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The impact of *Apocynum venetum* tea flavonoids on G422 glioma *in vivo*

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Abstract

This study looked at the inhibitory effects of *Apocynum venetum* tea flavonoids extract (AVTFE) on mouse glioma G422 cells. Through the use of high performance liquid chromatography, the flavonoids in AVTFE were examined (HPLC). Meanwhile, mice were injected with glioma G422 cells to create a mouse model of a transplanted tumor. AVTFE might significantly ($P < 0.05$) lower serum sIL-2R content and raise serum IFN-γ content in tumor-bearing mice, according to an analysis of mouse serum. In addition to reducing the rise in spleen weight and spleen index in mice brought on by transplanted tumors, AVTFE can limit the development of transplanted tumors in G422 cell mice. AVTFE can also boost immunity by encouraging T and B lymphocyte proliferation in tumor-bearing animals. AVTFE was therefore verified to have immunomodulatory properties and to limit the proliferation of glioma G422 cells.

Keywords: *Apocynum venetum* tea; G422 cells; glioma; immunity; spleen.

Practical Application: *Apocynum venetum* tea is a type of health tea that is thought to contain a variety of biological functions, but the study on its precise effects and processes is missing. According to this study, *Apocynum venetum* tea can limit the formation of gliomas, probably because it helps to regulate the immune system. The findings of the study can help the preparation and use of *Apocynum venetum* tea more effectively.

1 Introduction

The uncommon wild plant *Apocynum venetum*, also known as wild hemp, wild tea, camellia, etc., has been referred to as "fairy grass" for centuries. The name *Apocynum venetum* was given to this herb, which has been growing wild in the Luobu Plain in Yuli County, Xinjiang, China, for more than 15 years. In Northwest China, particularly in Dunhuang, Gansu, Hami, Korla, and other locations in Xinjiang, *Apocynum venetum* is very common (Feng et al., 2020). These locations have a unique soil composition and a very high salinity. The finest among them is the *Apocynum venetum* found in Xinjiang's arid region. Traditional tea manufacturing methods are used to create tea from *Apocynum venetum* leaves, which is convenient for everyday use. The flavonoids, organic acids, phenylpropane, various amino acids and minerals found in *Apocynum venetum* tea have been verified through animal experiments and human clinical experiments that *Apocynum venetum* tea has physiological and therapeutic effects on human body, including blood pressure control, cholesterol reduction, anti-aging, immune improvement and antidepressant effects (Zhang et al., 2010).

Glioma, referred to as glioma, is a tumor that occurs in the neuroectoderm. Glioma incidence is tightly correlated with both lifestyle and inheritance. In North America, Western Europe, Australia, and New Zealand, gliomas are quite common (Singh et al., 2012). According to certain research, the food habits of the locals may be connected to this geographic dispersion. Therefore, one of the most efficient ways to prevent glioma is by the consumption of natural plant active components (Wan & Huang, 2022). Immunity and cancer have a specific interaction. The normal immune system of the human body will promptly launch an immune response and swiftly eliminate cancer cells to prevent the development of cancer, hence maintaining the stability of the human body, when normal cells mutate to produce cancer cells. Cancer will develop when there is a significant flaw or when human immunity is lacking (Du et al., 2021). The formation of tumors continues as a result of the human body's inability to eradicate cancer cells in the early stages of the disease. The metastasis process of tumors is also influenced by a number of immunological factors in the body. It may be claimed that elements like immune response and immunological modulation of human immunity are not involved in the entire process of tumor occurrence, development, invasion, and metastasis. Malignant tumors like glial carcinoma pose a grave threat to the life of their victims (Vinay et al., 2015). This study used animal studies to perform a preliminary investigation into the anti-cancer properties of *Apocynum venetum* tea flavonoids extract (AVTFE), which gathered some theoretical support for the use and advancement of *Apocynum venetum* tea.

