds in Rv-PEM01 by HPLC analysis. The single dose toxicity study of Rv-PEM01 did not result in any deaths or abnormalities in daily behavior, body weight gain, or anatomical observations at necropsy. Thus, so we could not calculate the 50% lethal dose (LD₅₀) in mice, but it would be higher than 5.0 g/kg. Treatment with Rv-PEM01 at a dose of 2.5 g/kg tended to show antitumor activities on mice bearing Colon26 tumors compared with the control group. It was concluded that the formula was a safe

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antitumor agent with no side effects on mouse physiological function as judged by survival and organ weight.

Keywords

***Rhus verniciflua*, Antitumor, Antiproliferation, Acute Toxicity**

1. Introduction

Many phytochemicals from fruits, vegetables, and herbs which have antitumor activities may represent promising therapeutic and prophylactic treatment approaches against different types of cancers. The effects of phytochemicals on inhibition of tumor growth are well demonstrated both *in vitro* and *in vivo*. Many of these compounds, such as vinca alkaloids, have been reported to kill cancer cells [1].

The antitumor properties of medicinal herbs may be attributed to their antioxidant [2], anti-inflammatory [2], and immunomodulatory [3] properties. Many herbal extracts or their components also have been reported to induce apoptosis in cancer cells [4], so apoptosis-inducing activity of anticancer herbs may play an important role in tumor suppression.

The popularity of complementary and alternative medicine (CAM) is an international trend in cancer therapy. Due to the severe side effects and limited therapeutic efficacy of cancer chemotherapeutic agents used in conventional cancer treatment, CAM might improve clinical outcome and reduce adverse reactions to anticancer drugs. The World Health Organization has estimated that 80% of people worldwide are interested in traditional medicine [2]. Several Kampo (traditional Chinese or Japanese herbal) medicines, such as Keishi-ka-kei-to, Juzen-taiho-to, Shimotsu-to, Unsei-in, Hochu-ekki-to, Shosaiko-to and Shichimotsu-koka-to, have been reported to exhibit an antimetastatic effect, and among them, Keishi-ka-kei-to, Juzen-taiho-to, Shosaiko-to and Shichimotsu-koka-to also exert antiproliferative activity on cancer cell lines [5]. Tien-Hsien liquid (THL) is a Chinese herbal mixture consisting of extracts from 14 Chinese medicinal herbs that has been used as an anticancer dietary supplement for more than 20 years. THL is reported to have potent immunomodulatory effects [6] and antiproliferative activity [7].

We have been analyzing the antitumor activity of plant extracts, as well as their ability to maintain host immune capacity. *Rhus verniciflua* (*R. verniciflua*) is commonly known as the lacquer tree. The sap of this tree, which is collected by scratching the bark of the tree, has been used as a natural coating substance for wood carvings for several thousand years. In Korea, the bark, branch and stem of the tree are eaten with chicken and duck soups [8]. Urushiols, the major compounds of lacquer tree sap, have antioxidant and cytotoxic effects [9]. We recently reported that two novel urushiol derivatives, 1,2-dihydroxyphenyl-3-pentadeca-7'(E),9'(Z),11'(Z)-trien-14'-ol and 1,2-dihydroxyphenyl-3-pentadeca-8'(Z),10'(E),12'(E)-trien-14'-ol, isolated from the extract of leaves of *R. verniciflua* for the first time, showed inhibition of HIV-1 reverse transcriptase (RT) [10]. In our previous papers, we reported the anti-cell-proliferative activities of a plant extract mixture from six kinds of herbs containing *R. verniciflua* (Rv-PEM01) *in vitro*. Rv-PEM01 had an inhibitory effect on the proliferation of both human and mouse tumor cell lines, and we speculated that it might induce to apoptosis against these tumor cell lines [11]. However, toxicity studies and antitumor effects *in vivo* of Rv-PEM01 have not been investigated so far. In this paper, we report the antiproliferative activities of Rv-PEM01 on human and canine tumor cell lines *in vitro*, acute oral toxicity study using ddY mice, and antitumor effects using BALB/cAJcl-nu/nu mice *in vivo*.

