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ORIGINAL ARTICLE

In vivo Antitumor and Immune Effects of *Paris polyphylla* Rhizome and Tinosporae Radix Extracts Administered by Different Modes

Yonghong-Liang[1]*, Xiuying-Huang[2], Xunli-Xia[3], Huiying-Yao[3], Yu-Lu[1]

[1]Key Laboratory of Modern Preparation of TCM, Jiangxi University of Traditional Chinese Medicine, Ministry of Education, Nanchang 330004, [2]Library of Jiangxi University of Traditional Chinese Medicine, [3]College of Life Sciences, Jiangxi University of Traditional Chinese Medicine, Nanchang, China

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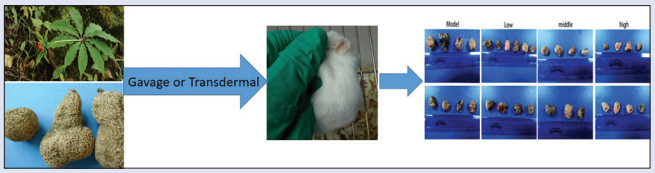
ABSTRACT

Objectives: To investigate the effects of gavage and transdermal administration on the anti-tumor activity of QJ623 (extracts of *Paris polyphylla* rhizome and Tinosporae Radix) on a murine H22 solid tumor transplantation model. **Materials and Methods:** H22 cell suspensions were diluted to a density of 4×10^5 cells/mL with sterile saline and then injected into 6-week-old mice subcutaneously (0.2 mL) into the right anterior axilla to create a murine model of liver cancer. Tumor tissues were collected after drug administration. The tumor growth-suppression rate was calculated, and the tumor tissues were stained with hematoxylin and eosin to detect necrosis. Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling assay was performed to observe the apoptosis. The expression of p-ACK1, p-AKT, Bax, Bcl-2, and Caspase3 in the tumor tissues was detected by western blotting. Bio-chemical kits were used to detect the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cre), blood urea nitrogen (BUN), and uric acid (UA) for liver and kidney function analyses. Serum levels of IL-6, TNF-α, IFN-γ, and IL-10 were determined using enzyme-linked immuno-sorbent assay. **Results:** Gavage and transdermal administration of QJ623 at different concentrations reduced the tumor mass and volume to different degrees; promoted tumor cell apoptosis; decreased the expression levels of the p-AKT and Bcl-2 proteins; increased the expression of the apoptotic proteins Bax and Caspase3; increased the number of Th1 (IFN-γ) cells; decreased the number of Th2 (IL-4) and Treg cells; reduced the serum AST, ALT, BUN, UA, IL-6, TNF-α, and IFN-γ levels; and increased the IL-10 levels. **Conclusion:** Both gavage and transdermal administration of QJ623 showed anti-tumor effects by promoting tumor cell apoptosis, decreasing the level of inflammatory factors, increasing the number of Th1 (IFN-γ) immune cells, and decreasing the numbers of Th2 (IL-4) and Treg immune cells.

Key words: Immune effect, liver cancer, *Paris polyphylla* Rhizome, *and Tinosporae Radix* extracts

SUMMARY

- Liver cancer is the most common cancer worldwide, with complex and diverse causative factors and a very poor prognosis. In this study, QJ623 administered by gavage and transdermally was found to promote apoptosis of tumor cells, reduce the level of related inflammatory factors, and increase immunity, thus reaching anti-tumor effects.



Abbreviations used:
AST: Aspartate transaminase; ALT: Alanine transaminase; Cre: Creatinine; BUN: Blood urea nitrogen; UA: Uric acid; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; IFN-γ: Interferon-γ; IL-10: Interleukin-10.

Correspondence to:

Dr. Yonghong-Liang,
Key Laboratory of Modern Preparation of TCM,
Jiangxi University of Traditional Chinese Medicine,
Ministry of Education, Nanchang - 330004, China.
E-mail: yonghong_liang2008@126.com
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INTRODUCTION

Liver cancer is the fifth deadliest cancer; its incidence continues to increase every year.[1] Many different and complex factors, such as liver disease and alcohol-related cirrhosis, are responsible for its onset.[2] The prognosis for hepatocellular carcinoma (HCC) is poor. Approximately 5–15% of patients in the early stages are suitable for surgical resection. The immune system participates in the killing of cancer cells.[3] In addition, targeting bio-markers of tumor growth and connective tissue proliferation is required to prevent tumor progression.[4] The poor prognosis of HCC has prompted researchers to search for new treatment options. Combining drugs and changing delivery methods open new avenues for improving malignancy.[3]

