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Original Research Article

Antitumor Effects of Ethanol Extract from Raw and Steaming Vietnamese *Panax notoginseng* Roots on Sarcoma 180 Tumor-Bearing Mice

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ABSTRACT

Panax notoginseng has been used in Vietnamese traditional medicine to treat hemoptysis, nosebleeds, hemorrhage, menorrhagia after giving birth, stagnation, abdominal pain, and dysentery. This research aimed to evaluate and compare the antitumor effects of 70% ethanol extracts of Vietnamese *P. notoginseng* roots with steaming (PNS) and without steaming (PNO) on sarcoma 180–bearing mice. The evaluation of tumor suppression effects was based on assessment of tumor size, histopathology and survival time of tumor-bearing mice. The results showed that PNS at both doses of 300 and 900 mg/kg/day had good antitumor effects by reducing the volume of tumors and prolonging survival time of tumor-bearing mice compared to the group not taking drugs in a dose-dependent manner, equivalent to the positive agent of lentinan at 240 mg/kg/day. PNO also showed good antitumor activity, but it was still inferior to PNS. Hence, the 70% ethanol extracts from *P. notoginseng* roots both with and without steaming showed good antitumor effects.

***Keywords*:** Panax notoginseng, steaming, antitumor, mice.

Introduction

P. notoginseng (Burk.) F.H. (*Tam thất* in Vietnamese) has long been proven as a valuable medicine in Eastern medicine, including in Vietnam. *P. notoginseng* has been known as *kim bất hoán*, meaning a medicine more precious than gold.[1] The main components of *P. notoginseng* have been identified as saponin (4.42–12.00%) of the protopanaxadiol and protopanaxatriol types.[1,2] In addition, alkaloids, polysaccharides, lipids, essential oils, free amino acids, carotene, and calcium have been found in the roots of *P. notoginseng*.[3,4] Many studies have proven that this plant has several pharmaceutical effects such as treating hemoptysis, nosebleeds, hemorrhage, menorrhagia after giving birth, stagnation, abdominal pain, and dysentery.[4-6] It has been used as a tonic to increase the body's adaptability. Several studies on *P. notoginseng* have concluded that this plant increased the sensitivity of cancer cells to radiation or chemical therapies, so it could be used in combination with these in the treatment of cancer patients.[7-9] According to ancient oriental documents, *P. notoginseng* could decrease the growth of tumors in cancer. According to clinical experience, many patients with fibroids such as in the breast, uterus, and prostate have been treated with *P. notoginseng* ventricular long-term doses, and the results have found that the tumor sizes were not greatly increased.[10,11] *P. notoginseng* root is one of the most important ingredients of the Chinese recipe *Yun Nan Bei Yao*, which was historically used to treat lymphoma and nasopharyngeal cancer in China.[12]

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In addition, water extracted from *P. notoginseng* has been confirmed to inhibit human lung carcinoma cells, with the inhibition rate reaching 89%.[13] Several studies have shown that steaming *P. notoginseng* roots increased the antitumor activity.[14-17]
In Vietnam, *P. notoginseng* grows wildly and is also grown in the high mountains in the north. However, research to evaluate the effect on tumors of raw or steamed *P. notoginseng* roots is lacking. The study evaluated and compared the antitumor effects of saponin-rich extracts from steamed and raw *P. notoginseng* roots collected in Vietnam on Swiss mice bearing solid sarcoma TG 180 tumors.

Materials and methods

Plant material
The roots used were from 4-year-old tuberous *P. notoginseng* (Burk.) F.H. grown in Simacai village, Lao Cai province, in the north of Vietnam. The roots were collected in July 2015 and indentified by Dr. Pham Thanh Huyen, Faculty of Medicinal Resources, Vietnam National Institute of Medicinal Materials. The voucher specimen number (HTV2-072015) was deposited at the Department of Plant Chemistry, Vietnam National Institute of Medicinal Materials.

Sample extraction and preparation
The 4-year-old roots of *P. notoginseng* were collected and washed, dried, and ground into powder. The processing met the standards of the Vietnamese Pharmacopeia V monograph. The powder (50 g, steamed or not steamed) was reflux-extracted with 500 mL of 70% ethanol, with a solvent–medicinal ratio of 10:1, 3 times, every 3 hours. The extract was pooled, and the solvent was recovered under reduced pressure to a high concentration. The concentrate was vacuum dried at 60ºC until a high dry yield was obtained for biological activity testing.

