

**Standard antitumour activity test in Sarcoma 180/mice**

The most commonly used method to assess the antitumour activity of the mushroom polysaccharides is with the Sarcoma 180/ICR-5 Ic mouse model. (at a dose of 1-100 mg/kg body weight). The following method is taken from Mizuno (1999).

“A group of female mice (5-7 of JCB/Jcl strain, 7 weeks old) were used. Sarcoma 180 tumour cells (ca.  $5 \times 10^6$ ) were collected from a mouse on the seventh day after transplantation under the skin on the back of the right groin. Once a day for 10 days, a test sample (1-100 mg/kg) dissolved in a physiological saline solution, was injected intraperitoneally. After transplantation, the size of the tumour (diameter in millimetres or weight in grams) was recorded each week. During the fifth week, the tumour was enucleated and weighed and the inhibitory rate (%) was calculated and compared to that of the untreated group. After 45 days the tumour completely disappeared in some mice. In addition, a 50% inhibitory dose ( $ID_{50}$ ) was calculated to compare the antitumour efficacies of the test samples. The dose (8 mg/kg) was plotted along the abscissa, and the tumour inhibitory ratio (%) was plotted along the ordinate on semilogarithmic paper in the logarithmic scale of base 10 ( $\log_{10}$ ) to show the inhibitory ratio (actual value) of 3 to 5 doses of each test sample. From the straight line obtained, a dose of test sample indicating 50% inhibition was determined as  $ID_{50}$ . Because the primary screening by intraperitoneal administration was shown to be effective, a secondary screening by the oral administration was performed as follows. Sarcoma 180 tumour cells ( $2 \times 10^6$ , or a piece about 2 mm in diameter in the case of a solid cancer) were grafted. The test sample, dissolved in physiological saline solution, was administered orally after 3, 4, 6, 7, 8, 9, 10, 11, 13 and 14 days – 10 times in total. The diameter of the tumour (millimetres) was measured 25 days later to calculate the tumour growth inhibitory ratio (%). After 45 days, the mice showing complete disappearance of the tumour were counted. A reduction in body weight 3 to 15 days after grafting was examined together with the level of toxicity”.

**TABLE 1 Appraisal judgements in antitumour activity in Sacroma 180/mice by i.p. or p.o. administration.**

Inhibition ratio (%) <sup>a</sup> of tumour proliferation	Judgement (symbol)
0-25	Noneffective (-)
26-50	Slightly effective (±)
51-75	Effective (+)
76-95	Considerably effective (++)
96-100	Remarkably effective (+++)

<sup>a</sup> Inhibition ratio (% = 100(1-B/A). A = average tumour size (diameter, cm or cm<sup>3</sup>) in control group. B = average tumour size (diameter, cm or cm<sup>3</sup>) in treatment group.

## Reference

Mizuno, T. 1999. The extraction and development of antitumour-active polysaccharides from medicinal mushrooms in Japan. *International Journal of Medicinal Mushrooms* **1**, 9-29.

