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Original Research Article

Effects of Yifukang Oral Liquid on Chemotherapy- and Radiotherapy-Induced Toxic and Side Effects of Myelosuppression, Leucopenia and Gastrointestinal Tract Disturbances

Xi-Kui Tian[1], Hong-Di Lv[1], Qing-Hua Zhao[2], Shao-Jun Hao[1], Xiu-Li Geng[1], Xi-Dong Wang[1], Zheng-Chen Zhang[1] and Jian-Chang Zhao[1]*

[1]The 371st Central Hospital of PLA, Xinxiang 453000, Henan, [2]The Jinan Military Institute for Drug Control, Jinan 250000, Shandong, PR China

For correspondence:* ***Email:* *jczhao_2015@163.com*

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Abstract

Purpose: *To investigate the effects of Yifukang oral liquid (YFKOL) on chemotherapy- and radiotherapy-induced myelosuppression, leucopenia and gastrointestinal tract disturbances.*
Methods: *The effects of YFKOL on myelosuppression, leucopenia and gastrointestinal tract disturbances were assessed by cyclophosphamide- and Co60-induced leucopenia in mice, copper sulfate-induced emesis in pigeons, ethanol-induced gastric mucosal lesions in rats, gastric emptying and intestinal propulsion in mice.*
Results: *In cyclophosphamide- and Co60-induced leucopenia assays, the mean white blood cell count (82.6 and 90.1 × 10^9/L; 7.3 and 8.2 × 10^9/L, respectively) and thighbone marrow granulocytes (66.1 % and 67.4 %; 60.8 and 66.5 %, respectively) were significantly ($p < 0.05$) increased after treatment with YFKOL (15 and 30 mL/kg), compared with the respective control (68.2 and 4.7 × 10^9/L; 58.2 and 53.1 %). In emesis, gastric mucosal lesions, gastric emptying and intestinal propulsion assays, the mean frequency of emesis (30.8 and 22.3 times, respectively) and ulcer index (39.6 and 26.5, respectively) significantly ($p < 0.05$) decreased, and the mean gastric emptying (25.0 and 24.0 %) and intestinal propulsion (81.9 and 82.8 %) were significantly ($p < 0.05$) promoted after treatment with YFKOL (10 and 20 mL/kg), compared with the respective control (54.7 times, 62.8, 42.0 and 68.9 %).*
Conclusion: *YFKOL may suppress chemotherapy- and radiotherapy-induced myelosuppression, leucopenia and gastrointestinal tract disturbances.*

Keywords: *Yifukang oral liquid, Gastrointestinal tract disturbances, Leucopenia, Myelosuppression, Tumor, Chemotherapy, Radiotherapy*

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INTRODUCTION

Currently, malignant tumor is the leading cause of death in human, and middle-late malignant tumor can cause the symptoms of anepithymia, muscle weakness, weight loss, hypoimmunity, pain, etc [1], which will become more obvious with the development of tumor. Chemotherapy and radiotherapy, the usual methods for treating tumor [2-5], does not only exacerbate the above symptoms, but also induce many toxic and side effects, such as myelosuppression, leucopenia, emesis and nausea [6-8].

Based on the knowledge of traditional Chinese medicine, a prescription named Yifukang oral liquid (YFKOL) was developed by our team to suppress the symptoms of myelosuppression, leucopenia and gastrointestinal tract disturbances induced by chemotherapy and radiotherapy. YFKOL is composed of 6 kinds of Chinese medicines including Ginseng Radix et Rhizoma (200 g), Pangolin Scales (100 g), Angelicae Sinensis Radix (150 g), Astragali Complanati Semen (300 g), Scrophulariae Radix (200 g) and Ophiopogonis Radix (200 g).

The objective of this study was to investigate the effects of YFKOL on chemotherapy- and radiotherapy-induced myelosuppression, leucopenia and gastrointestinal tract disturbances were assessed by cyclophosphamide- and Co60-induced leucopenia models in mice, copper sulfate-induced emesis model in pigeons, ethanol-induced gastric mucosal lesions model in rats, using gastric emptying and intestinal propulsion assays in mice.