Animals
A total of 130 adult Swiss mice, weighing 20–22 g, were supplied by the Animal Department of Vietnam Military Medical University. The mice were raised in an animal laboratory and fed normal mice food, with free access to clean water, for at least 1 week before the

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experiments. The experimental protocol was approved by the Vietnam Military Medical University, Hanoi, Vietnam (permission number IACUC-121/21, issued on January 12, 2021).

Sarcoma cell line

The sarcoma 180 cell line is a suspension cell derived from *Mus musculus* Swiss Webster mice with connective tissue cancer. The cells were purchased from ATCC and cultured at the Department of Cell Biology, Faculty of Biology, University of Natural Sciences, Vietnam National University in Hanoi.

Equipment and chemicals

Blunt-tip needles for the mice to drink, graduated cups, 1-mL syringes, calipers used to measure the size of the mouse tumors, and an analytical balance (10^{-4} g) were purchased from Sartorius (Germany). The reference drug, lentinan, was purchased from Carbosynth Limited UK (batch: FL 333211401; product code FL33321; CAS no. 37339-90-5).

Creation of solid sarcoma TG 180 tumors in mice

The sarcoma 180 cell line was used to induce tumors in the mice according to the improved method of Lapis.[18] In detail, the cells were stored in liquid nitrogen at -198 °C, thawed to 37°C, then cultured and proliferated to the desired density and number. Before injecting the tumor cells, they were centrifuged to remove the preservative solution, washed twice with phosphate-buffered saline (PBS) solution, and then diluted in PBS solution to obtain the final concentration of 5×10^6 cells/mL. The mice were injected subcutaneously in the right posterior thigh with 0.1 mL per mouse. At follow-up in the first 5 days after injection, the tumors were observed in the right thigh by palpation. The mice with tumor growth at the injection site in the thigh (usually 3 days after tumor detection) and a gradual increase in tumor volume over time were evaluated as having successful tumorigenesis and included in the study.

Evaluation of the antitumor effect of standardized PNS and PNO

Seventy mice were randomly assigned to 7 groups with 10 mice each. Group 1, the physiological control (PC), comprised non-tumor mice, drinking physiological saline (0.3 mL/20 g). Group 2, the cancer model (CM), comprised tumor-induced mice, drinking physiological saline (0.3 mL/20 g). Group 3, the positive control (LTN), comprised tumor-induced mice with lentinan administration (240 mg/kg/day). Group 4, PNO-1, comprised tumor-induced mice with PNO 300 mg/kg/day administration. Group 5, PNO-2, comprised tumor-induced mice with PNO 900 mg/kg/day administration. Group 6, PNS-1, comprised tumor-induced mice with PNS 300 mg/kg/day administration. Group 7, PNS-2, comprised tumor-induced mice with PNS 900 mg/kg/day administration. PNO, PNS, LTN, and physiological saline were given to the mice (according to groups) from day 6 after sarcoma 180 cell injection and continuously for 16 days. The evaluation of tumor suppression effects was based on observing the expression, activity, eating, locomotion, and weight of the mice.

The tumor size was measured with a caliper by determining the largest and smallest diameter of the tumor (Figure 1). The solid tumor volume was calculated according to the formula of Bagley *et al.*,[19] using (a) the smallest diameter of the tumor (mm), (b) the largest diameter of the tumor (mm), and (V) the tumor volume (mm^3).

