Anti-tumor Activity and Survival Impact of Whole Adlay Hot Water Extract in Sarcoma Mouse Model

Hirotaka OKUWA-HAYASHI1,*, Tomihisa OHTA2, Satoshi OHNO3, Satoru KYO4, Nobutaka SUZUKI5

1The Faculty of Food Culture, Kurashiki Sakuyo University
2Kanazawa University, Venture Business Laboratory
3Clinical Research Support Center, Shimane University, Medical School
4Department of Obstetrics and Gynecology, Shimane University, Medical School
5Department of Complementary and Alternative Medicine Clinical Research and Development, Kanazawa University Graduate School of Medical Science

【ABSTRACT】
Background: Adlay (Coix lacryma-jobi L. var. ma-yuen Stapf) has been used both in traditional Chinese medicine and as a nourishing food based on its unique biological effects and highly nutritional values. In the present study, we investigated the anti-tumor effect of a hot-water adlay extract in sarcoma mice model.

Materials and Methods: The hot water extract of whole adlay was orally administered to mice for one week, after which Sarcoma-180 cells (1 × 10^6) were subcutaneously implanted into the abdomen. Thereafter, the tumor growth was monitored and mouse survival was examined.

Results: Tumor weights measured at 18 days were significantly lower in mice treated with extract (100 and 300 mg/kg/day) than those in control group (p<0.01). Moreover, mice treated with extract (100 mg/kg/day) showed apparently longer survival than control group evaluated until 32 days (p<0.05).

Conclusion: These findings indicate that hot water adlay extract appears to have some anti-tumor effects in vivo in sarcoma cells.

【Key words】whole adlay, hot water extract, Sarcoma, anti-tumor effect, survival, mouse

INTRODUCTION
Adlay (Coix lacryma-jobi L. var. ma-yuen Stapf) is an annual crop in China. It has widely been used in traditional Chinese medicine or as a nourishing food with special biologic effects.

Emerging evidence has recently revealed that adlay extract have wide variety of anti-tumor activity in vitro and in vivo. The ethyl acetate-soluble fraction of methanol extract of adlay bran (ABM-EtOAc) exhibited a stronger anti-proliferative effect on various human lung and colorectal lines than other fractions confirmed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) assay. Woo et al. reported that the adlay extract emulsion exhibited growth inhibitory effect on xenografts of MDA-MB-231 breast cancer cells. The neutral lipid isolated from

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3515, Nagao, Kurashiki-shi, Okayama, 710-0292, Japan Tel: + 81-86-436-0299 E-mail: hirotaka.ohkuwa@ksu.ac.jp
the endosperm of adlay induced a dose- and time-dependent inhibition of proliferation in both PaTu-8988 and SW1990 human pancreatic cancer cells. Notably, the regulation of the specific protein expressions by adlay extract have been reported. For instance, Shih et al. reported that rats fed with diets containing 20% dehulled adlay seeds had lower expression of cyclooxygenase-2 (COX-2) in colon tumors. The methanol extract of adlay seed was also tested for its regulation of COX-2 expression of human lung cancer cells, in which the Western blot analysis showed the inhibition of basal and TPA-induced COX-2 expression in a dose-dependent fashion. Furthermore, the effects of the extract from different parts (hull, testa, bran, and polished adlay) of adlay on antiproliferative activity has been reported in malignant human cells. Numata et al. reported that the acetone extract of dehulled adlay inhibited growth of sarcoma 180 cell transplanted into mice and the active components were four free fatty acids; palmitic, stearic, oleic, and linoleic acids. Qian et al reported that coix seed extract could augment the efficacy of gemcitabine therapy in pancreatic cancer cells.

Based on these scientific backgrounds, we sought to investigate the anti-tumor activity of hot-water extract of husks, pellicles, astringent skin and grain of adlay in vivo.

MATERIALS AND METHODS

[Test sample preparation]

The hot water extract of the husks, pellicles, astringent skin and grains of adlay was used for test article (Fig.1). The hot water extract of the husks, pellicles and astringent skin of adlay can be obtained by any existing method. Specifically, adlay with husks (grains with husks) are washed well with water, and then dried sufficiently. The husks are then lightly ground with a rice-milling machine. After the dehulling treatment, adlay grains are separated into undeckled grains and dehulled grains using a sieve with a mesh size of approximately 3.5 (5.6 mm). The undeckled grains are treated again with a rice-milling machine, thereby obtaining the husks, pellicles or astringent skin. At this time, it is necessary to adjust the strength of rice-milling to a degree such that the grains are not broken. Seven liters of water is added to 1 kg of the grain, husks, pellicles and astringent skin, so that these parts are immersed in water for 1 hour. The solution containing these parts was gradually heated for 60 minutes to be boiled, followed by further boiling of 60 minutes. Subsequently, the solution is concentrated by vacuum centrifugation for 60 minutes while heating to 40°C to 50°C. The product is then cooled, sterilized 98°C for 30 minutes, then dried by a spray-dry method.
Experimental procedures were approved by Committee on Animal Experimentation in Kanazawa University, Ishikawa, Japan. Male C3H/HeN (age, 4-weeks) were obtained from Charles River Japan Inc., (Yokohama, Japan). Mice were kept in a room maintained at controlled temperature (23 ± 2 ºC), humidity (55 ± 10 %), and lighting (9:00 to 21:00 hours) and 3 or 4 rats were housed in one cage. The animals were given free access to solid feed (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water.

