Am J Transl Res 2022;14(9):6792-6804
www.ajtr.org /ISSN:1943-8141/AJTR0144432

Original Article

Efficacy of kangfuxin liquid on radiotherapy-induced oral mucositis for patients with head and neck squamous cell carcinoma and its effect on salivary glands and immune function

Hao Yuan[1], Jiajia Su[2], Junyin Tan[1], Yan Wei[1]

[1]*Department of Oncology, Guigang City People's Hospital, Guigang 537100, Guangxi Zhuang Autonomous Region, China;* [2]*Department of Echocardiography, Guigang City Hospital of Traditional Chinese Medicine, Guigang 537100, Guangxi Zhuang Autonomous Region, China*

Received May 28, 2022; Accepted August 13, 2022; Epub September 15, 2022; Published September 30, 2022

Abstract: Objective: To observe the efficacy of Kangfuxin liquid on radiotherapy-induced oral mucositis for patients with head and neck squamous cell carcinoma and its effects on salivary glands and immune function. Methods: A total of 97 patients with head and neck squamous cell carcinoma receiving radiotherapy in Guigang City People's Hospital from January 2019 to June 2021 were retrospectively analyzed and divided into a control group and a test group according to different treatment plans. The two groups received the same radiation therapy. Patients in the control group (n=46) were given borax-containing gargles, while those in the test group (n=51) were treated with Kangfuxin liquid. We observed the incidences and grades of oral mucositis and oral pain, changes in saliva flow rate, pH of saliva, levels of epidermal growth factor (EGF) and amylase, levels of $CD4^+/CD8^+$, $CD19^+/CD69^+$ and natural killer (NK) cells, and serum cytokine (TGF-β1, IL-6 and C-reactive protein (CRP)) levels in the two groups before radiotherapy, and after 21 d and 42 d of radiotherapy. Quality of Life Instruments for Cancer Patients-Head and Neck Cancer (QLICP-HN) scores were compared in both groups before radiotherapy, and after 42 d of radiotherapy. Results: No oral mucositis or oral pain was found before radiotherapy in both groups. The incidences of oral mucositis and oral pain after 21 d and 42 d of radiotherapy in the test group were not significantly different from those in the control group (all $P>0.05$). The grades of oral mucositis and oral pain in the test group after 21 d and 42 d of radiotherapy were lower than those in the control group (all $P<0.05$). The test group had higher saliva flow rate, pH of saliva, levels of EGF and amylase, and levels of $CD4^+/CD8^+$, $CD19^+/CD69^+$ and NK cells. The test group had lower serum levels of TGF-β1, IL-6, and CRP than the control group after 21 d and 42 d of radiotherapy (all $P<0.05$). The scores of each item of the QLICP-HN scale and total scores in the test group were higher than those of the control group after 42 d of radiotherapy (all $P<0.05$). Conclusion: Kangfuxin liquid effectively prevents the occurrence of radiotherapy-induced oral mucositis for patients with head and neck squamous cell carcinoma, reduces oral mucosal reactions and oral pain, improves salivary gland function, reduces inflammatory response, promotes cellular immune function, improves quality of life, and improves prognosis.

Keywords: Kangfuxin liquid, head and neck squamous cell carcinoma, radiotherapy, oral mucositis, salivary glands, immune function

Introduction

Radiation therapy is the major approach to treat head and neck cancer. Although radiation effectively kills tumor cells, it causes damage, necrosis, or apoptosis of mucosal epithelial cells, increases oral mucosal fragility, reduces saliva secretion, and lowers oral immune function, resulting in oral mucositis to varying degrees [1, 2]. Edema, mucosal hyperemia, ulceration, erosion, pain and secondary infection are the main clinical manifestations of oral mucositis, which not only seriously affects the normal eating of patients, causes weight loss and reduces quality of life, but also easily reduces the tolerance of normal tissues to radiation, finally affecting its efficacy [3, 4]. In 2010, the Clinical Guidelines for Oral Mucositis from the

European Society of Medical Oncology stated that patients with head and neck squamous cell carcinoma had a high risk (85%) of developing moderate to severe oral mucositis during radiotherapy, 15% of whom need hospitalization [5]. Therefore, exploring a reasonable and efficient treatment for the prevention and cure of radiation-induced oral mucositis is crucial to alleviating the patients' discomfort from radiotherapy and improving radiotherapy efficacy. Kangfuxin liquid is a dried insect extract of American cockroach, with mucinine, epidermal growth factor, polyols and other active substances, that can enhance cellular immunity, activate immune system, eliminate inflammatory edema, improve wound microcirculation, repair ulcer wounds, and accelerate wound granulation tissue production [6, 7]. At present, it has been confirmed that Kangfuxin liquid effectively prevents and treats radiation-induced oral mucositis in patients with malignant tumors and alleviates oral mucosal responses, but its effects on salivary gland function, immune function, and inflammatory response of patients with head and neck squamous cell carcinoma after radiation have been rarely reported [8]. In order to further prevent and treat radiation-induced oral mucositis, this study retrospectively analyzed the effects of Kangfuxin liquid on the occurrence of oral mucositis, salivary gland function, and immune function in head and neck squamous cell carcinoma patients.

Materials and methods

General data

A total of 97 patients with head and neck squamous cell carcinoma receiving radiotherapy in Guigang City People's Hospital from January 2019 to June 2021 were retrospectively analyzed in this study. Among them, there were 68 men and 29 women from 29 to 78 years old, with an average age of 59.94±2.89 years old. As for primary site, there were 32 cases in the oral cavity, 12 cases in oropharynx, 11 cases in hypopharynx, and 42 cases in larynx. According to the American Joint Committee Cancer (AJCC) staging system, 44 and 53 cases were at stage III and IV, respectively. This clinical study had been approved by the Ethics Committee of Guigang City People's Hospital (approval number: GYLLPJ-20210626-08). Patients or their family members voluntarily signed an informed consent form.

Inclusion criteria: (1) Patients met the diagnostic criteria for head and neck squamous cell carcinoma in the Expert Consensus on Comprehensive Treatment of Head and Neck Tumors [9]. (2) Patients were at stage III or IV in the AJCC staging system [10]. (3) Patients were undergoing radiation therapy for the first time. (4) Patients' Karnofsky scores were ≥70 points [11].

