Protective effect of Weikang decoction and partial ingredients on model rat with gastric mucosa ulcer

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Supported by the Education Commission of Hubei Province, No. (1996)028

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Received: 2004-03-20  Accepted: 2004-06-11

Abstract

AIM: To investigate the protective mechanisms of Weikang (WK) decoction on gastric mucosa.

METHODS: Ninety rats were randomly divided into nine groups of 10 each, namely group, model group, group with large WK dosage, group with medium WK dosage, group with small WK dosage, group with herbs of jianpiyiqi (strengthening the spleen and replenishing qi), group with herbs of yangxuehuoxue (invigorating the circulation of and nourishing the blood), group with herbs of qingrejiedu (clearing away the heat-evils and toxic materials), group with colloidal bismuth pectin (CBP) capsules. According to the method adopted by Yang Xuesong, except normal control group, chronic gastric ulcer was induced with 100% acetic acid. On the sixth day after moldmaking, WK decoction was administered, respectively at doses of 20, 10 and 5 g/kg to rats of the WK groups, or the groups with herbs of jianpiyiqi, yangxuehuoxue and qingrejiedu, 10 mL/kg was separately administered to each group every day. For the group with CBP capsules, medicine was dissolved with water and doses 15 times of human therapeutic dose were administered (10 mL/kg solution containing 0.35% CBP). Rats of other groups were fed with physiological saline (10 mL/kg every day). Administration lasted for 16 d. Rats were killed on d 22 after mold making to observe changes of gastric mucosa. The mucus thickness of gastric mucosa surface was measured. Levels of epidermal growth factor (EGF) in gastric juice, nitric oxide (NO) in gastric tissue, endothelin (ET) in plasma, superoxide dismutase (SOD) in plasma, malondialdehyde (MDA) in plasma and prostaglandin I\(_2\) (PGL\(_2\)) were examined.

RESULTS: Compared with control group, ulceration was found in gastric mucosa of model group rats. The mucus thickness of gastric mucosa surface, the levels of EGF, NO, 6-K-PGF\(_{1\alpha}\) and SOD decreased significantly in the model group (EGF: 0.818±0.18 vs 2.168±0.375, NO: 0.213±0.049 vs 0.601±0.081, 6-K-PGF\(_{1\alpha}\): 59.7±6.3 vs 96.6±8.30, SOD: 128.6±15.0 vs 196.6±35.3, P<0.01), the levels of ET (179.96±37.40 vs 46.6±21.20, P<0.01) and MDA (48.2±4.5 vs 15.7±4.8, P<0.01) increased. Compared with model group, the thickness of regenerative mucosa increased, glandular arrangement was in order, and cystic dilative glands decreased, while the mucus thickness of gastric mucosa surface increased (20 g/kg WK: 51.3±2.9 vs 23.2±8.4, 10 g/kg WK: 43.3±2.9 vs 23.2±8.4, 5 g/kg WK: 36.1±7.2 vs 23.2±8.4, jianpiyiqi: 35.4±5.6 vs 23.2±8.4, yangxuehuoxue: 33.1±8.9 vs 23.2±8.4, qingrejiedu: 31.0±8.0 vs 23.2±8.4 and CBP: 38.2±3.5 vs 23.2±8.4, P<0.05-0.01). The levels of EGF (20 g/kg WK: 1.364±0.12 vs 0.818±0.18, 10 g/kg WK: 1.359±0.24 vs 0.818±0.18, 5 g/kg WK: 1.245±0.31 vs 0.818±0.18, jianpiyiqi: 1.025±0.45 vs 0.818±0.18, yangxuehuoxue: 1.03±0.29 vs 0.818±0.18, qingrejiedu: 1.02±0.47 vs 0.818±0.18 and CBP: 1.237±0.20 vs 0.818±0.18, P<0.05-0.01), NO (20 g/kg WK: 0.480±0.026 vs 0.213±0.049, 10 g/kg WK: 0.390±0.055 vs 0.213±0.049, 5 g/kg WK: 0.394±0.026 vs 0.213±0.049, jianpiyiqi: 0.393±0.123 vs 0.213±0.049, yangxuehuoxue: 0.463±0.077 vs 0.213±0.049, qingrejiedu: 0.382±0.082 vs 0.213±0.049 and CBP: 0.395±0.053 vs 0.213±0.049, P<0.05-0.01), 6-K-PGF\(_{1\alpha}\) (20 g/kg WK: 86.8±7.6 vs 59.7±6.3, 10 g/kg WK: 77.9±7.0 vs 59.7±6.3, 5 g/kg WK: 70.0±5.4 vs 59.7±6.3, jianpiyiqi: 73.5±12.2 vs 59.7±6.3, yangxuehuoxue: 65.1±5.3 vs 59.7±6.3, qingrejiedu: 76.9±14.6 vs 59.7±6.3, and CBP: 93.7±10.7 vs 59.7±6.3, P<0.05-0.01) and SOD (20 g/kg WK: 186.4±19.9 vs 128.6±15.0, 10 g/kg WK: 168.2±21.7 vs 128.6±15.0, 5 g/kg WK: 155.6±21.6 vs 128.6±15.0, jianpiyiqi: 168.0±85.3 vs 128.6±15.0, yangxuehuoxue: 165.0±34.0 vs 128.6±15.0, qingrejiedu: 168.2±24.9 vs 128.6±15.0, and CBP: 156.3±18.1 vs 128.6±15.0, P<0.05-0.01) significantly increased. The levels of ET (20 g/kg WK: 81.30±17.20 vs 179.96±37.40, 10 g/kg WK: 83.40±25.90 vs 179.96±37.40, 5 g/kg WK: 93.87±20.70 vs 179.96±37.40, jianpiyiqi: 130.67±43.66 vs 179.96±37.40, yangxuehuoxue: 115.88±34.09 vs 179.96±37.40, qingrejiedu: 108.22±36.97 vs 179.96±37.40, and CBP: 91.96±19.0 vs 179.96±37.40, P<0.01) and MDA (20 g/kg WK: 21.6±7.4 vs 48.2±4.5, 10 g/kg WK: 32.2±7.3 vs 48.2±4.5, 5 g/kg WK: 34.2±6.2 vs 48.2±4.5, jianpiyiqi: 34.9±13.8 vs 48.2±4.5, yangxuehuoxue: 35.4±16.7 vs 48.2±4.5, qingrejiedu: 42.2±17.6 vs 48.2±4.5, and CBP: 30.1±6.1 vs 48.2±4.5, P<0.05-0.01) obviously decreased. The 20 g/kg WK group was better than 10 g/kg (the mucus thickness: 51.3±2.9 vs 43.3±2.9, NO: 0.480±0.026 vs 0.390±0.055, SOD: 186.4±19.9 vs 128.6±15.0).
INTRODUCTION