Paris polyphylla (P. polyphylla) is a perennial herb from the *Paris* genus (family, Liliaceae). It is effective in reducing swelling, relieving pain, clearing heat, de-toxifying the body, cooling the liver, and calming panic. *P. polyphylla* rhizome has anti-bacterial, anti-inflammatory, hemostatic, and immuno-modulatory effects.[5] The pharmacopeia records that *P. polyphylla* rhizome is the main source of Chonglou, and its chemical composition mainly includes steroidal saponins.[6,7] It exerts anti-cancer effects by inducing apoptosis, promoting anti-angiogenesis, and synergizing with chemotherapeutic drugs. The gracillin component of *P. polyphylla* rhizome can inhibit the growth of six types of human tumor cells: lung cancer (A-549), breast cancer (MCF-7), colon cancer (HT-29), kidney cancer (A-496), pancreatic cancer (PACA-2), and prostate cancer (PC-3).[8] The Chinese

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herbal medicine Tinosporae Radix is included in the "Pharmacopeia of the People's Republic of China". It can also clear heat, de-toxify the body, alleviate sore throat, and relieve pain. Moreover, it also has anti-inflammatory and anti-tumor effects[9] and is also known as "a natural alternative to antibiotics".

We aimed to investigate the *in vivo* anti-tumor and immunological effects of extracts of *Paris polyphylla* and *Tinosporae Radix* on H22 tumor-bearing mice; this experiment was performed to investigate the anti-tumor effects of gavage and transdermal administration of extracts of both drugs in a murine H22 solid tumor transplantation model.

MATERIALS AND METHODS

Chemicals and reagents

Paris polyphylla Rhizome *and* Tinosporae Radix extracts were obtained from Jiangxi Apsdal Biotechnology Co., China; Dulbecco's modified Eagle's medium (Hyclone, SH30022.01B) was obtained from Hyclone; an *in situ* Cell Death Detection Kit (11684817910) was obtained from Roche; IFN-γ-APC (505810) and IL-4-PE (504104) were obtained from Biolegend; CD4-FITC (85-11-0041-81), CD25-PE-Cyanine5 (15-0251-82), and Foxp3-PE (12-5773) were obtained from eBioscience; Neutral resin (PAB180017), a terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) apoptosis detection kit (PAB180028), a BCA kit (PAB180007), hematoxylin (PAB180015), eosin (PAB180016), phosphate-buffered saline (PAB180003), 0.25% trypsin (PAB180002), and enzyme-linked immuno-sorbent assay (ELISA) kits for IL-6 (MU30044), IFN-γ (MU30038), IL-10 (MU30055), and MU30055 (MU30030) were obtained from Bioswamp.

Cell lines

H22 mouse HCC cells were purchased from Procell Life Science and Technology Co., Ltd. (Wuhan, China). Cells were cultured and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C with 5% CO_2.

Construction of the mouse model

H22 cells were removed, digested with trypsin cell digest (containing ethylenediaminetetraacetic acid), centrifuged at 1000 × *g* for 5 min, and re-suspended in a serum-free medium to make a cell suspension. Next, the cells were counted, diluted with sterile saline to a concentration of 4×10^5 cells/mL, and then injected subcutaneously into the right anterior axilla of mice (0.2 mL each). Tumor volume was calculated using the following formula: V= (length × width^2)/2.

Experimental animals and grouping

Six-week-old male BALB/C mice weighing 18–22 g were used in the study. All animals were purchased from the Hubei Provincial Experimental Animal Research Center [Animal certificate no: 42000600031684, production license number SCXK (E) 2015-0018]. The mice were divided into eight groups of four mice each (n = 4). The eight groups include two model groups (gavage/transdermal), two low-dose groups (gavage/transdermal), two medium-dose groups (gavage/transdermal), and two high-dose groups (gavage/transdermal). The tumor cells were injected according to the grouping. The drug administration was started after 5 days. The drug was administered twice daily for 10 days; the model group was administered an equal amount of saline. Sampling was performed 24 h after drug administration on day 10. The experimental period lasted 16 days.

Preparation of drug delivery samples

Preparation of samples for gavage administration: 500 g of herbal powder of *Paris polyphylla* and 500 g of powder of Tinosporae Radix were taken and decoct with water at 10–12 times the total herbs for 1.5–2 hours. Eight to ten times the amount of water was added for the second time, and the mixture was decocted for 1–1.2 hours. Six to eight times the amount of water was added for the third time, and the mixture was decocted for 0.8–1.2 hours. Then, the three decoctions were combined and filtered; the filtrate was collected and concentrated to make a clear paste. The clear paste was left at room temperature, and ethanol was added to bring the ethanol content to 60–75% by weight. After standing for 24–48 h, the supernatant was extracted; the supernatant was recovered from ethanol, concentrated under reduced pressure, then centrifuged at 3000r/min, and spray-dried at 60–80°C to obtain the dry extract, ready for use. The infusion was divided into low (0.292 g/kg), medium (0.584 g/kg), and high dose (1.168 g/kg) groups. Preparation of transdermal topical drug delivery samples: 200 g of Hepatica spp. herb and 200 g of Gymnema Sylvestre herb were taken. Preparation was performed in the same way as described above. Before use, the infusion was divided into low (0.292 g/kg), medium (0.584 g/kg), and high dose (1.168 g/kg) groups. The preparation method is carried out with reference to the previous patent of the invention.[10]

Tumor inhibition rate

After drug administration on day 10, the mice were sacrificed by an overdose of sodium pentobarbital and sampled. The tumor mass inhibition and tumor volume inhibition were calculated as follows: tumor mass inhibition index = (W-L)/W × 100%, where W is the tumor mass of the model group and L is the tumor mass of the drug administration group; tumor volume inhibition tumor volume = 1/2 × a × b^2, where a is the maximum surface diameter of the tumor and b is the minimum surface diameter of the tumor.