Exclusion criteria: (1) Patients had oral mucositis, mouth ulcers, oral pain, periodontitis, Sjögren's syndrome, and infectious diseases before radiation therapy. (2) Patients had contraindications associated with radiation therapy to head and neck tumors. (3) Patients suffered from autoimmune diseases, severe organic diseases, abnormal bone marrow hematopoietic function, or other malignant tumors. (4) Patients were allergic to medications used in this study. (5) Patients had coagulation disorders. (6) Patients had cardiovascular and cerebrovascular diseases. (7) Patients had a previous history of salivary gland disease. (8) Pregnant or breast-feeding women. (9) Patients had poor compliance.

Methods

Radiotherapy regimen [12]: Intensity-modulated radiation therapy was carried out. 6 MV-X-rays were used, and a 7-9 wild IMRT radiotherapy plan was designed. The gross tumor volume included positive lymph nodes, primary lesions, 2 Gy/time, 5 times/week, with the prescribed dose of 66-74 Gy. The radiation doses for temporal lobe, temporomandibular joint, mandible, cervical cord, inner or middle ear and cartilagines larynges were limited to a dose of 60 Gy, 65 Gy, 65 Gy, 45 Gy, 50 Gy and 45 Gy, respectively. 50% volume of the bilateral parotid gland should be limited to a dose of 30 Gy. The radiation dose for oral mucosa without PTV was limited to 55 Gy. The radiation doses for optic nerve, optic chiasm and brainstem were all limited to 54 Gy.

Chemotherapy regimen: Intravenous infusion of cisplatin of 100 mg/m^2 was administered on the 1st day, 22nd day, and 43rd day of radiotherapy.

Treatment protocols: During radiotherapy and chemotherapy, gargle containing vitamin C, normal saline, lidocaine, vitamin B6, and dexa-

methasone was applied in both groups, 3 times/day. Gargle use was stopped after radiotherapy.

1) The control group was treated with borax-containing gargle (Shanghai Yunjia Huangpu Pharmaceutical Co., Ltd., 250 mL/bottle, H31022772), 15 mL/time. Patients were guided to rinse the mouth with water first, and then rinse with borax-containing gargle diluting with 5 times the amount of warm water. Patients were instructed to give the liquid as much exposure as possible to the oral mucosa, 3-4 times/day.

2) The test group was given 10 mL/time of Kangfuxin liquid from the 1st day to the end of radiotherapy (Kunming Sainuo Pharmaceutical Co., Ltd., 50 mL/bottle, Z53020054). Patients should rinse mouth with water first, and then rinse mouth with kangfuxin liquid and take it. Patients were instructed to give the liquid as much exposure as possible to the oral mucosa, and swallow slowly after about 3 minutes, 3 times/day.

Outcome measures

Oral mucositis and oral pain were observed and recorded before radiotherapy, and after 21 d and 42 d of radiotherapy. Based on CTCAE v3.0 criteria, 0 points was assigned for asymptomatic, 1 point for mucosal erythema, 2 points for plaque ulcers or pseudomembrane, 3 points for continuous ulcers or pseudomembrane, bleeding caused by small abrasions, and 4 points for tissue necrosis, obvious spontaneous bleeding, or life-threatening condition [13]. Verbal Rating Scale was used to describe pain intensity. "No pain" =0; "Mild pain which can be tolerated and had no effect on sleep or life" =1; "Moderate pain which can't be tolerated and had an effect on sleep and life" =2; "Severe pain which can't be tolerated and had serious effects on sleep and life, with postural or autonomic disturbances" =3.

Saliva flow rate and pH of saliva were evaluated before radiotherapy, and after 21 d and 42 d of radiotherapy [14]. A certain weight of yarn cotton was put in the patient's mouth and removed after 30 s. Saliva flow rate = increased weight of yarn cotton/0.5. The precision pH test strip (5.5-9.0; Beijing Kaishiyuan Biotechnology Co., Ltd.) was placed under the patient's tongue. After the front end of the test strip was soaked by patient's saliva, the value was read within 3-5 seconds.

The morning saliva of patients before radiotherapy, after 21 d and 42 d of radiotherapy was collected, and placed in a refrigerator (Guangzhou Kezhilan Instrument Co., Ltd.) at -4°C to precipitate [15]. After 24 h, the saliva was taken out and centrifuged on a high-speed centrifuge (415D, Eppendorf Company of Germany) for 10 min at a speed of 3,000 r/min with a centrifugal radius of 6 cm. The mucin and residue were removed, and 2-3 mL of filtrate was obtained. The levels of saliva epidermal growth factor (EGF, PC1828, Pufei Biotechnology Co., Ltd.) and amylase (YT6040, Beijing Ita Biotechnology Co., Ltd.) were determined by enzyme linked immunosorbent assay (ELISA), and the model 680 microplate reader (Bio Rad Company, USA) was used.

Fasting peripheral venous blood (3 mL) was collected from patients before radiotherapy, and after 21 d and 42 d of radiotherapy. The blood was centrifuged for 5 min, at 2,500 r/min, with the centrifugal radius of 6 cm. The supernatant was taken. A high-throughput flow cytometer (MACSQuant® Analyzer 16, Meitenbitech Co., Ltd., Germany) was used to determine the levels of $CD4^+$, $CD8^+$, $CD19^+$, $CD69^+$, and natural killer (NK) cells (M2704, Shanghai Mingjin Biological Technology Co., Ltd.). The $CD4^+/CD8^+$ values, and $CD19^+/CD69^+$ values were calculated. Serum levels of IL-6 (IB-E10049, Jiangxi Aiboin Biotechnology Co., Ltd.), TGF-β1 (EK-H12176, Shanghai Enzyme Research Biotechnology Co., Ltd.), and C-reactive protein (CRP, SEKH-0138, Beijing Solarbio Science & Technology Co., Ltd.) were determined by ELISA.

Quality of Life Instruments for Cancer Patients-Head and Neck Cancer scale was used to evaluate patients' quality of life before radiotherapy, and after 42 d of radiotherapy [16]. The scale includes 5 dimensions and 46 items, using a 5-point scale ranging from 1 to 5, with total scores of 230. There are 9 items for mental function, 8 items for physical function, 15 items for social function, 7 items for head and neck cancer specific modules, and 7 items for common symptoms and side effects. A higher score reveals higher quality of life.