At the end of the experiment on day 21, the tumor was dissected and removed from the thigh of each mouse. A scale balance (10^{-4} g) was used to accurately determine the weight of the tumor (W). Hematoxylin-Eosin (HE) staining was used for 4 tumors dissected from 4 mice randomly from each batch for evaluation. Histopathological specimens of the mouse tumors were made and examined at the Department of Pathology, Military Hospital 103, Vietnam Military Medical University. The evaluation of antitumor efficacy was according to Itokawa's criteria.[20]

Determination of survival time of mice

Tumors were induced in the mice by subcutaneous injection of sarcoma 180 cells as described. The successful tumorigenic mice were randomly divided into 6 groups of 20 each: Group 1, tumor-induced mice drinking physiological saline (0.3 mL/20 g, CMb); Group 2, tumor-induced mice with lentinan 240 mg/kg/day administration (LTNb); Group 3, tumor-induced mice with PNO 300 mg/kg/day administration (PNO-1b); Group 4, tumor-induced mice with PNO 900 mg/kg/day administration (PNO-2b); Group 5, tumor-induced mice with PNS 300 mg/kg/day administration (PNS-1b); Group 6, tumor-induced mice, with PNS 900 mg/kg/day administration (PNS-2b). PNO, PNS, LTN, and physiological saline were given to the mice (according to groups) from day 6 after the successful inoculation of sarcoma 180 cancer cells. The mice were cared for and monitored daily until they died. The survival time of each group was determined according to the established method.[21]

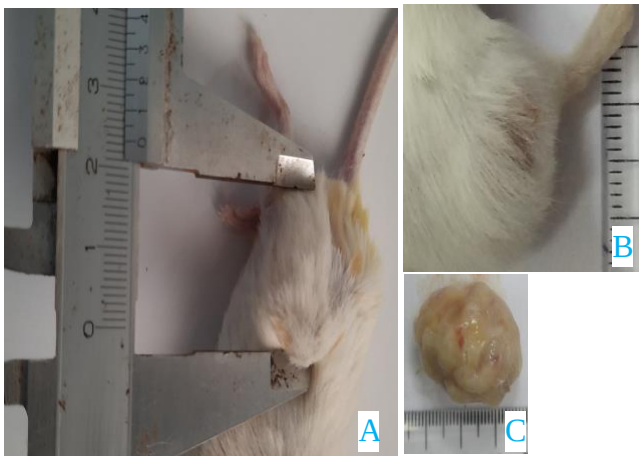

Figure 1: The sarcoma 180 cell line inducing the tumor in the thigh of mice. (A) Measure tumor size with caliper; (B) The tumor in mice; and (C) the tumor dissected from the mice thigh.

$$ILS\ (\%) = \left(\frac{Ts}{Cs} - 1\right) x\ 100$$

Ts: mean survival time of treated cancer mice; Cs: mean survival time of untreated cancer mice; ILS: increased life span.

Statistical analyses

Data were analyzed using one-way analysis of variance (One-way ANOVA) and post hoc least-significant differences (LSD) test and expressed as mean ± standard deviation to compare the difference between two mean values (using IBM SPSS Statistics 20.0 software). Differences were considered statistically significant when $p < 0.05$.

Results and Discussion

Tumor modeling results

The assessment of tumor growth at the right thigh injection site was observed by palpation and measurement of the tumor size with a caliper. The results of experimental modeling showed that 3 days after being injected with a suspension containing 5×10^6 sarcoma 180 cells under the skin of the right thigh, tumors were developed under the skin of the right and posterior thighs. Within 5 days, the tumors were clearly formed. The tumors were monitored during the first 5 days of tumorigenesis. Only the mice with well-formed tumors and marked tumor growth were evaluated as successful tumorigenic mice and included in the study. The success rate of tumor induction was 98%.

In the first 5 days after injecting the sarcoma 180 cells, the bodyweight of the mice in all groups increased steadily, with no difference between groups (Table 1, $p > 0.05$). In the CM group, the mice had on day 3. The average volume of tumors in this group ranged from 288.26 mm^3 to 303.35 mm^3. Tumors grew rapidly on day 5, and the average tumor volume in the CM group ranged from 508.84 mm^3 to 527.01 mm^3 (Table 1). Continued follow-up showed that the tumor volume gradually increased with the time since tumor induction

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(Table 1). Animals with late stage tumors showed signs of anorexia, exhaustion, and ruffled fur, and some began to die on day 22 after the injection of cancer cells.