Hematoxylin and eosin staining

Hematoxylin staining was used to observe the necrosis and disintegration of tumor cells. The tumors tissues were fixed in 10% neutral formalin for 1–2 days. Tumor tissues with a thickness of approximately 0.2–0.3 cm and a size of 1.5 cm × 1.5 cm × 0.3 cm were de-hydrated and embedded in immersion wax. Tissue sections (4–5 μm thick) were prepared and de-waxed in water, stained with hematoxylin solution for 3 min and 0.5% eosin solution for 3 min, sealed with neutral gum, and photographed by microscopy on a Leica Application Suite image system. The relevant sections of the samples were observed and analyzed.

TUNEL assay to detect apoptosis

The TUNEL apoptosis detection kit was used to observe tumor cell apoptosis. Tissues were washed, embedded in wax, sectioned (at a thickness of 4–7 μm), and then baked in an oven at 65°C for 1 h. Then, they were placed in xylene I and II for 15 min each, hydrated, and incubated with proteinase K at 21–37°C for 15–30 min. After the addition of 50 μL of the TUNEL reaction mix, they were covered in a wet box in the dark; then, the samples were rinsed, followed by the addition of 50 μL of transformed-POD (vial 3). After 30 min of incubation, 50–100 μL of the 3,3'-diaminobenzidine substrate was added. It was counter-stained with hematoxylin, dehydrated, and made transparent. Then, the relevant parts of the samples were analyzed microscopically and photographed on the Leica Application Suite imaging system.

Western blot

Proteins were extracted and quantified using the BCA kit. Then, twenty micrograms of protein were loaded onto the gel (12% resolving gel and

5% stacking gel) and ran at 80 V for 40 min on the stacking gel and at 120 V for 50 min on the resolving gel. The protein was transferred on the membrane at 90 V for 50 min. Next, the membrane was blocked with 5% skimmed milk overnight at 4°C and then incubated with a primary antibody [p-ACK1 (1:000, Orb191531, biorbyt), ACK1 (1:000, PAB43799, Bio-swamp), P-AKT (1:000, PAB43181-P, Bio-swamp), AKT (1:000, PAB30596, Bio-swamp), Bax (1:000, PAB30861, Bio-swamp), Bcl-2 (1:000, PAB30041, Bio-swamp), Caspase (1:000, PAB33236, Bio-swamp), and β-Actin (1:000, PAB36265, Bio-swamp)] at room temperature for 1 h. Afterward, the membrane was washed and incubated with a secondary antibody (Goat anti-rabbit IgG, 1:10000, SAB43711, Bio-swamp) for 1 h at room temperature. After adding the ECL luminescent solution, the membrane was developed on an X-ray film and analyzed using a fully automated chemiluminescent analyzer (Tanon-5200, TANON, Shanghai). The grayscale values of the relevant bands were measured in triplicates using the TANON GIS software.

Flow cytometry

Flow cytometry was performed to determine the number of immune cells in the spleen.

Procedure to detect Treg cells: After re-suspending in 100 μL of the flow buffer, 2 μL of anti-CD4 (85-11-0041-81, eBioscience) and anti-CD25 (15-0251-82, eBioscience) antibodies were added to each tube, and they were incubated for 30 min at 4°C in the dark. Two milliliters of the flow-staining buffer was added, washed, and incubated for 5 min at 4°C, protected from light, and incubated for 30 min at 4°C, protected from light. After incubation for 20 min at 4°C, they were centrifuged at 300 × *g* for 5 min; the cells in each tube were then re-suspended in 1 mL of the IX film-breaker fixative (562574, BD Biosciences) and again incubated for 5 min at 4°C. After incubation, they were again centrifuged at 300 × *g* at 4°C for 5 min. The cells were re-suspended in a 100 μL working Perm/wash buffer solution. Then, 2 μL of the Foxp3-PE antibody was added to each tube. Then, they were incubated at 4°C for 45 min in the dark. Next, 400 μL of the flow-staining buffer was added to re-suspend the cells. The percentage of Treg cells was analyzed by flow cytometry after setting the forward scatter angle (FSC) and lateral scatter angle (SSC) by adjusting the voltage with an empty standard tube. The lymphocyte population was identified, and CD4+ cells were gated in the FSC–SSC scatter plot with CD4 single-stained tubes. Then, CD4 + CD25 + FOXP3+/CD4 + percentages were analyzed by setting a gate in the CD4+-SSC scatter plot with CD4 + CD25 double-stained tubes. CD4 + Foxp3 double-stained tubes were used to adjust the compensation. The experimental results were analyzed using NovoCyte analysis software.