2. Materials and Methods

2.1. Preparation of Rv-PEM01

Rv-PEM01 was prepared using method of Hiruma W., *et al.* [11]; the six herbs for preparation of Rv-PEM01 are shown in Table 1. The leaves of *R. verniciflua* were collected from Guizhou, China, in June 2002, and identified by Prof. Zhu Shougian, College of Forestry of Guizhou University. The *Ulmus hollandica* (*U. hollandica*) were collected from Amsterdam, The Netherlands. The *Polygonatum sibiricum*, *Lycium chinense*, *Ganoderma japonicum* and *Panax ginseng* were purchased from Oofuna-Kanpodo Pharmacy (Kanagawa, Japan). Each of the six herbs was ground and mixed to one powder (total volume is 230 g), which was extracted with 10 vol of 70%

Table 1. Extraction of Rv-PEM01 from six herbs [11].

Herbs	Amount of herbs used for extraction (g)
<i>Rhus verniciflua</i>	90
<i>Ulmus hollandica</i>	60
<i>Polygonatum sibiricum</i>	50
<i>Lycium chinense</i>	10
<i>Ganoderma japonicum</i>	10
<i>Panax ginseng</i>	10
Total	230

The extract of Rv-PEM01 was prepared as follows. Each of the six herbs was ground and mixed to one powder (total volume is 230 g), which was extracted with 10 vol of 70% ethanol in water at room temperature. The extracted solutions were filtered, and the solvents were evaporated, filtered and lyophilized, yielding 60 g of lyophilized Rv-PEM01 from 230 g of herb powder.

ethanol in water at room temperature. The extracted solution was filtered and the solvent evaporated, after which the extract was lyophilized, yielding approximately 60 g of lyophilized Rv-PEM01. Urushiols were not detected in Rv-PEM01 by HPLC analysis (Figure 1).

2.2. Cell Culture and Treatment

Human prostate adenocarcinoma cell line PC-3, human lung adenocarcinoma cell line A549, and human normal lung fibroblast cell line MRC-5 were obtained from Health Science Research Resources Bank (Osaka, Japan). The canine osteosarcoma cell line D-17 was purchased from American Type Culture Collection (Virginia, USA). The PC-3 cell line was maintained in RPMI1640 medium containing 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml). The A549, MRC-5 and D-17 cell lines were maintained in Dulbecco's Modified Eagle medium (DMEM) containing 10% FBS, penicillin (100 U/ml), streptomycin (100 µg/ml), D-glucose (1 mg/ml), and sodium pyruvate (110 µg/ml). All cells were maintained in a humidified incubator containing 5% carbon dioxide at 37°C and cultured with various concentration of Rv-PEM01 for 72 hs.

2.3. Analysis of Cell Viability

Analysis of tumor cell growth *in vitro* was carried out using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium, monosodium salt (WST-1, DOJINDO, Kumamoto, Japan) and 1-methoxy-5-methylphenazinium methylsulfate (1-Methoxy PMS, DOJINDO, Kumamoto, Japan) colorimetric assay [12]. Cells were seeded onto 96 well microtiter culture plates at densities of 1000 cells per well (volume, 50 µl/well). After 16 hs, 50 µl of RPMI1640 medium or DMEM containing 1% ethanol (control wells), or various concentrations of Rv-PEM01 were added and the cells were incubated for 72 hs at 37°C. The incubation was terminated using WST-1 and 1-Methoxy PMS mixture 10 µl per a well, after which absorbance was recorded on BIO-RAD Benchmark microplate reader at 450 nm/655 nm wavelength. The IC₅₀ values were determined from Rv-PEM01 dose versus control growth curves.