Table 1. Comparison of general data (n, $\bar{x}$ ± sd)

Group	Male/ Female	Age (years)	Course of disease (years)	Body mass index (kg/m^2)	Primary site	AJCC staging
					Oral cavity/oropharynx/hypopharynx/larynx	III/IV
Control group (n=46)	30/16	59.6±3.3	5.84±1.02	22.84±1.65	15/6/5/20	20/26
Test group (n=51)	38/13	60.2±2.7	5.97±1.35	22.98±1.52	17/6/6/22	24/27
χ^2/t	0.996	0.890	0.531	0.435	0.003	0.125
P	0.318	0.376	0.597	0.665	0.956	0.724

Note: The incidence of radiotherapy-reduced oral mucositis among male/female, primary site, and AJCC staging ratio was compared between the two groups, using Pearson's chi-square test. The averages of age, disease course, and body mass index of the two groups were compared with independent samples t-test. AJCC: American Joint Committee on Cancer.

Table 2. Comparison of oral mucositis before radiotherapy, after 21 d and 42 d of radiotherapy (n, %)

Group	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Total incidence
Before radiotherapy						
Control group (n=46)	46 (100.00)	0	0	0	0	0 (0.00)
Test group (n=51)	51 (100.00)	0	0	0	0	0 (0.00)
χ^2/Z			Z=0.000			χ^2=0.000
P			1.000			1.000
After 21 d of radiotherapy						
Control group (n=46)	0	5	15	26	0	46 (100.00)
Test group (n=51)	1	12	18	20	0	50 (98.04)
χ^2/Z			Z=2.023			χ^2=0.000
P			0.043			1.000
After 42 d of radiotherapy						
Control group (n=46)	0	6	14	26	0	46 (100.00)
Test group (n=51)	4	13	15	19	0	47 (92.16)
χ^2/Z			Z=2.422			χ^2=2.041
P			0.016			0.153

Note: Pearson's chi-square test was used for comparison of oral mucositis between the two groups. Z-test was adopted to test the grades of oral mucositis between the two groups.

Three months after radiotherapy, the recurrence of stomatitis was evaluated.

Statistical analysis

Statistical analyses were conducted using SPSS 23.0 software. Measured data with a normal distribution were expressed as mean ± standard deviation ($\bar{x}$ ± sd). Independent sample t-test was adopted for the comparison between groups. Paired t-test was used for the comparison in the same group. Wilcoxon signed-rank test was used to analyze measurement data with non-normal distribution. Counted data were represented as case/percentage (n/%) and tested by Pearson's chi-square test. Ordinal data were analyzed by Rank-sum test. P<0.05 was considered a significant difference.

Results

Comparison of general data

There was no significant difference in general data between both groups (P>0.05). See **Table 1**.

Comparison of oral mucositis

No oral mucositis was found before radiotherapy in both groups. The incidences of oral mucositis (98.04%, 92.16%) after 21 d and 42 d of radiotherapy in the test group were not significantly different from those of the control group (100.00%, 100.00%; both P>0.05). The grades of oral mucositis in the test group after 21 d and 42 d of radiotherapy were lower than those of the control group (both P<0.05). See **Table 2**.

Table 3. Comparison of oral pain before radiotherapy, and after 21 d and 42 d of radiotherapy (n, %)

Time	Group	Grade 0	Grade 1	Grade 2	Grade 3	Total incidence
Before radiotherapy	Control group (n=46)	46 (100.00)	0	0	0	0 (0.00)
	Test group (n=51)	51 (100.00)	0	0	0	0 (0.00)
	χ^2/Z		0.000			0.000
	P		1.000			1.000
After 21 d of radiotherapy	Control group (n=46)	0	11	30	5	46 (100.00)
	Test group (n=51)	2	24	24	1	49 (96.08)
	χ^2/Z		3.055			0.005
	P		0.002			0.943
After 42 d of radiotherapy	Control group (n=46)	1	12	31	2	45 (97.83)
	Test group (n=51)	6	21	23	1	45 (88.24)
	χ^2/Z		2.625			2.045
	P		0.009			0.153

Note: Pearson's chi-square test was used for comparison of oral pain between the two groups. Z-test was adopted to test the grades of oral pain between the two groups.

Table 4. Comparison of saliva flow rate and pH of saliva before radiotherapy, and after 21 d and 42 d of radiotherapy ($\bar{x}$ ± sd)

Time	Group	Saliva flow rate (mg/min)	pH of saliva
Before radiotherapy	Control group (n=46)	229.65±21.54	5.96±0.74
	Test group (n=51)	231.05±22.49	6.02±0.84
	t	0.312	0.372
	P	0.756	0.711
After 21 d of radiotherapy	Control group (n=46)	302.45±28.96***	6.48±0.87***
	Test group (n=51)	349.67±31.78***	6.89±0.82***
	t	7.620	2.389
	P	<0.001	0.019
After 42 d of radiotherapy	Control group (n=46)	396.74±33.75***,###	6.96±0.76***,##
	Test group (n=51)	493.87±48.52***,###	7.35±0.69***,###
	t	11.326	2.649
	P	<0.001	0.001

Note: Paired t-test was used for the comparison in the same group before radiotherapy, ***P<0.001. Paired t-test was used for the comparison in the same group after 21 d of radiotherapy, ##P<0.01, ###P<0.001.

Comparison of oral pain

There was no oral pain before radiotherapy in both groups. The incidences of oral pain (96.08%, 88.24%) after 21 d and 42 d of radiotherapy in the test group were not significantly different from those of the control group (100.00%, 97.83%; both P>0.05). The grades of oral pain in the test group after 21 d and 42 d of radiotherapy were lower than those of the control group (P<0.05). See **Table 3**.

Comparison of saliva flow rate and pH of saliva

Before radiotherapy, compared to the control group, there were no significant differences in saliva flow rate or pH of saliva in the test group (both P>0.05). Compared to before radiotherapy, the saliva flow rate and pH of saliva were higher in both groups after 21 d and 42 d of radiotherapy (both P<0.05). The test group had a higher saliva flow rate and pH of saliva than the control group (both P<0.05). See **Table 4**.