Th1 and Th2 cell assays: Cells were re-suspended in 1 mL of the cell culture medium. Then, stimulants, 2 μL of para-methoxyamphetamine (50 ng/mL) and an ionophore (ionomycin, 2 μg/mL), were added to each tube. After the addition of monensin (3 μg/mL), trans-Golgi inhibitor), the tubes were incubated for 6 h at 37°C in a CO_2 incubator. Then, cells were washed using 2 mL of the flow-staining buffer and centrifuged at 300 g at 4°C for 5 min. The cells were re-suspended in 100 μL of the flow buffer. Then, 2 μL of the CD4 antibody was added to each tube and incubated for 30 min at 4°C in the dark. Next, 1 mL of the 1X membrane breaker fixative was added to each tube to re-suspend the cells. After incubating for 20 min in the dark, the cells were centrifuged at 300 × *g* for 5 min at 4°C. The membrane breaker master mix (10X) was diluted with de-ionized water. Then, 100 μL of the flow-staining buffer was used to re-suspend cells, and 2 μL of anti-IFN-γ and anti-IL4 antibodies were added to each tube. The tubes were incubated for 45 min at 4°C in the dark. Cells were re-suspended in 400 μL of the flow-staining buffer at 4°C in the dark and stored for flow-through detection. Another cell empty standard tube was set up as a single staining tube.

Analysis of the percentage of cell expression by flow cytometry: first, the FSC and SSC were set to determine the lymphocyte population with the empty standard tube, and then, the compensation was adjusted with the single stain tube. The experimental results were analyzed using NovoCyte analysis software.

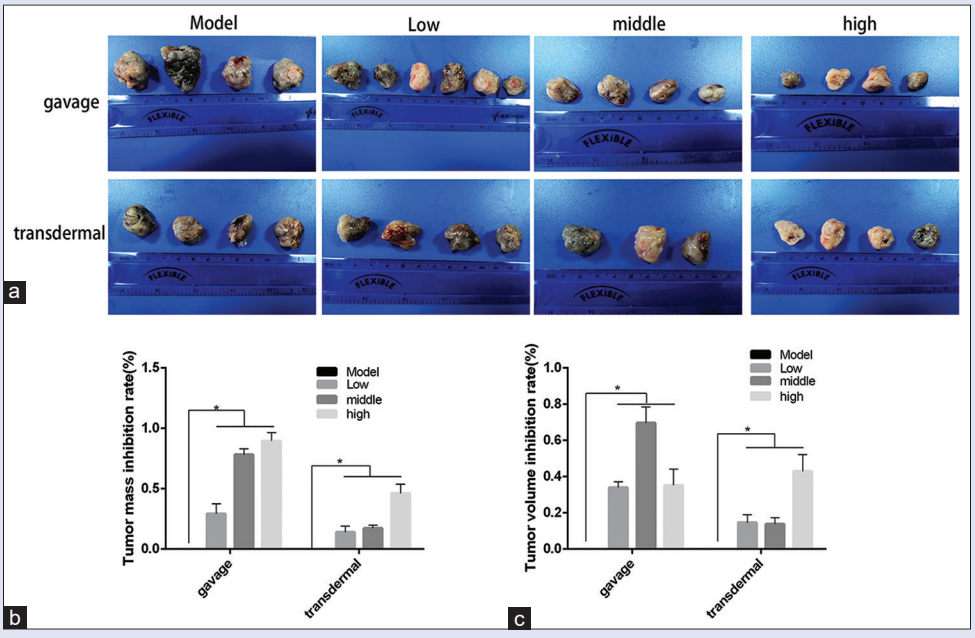

Figure 1: Effects of different concentrations and administration methods of QJ623 on tumor inhibition rate. (a) Photograph of the tumor; (b) Tumor mass inhibition rate; (c) Tumor volume inhibition

Renal function analysis

Blood from the orbital venous plexus was collected and centrifuged at 3000 × *g*. Serum was collected from the blood. Bio-chemical analyses were performed using an Aeroset-2000 automatic bio-chemical analyzer to determine the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cre), blood urea nitrogen (BUN), and uric acid (UA).

ELISA analysis

The serum was used for ELISA to detect levels of inflammation-related factors, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and IL-10. All ELISA kits were purchased from Bioswamp. The experiments were performed according to the manufacturer's instructions.