2 Materials and methods

2.1 Apocynum venetum tea flavonoids extract

In preparation for usage, FL-3 macroporous resin was prepared with a certain amount of ethanol and hydrochloric acid, then soaked in distilled water. 500 g of Apocynum

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venetum tea were crushed and sieved, 70 percent ethanol (20:1) was added, and the extract was heated to 60 degrees Celsius for three hours. In order to acquire the flavonoid extract, the macroporous resin was first eluted with 70% ethanol until it was colorless. The flavonoid extract was then spun for three hours using a rotary evaporator, dried, and crushed to create sample powder.

2.2 HPLC analysis of sample components

Make a mixed standard solution by precisely weighing the standard product, dissolving it with chromatographic pure methanol, and adding it. AVTFE powder was accurately weighed into a 10 mL volumetric flask using a methanol bandwidth assessment, sonicated, and then filtered via a 0.45 m filter membrane at the same time. After that, component analysis was carried out using HPLC. Column AgilentzorbaxSB-C18 (5 μm, 4.6 × 250 mm), layer elution, acetonitrile as mobile phase A and 0.5% acetic acid solution as mobile phase B, flow rate of 0.6 mL/min, column temperature of 35 °C, detection wavelength of 359 nm, and injection volume of 10 μL were the chromatographic parameters.

2.3 Modeling, grouping and gavage of experimental animals

Five healthy ICR mice were reared for two weeks after receiving a subcutaneous injection of in vitro glioma G422 tumor cell suspension in the right axilla. After that, the G422 tumor tissue that had been subcutaneously injected into the right axillary of the mice was chopped, crushed, and sieved. The tumor cells were then resuspended in five times the volume of regular saline after being spun at 1000 rpm for five minutes. Six-week-old, healthy ICR mice were divided into four groups: normal group, model group, AVTFE low concentration (L-AVTFE) group, and AVTFE high concentration (H-AVTFE) group. A total of 40 mice were used for each group. All of the mice in the other groups, except from those in the normal group, had 0.2 mL/mice of glioma G422 tumor tissue cell solution injected beneath their right axillary skin (Zhang et al., 2013). Mice in the L-AVTFE and H-AVTFE groups were gavaged with AVTFE at 50 and 100 mg/kg per day for two weeks, respectively, whereas mice in the normal group and model group received 0.2 mL of distilled water daily.

2.4 Serum sIL-2R content in mice

On the second day following the gavage sample, blood was drawn using the eyeball removal procedure, the serum was regularly separated, and the presence of sIL-2R was assessed using a double-antibody sandwich ABC-ELISA in accordance with the kit's instructions (Long et al., 2022).

2.5 Mouse serum IFN-γ content

On the second day following the gavage sample, blood was drawn using the eyeball removal technique. Serum was regularly separated and, using a mouse enzyme-linked immunosorbent assay (ELISA) IFN-γ kit, the amount of IFN-γ was measured in the mouse serum (Hu et al., 2022).

2.6 Determination of tumor inhibition rate and spleen index

The tumor weight was immediately fully excised after blood was collected, the mice were slaughtered, the cervical spine was dislocated, the tumor mass' weight was determined, and the average tumor weight and tumor inhibition rate of each group were computed. The spleen was fully removed during the tumor removal process, and its weight was measured. The spleen index was computed by comparing the differences in spleen weight between each group and the model group. Tumor inhibition rate (%) = [1- (average tumor weight in AVTFE group/average tumor weight in model group)] × 100%; spleen index = spleen weight (mg)/body weight (g).

2.7 Spleen lymphocyte proliferation assay

After blood was drawn, mice were killed by cervical dislocation, their spleens were aseptically separated, and splenic lymphocytes were produced as per standard procedure. Using RPMI 1640 medium with 10% calf serum, the cell density was increased to 1×10^7/mL. The splenocytes were mixed with ConA, and the aforementioned cell suspension was added to each well of the 96-well culture plate, with one control well remaining empty. The culture plate was cultured for 48 hours at 37 °C with saturated humidity of 5% CO_2 to determine the cell proliferation response using MTT method (Zhang et al., 2013). With the use of a microplate reader, the OD value of each well was determined at 570 nm.