Effect of PNS and PNO on bodyweight of the mice bearing solid sarcoma

After successful tumorigenesis was determined on day 5, the mice were given drugs according to their group starting from day 6, then their weights were measured every 2 days. The results are presented in Table 2. The weights of the mice in the PC (non-tumor) and CM (tumor with no drug) groups increased steadily from 31.09 ± 0.79 g to 45.32 ± 1.44 g, and from 30.97 ± 1.22 g to 44.51 ± 1.48 g, respectively. The bodyweight of the mice with tumors increased from day 7 to day 17. On day 19, the bodyweight of the mice tended to decrease slightly and then increase again on day 21. Comparison between all groups at the same time of measurement showed that the bodyweight of rats between groups was not statistically significant ($p > 0.05$).

Effect of PNS and PNO on solid tumor growth

The mice began receiving the drug on day 6 after the tumor cell injection. On day 7, the tumor volume in all treated groups was not statistically significantly different from that of the CM group. In the following days, the tumor volumes of the mice in the CM group increased continuously and very quickly with each measurement (Table 3). In the LTN, PNO-1, PNO-2, PNS-1 and PNS-2 groups, the tumor volumes also increased but slowly. On days 9 and 11, the tumor volume of the drug-treated groups was significantly smaller than that of the non-drug-treated group ($p < 0.05$ and $p < 0.01$, respectively). From day 13 (day 8 of drug administration) onwards, the tumor volume of all drug-treated groups was statistically significantly smaller than that of the non-medicated cancer CM group ($p < 0.001$). The PNS-1 and PNS-2 groups also showed increasing tumor volume, but the growth was significantly slower. In the PNS-1 group, the tumor volumes were measured on days 15, 17, 19, and 21, as shown in Table 3. The average tumor volumes were from 893.57 to 1,099.03 mm^3, smaller than in the PNO-1 and PNO-2 groups ($p < 0.01$ and $p < 0.05$, respectively). Similarly, the mean tumor volume of the high-dose PNS-2 group was smaller than in the PNO-1 and PNO-2 groups ($p < 0.001$ and $p < 0.01$, respectively). On day 21, the average tumor volume of the groups with high doses of both steamed and non-steamed root was smaller than in the low-dose groups ($p < 0.05$). Interestingly, the tumor volumes of the PNS-1, PNS-2, and PNO-2 groups were similar to the positive lentinan administration (LTN) group ($p > 0.05$). *Evaluation of antitumor efficacy of PNS and PNO*

The results of the antitumor efficacy assessment on day 21 are presented in Table 4. On day 21 post tumor injection (after 16 days of drug administration), the tumor volumes and tumor weights in the PNS group were smaller than those in the PNO group with the same dose administration ($p < 0.01$ and $p < 0.001$). The tumor volumes and tumor weights in the PNS-2 group were smaller than those in the PNS-1 group ($p < 0.05$). The LTN, PNS-1, and PNS-2 groups had tumor suppression ratios of 64.31%, 66.18%, and 72.09%, respectively, and the tumor suppression ratios by weight were 64.56%, 66.59%, and 72.24%, respectively. These entire groups achieved the antitumor effect (++) according to the rating scale.[19] The tumor suppression ratio of the PNS-2 group was the largest, at 72.09% by tumor weight. Groups PNO-1 and PNO-2 had tumor suppression ratios by volume of 53.66% and 59.54%, respectively, and the tumor suppression ratios by weight were 53.90% and 59.37%, respectively.

Table 1: Bodyweight and tumor volume in the tumor-inducing stage

Groups	Mice weight (g)			Tumor volume (mm^3)	
	Day 1	Day 3	Day 5	Day 3	Day 5
PC	21.28 ± 0.42	25.34 ± 0.54	27.65 ± 0.65	-	-
CM	21.31± 0.67	25.26 ± 1.14	27.31 ± 1.06	303.35 ± 44.60	527.01 ± 73.70
LTN	21.22 ± 0.74	24.81 ± 0.92	26.48 ± 1.22	289.85 ± 38.13	516.65 ± 82.03
PNO-1	21.36 ± 0.42	25.18 ± 0.99	26.81 ± 1.06	299.10 ± 28.99	519.45 ± 55.92
PNO-2	21.39 ± 0.46	24.72 ± 0.70	26.32 ± 1.02	293.27 ± 29.29	508.84 ± 63.20
PNS-1	21.24 ± 0.43	24.95 ± 0.48	26.71 ± 0.48	296.80 ± 33.16	522.91 ± 71.66
PNS-2	21.13 ± 0.39	24.32 ± 0.73	26.13 ± 1.12	288.26 ± 46.50	524.91 ± 60.58
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

PC: physiological control; CM - cancer model; LTN - positive control; PNO - *P. notoginseng* roots without steaming; PNS - *P. notoginseng* roots with steaming; Data are mean ± SD; n = 10; *p* - comparison between groups at the same time of evaluation.