UHPLC-MS/MS analysis

The contents of the active ingredients in the two herbal extracts were determined using ultra-high-pressure liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). The experiment was performed at Wuhan Pernice Biotechnology Co., and data were processed using Shimadzu LC-MS LabSolutions version 5.65 SP4 (Shimadzu Corp.). The UHPLC conditions were as follows: chromatographic column, poroshell 120 EC-C_{18} reversed-phase column (2.1 × 150, 2.7 μm); column temperature, 35°C; mobile phase A, 0.1% formic acid in water; mobile phase B, methanol; flow rate, 0.3 mL/min; injection volume, 1 μL. The mass spectrometry parameters were as follows: ionization mode, electro-spray ionization positive and negative ion modes are monitored; scan type, MRM; air curtain gas, 15 psi; spraying voltage, +5000 V, -4500 V; nebulizing gas pressure, 60 psi; auxiliary gas pressure, 60 psi; atomization temperature, 400°C.

Statistical analysis

All data are expressed as the mean ± standard deviation. SPSS 19.0 was used for data analysis, and GraphPad Prism6 was used for constructing graphs. Differences between groups were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test. Differences were considered statistically significant at $P < 0.05$.

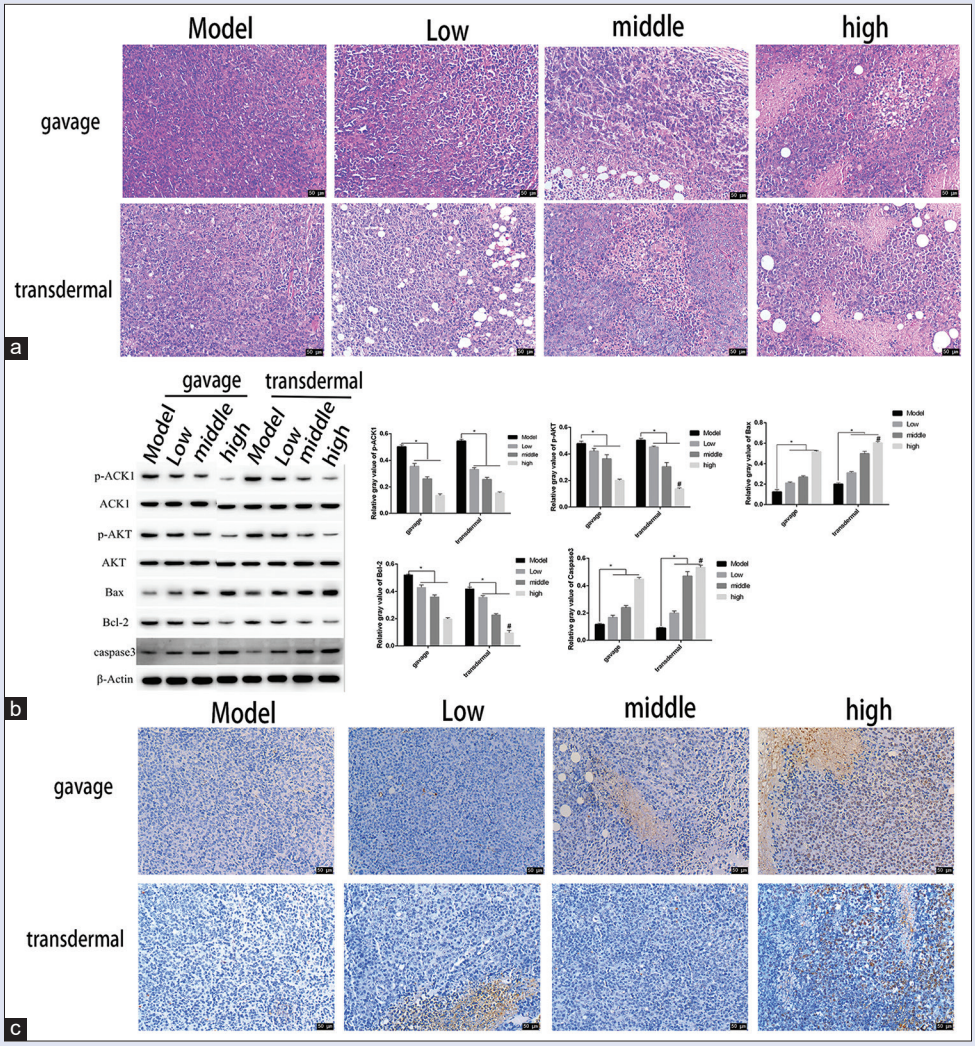

Figure 2: The effects of different concentrations and administration methods of QJ623 on tumor cell apoptosis and expression of related proteins. (a) The appearance of necrosis and disintegration of tumor cells, as observed by hematoxylin and eosin staining; (b) Expression of apoptosis-related proteins, as observed by western blotting; (c) Detection of apoptosis in tumor cells by the TUNEL method

Results

Effect of different concentrations and delivery modes of QJ623 on tumor growth inhibition

As shown in Figure 1, gavage and transdermal administration of different concentrations of QJ623 significantly inhibited the tumor growth rate (Figure 1b) and tumor volume (Figure 1c) compared to the model group. The results indicate that QJ623 can inhibit the increase in tumor mass and volume in the H22 transplantation model mice.