Comparison of levels of EGF and amylase

Compared to the control group, there was no significant difference between levels of EGF and amylase before radiotherapy in the test group (both P>0.05). The levels of EGF and amylase were increased in the two groups after 21 d and 42 d of radiotherapy (both P<0.05).

Table 5. Comparison of levels of EGF and amylase before radiotherapy, and after 21 d and 42 d of radiotherapy ($\bar{x}$ ± sd)

Time	Group	Amylase (ng/mL)	EGF (ng/L)
Before radiotherapy	Control group (n=46)	211.36±46.84	69.63±10.35
	Test group (n=51)	213.94±44.76	70.16±9.68
	t	0.277	0.261
	P	0.782	0.795
After 21 d of radiotherapy	Control group (n=46)	273.95±36.98***	99.63±12.74***
	Test group (n=51)	328.74±40.09***	143.86±20.47***
	t	6.974	12.613
	P	<0.001	<0.001
After 42 d of radiotherapy	Control group (n=46)	342.74±51.29***,###	186.63±20.41***,###
	Test group (n=51)	417.96±58.62***,###	257.83±31.48***,###
	t	6.693	13.060
	P	<0.001	<0.001

Note: Paired t-test was used for the comparison in the same group before radiotherapy, ***P<0.001. Paired t-test was used for the comparison in the same group after 21 d of radiotherapy, ###P<0.001. EGF: epidermal growth factor.

Table 6. Comparison of cellular immune function before radiotherapy, and after 21 d and 42 d of radiotherapy ($\bar{x}$ ± sd)

Time	Group	$CD4^+/CD8^+$	$CD19^+/CD69^+$	NK cell (%)
Before radiotherapy	Control group (n=46)	1.95±0.24	15.68±2.28	59.63±6.25
	Test group (n=51)	1.93±0.35	16.15±3.34	58.49±7.72
	t	0.325	0.801	0.794
	P	0.746	0.425	0.429
After 21 d of radiotherapy	Control group (n=46)	0.80±0.22***	5.86±1.28***	31.02±4.15***
	Test group (n=51)	0.99±0.35***	8.96±1.37***	39.64±6.02***
	t	3.161	11.479	8.124
	P	0.002	<0.001	<0.001
After 42 d of radiotherapy	Control group (n=46)	1.19±0.19***,###	9.36±2.02***,###	39.63±5.52***,###
	Test group (n=51)	1.76±0.38***,###	13.38±3.38***,###	45.96±6.32***,###
	t	9.187	7.014	5.228
	P	<0.001	<0.001	<0.001

Note: Paired t-test was used for the comparison in the same group before radiotherapy, ***P<0.001. Paired t-test was used for the comparison in the same group after 21 d of radiotherapy, ###P<0.001. NK: natural killer.

The levels of EGF and amylase were higher in the test group than in the control group (both P<0.05). See **Table 5**.

Comparison of cellular immune function

No significant differences between the levels of $CD4^+/CD8^+$, $CD19^+/CD69^+$, or NK cells were found in the test group compared to the control group before radiotherapy (all P>0.05). The levels of $CD4^+/CD8^+$, $CD19^+/CD69^+$, and NK cell counts after 21 d of radiotherapy were decreased in the two groups, and then were increased after 42 d of radiotherapy (all P<0.05). The levels of $CD4^+/CD8^+$, $CD19^+/CD69^+$, and NK cells in the test group after 21 d and 42 d of radiotherapy were higher than those of the control group (all P<0.05). See **Table 6**.

Comparison of serum cytokine levels

Serum cytokine (TGF-β1, IL-6 and CRP) levels before radiotherapy in the test group were not significantly different from those in the control group (all P>0.05). Serum cytokine levels were reduced after 21 d and 42 d of radiotherapy in the two groups (all P<0.05). The serum levels of TGF-β1, IL-6, and CRP in the test group after 21 d and 42 d of radiotherapy were lower than

Table 7. Comparison of serum cytokine levels before radiotherapy, and after 21 d and 42 d of radiotherapy ($\bar{x} \pm sd$)

Time	Group	TGF-β1 (ng/mL)	IL-6 (ng/L)	CRP (ng/L)
Before radiotherapy	Control group (n=46)	15.65±3.32	18.63±3.12	21.69±5.25
	Test group (n=51)	14.49±2.98	19.68±2.96	22.39±4.15
	t	1.814	1.700	0.732
	P	0.073	0.092	0.466
After 21 d of radiotherapy	Control group (n=46)	11.25±2.74***	15.98±2.18***	17.11±4.36***
	Test group (n=51)	9.02±2.19***	12.02±3.32***	13.39±5.28***
	t	4.448	6.863	3.760
	P	<0.001	<0.001	<0.001
After 42 d of radiotherapy	Control group (n=46)	8.19±1.65***,###	11.98±2.38***,###	13.84±5.84***,###
	Test group (n=51)	6.63±1.02***,###	9.02±1.74***,###	9.63±3.38***,###
	t	5.660	7.039	4.440
	P	<0.001	<0.001	<0.001

Note: Paired t-test was used for the comparison in the same group before radiotherapy, ***P<0.001. Paired t-test was used for the comparison in the same group after 21 d of radiotherapy, ###P<0.001. CRP: C-reactive protein.

those of the control group (all P<0.05). See **Table 7** and **Figure 1**.

Comparison of quality of life

Before radiotherapy, there was no significant difference in the QLICP-HN scores in the test group compared to the control group (P>0.05). The scores of each dimension of the QLICP-HN scale and total scores after 42 d of radiotherapy were increased in both groups (all P<0.05). All the scores of the test group were higher than those of the control group after 42 d of radiotherapy (all P<0.05). See **Table 8** and **Figure 2**.

Comparison of prognosis

Three months after radiotherapy, a recurrence of stomatitis was observed in both groups. The recurrence rates of the two groups were both 0, indicating a good prognosis in both.

Discussion

Currently, the pathogenesis of radiotherapy-induced oral mucositis has not been fully elucidated. It is generally believed to be related to inhibition of mucosal epithelial cell division and proliferation, an oral microflora disorder, weakened immune function, and activation of inflammatory factors due to radiation [17]. At this stage, the main prevention and control measures include cell protective agents, laser therapy, and cryotherapy, but the prevention and treatment effects vary greatly with no unified treatment standard. Borax-containing gargles are commonly applied in the treatment of oral mucositis, which protect the oral mucosa, have antibacterial and anti-inflammatory effects, but cannot promote mucosal repair. Additionally, long-term use of them may induce adverse reactions such as dry mouth and oral pigmentation [18].