Table 2: Bodyweight of the mice during the oral administration

Groups	Bodyweight (g)							
	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
PC	31.09 ± 0.79	34.18 ± 1.55	37.62 ± 1.56	41.51 ± 1.42	43.36 ± 1.37	44.53 ± 1.37	44.96 ± 1.44	45.32 ± 1.44
CM	30.97 ± 1.22	34.02 ± 1.45	37.46 ± 2.28	41.33 ± 2.27	43.14 ± 1.95	44.12 ± 1.74	44.36 ± 1.63	44.51 ± 1.48
LTN	30.23 ± 1.43	33.09 ± 1.58	36.35 ± 1.55	40.63 ± 1.08	42.33 ± 1.06	43.67 ± 1.12	43.28 ± 0.99	43.45± 1.04
PNO-1	30.72 ± 1.36	33.75 ± 1.33	36.83 ± 1.48	41.02 ± 1.59	42.51 ± 1.68	43.83 ± 1.89	43.66 ± 1.95	43.83 ± 1.94
PNO-2	30.18 ± 1.46	33.12 ± 0.97	36.21 ± 0.89	40.88 ± 0.91	42.19 ± 0.94	43.45 ± 1.06	43.12 ± 1.08	43.36 ± 1.04
PNS-1	30.57 ± 0.97	33.51 ± 0.74	36.62 ± 0.82	40.97 ± 1.43	42.34 ± 1.38	43.61 ± 1.14	43.34 ± 1.03	43.54 ± 1.04
PNS-2	30.06 ± 0.81	33.03 ± 0.84	36.05 ± 0.86	40.18 ± 1.13	42.03 ± 1.08	43.16 ± 1.08	43.02 ± 0.97	43.16± 1.02
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

PC: physiological control; CM - cancer model; LTN - positive control; PNO - *P. notoginseng* roots without steaming; PNS - *P. notoginseng* roots with steaming. Data are mean ± SD; n = 10; *p* - comparison between groups at the same time of evaluation.

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These groups achieved the antitumor effect (+) according to the rating scale.[19] The histopathological results showed many signals of tumor cells with mitotic nuclei, with tumor cells more common in the CM (A) group than the other treated groups (B–F). No significant differences were found in tumor histopathology between the treated groups (C–F) and the positive control group (B) (Figure 2).

Effect of prolonging survival time of PNS and PNO in mice bearing solid sarcoma 180 tumors

In the untreated cancer-induced (CMb) group, the first mouse died on day 22, with more deaths over the following days than the other treated groups. In the treated groups, some mice died on later days, and fewer mice died than in the CMb group. In particular, on day 142, 1 survivor remained in each of the PNS-1b and PNS-2b groups, and on day 122, 1 survivor remained in each of the LTNb and PNO-2b groups. No survivors remained on day 82 in the CMb group. Interestingly, on day 145, only 1 mouse in the PNS-2b group remained, and on day 149, this last mouse died (Tables 5, 6 and Figure 3). The mice in the LTNb, PNO-1b, and PNO-2b groups had a longer average survival time than those in the CMb group ($p < 0.05$). Similarly, the mice in the PNS-1b and PNS-2b groups had a longer survival time than those in the CMb group ($p < 0.01$). Overall, the mice in the PNS-2b group had the longest extended survival time (55.12%). However, the average survival time of mice between the different tumor-induced groups was not statistically significant.