2.8 Statistical method

Software called SPSS 22.0 was utilized for the statistical evaluation. The measurement information was written as (x ± s). One-way analysis of variance was used, and $P < 0.05$ was used to determine statistical significance of the difference.

3 Results

3.1 Composition of Apocynum venetum tea flavonoids extract

Rutin and isoquercitrin were the two primary flavonoids identified by HPLC analysis in the AVTFE, with isoquercitrin having a substantially greater concentration than rutin (Figure 1).

3.2 Serum sIL-2R content in tumor-bearing mice

The model group had the greatest concentration of sIL-2R, as seen in Table 1. AVTFE can lower the level of sIL-2R in tumor-bearing mice's blood when compared to the model group ($P < 0.05$). Additionally, the content of sIL-2R was closer to the normal group as AVTFE concentration increased.

Table 1. Effects of *Apocynum venetum* tea flavonoids on serum sIL-2R levels in tumor-bearing mice.

Group	Sample dose (mg/kg b.w.)	sIL-2R (pmol/L)
Normal	/	24.16 ± 2.34^{D}
Model	/	73.58 ± 4.11^{A}
L-AVTFE	50	51.89 ± 3.92^{B}
H-AVTFE	100	42.35 ± 1.71^{C}

A-D: Significant differences between the related groups are denoted by different letters ($p < 0.05$).

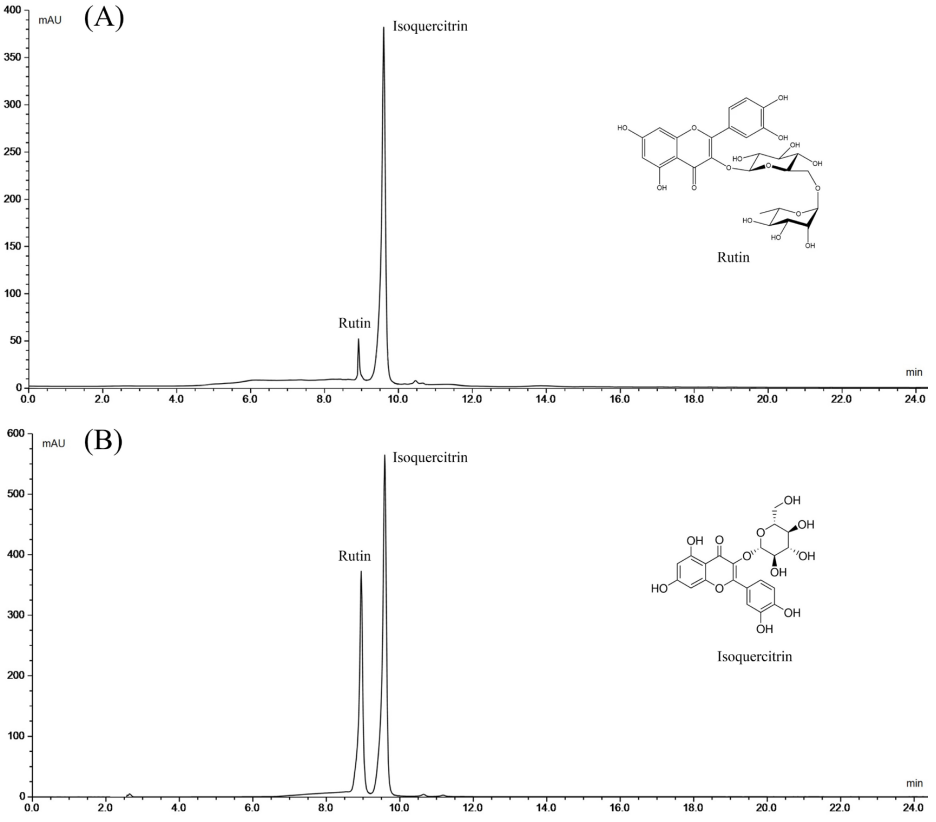

Figure 1. HPLC chromatograms of *Apocynum venetum* tea flavonoids (A) and mixed standards (B).