Kangfuxin liquid, as an ethanol extract of American cockroach with polyols, mucous acids, peptides, a variety of growth factors and 18 kinds of amino acids, has the pharmacological effect of enhancing immunity, promoting blood circulation, nourishing yin and promoting granulation. It is widely used in the treatment of nasal diseases, digestive system diseases, and mouth ulcers. [19]. Qu et al. reported in a meta-systematic review that Kangfuxin liquid can effectively prevent diabetic foot ulcers and shorten treatment time [20]. Tang et al. have found that Kangfuxin liquid can alleviate acute ulcerative colitis in mice by inhibiting the inflammatory response and regulating the immune response [21]. However, there are few reports on the use of Kangfuxin liquid in the prevention and treatment of radiotherapy-reduced oral mucositis. In this study, the test group had a lower incidence of oral mucositis and oral pain than the control group after 21 d and 42 d of radiotherapy. The test group had increased scores of each dimension of the QLICP-HN scale and total scores in comparison to the control group after 42 d of radiotherapy. This

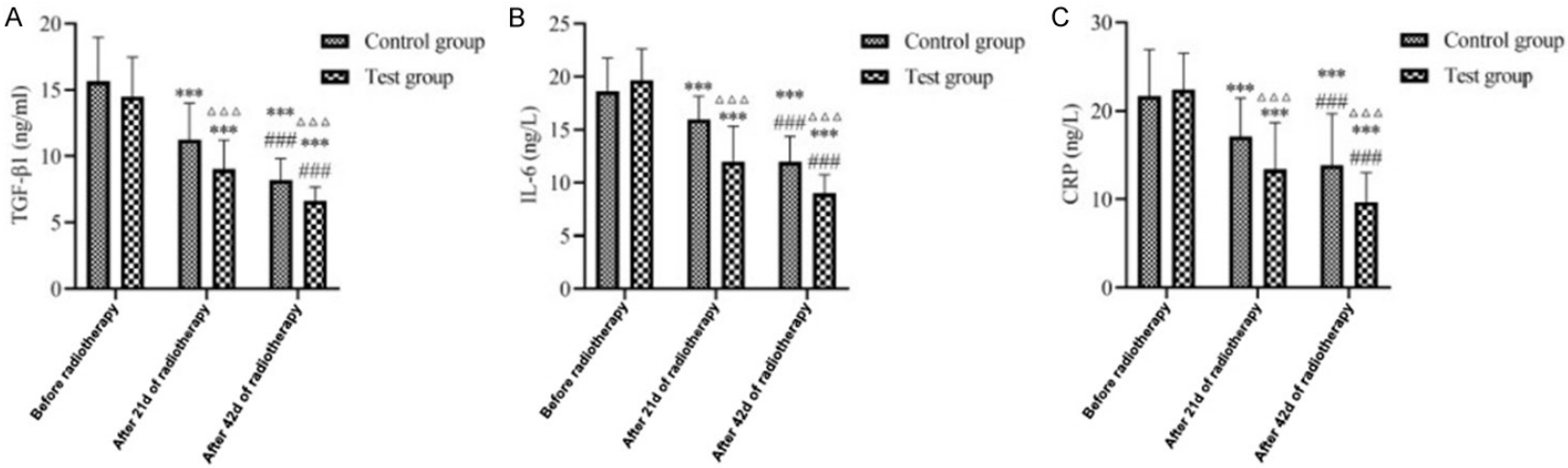

Figure 1. Comparison of serum cytokine levels before radiotherapy, after 21 d of radiotherapy, and after 42 d of radiotherapy. A: TGF-β1; B: IL-6; C: CRP. Compared to before radiotherapy in the same group, ***P<0.001; compared to after 21 d of radiotherapy in the same group, ###P<0.001; compared to the control group at the same time, ΔΔΔP<0.001. CRP: C-reactive protein.

Table 8. Comparison of quality of life before radiotherapy, and after 42 d of radiotherapy ($\bar{x}$ ± sd)

Time	Group	Mental function	Physical function	Social function	Head and neck cancer specific module	Common symptoms and side effects	Total score
Before radiotherapy	Control group (n=46)	21.03±4.58	20.65±3.98	45.63±6.32	20.22±4.15	23.65±4.11	131.18±28.65
	Test group (n=51)	22.16±4.96	19.65±4.02	46.18±5.52	19.68±3.95	22.98±3.96	130.62±29.32
	t	1.162	1.229	0.458	0.656	0.817	0.095
	P	0.248	0.222	0.648	0.513	0.416	0.925
After 42 d of radiotherapy	Control group (n=46)	28.96±5.52***	27.69±4.42***	51.16±4.96***	23.86±5.52***	26.48±3.02***	158.15±31.05***
	Test group (n=51)	34.96±6.32***	32.35±5.52***	56.39±3.25***	27.15±2.52***	31.15±2.14***	182.00±35.78***
	t	4.956	4.557	6.200	3.838	8.853	3.489
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Paired t-test was used for the comparison in the same group before radiotherapy, ***P<0.001.