EXPERIMENTAL

Plant materials

Ginseng Radix et Rhizoma, Pangolin Scales, Angelicae Sinensis Radix, Astragali Complanati Semen, Scrophulariae Radix and Ophiopogonis Radix were obtained from Beijing TongRenTang, Co, Ltd and the plant materials were identified by Shao-Jun Hao, a taxonomist, the 371st Central Hospital of PLA's Pharmacy Department, Xinxiang Henan, China. A series of voucher specimen (voucher no. 201381/CPLA371, 201382/CPLA371, 201383/CPLA371, 201384/CPLA371, 201385/CPLA371 and 201386/CPLA371) was stored in dispensary of traditional Chinese medicine of our hospital for future reference.

Chemicals and reagents

Cyclophosphamide was purchased from the 12th Shanghai pharmaceutical factory (batch number: 080330). Copper sulfate was obtained from Zhengzhou chemical reagent first factory (batch number: 920429). Methyl orange was purchased from Shanghai reagent third factory (batch number: 930924). Fu Fang E Jiao Jiang (FFEJJ) was purchased from Shan Dong Dong-E E-Jiao Co., Ltd (batch number: 080112). Oulishu oral liquid (OLSOL) was obtained from Xinxiang yuanda pharmaceutical factory in China (batch number: 960607).

Preparation of YFKOL

YFKOL was provided by the 371st Central Hospital of PLA's manufacturing laboratory (batch number: 970203). The preparation of YFKOL was described as follows. Ginseng Radix et Rhizoma (200 g), Pangolin Scales (100 g), Angelicae Sinensis Radix (150 g), Astragali Complanati Semen (300 g), Scrophulariae Radix (200 g) and Ophiopogonis Radix (200 g) were decocted with water for 40 min for three times. The combined decoction filtrate was concentrated to reach a relative density of 1.15 - 1.30 (80 °C), then concentrate was diluted by ethanol to obtain 60 % ethanol solution followed by standing at room temperature for 24 h. After filtration, the obtained ethanol solution was condensed to remove alcohol, and simple syrup was added into the solution. The above solution was diluted by water to get 1000 mL (pH = 5.0 - 7.0) solution which was filtrated and sterilized to gain YFKOL.

Animals

All animal treatments in this study were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [9] and experiments were approved by the ethics committee of the 371st Central Hospital of PLA (protocol no. PLA 371 CH 2013). In this research, a lot of measures were performed to reduce the amount of animals and relieve the suffering of animals. Male and female pigeons (300 - 450 g), SD rats (200 ± 20 g) and ICR mice (20 ± 2 g) were provided by Henan medical university laboratory animal center. Animals were bred with constant room temperature (25 ºC) and appropriate humidity (55 – 60 %). Animals had free access to food and water.

Acute toxicity study of YFKOL

The result of acute toxicity studies of YFKOL showed that maximum tolerated dose (MTD) of YFKOL was more than 0.03 mL/g, which was far more than the maximum clinical dosage of YFKOL.

Cyclophosphamide-induced leucopenia assay

ICR mice were randomly divided into normal, control, YFKOL (high and low dose) and FFEJJ groups (n = 10). Control, YFKOL (high and low dose) and FFEJJ groups were treated with 80 mg/kg cyclophosphamide once a day (3 days) by intraperitoneal injection (ip) and normal group was treated with isopyknic normal saline once a

day (3 days) by ip. Meanwhile, control, YFKOL (high and low dose) and FFEJJ groups were separately treated with normal saline, YFKOL (30 and 15 mL/kg) and FFEJJ (30 mL/kg) once a day (7 days) by intragastric administration (ig) and normal group was treated with isopyknic normal saline once a day (7 days) by ig. Finally, mice were directly sacrificed to obtain orbital blood and thighbone marrow after 2 h of drugs treatment on the seventh day. Orbital blood was used to determine white blood cell count according to the existed method [10]. Thighbone marrow smears, which were stained by Wright's stain, were monitored by a microscope. Thighbone marrow granulocytes percentage was used to evaluate the effect of drugs on cyclophosphamide-induced leucopenia [11].

Co60-induced leucopenia assay

In this assay, all groups' treatments and tests were the same as cyclophosphamide-induced leucopenia assay except that the modeling for control, YFKOL (high and low dose) and FFEJJ groups were induced by Co60.