PU is a common disease with a high clinical incidence, which features predicted curative ratio up to 95%, but high relapse rate at about 65-80% one year after withdrawal and up to nearly 100% within two years. Such being the case, treatment of PU and prevention of its relapse still is a problem in the medical field[1-5]. When the gastric mucosa is normal, the gastric wall can prevent digestion in the stomach by the mucus with hydrogen ion’s consistency being 3-4 million times higher than that in the blood. Furthermore, most of the ulcerations are caused by the weakening of the protective ability of mucus than by the increasing of the attacking factors[6-8]. In recent years, many experts have proved, by virtue of modern approaches, that herbs for strengthening the spleen can remove the damage to gastric mucosa in experiments, and can withstand ulcerations by way of consolidating the barrier of gastric mucus, speeding up the mucosal blood flow, quickening the reproduction of PGI_{2}, and holding back the damage by free radicals[9,10]. Protective effects of traditional Chinese medicine on gastric and intestinal mucosa offer a new method. WK decoction can treat PU and prevent relapse of PU according to clinical research[9,10]. The experiment was carried out to investigate the possible mechanisms and find a new traditional Chinese medical recipe on mucosal protection.

MATERIALS AND METHODS

Materials

Ninety pure wistar rats of either sex (180-250 g), provided by Tongji Medical University Experimental Animal Center, were caged by layer, male and female separated. After 1 wk of feeding, if no unfavorable reaction was inspected, the experiment would begin. Entire herbs consisting of huangqi and pugongying 30 g each, guizhi, zhihancao and saiqi 6 g each, baishao, yuanyu 15 g each, dahuang 12 g, jianpiyi group herbs consisting of huangqi 30 g, guizhi and zhihancao 6 g each, yangxuehuoxue herbs consisting of yuanyu and banbio 15 g each, saiqi 6 g, qingrejiedu herbs consisting of dahuang 12 g and pugongying 30 g. The above herbs were boiled with 400 mL deionized water twice, 30 min each, then, the filtrate was condensed to a concentration of 1 g/mL, and was put in a 500-mL glass bottle kept at low temperature. The CBP capsules were produced by Shanxi Ante Biological Pharmaceutical Co., Ltd., 92 Wei Yao Zhun Zi X-47-1.