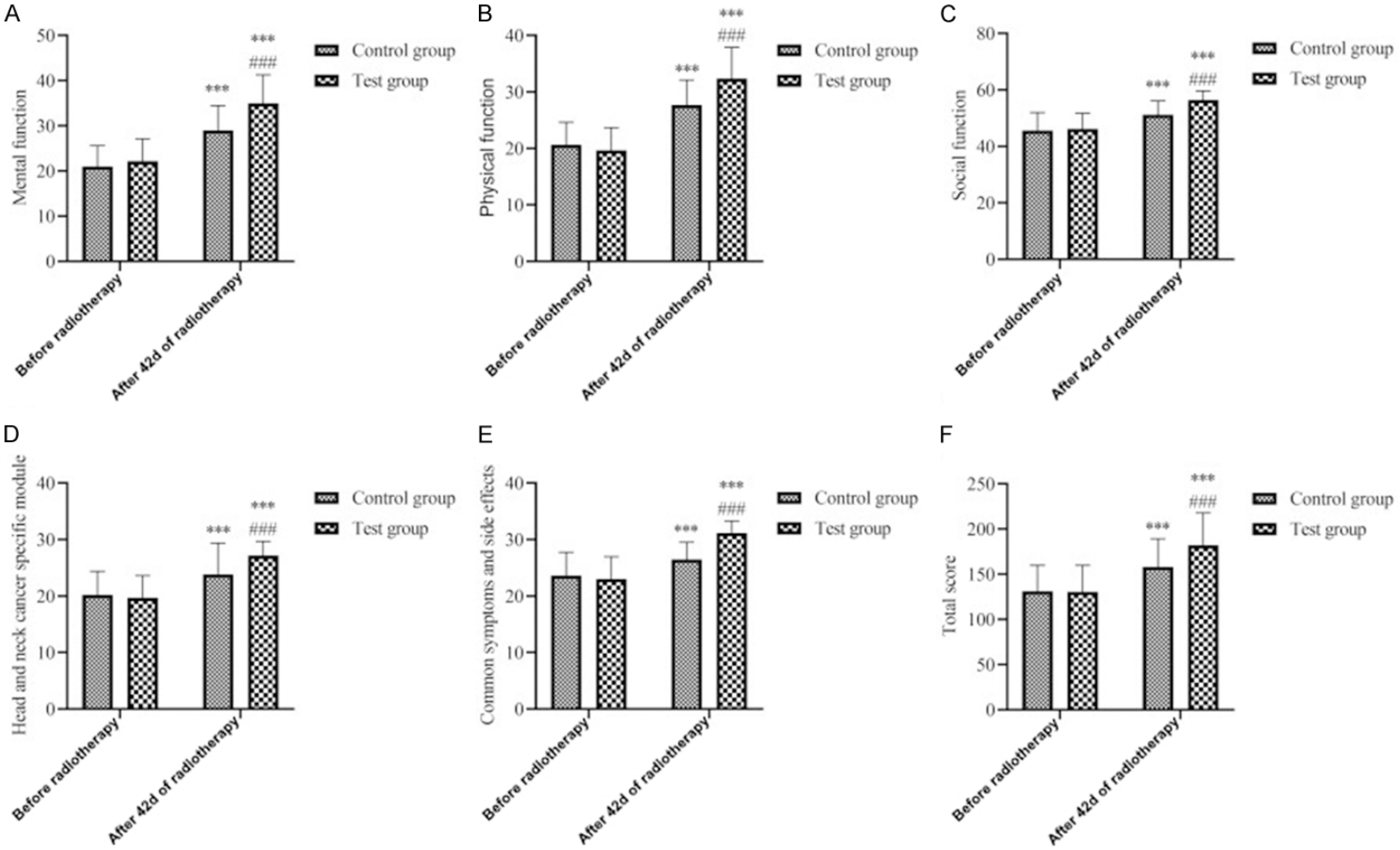

Figure 2. Comparison of quality of life before radiotherapy and after 42 d of radiotherapy. A: Mental function; B: Physical function; C: Social function; D: Head and neck cancer specific module; E: Common symptoms and side effects; F: Total score. Compared to before radiotherapy in the same group, $^{***}P<0.001$; compared to control group, $^{###}P<0.001$.

result was similar to some Chinese reports. For example, Bai et al. have reported that Kangfuxin liquid can significantly reduce the incidence of oral mucositis and oral pain in patients with nasopharyngeal cancer during radiotherapy [22]. Possible mechanisms of the Kangfuxin fluid are as follows. (1) The peptides in Kangfuxin fluid promote the synthesis and secretion of nucleic acids in the oral mucosa cells, thereby accelerating the growth of the new granulation tissue and promoting the repair of ulcer wounds. (2) The polyols in Kangfuxin liquid promote angiogenesis, granulation tissue proliferation, and epidermal cell proliferation, regulate the blood microcirculation of the wound, and accelerate the shedding of necrotic tissue, thereby repairing the wound [23]. (3) EGF is a stimulator of cellular mitosis. It plays an important role in promoting ulcer healing and extracellular matrix formation by influencing the proliferation and metabolism of epithelial cells and fibroblasts, regulating the inflammatory response, and changing the synthesis and secretion of extracellular matrix. Kangfuxin liquid is rich in epidermal growth factors that promote tissue regeneration and cell growth, which can enhance the production, differentiation, and proliferation of epidermal cells, promote the shedding of wound necrotic tissues, and then accelerate the healing of oral mucosa.

The main function of the salivary gland is to secrete saliva to maintain the normality and stability of digestive function, feeding, and speech. Excessive radiation exposure can cause apoptosis of glandular cells, a decrease in the total number of cells, and a weakening of secretory function, leading to a decrease in the amount of glandular secretion [24]. EGF is a single-chain polypeptide composed of 53 amino acids, which can aggregate many growth factors in wounds, stimulate epidermal growth and keratinization, promote the growth of epithelium and interstitial matter, and maintain the stability of the oral mucosa. Thereby, EGF plays a key role in promoting the healing of damaged mucosa and maintaining the integrity of the epithelium. When the content of EGF in saliva decreases, this may indicate a decrease in the thickness of mucus on the surface of the oral epithelium and the loss of the first protective barrier function of the epithelial tissue, thereby increasing the risk of oral ulcers and oral mucositis. Amylase is an indicator of the synthesis of proteins in the serous acinar cells of the salivary glands, which reflects sympathetic activity and glandular cell damage. Kawahara et al. found that baseline amylase levels were inversely correlated with the amount of stimulated saliva 1 month after chemoradiotherapy and positively correlated with EGF levels, indicating a decrease in amylase levels may increase the risk of oral mucositis during chemoradiotherapy [25]. When buffer systems such as phosphate and bicarbonate are intact, the pH of saliva is generally maintained within a neutral range. After the saliva buffer system is affected by external factors such as radiotherapy, it leads to abnormal oral pH and disrupts the homeostasis of oral flora. In this study, the saliva flow rate, pH of saliva, and levels of EGF and amylase were higher in the test group than in the control group after 21 d and 42 d of radiotherapy. This suggests that Kangfuxin liquid may improve salivary gland function in patients with head and neck squamous cell carcinoma undergoing radiotherapy by increasing salivary amylase and EGF levels, and maintaining oral pH, to prevent oral mucositis.