Copper sulfate-induced emesis assay

Pigeons were randomly divided into control, YFKOL (high and low dose) and OLSOL groups (n = 10). Control, YFKOL (high and low doses) and OLSOL groups were separately treated with normal saline, YFKOL (20 and 10 mL/kg) and OLSOL (8 mL/kg) once a day (3 days) by ig. Then, copper sulfate was administered orally to pigeons of control, YFKOL (high and low dose) and OLSOL groups at a dose of 300 mg/kg after 0.5 h of drugs treatment on the third day. Finally, the effects of drugs on copper sulfate-induced emesis were analyzed by incubation period and number of emesis within 1 h.

Ethanol-induced gastric mucosal lesions assay

SD rats were randomly divided into control, YFKOL (high and low dose) and OLSOL groups (n = 10). The treatments of all groups were the same as copper sulfate-induced emesis assay. All rats were managed with abrosia, but had free access to water for 12 h on the third day. 75 % ethanol (0.8 mL) was administered orally to each rat after 1.5 h of drugs treatment on the third day. After another one hour, mice were directly sacrificed for gathering stomach, which was then fixed by 1 % formaldehyde for 30 min. The situation of gastric mucosal lesions was directly observed. Based on gastric mucosal lesions area (mm^2), ulcer index was calculated according to existing method [12]. Damage inhibition rate, calculated as in Eq 1, and ulcer index was used to evaluate the effect of drugs on 75% ethanol-induced gastric mucosal lesions [13].

Damage inhibition rate (%) = {(A – B)/A}100 ……. (1)

Where A and B stands for the ulcer index of control group and treatment group, respectively.

Gastric emptying assay

ICR mice were randomly divided into control, YFKOL (high and low dose) and OLSOL groups (n = 10). The treatments of all groups were the same as copper sulfate-induced emesis assay. All mice were managed with abrosia, but had free access to water for 12 h on the third day. Methyl orange aqueous solution (0.1 %, 0.2 mL) was administered orally to each mouse after 1 h of drugs treatment on the third day. Then, mice were directly sacrificed for gathering the stomach after 20 min of methyl orange aqueous solution treatment. Gastric content was washed into test tube by 10 mL distilled water, and the pH of above solution was adjusted to 6.0 - 6.5 by 5 % sodium bicarbonate. After filtration, the filtrate was analyzed by spectrophotometer at 420 nm. The gastric residual rate of methyl orange, used to evaluate the effect of drugs on gastric emptying, was calculated as in Eq 2 [14].

G (%) = (C/D)100 …….. (2)

where C and D stands for the optical density (OD) of methyl orange in stomach and methyl orange aqueous solution (0.1 %, 0.2 mL), dilute by 10 mL distilled water, respectively.

Intestinal propulsion assay

ICR mice were randomly divided into control, YFKOL (high and low dose) and OLSOL groups (n = 10). The treatments of all groups were the same as copper sulfate-induced emesis assay. All mice were managed with abrosia, but had free access to water for 12 h on the third day. Carbon powder suspension composed of 10 % arabic gum and 5 % active carbon (0.2 mL) was administered orally to each mouse after 1 h of drugs treatment on the third day. Then mice were directly sacrificed for gathering total gastrointestinal tract after 20 min of carbon powder suspension treatment. The distance between pylorus and ileocecum (E), and between pylorus and carbon powder forefront (F) were determined. Further, the propulsive rate of carbon powder, calculated as in Eq 3, was used to evaluate the effect of drugs on intestinal propulsion.

Propulsive rate (%) = {(E – F)/E}100 ……(3)

Statistical analysis

All data are presented as mean ± standard deviation (SD, n = 10) and were analyzed by one-way ANOVA of Statistical Package and Social Sciences (SPSS), version 21.0. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of YFKOL on cyclophosphamide-induced leucopenia in mice

The effects of YFKOL and FFEJJ on cyclophosphamide-induced leucopenia are shown in Figures 1 and 2 separately. After treatment with cyclophosphamide, the white blood cell count and thighbone marrow granulocytes percentage of control group significantly ($p < 0.05$ or 0.01) were inhibited, compared with normal group, which indicated the modeling was successful. The results shows that YFKOL (15 and 30 mL/kg) and FFEJJ (30 mL/kg) significantly ($p < 0.05$ or 0.01) increased the white blood cell count and thighbone marrow granulocytes percentage, compared with control group.