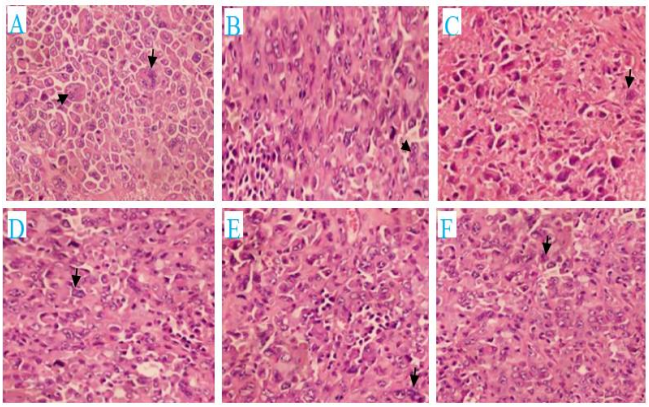

Figure 2: Histopathological image of sarcoma 180 tumor in mice. A, B, C, D, E, and F are symbols for groups CM, LTN, PNO-1, PNO-2, PNS-1, and PNS-2, respectively. Arrows indicate the tumor cells with a mitotic nucleus

Table 3: Tumor volume of mice during the oral administration (day 7 to day 21)

Groups	Tumor volume (mm^3)							
	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
CM (1)	858.99 ± 196.05	1016.88 ± 188.69	1315.94 ± 404.59	1741.88 ± 502.20	1963.01 ± 464.01	2480.70 ± 386.12	3184.01 ± 536.99	3254.29 ± 544.01
LTN (2)	791.00 ± 192.47	839.37* ± 181.94	$855.70^{▲}$ ± 195.11	$892.00^{Δ}$ ± 243.74	$973.99^{Δ}$ ± 287.85	$1017.08^{Δ}$ ± 301.38	$1085.11^{Δ}$ ± 314.85	$1164.54^{Δ}$ ± 393.88
PNO-1 (3)	829.00 ± 158.76	862.00* ± 129.21	$884.00^{▲}$ ± 145.67	$958.98^{Δ}$ ± 141.98	$1157.00^{Δ}$ ± 178.71	$1296.02^{Δ†}$ ± 207.68	$1452.02^{Δ†}$ ± 275.00	$1507.62^{Δ†}$ ± 273.95
PNO-2 (4)	771.90 ± 165.45	837.98* ± 153.41	$890.70^{▲}$ ± 152.34	$938.99^{Δ}$ ± 138.43	$1041.86^{Δ}$ ± 138.74	$1247.01^{Δ}$ ± 286.84	$1288.02^{Δ}$ ± 254.56	$1316.19^{Δ}$ ± 254.24
PNS-1 (5)	797.99 ± 138.35	834.01* ± 140.05	$852.03^{▲}$ ± 157.84	$861.34^{Δ}$ ± 156.57	$893.57^{Δ‖}$ ± 163.48	$936.99^{Δ‖}$ ± 191.89	$998.99^{Δ‖}$ ± 198.23	$1099.03^{Δ‖}$ ± 197.65
PNS-2 (6)	743.05 ± 143.11	808.00* ± 134.36	$813.94^{▲}$ ± 134.90	$826.10^{Δ}$ ± 153.34	$858.41^{Δ¥}$ ± 145.19	$885.99^{Δ¥}$ ± 144.08	$896.00^{Δ¥}$ ± 141.90	$913.44^{Δ¥β}$ ± 150.99

CM - cancer model; LTN - positive control; PNO - *P. notoginseng* roots without steaming; PNS - *P. notoginseng* roots with steaming; Data are mean ± SD; n = 10; $*^{,▲,Δ}$ – $p < 0.05$. $p < 0.01$ and $p < 0.001$ compared to (1); † = $p < 0.05$ compared to (2); ‖ = $p < 0.01$ compared to (3), and $p < 0.05$ compared to (4); $^{¥}$ = $p < 0.001$ compared to (3) and $p < 0.01$ compared to (4); β = $p < 0.05$ compared to (5)..