Immunosuppression is another important mechanism for the occurrence of radiotherapy-reduced oral mucositis. While killing tumor cells, radiation also damages normal cells and tissues to a certain extent, inhibits the immune system, causes neutropenia, leads to lymphocyte and B cell functional activity disorders, weakens the proliferation of immune cells, and thus promotes oral mucositis [26]. Under normal circumstances, $CD4^+/CD8^+$ is in a relatively balanced state, which helps maintain the body's immune function. When the $CD4^+/CD8^+$ value decreases, the body's anti-tumor ability is reduced, indicating disturbed immune function. NK cells, whose activity is closely related to patient prognosis, play an important role in immune regulation, immune surveillance, and killing target cells. $CD19^+$ is a totally specific antigen for B lymphocytes, which is closely involved in the physiological processes of B lymphocyte activation and signaling. $CD69^+$ is the earliest surface antigen expressed by T lymphocytes after activation, and can be used as a co-stimulation signal to promote further proliferation and activation of T cells. Yang et al. point out that kangfuxin liquid increases the

levels of $CD4^+/CD8^+$, and $CD19^+/CD69^+$ cells after radiotherapy and chemotherapy for nasopharyngeal cancer, and improves patients' cellular immune function [27]. In this study, the levels of $CD4^+/CD8^+$, $CD19^+/CD69^+$, and NK cells after 21 d of radiotherapy were decreased in the two groups, and then were increased after 42 d of radiotherapy. The levels of $CD4^+/CD8^+$, $CD19^+/CD69^+$, and NK cells in the test group after 21 d and 42 d of radiotherapy were higher than those of the control group, which was consistent with the above finding. In addition, in this study, after 21 d and 42 d of radiotherapy, the serum levels of TGF-β1, IL-6, and CRP in the test group were lower than those in the control group. It could be seen that Kangfuxin liquid improved the cellular immune function during radiotherapy and chemotherapy in patients with head and neck squamous cell carcinoma, reduced the inflammatory response, improved the tolerance, and reduced radiation injury and infection. The reasons are related to the following points. (1) The mucosulline contained in Kangfuxin liquid has the effect of activating non-specific immune function cells, which can enhance the direct phagocytosis of pathogens by NK cells, macrophages, and polymorphonuclear leukocytes, thereby enhancing serum lysozyme activity and lymphocyte activity, releasing free radicals, and killing microorganisms [28, 29]. (2) Kangfuxin liquid can reduce the effect of effector T cells on local accumulation of tissue by regulating the cytokine network and cytokine secretion, and enhance wound immunity. (3) Kangfuxin liquid increases the number of neutrophils in the wound and improves the function of neutrophil actin, thereby enhancing the body's immune function. (4) In addition, kangfuxin liquid promotes the body to produce leukotrienes, interleukins, and prostaglandins, accelerates the elimination of edema, and weakens the inflammatory response [30].

However, some limitations existed in this study. This was a single-center study with small sample size, and short observation time. A multicenter study should be further carried out, the number of cases should be expanded, and the observation time should be extended in the future.

To sum up, Kangfuxin liquid effectively prevents the occurrence of radiotherapy-induced oral mucositis for patients with head and neck squamous cell carcinoma, reduces oral mucosal reactions and oral pain, improves salivary gland function, reduces inflammatory response, promotes cellular immune function, improves quality of life, and improves prognosis.

Disclosure of conflict of interest

None.

Address correspondence to: Yan Wei, Department of Oncology, Guigang City People's Hospital, No.1 Zhongshan Middle Road, Gangbei District, Guigang 537100, Guangxi Zhuang Autonomous Region, China. Tel: +86-0775-4200219; E-mail: drhaoji@163.com

References

[1] Sio TT, Le-Rademacher JG, Leenstra JL, Loprinzi CL, Rine G, Curtis A, Singh AK, Martenson JA Jr, Novotny PJ, Tan AD, Qin R, Ko SJ, Reiter PL and Miller RC. Effect of doxepin mouthwash or diphenhydramine-lidocaine-antacid mouthwash vs placebo on radiotherapy-related oral mucositis pain: the alliance A221304 randomized clinical trial. JAMA 2019; 321: 1481-1490.

[2] Rades D, Narvaez CA, Doemer C, Janssen S, Olbrich D, Tvilsted S, Conde-Moreno AJ and Cacicedo J. Radiotherapy-related skin toxicity (RAREST-02): a randomized trial testing the effect of a mobile application reminding head-and-neck cancer patients to perform skin care (Reminder App) on radiation dermatitis. Trials 2020; 21: 424.

[3] Faustino ISP, Georgaki M, Santos-Silva AR, Vargas PA and Lopes MA. Head and neck radiotherapy leading to extensive late oral soft-tissue necrosis. Oral Oncol 2022; 125: 105710.

[4] Nayar S, Greer A, Mosaku A and Vere J. The use of intraoral devices in reducing oral and dental side effects in head and neck cancer patients undergoing radiotherapy - a systematic review. Int J Prosthodont 2022; 35: 233-239.

[5] Rodríguez-Caballero A, Torres-Lagares D, Robles-García M, Pachón-Ibáñez J, González-Padilla D and Gutiérrez-Pérez JL. Cancer treatment-induced oral mucositis: a critical review. Int J Oral Maxillofac Surg 2012; 41: 225-238.

[6] Zou JB, Zhang XF, Shi YJ, Tai J, Wang Y, Liang YL, Wang F, Cheng JX, Wang J and Guo DY. Therapeutic efficacy of Kangfuxin liquid combined with PPIs in gastric ulcer. Evid Based Complement Alternat Med 2019; 2019: 1324969.