2.4. HPLC Analysis

The Rv-PEM01 was analyzed by HPLC using a Shimadzu LC6A system (Kyoto, Japan) equipped with a SPD-6AV UV-visible spectrophotometric detector and TSK gel ODS-80TM (7.8 i.d. × 300 mm) column (Tosoh, Tokyo, Japan). The mobile phase consisted of 50% methanol, and flow rate was 0.8 ml/min with UV detection at 254 nm. Measurement of urushiols was performed using the method of Du Y., *et al.* [13] with some modification. The urushiols were measured using Develosil ODS-5 (4.6 i.d. × 250 mm) column (Nomura Chemical, Aichi, Japan). The mobile phase consisted of acetonitrile (90:10) containing 2% acetic acid, and flow rate was 1 ml/min with UV detection at 272 nm.

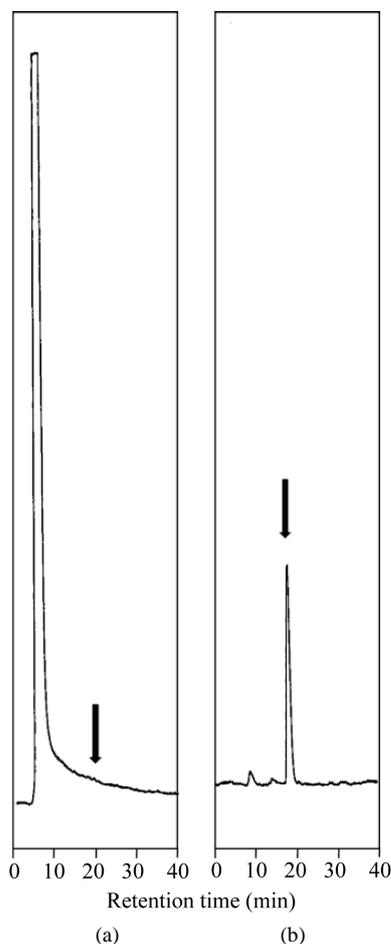


Figure 1. HPLC chromatogram of Rv-PEM01. (a) 10 mg/ml of Rv-PEM01 (20 μ l injection); (b) 1 mg/ml urushiol (5 μ l injection). Urushiols were measured using develosil ODS-5 (4.6 i.d. \times 250 mm) column (Nomura Chemical, Aichi, Japan). The mobile phase consisted of acetonitrile (90:10) containing 2% acetic acid, and flow rate was 1 ml/min with UV detection at 272 nm. Arrows indicate the peak for urushiol.

2.5. Single Dose Toxicity Study

Six male and female seven-week-old ddY mice, were used for the acute oral toxicity study [14]. The tests were carried out according to Ethics of the Organization for Economic Co-operation and Development (OECD) Test Guideline 401. The mice were housed at $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$, 50% relative humidity. The Rv-PEM01 was suspended in sterile water and administered to mice in single oral doses of 2 and 5 g/kg body weight. Mice were weighted at 0 - 7 days after administration, and clinical observations were made once a day. Necropsy was performed on all mice seven days after administration.

2.6. Antitumor Activity Test *in Vivo*

BALB/cAJcl-nu/nu mice were used to investigate the antitumor effects of Rv-PEM01 *in vivo* using the method of Kiyama S., *et al.* [15]. Mouse colorectal tumor cell line (Colon26; 1×10^6 cells in 0.1 ml of PBS) was injected into the right flank of six-week-old male BALB/cAJcl-nu/nu mice (CLEA Japan Inc., Tokyo, Japan). After ten days of tumor cell inoculation, the mice were treated with Rv-PEM01 for twenty eight days at a dose 0.0025 g/kg and 2.5 g/kg body weight per day and tumor size was measured daily. Tumor volumes were calculated by the formula of $[1/2 \times \text{longest dimension} \times (\text{shortest dimension})^2]$.