Effect of YFKOL on Co60-induced leucopenia in mice

After treatment with Co60, the white blood cell count and thighbone marrow granulocytes percentage of control group were significantly ($p < 0.05$ or 0.01) inhibited, compared with normal group, which indicated the modeling was successful. The effects of YFKOL (15 and 30 mL/kg) and FFEJJ (30 mL/kg) on Co60-induced leucopenia indicated that they significantly ($p < 0.05$ or 0.01) increased the white blood cell count and thighbone marrow granulocytes percentage,

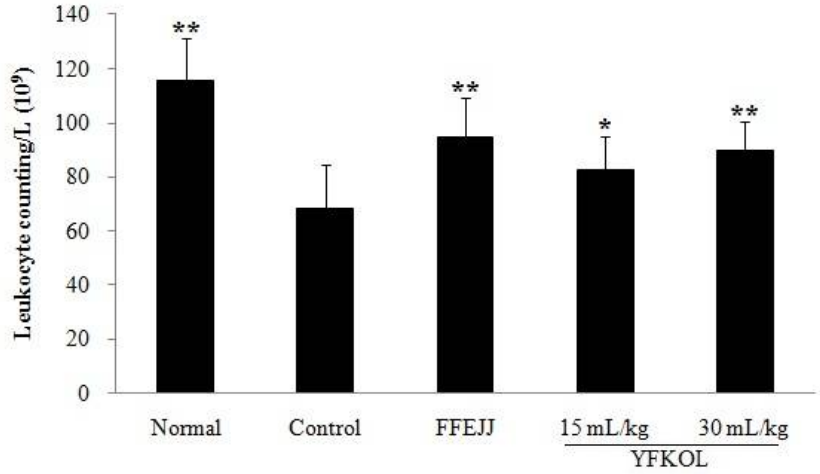

Figure 1: Effects of YFKOL and FFEJJ on cyclophosphamide-induced leucopenia model in ICR mice (n = 10); *$p < 0.05$, **$p < 0.01$, compared with control group

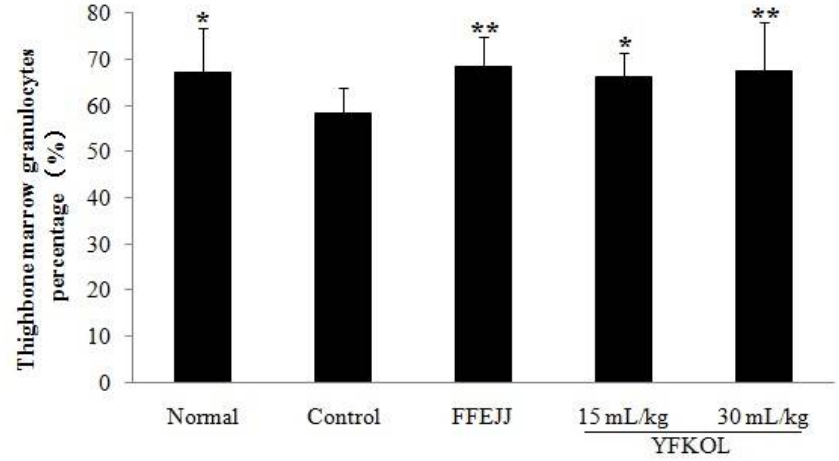

Figure 2: Effects of YFKOL and FFEJJ on thighbone marrow granulocytes of cyclophosphamide-induced leucopenia model in ICR mice (n = 10); *$p < 0.05$, **$p < 0.01$, compared with control group

compared with control group, except that YFKOL (15 mL/kg) cannot raise thighbone marrow granulocytes percentage. The results are depicted in Figures 3 and 4.

Effects of YFKOL on copper sulfate-induced emesis in pigeons

The results (Table 1) indicate that YFKOL (10 and 20 mL/kg) and OLSOL (8 mL/kg) significantly ($p < 0.05$ or 0.01) decreased the number of emesis induced by copper sulfate within 1 h, compared with control group. Moreover, YFKOL (20 mL/kg) significantly ($p < 0.05$) inhibited incubation period of emesis induced by copper sulfate, compared with control group.