[7] Li HB, Chen MY, Qiu ZW, Cai QQ, Li DT, Tang HM and Chen XL. Efficacy and safety of kangfuxin liquid combined with aminosalicylic acid for the treatment of ulcerative colitis: a systematic review and meta-analysis. Medicine (Baltimore) 2018; 97: e10807.
[8] Wang J, Zhou GH, Long B, Luo WM and Liu ZH. Value analysis of selenium yeast combined with rehabilitation new solution for the prevention and treatment of oral mucositis caused by simultaneous radiotherapy and chemotherapy for nasopharyngeal carcinoma. Pract Onco J 2020; 35: 550-554.
[9] Radiation Oncology Committee of Chinese Anti-Cancer Association, Head and Neck Tumor Professional Committee of Chinese Anti-Cancer Association. Expert consensus on comprehensive treatment of head and neck tumors. Chin J Otolaryngol Head Neck Surg 2010; 45: 535-541.
[10] Guo W, Yin GF, Duan HY, Liu HF, Huang JW, Yang Z, Xu HB and Huang ZG. Factors related to tumor mutation burden in head and neck squamous cell carcinoma. Chin Department Otolaryngol Head Neck Surg 2020; 27: 13-16.
[11] Gu L, Zhang W and Wang JY. Effects of kidney and spleen shengjin formula on saliva secretion and quality of life in patients with radiotherapy for head and neck malignant tumors. Int J Tradit Chin Med 2020; 42: 950-954.
[12] Ban XL, Wang HT and Shen PY. Riboflavin sodium phosphate combined with rehabilitation new solution for the prevention and treatment of oral mucositis caused by radiotherapy and chemotherapy of head and neck tumors. J Pract Med 2020; 37: 990-992.
[13] Liu YJ, Zhu GP and Guan XY. Comparison of the NCI-CTCAE version 4. 0 and version 3. 0 in assessing chemoradiation-induced oral mucositis for locally advanced nasopharyngeal carcinoma. Oral Oncol 2012; 48: 554-559.
[14] Liao SQ, Qiu SH, Li BX and Chen PY. Changes in Saliva flow rate and subjective oral feelings in patients with radiotherapy for nasopharyngeal carcinoma. EJL Clin Nur 2020; 53-54.
[15] Dong KC, Zhang M, Liang Y, Zhang MJ and Fei XX. Therapeutic effect of rehabilitation new liquid on severe radiation oral mucositis after radiotherapy for head and neck tumors and protective effect on salivary glands. World J Integr Med 2021; 16: 123-127.
[16] Li ZQ. Development and evaluation of head and neck cancer scale QLICP-HN(V2.0T) of cancer patient quality of life measurement scale system. Kunming Med Univ 2017.
[17] Wu M, Ou D, He X and Hu CS. Long-term results of a phase II study of gemcitabine and cisplatin chemotherapy combined with intensity-modulated radiotherapy in locoregionally advanced nasopharyngeal carcinoma. Oral Oncol 2017; 73: 118-123.
[18] Judge LF, Farrugia MK and Singh AK. Narrative review of the management of oral mucositis during chemoradiation for head and neck cancer. Ann Transl Med 2021; 9: 916.
[19] Wang T, Lu H, Li F and Zhang Q. Effect of Kangfuxin liquid enema combined with mesalazine on gestational outcomes and quality of life in child-bearing female with active ulcerative colitis: a protocol for randomized, double-blind, controlled trial. Medicine (Baltimore) 2021; 100: e23915.
[20] Qu KS, Li Y, Liang Y, Hu XJ, Wang XY, Chen X and Que HF. KangFuXin liquid in the treatment of diabetic foot ulcer: a systematic review and meta-analysis. J Clin Oncol 2014; 32: 1571-1577.
[21] Tang M, Ni LL, Xu JL, Wang YJ, Zhang CG, Ali T, Wu XM, Liu H and He M. Kangfuxin liquid ameliorates Dextran Sulfate Sodium (DSS)-induced acute ulcerative colitis in mice by modulating immune response and suppressing inflammation. Med Sci Monit Basic Res 2021; 27: e930887.
[22] Bai HF, Jiang QH, Zeng WQ, Li B, Xiang KH and Lang JY. Effect of rehabilitation new solution in the prevention and treatment of oral mucositis caused by radiotherapy for nasopharyngeal carcinoma. Cancer Prev Treat 2017; 30: 43-48.
[23] Hu Q and Ke G. Intravesical instillation of Kangfuxin liquid combined with thrombin and epidermal growth factor for radiation-induced hemorrhagic cystitis in patients with cervical cancer: a report of 34 cases. Bioengineered 2021; 12: 815-820.
[24] Ma J, Yang YC, Su LQ, Qin DM, Yuan K, Zhang Y and Wang RR. The Liquid Kangfuxin (KFX) has efficient antifungal activity and can be used in the treatment of vulvovaginal candidiasis in mice. Lett Appl Microbiol 2022; 74: 564-576.
[25] Kawahara K, Hiraki A, Arita H, Takeshita H, Hirosue A, Matsuoka Y, Sakata J, Obayashi Y, Nakashima H, Hirayama M, Nagata M, Yoshida R, Shinohara M and Nakayama H. Role of serum amylase and salivary cytokines in oral complications during chemoradiotherapy. Oral Dis 2021; 27: 1564-1571.
[26] Ma PT, Wu NN and Pei R. Effect of Kangfuxin liquid combined with garlicin capsules in treatment of children with recurrent oral ulcer and on immune regulation. Shanghai Kou Qiang Yi Xue 2018; 27: 526-529.
[27] Yang YP, Liu HT, Song XF, Yang SJ, Dong GN and Gu JQ. Application of sanshendi soup combined with new rehabilitation solution in radiotherapy treatment of nasopharyngeal carcinoma and its effects on oral mucosal response,

cellular immune function and quality of life. Sichuan J Trad Chin Med 2019; 37: 180-183.
[28] Xue P, Wang L, Xu J, Liu JY, Pan XH, Zhao YZ and Xu HL. Temperature-sensitive hydrogel for rectal perfusion improved the therapeutic effect of kangfuxin liquid on dss-induced ulcerative colitis mice: the inflammation alleviation and the colonic mucosal barriers repair. Int J Pharm 2020; 589: 119846.
[29] Lu S, Wu D, Sun G, Geng FN, Shen YM, Tan J, Sun XB and Luo Y. Gastroprotective effects of kangfuxin against water-immersion and restraint stress-induced gastric ulcer in rats: roles of antioxidation, anti-inflammation, and pro-survival. Pharm Biol 2019; 57: 770-777.
[30] Wei J, Wu J, Wang H, Wang B, Zhao TT, Meng LB, Dong LH and Jiang X. A bioadhesive barrier-forming oral liquid gel improved oral mucositis and nutritional status in patients with head and neck cancers undergoing radiotherapy: a retrospective single center study. Front Oncol 2021; 11: 617392.