2.7. Statistical Analyses

The data are expressed as the mean \pm standard deviation (SD.). The significance of differences between groups was assessed using the Student's *t*-test. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Antiproliferative Activity of Rv-PEM01 on Tumor Cell Proliferation

The Rv-PEM01 treatment resulted in a dose-dependent decrease in cell growth in all cell lines (**Figure 2**). Calculated IC_{50} values for each cell line were 0.328 ± 0.081 , 0.520 ± 0.070 , 0.124 ± 0.037 mg/ml, and 0.505 ± 0.058 mg/ml for PC-3, A549, D-17 and MRC-5, respectively (**Table 2**). The antiproliferative activity data of Rv-PEM01 against the other eight tumor cell lines presented in **Table 2** (MOLT-3, KG-1, HeLa, DLD-1, MCF-7, K-562, Colon26 and B16) were reported in our previous paper [11]. The antiproliferative activities of Rv-PEM01 in 12 different cell lines are presented in **Figure 3**. The values are calculated as follows: $\log(IC_{50} \text{ each cell line}) - \log(IC_{50} \text{ average})$ [16]. Negative values showed that the cell line was more sensitive than the average. Positive values showed that the cell line was more resistant than the average. HeLa, DLD-1, MCF-7, K-562, B16, A549 and MRC-5 were more resistant, and MOLT-3, KG-1, Colon26, PC-3 and D-17 were sensitive to Rv-PEM01 than other cell lines.

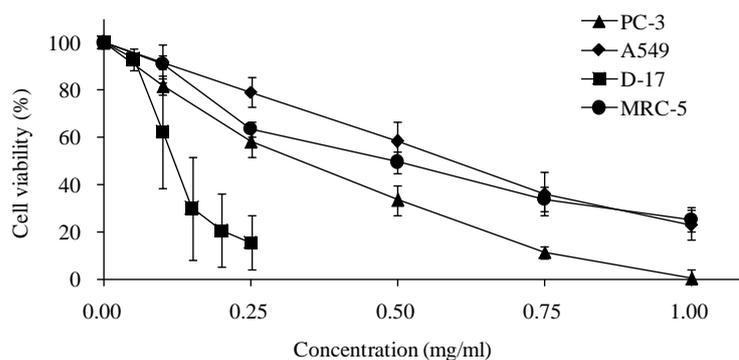


Figure 2. Dose dependent inhibitory effects of Rv-PEM01 on PC-3 (triangles), A549 (diamonds), D-17 (squares) and MRC-5 (circles). Results are means \pm SD. (n = 3).

Table 2. Antiproliferative activity of Rv-PEM01 in human, mouse and canine tumor cell lines.

Cell lines	IC_{50} (mg/ml) [*]	Cell origin	Reference
MOLT-3	0.208 ± 0.022	Human tumor cell	Hiruma, <i>et al.</i> [11]
KG-1	0.293 ± 0.007	Human tumor cell	Hiruma, <i>et al.</i> [11]
HeLa	0.433 ± 0.043	Human tumor cell	Hiruma, <i>et al.</i> [11]
DLD-1	0.510 ± 0.030	Human tumor cell	Hiruma, <i>et al.</i> [11]
MCF-7	0.580 ± 0.054	Human tumor cell	Hiruma, <i>et al.</i> [11]
K-562	0.610 ± 0.141	Human tumor cell	Hiruma, <i>et al.</i> [11]
Colon26	0.389 ± 0.093	Mouse tumor cell	Hiruma, <i>et al.</i> [11]
B16	0.565 ± 0.028	Mouse tumor cell	Hiruma, <i>et al.</i> [11]
PC-3	0.328 ± 0.081	Human tumor cell	This work
A549	0.520 ± 0.070	Human tumor cell	This work
D-17	0.124 ± 0.037	Canine tumor cell	This work
MRC-5	0.505 ± 0.058	Human normal diploid fibroblast cell	This work

^{*}Results are means \pm SD. (n = 3).