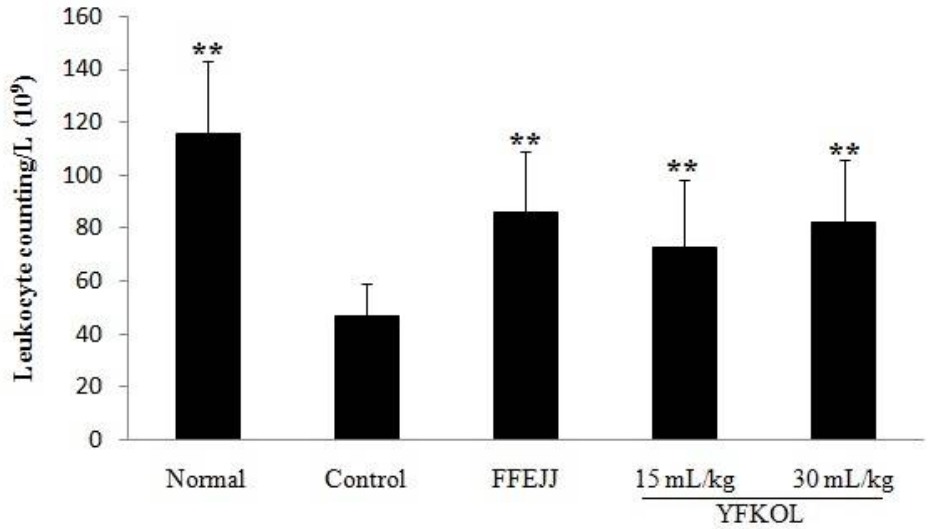

Figure 3: Effects of YFKOL and FFEJJ on Co60-induced leucopenia model in ICR mice (n = 10); *$p < 0.05$, **$p < 0.01$, compared with control group

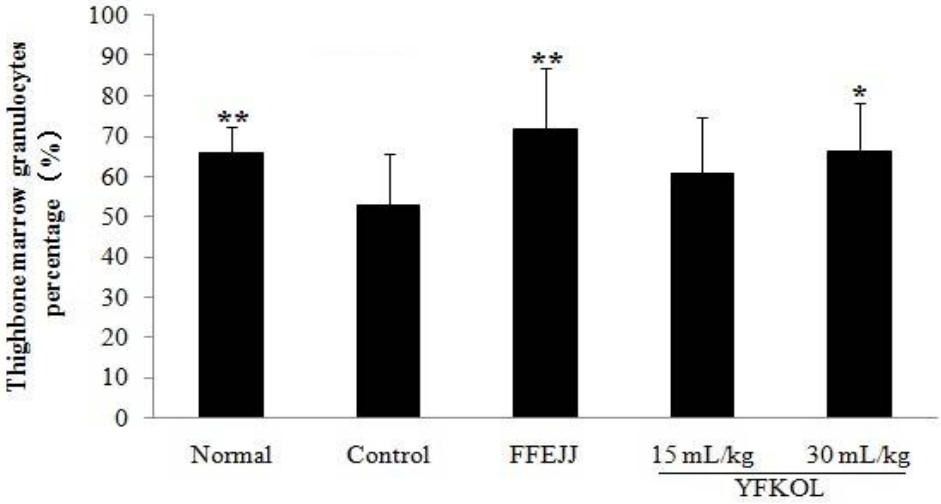

Figure 4: Effects of YFKOL and FFEJJ on thighbone marrow granulocytes of Co60-induced leucopenia model in ICR mice (n = 10); *$p < 0.05$, **$p < 0.01$, compared with control group

Table 1: Effect of YFKOL on copper sulfate-induced emesis model

Group	Dose (mL/kg)	Incubation period of emesis (min)	Number of emesis (time/h)
Control		16.4 ± 5.2	54.7 ± 28.9
OLSOL	8	18.0 ± 6.9	27.4 ± 14.4*
YFKOL	10	19.5 ± 8.1	30.8 ± 14.7*
YFKOL	20	26.2 ± 10.5*	22.3 ± 13.0**

*All data stood for mean ± standard deviation (n = 10); *p < 0.05, **p < 0.01 compared with control group*

Effect of YFKOL on 75 % ethanol-induced gastric mucosal lesions in rats

The results (Table 2) indicated that YFKOL (10 and 20 mL/kg) and OLSOL (8 mL/kg) significantly ($p < 0.01$) decreased ulcer index induced by 75 % ethanol, compared with control group. Furthermore, the damage inhibition rate (%) of OLSOL, 10 mL/kg YFKOL and 20 mL/kg YFKOL were 28.7, 36.9 and 57.8 separately.