Effects of different concentrations and delivery modes of QJ623 on tumor cell apoptosis and related proteins

The results showed that in the gavage and transdermal model groups, tumor cells were intact, and no tumor necrosis occurred [Figure 2a]. In contrast, tumor cells underwent coagulative necrosis after the gavage or transdermal administration of different concentrations of QJ623. The expression levels of p-ACK1, p-AKT, and Bcl-2 were significantly reduced, and those of Bax and Caspase3 were significantly increased after gavage or transdermal administration of different concentrations of QJ623, compared to the model group [Figure 2b]. Moreover, the most prominent effects on the expression levels of p-AKT, Bax, Bcl-2, and Caspase3 were observed when QJ623 was administered transdermally at 1.168 g/kg. As shown in Figure 2c, apoptosis was detected in the gavage and transdermal model groups, and tumor cells showed different degrees of apoptosis when treated with different concentrations of QJ623 (via both ways). This indicated that QJ623 can markedly improve the tumor status in murine liver cancer *in vivo* by promoting the apoptosis of tumor cells.

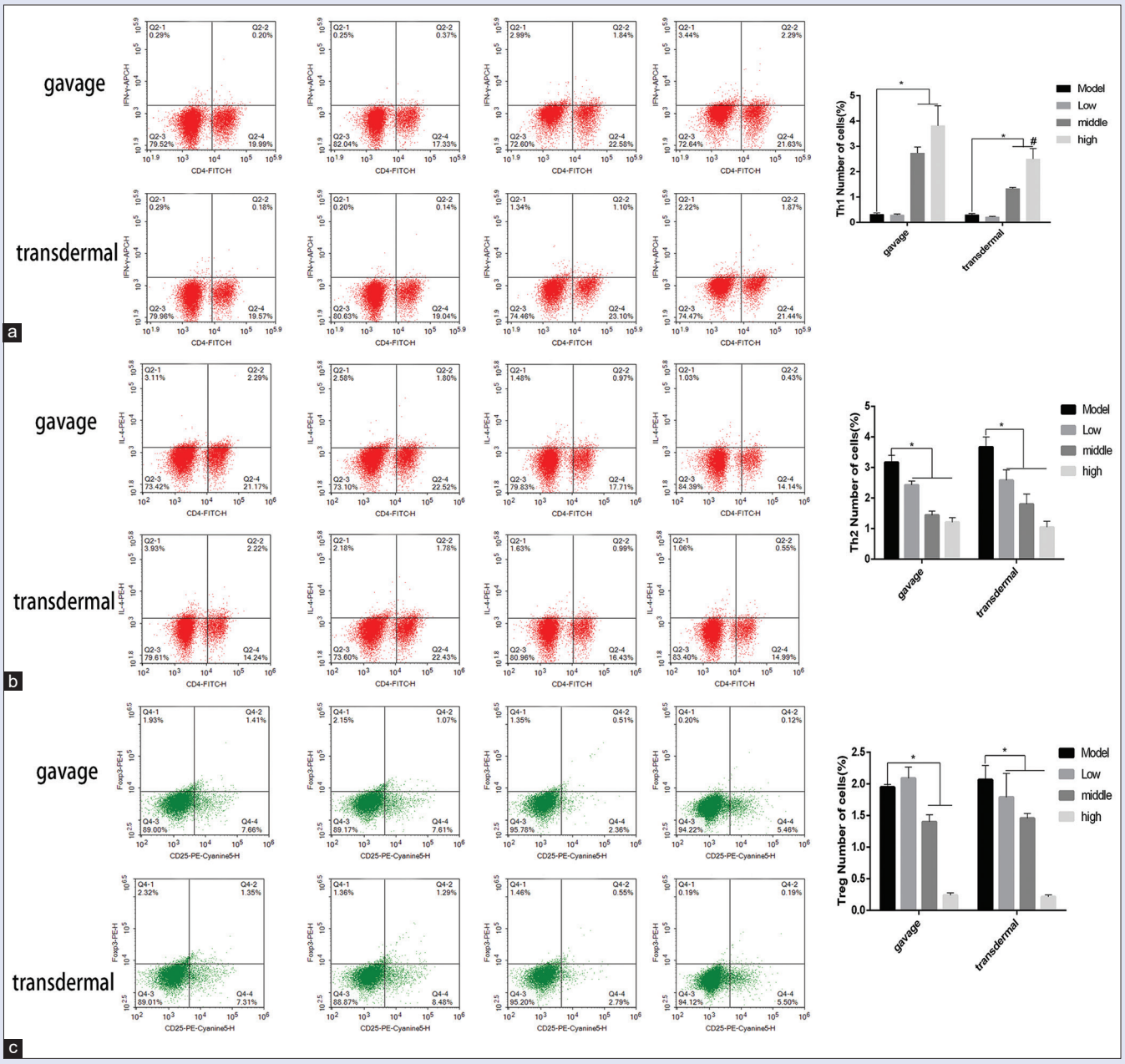

Figure 3: Flow cytometry analysis of the number of immune cells in the spleen. Analysis of the number of (a) Th1 cells, (b) Th2 cells, and (c) Treg cells