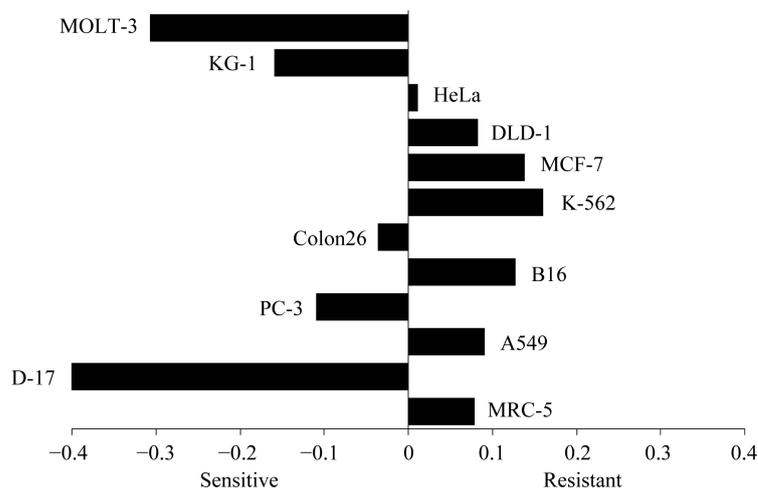


Figure 3. Antiproliferative activity of Rv-PEM01 in human, mouse and canine tumor cell lines. The values are calculated as follows: $\log(\text{IC}_{50} \text{ each cell line}) - \log(\text{IC}_{50} \text{ average})$ [16]. Negative values showed that the cell line was more sensitive than the average. Positive values showed that the cell line was more resistant than the average.

3.2. Identification of Active Compounds Contained in Rv-PEM01

The HPLC chromatogram of Rv-PEM01 is illustrated in **Figure 4**. Among the five peaks observed in the chromatogram, peaks 2 and 4 showed antiproliferative activity against MOLT-3, KG-1, Colon26, PC-3 and D-17 cell lines (**Figure 4**, **Table 3**). Peaks 2 and 4 were identified as luteolin 7- β -D-glucopyranoside and apigenin 7- β -D-glucopyranoside, respectively.

3.3. Single Dose Toxicity Study of Rv-PEM01

No deaths or abnormalities of body weight, water and food consumption, or coat condition were observed in the treated mice. Necropsy evaluation of the mice did not reveal any significant differences in thymus, liver, spleen, kidney, adrenal gland and testicle weights between the control group and the Rv-PEM01 treatment groups, or between males and females (**Table 4**).

3.4. In Vivo Antitumor Effects of Rv-PEM01 on Nude Mice

No deaths or significant differences in the body weight gain, tumor volume, or tumor size were observed between the control group and Rv-PEM01 treatment group (**Figure 5**). The Rv-PEM01 treatment at dose of 2.5 g/kg tended to have antitumor activities compared with the control group, but the difference did not reach statistical significance. Additional studies of antitumor effects of Rv-PEM01 *in vivo* are currently under investigation.

4. Discussion

In this study, Rv-PEM01 exhibited antitumor activities against human, canine and mouse tumor cell lines *in vitro*. These results confirmed our previous data, which suggested the main active herb in Rv-PEM01 was *R. verniciflua*. The branches and the sap from *R. verniciflua* contain active compounds such as urushiol, fustin, quercetin, butein and sulfuretin, and the antioxidant [17] [18], antitumorigenic [19] and cytotoxic [20] effects of these compounds have been reported. Urushiols, which are mixture of olefinic catechols with alkyl side chain [21], are characteristic compounds in *R. verniciflua* branches and sap. These compounds have shown the biological activities including cytotoxic effects on human cancer cell lines [22] and antioxidant properties [23]. Recently, two novel urushiol derivatives, 1,2-dihydroxyphenyl-3-pentadeca-7'(E),9'(Z),11'(Z)-trien-14'-ol and 1,2-dihydroxyphenyl-3-pentadeca-8'(Z),10'(E),12'(E)-trien-14'-ol, were isolated from leaves of *R. verniciflua*, and shown to inhibit HIV-1 RT [10]. In addition to these activities of *R. verniciflua*, urushiol compounds are well known as allergenic compounds that can induce contact dermatitis [24]. Therefore, urushiols and their deri-