3.3 Serum IFN-γ content in tumor-bearing mice

Table 2 demonstrates that there was a statistically significant ($P < 0.05$) difference in the levels of IFN-γ in the model group and the normal control group. It was statistically significant ($P < 0.05$) that the levels of IFN-γ in the L-AVTFE and H-AVTFE groups were greater than those in the model group, with the H-AVTFE group being closest to the normal group.

3.4 Tumor weight in tumor-bearing mice

AVTFE considerably ($P < 0.05$) decreased the tumor weight of the tumor-bearing mice as compared to the model group (Table 3), with H-AVTFE having a higher effect than L-AVTFE in doing so. The tumor suppression rate was thus greater in H-AVTFE.

3.5 Spleen weight and spleen index in tumor-bearing mice

According to Table 4, there was a statistically significant ($P < 0.05$) difference between the spleen weight and spleen index of the model group and the normal group. In tumor-bearing mice (the model group), AVTFE can lower spleen weight and spleen index, and its concentration is favorably connected with these changes.

3.6 Proliferation of T and B lymphocytes in tumor-bearing mice

The difference between the OD values of T and B lymphocytes in the model group and those in the healthy control group was statistically significant ($P < 0.05$, Table 5). T and B lymphocyte OD values were substantially ($P < 0.05$) greater in the low and high dosage AVTFE groups (L-AVTFE and H-AVTFE) than in the model group, but significantly ($P < 0.05$) lower than in the normal group.

Table 2. Effects of *Apocynum venetum* tea flavonoids on serum IFN-γ levels in tumor-bearing mice.

Group	Sample dose (mg/kg b.w.)	sIL-2R (pmol/L)
Normal	/	1254.59 ± 35.12^{A}
Model	/	835.78 ± 22.67^{D}
L-AVTFE	50	912.78 ± 24.65^{C}
H-AVTFE	100	1081.55 ± 36.42^{B}

A-D: Significant differences between the related groups are denoted by different letters ($p < 0.05$).

Table 3. Effects of *Apocynum venetum* tea flavonoids on tumor weight in tumor-bearing mice.

Group	Sample dose (mg/kg b.w.)	Tumor weight (g)	Tumor inhibitory rate (%)
Model	/	2.25 ± 0.27^{A}	/
L-AVTFE	50	1.73 ± 0.24^{B}	23.06 ± 7.84^{B}
H-AVTFE	100	0.83 ± 0.14^{C}	51.78 ± 7.74^{A}

A-C: Significant differences between the related groups are denoted by different letters ($p < 0.05$).

Table 4. Effects of *Apocynum venetum* tea flavonoids on spleen weight and spleen index in tumor-bearing mice.

Group	Sample dose (mg/kg b.w.)	Spleen weight (mg)	Spleen index rate (mg/g)
Normal	/	128.65 ± 7.62^{D}	4.01 ± 0.42^{C}
Model	/	210.55 ± 8.33^{A}	6.88 ± 0.69^{A}
L-AVTFE	50	172.05 ± 8.91^{B}	5.55 ± 0.65^{B}
H-AVTFE	100	147.31 ± 6.88^{C}	4.64 ± 0.35^{C}

A-D: Significant differences between the related groups are denoted by different letters ($p < 0.05$).

Table 5. Effects of *Apocynum venetum* tea flavonoids on T and B lymphocyte proliferation in tumor-bearing mice.