Group condition and dose levels: at 5-weeks of age, mice were randomly divided into four groups; control (aqueous solution of dextrin), 50, 100 and 300 mg/kg of the test article administered (n=8). The test article was dissolved in distilled water and was administered intragastrically by direct stomach intubation from 6 weeks of age.

[In vivo growth and evaluation of anti-tumor effect]
Sarcoma 180 cell (1×10^6 cells) were implanted subcutaneously into the abdomen of mice at 6 weeks of age. At 18 days after implantation of Sarcoma 180 cells, the subcutaneous tumors formed under the skin of abdomen were enucleated and weighted.

[Evaluation of life duration]
At 5-weeks of age, mice were randomly divided into two groups: control (aqueous solution of dextrin) and 100 mg/kg of the test diet administered (n=8). Sarcoma 180 cells (1×10^6) were implanted subcutaneously onto the abdomen of mice at 6 weeks of age. After implantation, the survival of each mouse was observed. The increase in life-span (ILS) was calculated according to the following formula: 

\[ ILS (%) = \frac{\text{mean survival days of test sample group}}{\text{mean survival days of control mice}} - 1 \] \times 100.

RESULTS
Mice fed with hot water extract of adlay diet appeared to be healthy, lacking any signs of disease or abnormalities during the period of diet administration. There was no significant difference in body weight gain between the control and the diet groups fed with the 50 mg/kg extract, 100 mg/kg or 300 mg/kg extract (Fig.2). Moreover, the weight of liver, kidney and spleen collected after ventrotomy did not differ among the three groups (date not shown). These data suggest that the test dose of adlay hot water extract used in the present study was not toxic and can safety be used for the subsequent analysis.

Then, we examined the antitumor effect of hot water extract of adlay on the subcutaneously implanted Sarcoma 180 cells in mice. The tumor weight at 18 days after implantation was measured and found to be significantly lower in 100 mg/kg or 300 mg/kg adlay-extract group compared with that in the control group (p<0.01, p<0.01) (Fig.3). However, there was no statistically significant difference of the tumor weight
between both groups.

Survival impact of the extract was then analyzed treated (100 mg/kg/day) and untreated groups (Fig.4). The observation was conducted for 32 days after the tumor implantation. In the control group, mice began to die at 20 days after the tumor implantation, and the 57% of control group mice were dead at 32 days. In contrast, treated group have not been dead until 32 days and only 10% of the treated group died at 32 days. Statistical comparisons of the survival curves revealed that survival rates of treated mice were significantly improved compared with the untreated group until 32 days (p<0.05). There is no significant difference compared between treated group and control groups after 32 days. The increase in life-span of the mice treated with extract (%) was 15.34%.

**DISCUSSION**

Recently, the prevention of diseases using food has been started to garner attention. The purpose of our study is to examine the antitumor or cancer preventive effect of adlay hot water extract. Some studies have indicated that adlay possesses various pharmacological effects including...
anti-cancer, anti-obesity, hypolipemic, anti-diabetic, and osteoporosis prevention. Numata et al. reported that the four fatty acids; palmitic, stearic, oleic, and linoleic acids; isolated from adlay seeds by the acetone extraction have life-extending effect on mice implanted Sarcoma 180 cells mice in a dose dependent manner. In the present study, we demonstrated that hot-water extract of adlay have anti-tumor activity and life-extending effect on mice implanted with Sarcoma 180. However, the activity appeared to be lower than the acetone extract. It has been reported that the alcohol and acetone extract of adlay seed contains fatty acids, which may be critical for the antitumor activity.

We tested the anti-tumor activity of hot water extract of adlay using other cell types. Interestingly, the effects significantly differed in cell types; the proliferation of Caski cells derived from cervical cancer was not inhibited by the extract using in the present study (data not shown). These findings indicate that the effects of hot water extract of adlay were cell-type specific. Although the species mechanisms of these diversity remain unclear, we should pay special attention to the experimental design for dissecting the roles extract in tumor biology. In the present study, we propose that these extract should be used as food with tumor preventive actions, rather than that with therapeutic actions, because the tumor activity is relatively low and the actions appear to be cell-type specific. Nevertheless, our data suggests that hot water extract of adlay have anti-tumor activity and exhibited survival impact in vivo without any significant adverse effects. It is possible that new bioactive ingredients are contained in the extract. We are currently identifying such active ingredients obtained from the hot water extract of adlay.

CONCLUSION

Hot water extract of whole adlay (Coix lacryma-jobi L. var. ma-yuen Stapf) have anti-tumor activity and survival impact in vivo.

SUBVENTION

No special mention.

CONFLICTS OF INTEREST

No special mention.

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ハトムギ全粒熱水抽出物は、抗腫瘍効果を有することが示唆された。

キーワード：ハトムギ全粒, 熱水抽出物, 肉腫, 抗腫瘍効果, 生存, マウス