Table 4: Antitumor efficacy on day 21 post-tumor injection in treatment groups

Groups	Average tumor volume at day 21 (mm^3)	Percent inhibition (%)	Tumor weigh at day 21 (g)	Inhibition ratio per weight (%)	Anti-tumor efficacy
CM (1)	3254.29 ± 544.01	-	3.451± 0.575	-	-
LTN (2)	$1164.54^{Δ}$ ± 393.88	64.31	$1.223^{Δ}$ ± 0.415	64.56	++
PNO-1 (3)	$1507.62^{Δ†}$ ± 273.95	53.66	$1.591^{Δ†}$ ± 0.288	53.90	+
PNO-2 (4)	$1316.19^{Δ}$ ± 254.24	59.54	$1.402^{Δ}$ ± 0.269	59.37	+
PNS-1 (5)	$1099.03^{Δ‖}$ ± 197.65	66.18	$1.153^{Δ‖}$ ± 0.206	66.59	++
PNS-2 (6)	$913.44^{Δ¥β}$ ± 150.99	72.09	$0.958^{Δ¥β}$ ± 0.160	72.24	++

CM - cancer model; LTN - positive control; PNO - *P. notoginseng* roots without steaming; PNS - *P. notoginseng* roots with steaming; ^{Δ} – $p < 0.001$ compared to (1); † = $p < 0.05$ compared to (2); ‖ = $p < 0.01$ compared to (3) and $p < 0.05$ compared to (4); $^{¥}$ = $p < 0.001$ compared to (3) and $p < 0.01$ compared to (4); β = $p < 0.05$ compared to (5); n = 10

This study evaluated the antitumor effect of Vietnamese *P. notoginseng* roots with steaming (PNS) and without steaming (PNO) using 2 doses: 300 and 900 mg/kg. The doses used in the study were based on the results of acute toxicity assessment. In our previous study, we evaluated the acute oral toxicity of PNS in Swiss mice, resulting in a maximum tolerated dose of 6,000 mg/kg in mice.[22] According to the pharmacological dose estimation principle, the doses with pharmacological effects ranged from 1/20 to 1/5 of LD_{50} or the maximum tolerated doses.[23] The dose of 300 mg/kg is equal to 1/20 of the maximum tolerated doses.[20] PNS extract has 25.51 ± 0.20% total saponins, so 300 mg of PNS contains around 76.53 mg of total saponin, equivalent to the effective dose range according to research by Zhang *et al*.[24]

In our experiment, the tumor growth was assessed by measuring its size using a caliper with an accuracy of 0.1 mm. The tumor volume can be estimated relatively accurately from the largest and smallest diameter according to the calculation formula.[19] On day 21, the exact weight of the tumor was determined after dissecting it. The results (Table 4) show that the correlation between the measured tumor volume and tumor weight in all the groups changed significantly. The assessment of tumors by size and volume measurement was almost no different from the assessment by weight. Thus, the measurement of

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tumor size and volume to evaluate tumor growth used in the study was appropriate and highly accurate.

As shown in Figure 2 and Table 2, the tumor volumes of the LTN, PNO-1, PNO-2, PNS-1 and PNS-2 groups increased significantly more slowly than in the non-treatment group CM (Figure 2 and Table 2). Both PNS and PNO at both doses of 300 mg/kg and 900 mg/kg showed inhibitory effects on tumor growth (Tables 3 and 4). However, the steamed groups PNS-1 and PNS-2 showed the most obvious antitumor effect, which was statistically different from the non-steamed PNO-1 and PNO-2 groups ($p < 0.01$) (Table 2). This result agrees with previous studies of the *in vitro* antitumor effects of Vietnamese *P. notoginseng*.[17,25] Steamed *P. notoginseng* extract has consistently shown a better effect than non-steamed *P. notoginseng* extract on the growth inhibition of several cancer cells. This result is explained by the content of saponins with good antitumor effects, such as Rg3 and Rh1, being much higher in steamed samples than in non-steamed samples.[14,15]

The survival time after treatment is a clinically significant indicator, considered the most important factor to evaluate the overall effectiveness of cancer therapies. The results in Tables 5 and 6 and Figure 3 show that the first mouse died in the CMb group on day 22 after tumor induction, and the greatest number of mice died earliest in this group. The survival time of the mice in the lentinan and non-steamed PNO-1 and PNO-2 groups was longer than that of the mice without treatment ($p < 0.05$). The 2 steamed groups had a longer survival time than the untreated mice ($p < 0.01$), and PNS-2b had the greatest survival, at 55.12% (Table 6). Previously, *P. notoginseng* has been identified as having immune-enhancing and antioxidant effects, which are important in cancer treatment. These effects were stronger when *P. notoginseng* was used in the steamed form.[14,26]

