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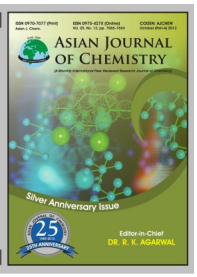

Antidiarrheal Activity of Extract from *Herba kummerowiae* in Mice

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Herba kummerowiae is a traditional Chinese herb with functions of heat-clearing, detoxicating, promoting urination and antidiarrhea and have been used in the treatment of gastroenteritis, dysentery and urinary system infection. There is few research on chemical composition activities, pharmacology, *etc*. In this study, the extract from *Herba kummerowiae* was analyzed by HPLC, two kinds of compouds including genistein, kaempferol were identifed. The content was respectively 0.16 and 0.37 %. Through the senna leaf diarrhea model, antidiarrheal activity of the extract in mice was studied. Compared with the model control group, the loose stools rate and diarrhea index were reduced by *Herba kummerowiae*, xiaoer xiesuting granule and montmorillonite powder ($p < 0.05$). The effect of inhibitting peristalsis was related with the dose of *Herba kummerowiae* and the effect was weaker than montmorillonite powder. The results show that *Herba kummerowiae* is effective in diarrhea and more suitable for children or the aged.

Key Words: *Herba kummerowiae*, Kaempferol, Genistein, HPLC, Diarrhea.

INTRODUCTION

Two species of genus *Kummerowia* (Leguminosae), *Kummerowia striata* Thunb. and *K. stipulacea* (Maxim.) Makino, are used for medicinal purposes in Traditional Chinese-system of Medicine (TCM) and else-where as a cure for different diseases of digestive system. Herb of the genus plants are as Traditional Chinese drugs called *Herba kummerowiae* (Jiyancao). These plants have a wide distribution in China. Based on the theories describing the medicinal properties of this plant in the traditional Chinese Medicine, *Herba kummerowiae* has functions of heat-clearing, detoxicating, promoting urination and antidiarrhea and has been used in the treatment of gastroenteritis, dysentery and urinary system infection. Nevertheless, there is few research on chemical composition, activities, pharmacology, *etc*. Luteolin 4′-O-glucopyranoside was identified as the IL-5 inhibitor from *Kummerowia striata* Thunb[1]. *K. striata* (Thunb.) Schindl had a good antiinflammatory effect on LPS-stimulated RAW264.7 cell. On one hand, it could significantly inhibit the production of IL-1β, IL-6, NO, TNF-α, COX-2 in LPS-stimulated cell than that of single LPS stimulated cell[2]. Clinical researches are more relatively and more indications are infant chronic bacillary diarrhea[3-5], rotavirus enteritis[6]. Epidemic keratoconjunctivitis[7] and anaphylactoid purpura[8], especially, the first indication.

In this article, the extract of *Herba kummerowiae* was studied on the antidiarrheal activity in mice.

EXPERIMENTAL

The herb of *Kummerowia striata* (Thunb.) Schindl. were collected from Anguo city of Hebei province, China. The collection process was authenticated by Prof. Changji Yuan, School of Pharmaceutical Scince, China Medical University. It must be noted that a voucher specimen has been deposited in pharmacognosy laboratory under specimen number SY006.

Preparation of extract of *Herba kummerowiae* and HPLC analyses: *Herba kummerowiae* was ground into powder and refluxed in water (v/v) (100 g/1000 mL, 1.5 h × 2 h). The filtrate was concentrated under reduced pressure, the residue was dryed in an oven. The resulting powder was used. A Waters 2695 series HPLC system, equipped with a UV-visible detector, was used for liquid chromatography analysis of the powders. A stainless steel column (4.6 mm × 150 mm) packed with C_{18}, 5 μm particle diameter, was used. The mobile phase contained methanol-0.1 % phosphonic acid (63:37). The sample (10 μL) was injected into the column. The flow rate was maintained at 1 mL/min and UV absorbance detection was carried out at 370 nm.