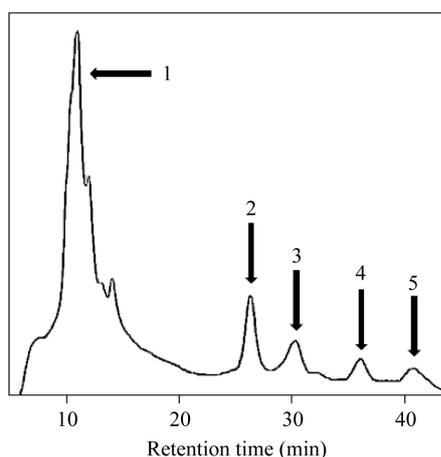


Figure 4. HPLC chromatogram of Rv-PEM01. Rv-PEM01 was analyzed by HPLC using Shimadzu LC6A system (Kyoto, Japan) equipped with a TSK gel ODS-80TM (7.8 i.d. × 300 mm) column (Tosoh, Tokyo, Japan) and a SPD-6AV UV-visible spectrophotometric detector. The mobile phase consisted of 50% methanol, and flow rate was 0.8 ml/min with UV detection at 254 nm.

Table 3. Antiproliferative activity of fraction No.1-5 from Rv-PEM01 in human, mouse and canine tumor cell lines.

Peak No.	MOLT-3 IC ₅₀ (mg/ml)	KG-1 IC ₅₀ (mg/ml)	Colon26 IC ₅₀ (mg/ml)	PC-3 IC ₅₀ (mg/ml)	D-17 IC ₅₀ (mg/ml)
1	0.035 ± 0.009	>0.750	>0.750	>0.750	>0.750
2	0.010 ± 0.001	0.022 ± 0.003	0.030 ± 0.005	0.023 ± 0.004	0.011 ± 0.004
3	0.063 ± 0.025	0.089 ± 0.032	0.184 ± 0.025	0.182 ± 0.034	0.162 ± 0.028
4	0.013 ± 0.005	0.007 ± 0.003	0.013 ± 0.002	0.011 ± 0.003	0.009 ± 0.004
5	0.055 ± 0.012	0.046 ± 0.014	0.127 ± 0.030	0.073 ± 0.011	0.096 ± 0.033

*Results are means ± SD. (n = 3).

Table 4. Absolute organ weights of mice after 7 days oral administration of Rv-PEM01.

Dose (g/kg)	Male (n = 6)			Female (n = 6)		
	0	2.5	5.0	0	2.5	5.0
Body weight (g)	38.0 ± 1.0	38.0 ± 1.7	38.0 ± 0.9	30.3 ± 0.4	31.3 ± 1.1	30.8 ± 0.5
Thymus (mg)	59.8 ± 13.0	68.0 ± 17.4	66.7 ± 21.1	82.6 ± 20.6	78.3 ± 6.7	81.2 ± 15.6
Liver (g)	2.1 ± 0.3	1.9 ± 0.2	2.0 ± 0.2	1.6 ± 0.1	1.6 ± 0.2	1.6 ± 0.1
Spleen (mg)	135.0 ± 15.3	159.4 ± 12.6	161.7 ± 31.9	147.1 ± 28.8	170.3 ± 23.2	154.6 ± 17.8
Left kidney (mg)	335.1 ± 76.9	338.1 ± 41.5	333.7 ± 28.2	227.0 ± 42.6	219.9 ± 23.9	206.6 ± 18.7
Right kidney (mg)	280.7 ± 21.0	310.6 ± 42.8	340.3 ± 52.5	227.0 ± 42.6	210.1 ± 20.6	205.8 ± 18.9
Adrenal gland (mg)	17.4 ± 6.2	18.7 ± 6.3	15.6 ± 4.3	19.7 ± 2.7	21.3 ± 3.0	20.6 ± 1.5
Testicles (mg)	264.6 ± 25.1	273.2 ± 16.3	281.5 ± 18.3	33.8 ± 12.2	41.9 ± 13.2	36.7 ± 6.9

*Results are means ± SD. (n = 6).

vatives from *R. verniciflua* were not contained in Rv-PEM01 (Figure 1), and antiproliferative activities of these on human and mouse tumor cell lines were investigated in previous our paper [11]. In the present study, the antiproliferative activity of Rv-PEM01 was evaluated in human and canine tumor cell lines that were different from the previous tumor cell lines used *in vitro*.