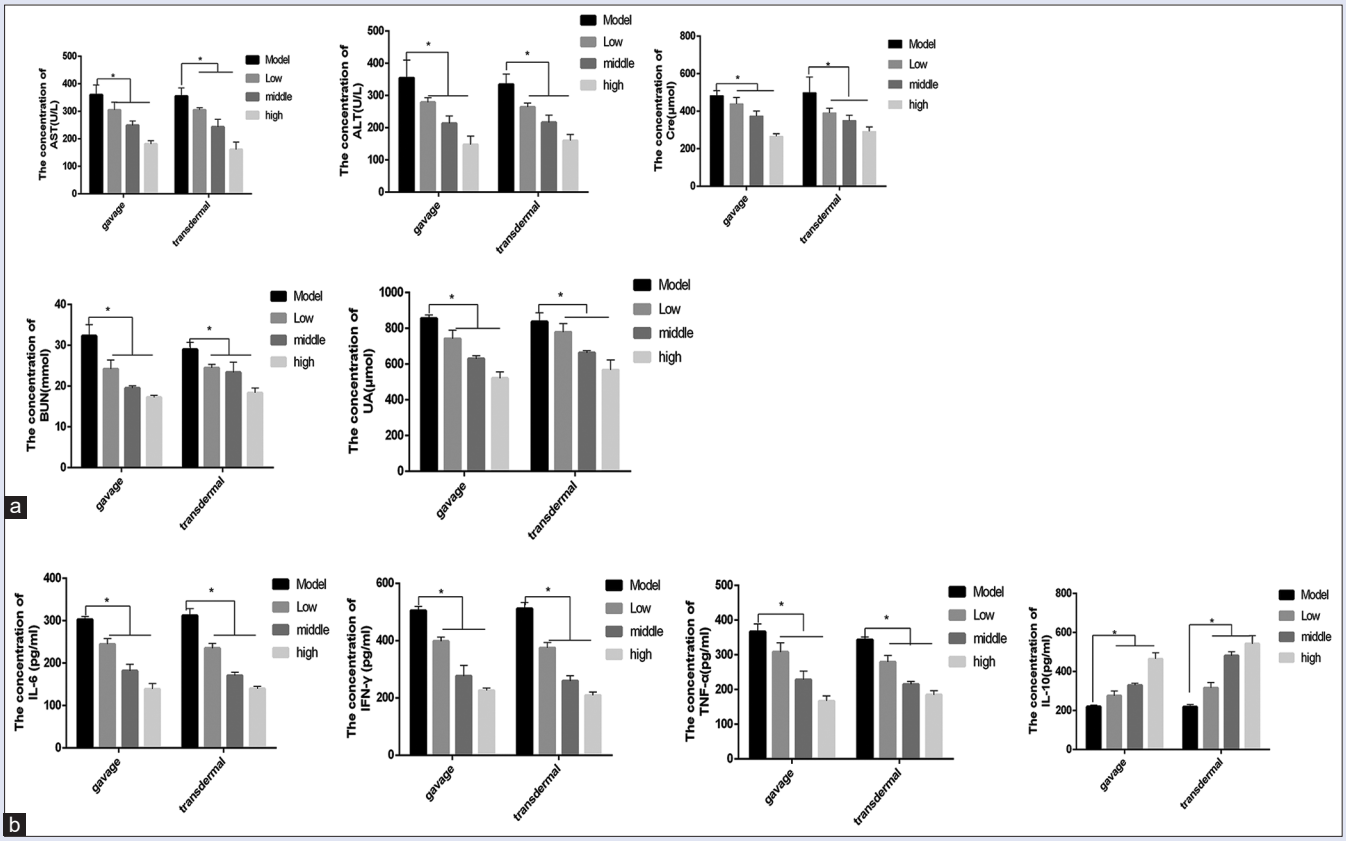

Figure 4: Effects of different concentrations and administration methods of QJ623 on liver and kidney functions and inflammation-related factors. (a) The serum levels of AST, ALT, BUN, and UA; (b) The serum levels of IL-6, TNF-α, IFN-γ, and IL-10

Effects of different concentrations and delivery modes of QJ623 on immune cells in the spleen

Compared to the model group, QJ623 administration by gavage or transdermally at 0.584 g/kg or 1.168 g/kg significantly increased the abundance of Th1 (IFN-γ) cells among CD4 cells [Figure 3a] and decreased the abundance of Th2 (IL-4) [Figure 3b] and Treg cells among CD4 cells [Figure 3c]. Thus, QJ623 acts as an anti-tumor agent *in vivo* by enhancing the immune action in mice with liver cancer.