Grouping of animals and diarrhea model: Fifty six male and female KunMing (KM) mice (20 ± 2 g) were supplied by the Experimental Animal Centre, China Medical University. The animals were housed in an airconditioned room at an ambient temperature of 24 ºC and 50-65 % relative humidity with automatic 12 h light/12 h dark cycles. Fifty six mice were randomly divided into 7 groups (n = 8): normal control group

TABLE-1
EFFECT OF *Herba kummerowiae* ON SENNA-INDUCED DIARRHEA IN MICE

Groups	Diarrhea rate (%)	Loose stools rate (%)	Loose stools level	Diarrhea index
Control	0	0	0	0
Model	100	83.08 ± 18.76	2.01 ± 0.95	1.75 ± 0.92
HK-1.93 g/kg	100	79.03 ± 6.78^{bcd}	1.28 ± 0.65^{bcd}	0.98 ± 0.47^{bcd}
HK-3.86 g/kg	100	56.90 ± 9.61^{bcd}	0.95 ± 0.47^{bcd}	0.56 ± 0.32^{bcd}
HK-7.72 g/kg	100	6.41 ± 7.99^{bcd}	0.09 ± 0.11^{b}	0.01 ± 0.01^{b}
XX	100	10.35 ± 9.78^{b}	0.10 ± 0.09^{b}	0.016 ± 0.015^{b}
MT	100	9.93 ± 10.38^{b}	0.09 ± 0.08^{b}	0.015 ± 0.018^{b}

All values are expressed as mean ± SE, $^{b}p < 0.05$, when compared with model mice. $^{c}p < 0.05$, when compared with XX mice, $^{d}p < 0.05$, when compared with MT mice.

(NC), model group, Xiaoer Xiesuting granule group (XX), montmorillonite powder group (MT), *Herba kummerowiae* (HK)-1.93 g/kg, HK-3.86 g/kg and HK-7.72 g/kg. Normal mice were given only water. All mice except those in normal control group were given sennae leaf decoction (0.25 g/20 g) to make diarrhea models for 3 days[9] and calculated the diarrhea rate and then, sennae leaf decoction or water (NC group) was given for three days in the morning and observed 3 h to record total number of stools, loose stools and diameter of loose stools stains. In the afternoon, three kinds of drugs including Xiaoer Xiesuting, montmorillonite and *Herba kummerowiae* were dissolved in water and orally administered to the mice and observed 3 h to record all kinds of data. After administration of 0.5 h of the last, each mouse fed carbon powder (0.2 mL/20 g), after 20 min. The mice were sacrificed by cervical dislocation method. The digestive tract from the pylorus to the terminal rectum was completely removed. Measured the total length and the distance from pylorus to the forefront of carbon powder. The charcoal propulsion rate was calculated. All animals received humane care during the study with unlimited access to chow and water.

Statistical analysis: All results were expressed as mean ± SE. The data were analyzed by using one-way analysis of variance (ANOVA) followed by Student's *t*-test using SPSS computer software version 16.0, Level of significance was fixed at 0.05.

RESULTS AND DISCUSSION

HPLC analyses of *Herba kummerowiae*: Two known compounds were yielded from the extract and the structure was confirmed by chemical reference substances. The compounds were identified as genistein and kaempferide, their structures were illustrated in Fig. 1. Based on the results obtained through HPLC analysis, it was found that the content of genistein and kaempferide was, respectively 0.16 and 0.37 % (Fig. 2).

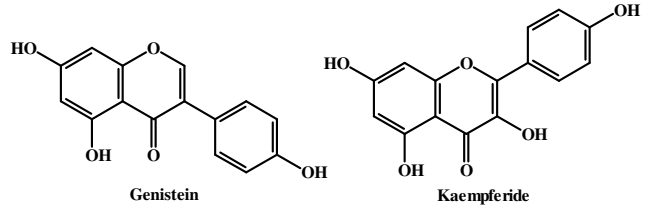

Fig. 1. Structure of genistein and kaempferide

Herba kummerowiae is one of medicinal plants with richer flavones including kaempferide, genistein. In this study, we

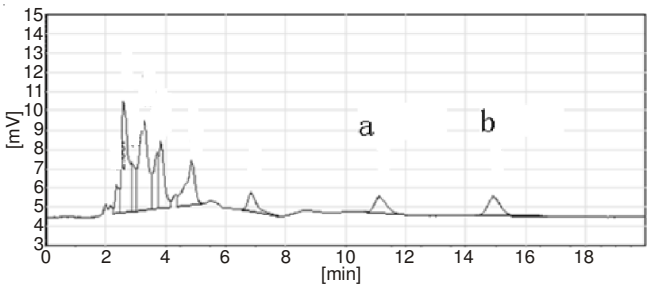

Fig. 2. HPLC chromatogram of extract of *Herba kummerowiae*

expanded the horizons of our research. HPLC technique also made it clare for us to further characterize more chemical informations.