Table 5: Number of the mice that survived in the study groups

Groups		Days after administration												
		1	22	32	42	52	62	72	82	92	102	122	142	145	149
CMb	n	20	19	17	12	8	3	1	0	0	0	0	0	0	0
	%	100	95	85	60	40	15	5	0	0	0	0	0	0	0
LTNb	n	20	20	19	16	14	11	9	8	6	3	1	0	0	0
	%	100	100	95	80	70	55	45	40	30	15	5	0	0	0
PNO-1b	n	20	20	18	16	13	11	8	6	5	2	0	0	0	0
	%	100	100	90	80	65	55	40	30	25	10	0	0	0	0
PNO-2b	n	20	20	19	17	14	12	9	7	5	3	1	0	0	0
	%	100	100	95	85	70	60	45	35	25	15	5	0	0	0
PNS-1b	n	20	20	20	17	15	12	9	8	6	4	1	1	0	0
	%	100	100	100	85	75	60	45	40	30	20	5	5	0	0
PNS-2b	n	20	20	20	18	16	13	10	8	6	4	2	1	1	0
	%	100	100	100	90	80	65	50	40	30	20	10	5	5	0

CM - cancer model; LTN - positive control; PNO - *P. notoginseng* roots without steaming; PNS - *P. notoginseng* roots with steaming; n - number of mice that survived; % - % mice that survived.

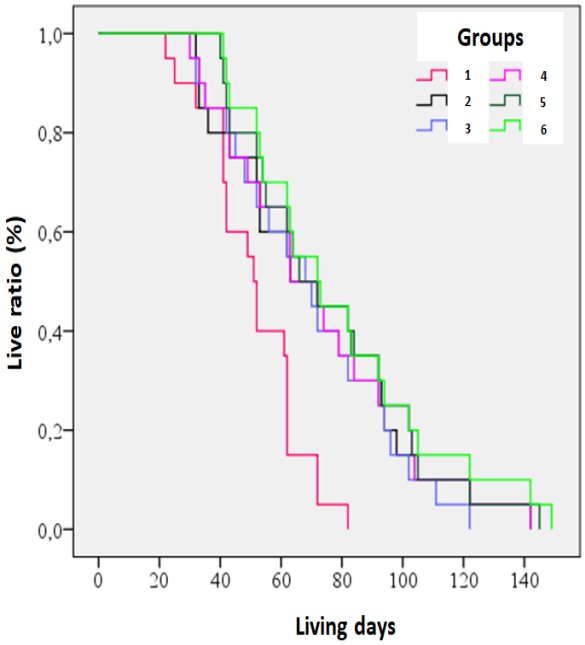

Figure 3: Ratios of the mice surviving over time

Table 6: Average survival time and extended survival of the mice

Groups	Survival time (day)	ILS (%)	p values
CMb (1)	51.25 ± 15.85	-	$p_{2.3.4\text{-}1} < 0.05$
LTNb (2)	72.10 ± 31.40	40.68	$p_{5.6\text{-}1} < 0.01$
PNO-1b (3)	69.50 ± 27.26	35.61	$p_{3.4.5.6\text{-}2} > 0.05$
PNO-2b (4)	72.40 ± 31.09	41.27	$p_{4.5.6\text{-}3} > 0.05$
PNS-1b (5)	75.95 ± 29.46	48.20	$p_{5.6\text{-}4} > 0.05$
PNS-2b (6)	79.50 ± 31.90	55.12	$p_{6\text{-}5} > 0.05$

CM - cancer model; LTN - positive control; PNO - *P. notoginseng* roots without steaming; PNS - *P. notoginseng* roots with steaming; Data of survival time are mean ± SD; n = 20; ILS - increased life span.

Conclusion

Extracts from Vietnamese *P. notoginseng* roots with steaming and without steaming at the doses studied had good antitumor effects on sarcoma 180 tumor-bearing Swiss mice. They reduced tumor volume and tumor weight and extended the survival time of mice. PNS had better antitumor activity than PNO, which increased with dose. The antitumor effect of steamed samples may be better than non-steamed samples due to the changed active saponin composition.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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