Effects of different concentrations and administration methods of QJ623 on liver and kidney functions and inflammation-related factors

The results showed that compared with the model group, different concentrations of QJ623, administered by gavage or transdermally, reduced the levels of AST, ALT, BUN, and UA to different degrees [Figure 4a]; decreased the levels of IL-6, TNF-α, and IFN-γ to different degrees; and increased the levels of IL-10 [Figure 4b].

Detection of active components

The contents of the five active ingredients in the herbal extracts were determined using UHPLC-MS/MS. The results are presented in Table 1. The contents of ferulic acid, astragaloside, calycosin isoflavone, formononetin, and ligustilide were 41537.83 ng/g, 42791.21 ng/g, 92687.91 ng/g, 87203.31 ng/g, and 97781.74 ng/g, respectively.

DISCUSSION

Most HCCs are diagnosed at an advanced stage as highly malignant and aggressive solid tumors, and thus, their treatment is limited.[11,12] The occurrence and development of liver cancer are closely related to the tumor micro-environment. Immune cells are the key players in the HCC micro-environment and exhibit complex crosstalk with cancer cells.[12] A variety of tumor-promoting cytokines, such as IL-1β, IL-6, TGF-β, and tumor necrosis factor-alpha (TNF-α), create a tumor-prone inflammatory micro-environment.[13,14]

T-lymphocytes are sub-divided into two sub-populations, Th1 and Th2, which play key roles in the development and progression of primary liver cancer.[15] Th1 secretes IL-2 and TNF-α, whereas Th2 cells secrete IL-4, IL-6, and IL-10. Under normal conditions, Th1 and Th2 maintain a dynamic balance.[16] The Th1 cells promote the toxic effects of killer cells and induce cellular immunity, whereas Th2 cells promote antibody production, suppress the activity of Th1 cells, and mediate humoral immunity.[15] As HCC progresses, Th2 cells inhibit the activity of Th1 cells, suppressing the anti-tumor activity of the body. In the present study, gavage or transdermal administration of QJ623 at a dose of 0.584 g/kg or 1.168 g/kg to mice with liver cancer showed a significant increase in the number of Th1 cells and a decrease in the number of Th2 and Treg cells in the spleen tissue samples. The ELISA results showed that the serum levels of the inflammation-related factors IL-6, TNF-α, and IFN-γ were significantly reduced and those of IL-10 increased after the gavage or transdermal administration of QJ623 in mice with HCC. These results suggest that QJ623, administered by different modes, exerted an anti-tumor effect through Th1 cell-mediated cellular immunity. It enhanced the toxic effects of killer cells and the immunity of the organism, ultimately suppressing the tumor growth rate in mice.

Apoptosis, unlike cell necrosis, is a genetically regulated process of autonomous death occurring during the growth and development of

Table 1: Detection of active ingredients

Name	Dissolution volume (ml)	Weighing mass (g)	Dilution times	Detection concentration	Content per gram of sample (ng/g)
Ferulic acid	10	1.004	10	417.03	41537.83
Astragaloside	10	1.004	10	411.67	42791.21
Calycosin Isoflavone	10	1.004	10	930.57	92687.91
Formononetin	10	1.004	10	875.50	87203.31
Ligustilide	10	1.004	10	969.77	97781.74

an organism, cell differentiation, and pathological states. Apoptosis is the main signaling pathway involved in hepatocarcinogenesis and development. Therefore, the treatment of HCC can be achieved by inducing apoptosis in tumor cells.[17] We examined the effect of QJ623 on the apoptosis of HCC cells. QJ623 treatment promoted necrosis and disintegration of tumor cells in mice, as observed by hematoxylin and eosin staining. TUNEL assay revealed that QJ623 promoted apoptosis in tumor cells. The western blot assay showed that the expression of the pro-apoptotic proteins Bax and Caspase3 was significantly up-regulated and that of the anti-apoptotic protein Bcl-2 was significantly down-regulated.

Paris forrestii has been reported to show *in vivo* anti-tumor effects;[18] thus, it is a potential candidate for cancer treatment. Moreover, Yu *et al.*[19] found that the oral administration of polyphyllin I alleviated protein-calorie malnutrition. It has also been found that Tinosporae Radix has bactericidal effects *in vivo* and *in vitro.*[20] However, very few studies have reported the effects of the mixed administration of these two drugs. The novelty of this study is that it describes a unique formulation as a potential chemotherapeutic agent for HCC.

CONCLUSION

In conclusion, this study demonstrates that gavage or transdermal administration of QJ623 can promote apoptosis, enhance cellular immunity, and reduce the level of cellular inflammation, exerting anti-tumor effects in mice with HCC. Thus, QJ623 may be a potential anti-tumor chemotherapeutic agent against HCC. However, the active components of QJ623 must be specifically explored for elucidating the mechanism underlying their action against tumors. We acknowledge that there are limitations to this study and that further research is needed to establish the effectiveness of the specific drug components.

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Conflict of interest

There are no conflicts of interest.

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