Antidiarrheal activity of extract from *Herba kummerowiae* in mice: In addition to the normal control mice, the others suffered from diarrhea induced by senna leaf. The manifestations were as follows: increasing in frequency of defecation, loose stool, mice activity being significantly reduced, appearing weak, apathetic, *etc*.

TABLE-2
EFFECT OF *Herba kummerowiae*
ON INTESTINAL TRANSIT IN MICE

Groups	Full-length of intestine (cm)	Advance distance of carbon powder (cm)	Advance rate (%)
Control	60.88 ± 7.99	18.61 ± 4.07	30.63 ± 5.71
Model	59.50 ± 6.30	23.00 ± 6.59	39.28 ± 12.71^{a}
HK-1.93 g /kg	53.75 ± 3.58	23.00 ± 5.24	43.08 ± 10.88^{ad}
HK-3.86 g/kg	60.38 ± 4.60	22.00 ± 3.46	36.52 ± 5.93^{abd}
HK-7.72 g/kg	59.38 ± 5.55	19.63 ± 3.85	33.19 ± 7.12^{b}
XX	60.12 ± 4.94	21.12 ± 5.77	35.55 ± 10.46^{ab}
MT	60.50 ± 3.46	17.88 ± 8.18	30.13 ± 15.13^{b}

All values are expressed as mean ± SE, $^{a}p < 0.05$, when compared with control mice, $^{b}p < 0.05$, when compared with model mice. $^{c}p < 0.05$, when compared with XX mice , $^{d}p < 0.05$, when compared with MT mice.

Diarrhea represents an increase in the water content of the stool and in the frequency of evacuation and mainly results from dysregulation of either intestinal secretory function or colonic motor function[10]. In this study, After administration, compared with the model control group, the loose stools rate and diarrhea index of the diarrhea were reduced by *Herba kummerowiae*, Xiaoer Xiesuting and montmorillonite ($p < 0.05$) (Table-1). The data including loose stools rate and diarrhea index, *etc*. as objective evaluation index of stool characters reflected the degree of loose stools. The results

shown that *Herba kummerowiae* might be have the function of reducing intestinal secretion. Compared with the model control mice, the effect of inhibitting peristalsis was related with the dose of *Herba kummerowiae* and the effect was weaker than montmorillonite (Table-2), but, that might be favorable to diarrhea in children or the aged.

Conclusion

Herba kummerowiae is effective in senna-leaf-induced diarrhea in mice. The underlying mechanism may be related with its inhibition on the intestinal propulsive motility and secretion. It is effective in diarrhea and suitable for children or the aged person.

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REFERENCES

1. K.Y. Park, S.H. Lee, B.K. Min, K.S. Lee, J.S. Choi, S.R. Chung, K.R. Min and Y. Kim, *Planta Med*., **65**, 457 (1999).
2. J.Y. Tao, L. Zhao, Z.J. Huang, X.Y. Zhang, S.L. Zhang, Q.G. Zhang, B.H. Zhang, Q.L. Feng and G.H. Zheng, *Inflammation*, **31**, 154 (2008).
3. R. Luo, X.Q. Zhang, E. Shang and J. Sun, *Hubei J. Trad. Chin. Med.*, **24**, 26 (2002).
4. E. Shang and R. Luo, *J. Nursing Sci*., **18**, 203 (2003).
5. J. Sun, Y. Wang and X. Ding, *Modern J. Integr. Trad. Chin. Western Med*., **19**, 55 (2010).
6. J. Sun, X. Wang, Y. Wang and Z. Li, *J. Emergency Trad. Chin. Med.*, **18**, 1520 (2009).
7. Y. Yuan, *J. Zhengzhou Univ. (Med. Sci.)*, **2**, 16 (1976).
8. X. Mai, J. Guo and L. Guo, *China J. Chin. Meter. Med*., **21**, 121 (1996).
9. F. Tang, H. Wang, Y. He and W. Liu, *Chin. J. Hospital Pharm*., **28**, 1355 (2008).
10. R. Moriya, T. Shirakura, H. Hirose, T. Kanno, J. Suzuki and A. Kanatani, *Peptides*, **31**, 671 (2010).