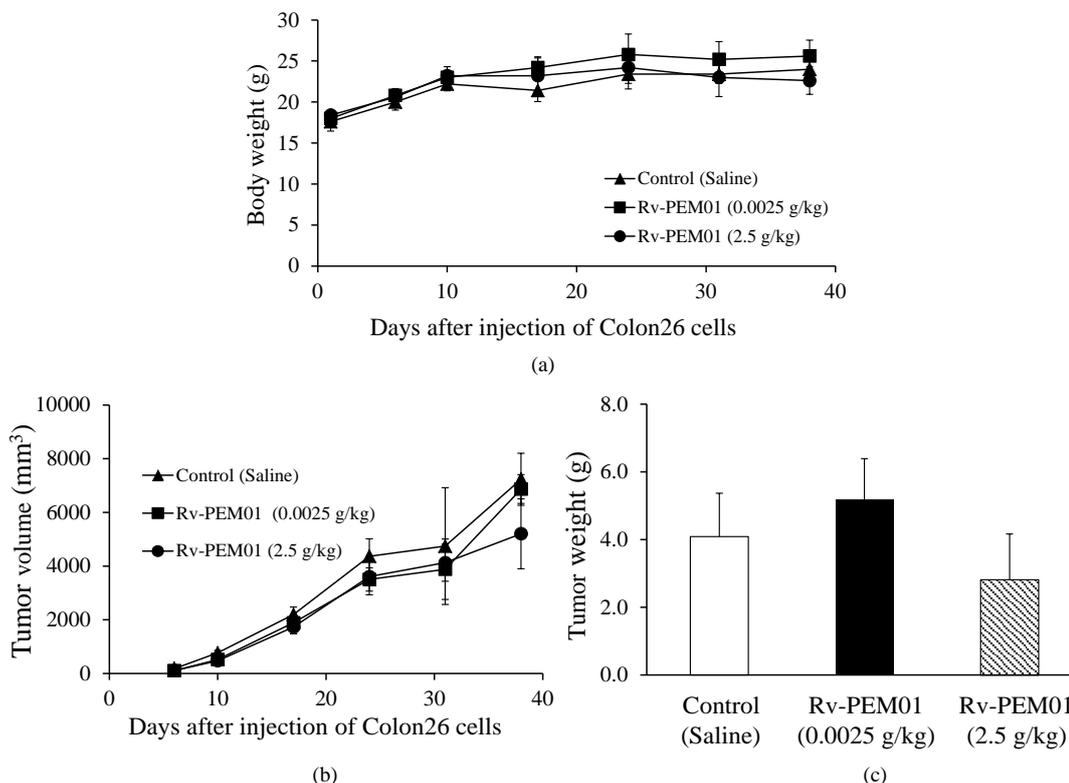


Figure 5. Effects of Rv-PEM01 on BALB/cAJcl-nu/nu mice bearing Colon26 tumor cells. Mouse colorectal tumor cell line (Colon26; 1×10^6 cells in 0.1 ml of PBS) was injected into the right flank of six-week-old male BALB/cAJcl-nu/nu mice. After 10 days of inoculation, the mice were treated with Rv-PEM01 for 28 days. (a) Change of body weight (g); (b) Change in tumor volume (mm³); (c) Tumor weight (g) after 38 days. Results are means \pm SD. (n = 5).