Group	Sample dose (mg/kg b.w.)	T lymphocyte OD value	B lymphocyte OD value
Normal	/	0.625 ± 0.034^{A}	0.595 ± 0.017^{A}
Model	/	0.471 ± 0.031^{D}	0.325 ± 0.015^{D}
L-AVTFE	50	0.525 ± 0.017^{C}	0.442 ± 0.025^{C}
H-AVTFE	100	0.590 ± 0.023^{B}	0.527 ± 0.019^{B}

A-D: Significant differences between the related groups are denoted by different letters ($p < 0.05$).

4 Discussion

Flavonoids are abundant in plant foods such as fruits, vegetables, tea leaves, and so on. Natural flavonoids have been proven in studies to be a class of chemicals with considerable anti-glioma efficacy, as well as low toxicity and side effects and excellent safety (Hardinasinta et al., 2022; Yin et al., 2022). Natural phytoflavonoids can suppress gliomas across the blood-brain barrier and can have a role in glioma resistance reversal. The drug's impact as an adjuvant in the treatment of gliomas (Kiskova et al., 2020). As a result, the flavonoids in *Apocynum venetum* tea were also investigated in this study.

The polypeptide SIL-2R is generated by activated lymphocytes and core solitary cells. It is a significant immunosuppressive agent. It can compete for IL-2 binding with the membrane interleukin-2 receptor (mIL-2R). Neutralize activated T cells and surrounding IL-2, operate as blocking factors, restrict T cell growth, and impair immunological activity in the body (Zhang et al., 2022). IFN-γ is a glycoprotein with significant immunomodulatory properties that can increase cellular performance, speed up immunological complex clearance, and improve phagocytic foreign body function, as well as having a bidirectional regulatory influence on lymphocytes (Shao et al., 2017). Both the AVTFE low-dose and high-dose groups dramatically reduced the serum sIL-2R level of tumor-bearing mice in this experiment, and the AVTFE high-dose group additionally increased the serum IFN-γ content of tumor-bearing mice, indicating that it was immune to tumor-bearing mice. The function has an influence on improvement and adjustment.

The spleen is a vital immunological organ in the human body, with the primary role of regulating immunity. Physical lesions, particularly the influence on the immune system following the formation of malignancies, can be represented in splenic lesions (Shakoor et al., 2022). The spleen index is a test for detecting spleen lesions in animals. A divergence from the index's normal state indicates that the spleen is sick (Aghili et al., 2014). This study also proved that the immune system of mice has issues as a result of glioma cell inoculation, which affects the spleen and alters the spleen index, and AVTFE can suppress this spleen alteration.

When a tumor develops in the body, the immune system can respond to tumor cells in a variety of ways and eliminate malignant cells (An et al., 2022; Shafay et al., 2022; Kesika et al., 2022). T lymphocyte-mediated cellular immunity and B lymphocyte-mediated humoral immunity both play essential roles in the body's anti-tumor response (Mediavilla-Varela et al., 2017). The low and high dose groups of AVTFE were shown to considerably stimulate the proliferation of T and B lymphocytes in tumor-bearing mice caused by ConA, and when the dose was increased, the amount of tumor weight gradually reduced, indicating that AVTFE can improve the body's immune function. The function has anti-glioma properties.

Rutin and isoquercitrin are both flavonoids with good antioxidant activity. Due to their excellent antioxidant capacity, they play an important role in enhancing immunity and inhibiting tumor by regulating the antioxidant stress of the body (Lin et al., 2012; Shimada et al., 2010). Rutin and isoquercitrin are the core compounds of AVTFE, and the effect of AVTFE on glioma is derived from the combination of these two flavonoids

5 Conclusion

In this study, the flavonoids, which are the primary active components of *Apocynum venetum* tea's health benefits, were examined. Animal studies were used to examine the effect of *Apocynum venetum* tea flavonoids on glioma, and it was discovered that these flavonoids may reduce glioma through modulating immunity. As a result, it can be said that *Apocynum venetum* tea is a premium health beverage with glioma-related properties.

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