Effect of YFKOL on gastric emptying and intestinal propulsion in mice

The results (Table 3) indicated that YFKOL (10 and 20 mL/kg) and OLSOL (8 mL/kg) significantly ($p < 0.05$ or 0.01) decreased gastric residual rate of methyl orange and improved intestinal propulsion, compared with control group.

DISCUSSION

Based on the clinical observation, myelosuppression, leucopenia and gastrointestinal tract disturbances are the usual toxic and side effects of cancer chemotherapy and radiotherapy, which do great damage to immune system and digestive system of patients [15-17]. Thus, reducing these toxic and side effects were vital in the treatment of cancer. According to the knowledge of traditional Chinese medicine, YFKOL was developed to reduce chemotherapy- and radiotherapy-induced toxic and side effects of myelosuppression, leucopenia and gastrointestinal tract disturbances.

Cyclophosphamide-based chemotherapy and Co60-based radiotherapy are extensively used to treat tumor in clinic [18,19]. Thus, cyclophosphamide and Co60 were used to induce leucopenia in this research [20]. Similarly, FFEJJ and OLSOL were selected as positive control in this study [21-23].

The cyclophosphamide-induced and Co60-induced leucopenia models in mice are two common methods used to study the therapeutic effects of drugs on leucopenia [24,25]. In this work, the two models were used to study the immune-enhancement effects of YFKOL. As depicted in Figures 1 - 4, chemotherapy- and radiotherapy-induced toxic and side effects of myelosuppression and leucopenia can be significantly decreased by YFKOL through increasing white blood cell count and thighbone marrow granulocytes percentage.

The copper sulfate-induced emesis model in pigeon and ethanol-induced gastric mucosal lesions model in rat are commonly used to explore the effects of drugs on emesis and gastric mucosal lesions [26,27]. In this work, the two models were used to study the effects of YFKOL on emesis and gastric mucosal lesions. As shown in Tables 1 and 2, YFKOL can significantly inhibit copper sulfate-induced emesis and ethanol-induced gastric mucosal lesions by decreasing the number of emesis and damage to gastric mucosa.

The gastric emptying and intestinal propulsion assays in mice are commonly used to study the effects of drugs on gastric emptying and intestinal propulsion functions [28]. In this research, the two assays were performed to study the effects of YFKOL on gastric functions. As shown in Table 3, YFKOL can significantly improve gastric functions by promoting gastric emptying and intestinal propulsion.

Table 2: Effect of YFKOL on 75 % ethanol-induced gastric mucosal lesions model

Group	Dose (mL/kg)	Ulcer index	Damage inhibition rate (%)
Control		62.8 ± 13.4	
OLSOL	8	44.8 ± 11.8**	28.7
YFKOL	10	39.6 ± 11.5**	36.9
YFKOL	20	26.5 ± 9.0**	57.8

*All data stood for mean ± standard deviation (n = 10). **p < 0.01, compared with control group*

Table 3: Effect of YFKOL on gastric emptying and intestinal propulsion

Group	Dose (mL/kg)	Methyl orange residual rate (%)	Propulsive rate (%)
Control		42.0 ± 10.0	68.9 ± 9.8
OLSOL	8	28.0 ± 8.0**	77.3± 5.8*
YFKOL	10	25.0 ± 7.0**	81.9 ± 16.2**
YFKOL	20	24.0 ± 10.0**	82.8 ± 7.2**

*All data stood for mean ± standard deviation (n = 10); *p < 0.05, **p < 0.01, compared with control group*

CONCLUSION

YFKOL suppresses symptoms of myelo-suppression, leucopenia and gastrointestinal tract, induced by chemotherapy and radiotherapy, by increasing white blood cell count and thighbone marrow granulocytes percentage, minimizing emesis and damage to gastric mucosa, and promoting gastric emptying and intestinal propulsion in animal models. This study may lead to another approach to tackling the toxic and side effects of myelosuppression, leucopenia and gastrointestinal tract disturbances induced by chemotherapy and radiotherapy. Thus, YFKOL may be used to help cancer patients to go through the undesirable effects of chemotherapy and radiotherapy, and therefore further efforst are required to investigate YFKOL in this regard.

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