Rv-PEM01 had antiproliferative activities on PC-3, A549, D-17 and MRC-5 cell lines (Figure 2, Table 2). Evaluation of the antiproliferative activities of Rv-PEM01 in 12 different cell lines, indicated that HeLa, DLD-1, MCF-7, K-562, B16, A549 and MRC-5 were more resistant to Rv-PEM01, whereas MOLT-3, KG-1, Colon26, PC-3 and D-17 were more sensitive to it (Figure 3). The antiproliferative activity of Rv-PEM01 was more potent on D-17 than on any of the other tumor cell lines. Our previous investigation found that Rv-PEM01 induced apoptosis in MOLT-3, KG-1 and K-562 leukemia cell lines by measurement of DNA fragmentation and caspase-3 and caspase-9 activities [11]. The mechanisms of Rv-PEM01's antiproliferative effects on Colon26, PC-3 and D-17 are still unclear, but it may induce apoptosis on these tumor cell lines as it did in the leukemia cell lines in our previous report. Spontaneous canine tumors show many clinical and molecular similarities to human tumors, and such offer an attractive model for preclinical investigations [25]. This study is the first to our knowledge to evaluate the effects of Rv-PEM01 on canine tumor cell lines. Additional investigations of the effectiveness of Rv-PEM01 on canine tumor cell lines are indicated.

HPLC analysis showed five major peaks (Figure 4). The antiproliferative activity of peaks 2 and 4 on MOLT-3, KG-1, Colon26, PC-3 and D-17 cell lines were more potent than that of other peaks (Table 3). Peaks 2 and 4 were identified as luteolin 7- β -D-glucopyranoside and apigenin 7- β -D-glucopyranoside, respectively. Rashed KN., *et al.* recently reported that a hydromethanolic extract of *Sapindus saponaria* showed cytotoxicity against human carcinoma cell lines, and that the active compounds were luteolin 8-C- β -glucoside, luteolin 6-C- β -glucoside, luteolin 7-O- β -glucuronide, and rutin [26]. Nakazaki E., *et al.* found that the apigenin 7-glucoside inhibited HL-60 leukemia cell line [27]. Kim S., *et al.* reported that extraction of *R. verniciflua* leaf with 70% methanol showed the neuroprotective effects, and that the active compounds were fisetin, sulfuretin, quercetin and butein [28]. There was a report that mansonone E and F, which were extracted with 80% ethanol from *Ulmus pumila*, showed potent antiproliferative effects on human tumor cell lines [29]. Ahn MJ., *et al.* also reported that neosibiricoside A-D isolated from the rhizomes of *Polygonatum sibiricum* had cytotoxic activity on MCF-7

breast cancer cells [30]. From these reports, we expected that antitumor effects of the Rv-PEM01 may be related to the luteolin 7- β -D-glucopyranoside, apigenin 7- β -D-glucopyranoside and some other compounds such as fisetin, sulfuretin, quercetin, butein, mansonone species and neosibiricoside species that are contained in the extract.

In the acute oral toxicity study, there were no changes attributable to Rv-PEM01 administration at doses of 2.5 or 5.0 g/kg. The 50% mortality rate LD₅₀ could not be calculated, but must be higher than 5.0 g/kg. In the chronic study, we found no adverse effects of Rv-PEM01 at the doses used, suggesting longer-term safety at these doses. Unfortunately, no statistically significant difference of antitumor efficacy from the control group was found, although Rv-PEM01 treatment at dose of 2.5 g/kg resulted in a trend toward antitumor activity compared with control. Additional studies, using different doses and model systems, as well as a study of potential prophylactic antitumor effects of Rv-PEM01 *in vivo* currently are in progress.

5. Conclusion

Biological activities of Rv-PEM01, as well as its antitumor effects, safety, and toxicity, were investigated. Rv-PEM01 exhibited antiproliferative activities against PC-3, A549, D-17 and MRC-5 *in vitro*, and tendency toward tumor growth inhibition *in vivo*. The main active compounds of Rv-PEM01 were luteolin 7- β -D-glucopyranoside and apigenin 7- β -D-glucopyranoside. The safety studies did not identify any adverse reactions in mice. Therefore, it could be useful as a novel functional food material and/or nutritional supplement. Additional studies of the antitumor effects of Rv-PEM01 *in vivo* are needed.

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