Methods

Ninety rats were randomly divided into nine groups of 10 each, respectively marked with control group, model group, group with large WK dosage, group with medium WK dosage, group with small WK dosage, jianpiyiqi group, yangxuehuoxue group, qingrejiedu group and group with CBP capsules. According to the method adopted by Yang Xuesong14, except for the control group, food was withheld for 24 h before modeling while water was allowed. Anesthetized with ether, these rats had their furs cut. Then, along the middle line under the xiphoid process, they were cut open from the belly (the cut was about 2 cm long). The stomach was exposed. Subsequently, filter paper with a diameter of 5 mm immersed in 100% acetic acid was pasted at the serosa at the intersection of antrum phlori and the stomach body twice of 30 s each. After that, new filter paper was used to absorb extra acetic acid on the surface of the stomach. This part was covered with omentum. The stomach was resumed to normal shape. Finally, cuts were sutured by layers, and coated with a layer of diluted ceiba acid for protection purpose. Administration was made to lavage the stomach from the sixth day after modeling. The doses of WK decoction were calculated by extrapolating the human therapeutic dose. Medium dosage was 15 times of human dose (10 mL/kg every d), large dosage was twice as much (20 mL/kg every d) while small dosage of one second (5 mL/kg every d), jianpiyiqi group, yangxuehuoxue group, qingrejiedu group (10 mL/kg every d) was administrated to each group, for the group with CBP capsules, medicine was dissolved with water and doses 15 times of human therapeutic dose were administrated (10 mL/kg solution containing 0.35% pectin bismuth), rats of other groups were fed with physiological saline (10 mL/kg every d). Administration lasted 16 d. From the 17 d, these rats were fasted for 24 h, water allowed. Before the experiment, the animals received 1 mL physiological saline. Two hours later, they were anesthetized with 1% sodium pentobarbital at a dose of 40-50 mg/kg on the
RESULTS

Overview
When coming around after modeling, the rats began to eat but lost their appetite dramatically. They were not as active as before, their furs and skins were dry and urine volume also decreased. About 5-7 d after modeling, they became active gradually, with appetite getting better, their skins and furs resumed lustrousness and the urine volume also rose. During the experiments, four rats died, three from the jianpiyiqi group, and one from the qingrejiedu group.

Pathological observations
Compared with the control group, rats in the model group suffered from damaged gastric mucosa. Ulcerations were deep into the muscular layer. Additionally, the holes were covered only with little reproduced mucosa, and glands were arranged disorderly, presenting a cystic dilatation. For the rats in the groups respectively with large, medium and small WK dosage, as well as the group with CBP capsules, the mucosa reproduced at the holes of gastric mucosa was thinner than the model group, and the damage area in the muscular layer was smaller. Glands tended to arrange orderly, with those of cystic dilation decreased.

Effect on the thickness of mucus on gastric mucosa
If arranged by the thickness of mucus on the gastric mucosa in ascending order, then the sequence is as follows: model group (P<0.01), group with small WK dosage and group with CBP capsules (P<0.01), group with medium WK dosage (P<0.05), group with large WK dosage (P<0.01), and the control group. There is little difference between the group with small WK dosage and the group with CBP capsules. Moreover, the group of the mucus with partial ingredients is thicker than the model group (P<0.05-0.01) but thinner than the group with entire ingredients. The results are summarized in Table 1.

| Table 1 Thickness of mucus on gastric mucosa surface and the content of EGF in gastric juice (mean±SD) |
|---------------------------------|---------------------|------------------|
| Group                          | Specimens | Thickness of mucus (µm) | EGF (pg/g) |
|--------------------------------|-----------|-------------------------|------------|
| Control                        | 10        | 64.19±3.9a,d            | 2.168±0.375a |
| Model                          | 10        | 23.2±8.4                | 0.818±0.18            |
| WK (20 g/kg)                   | 10        | 51.3±2.9d              | 1.364±0.12c         |
| WK (10 g/kg)                   | 10        | 45.3±2.9e              | 1.359±0.24c         |
| WK (5 g/kg)                    | 9         | 36.4±7.2               | 1.245±0.31c         |
| Jianpiyiqi                     | 7         | 35.4±5.6               | 1.025±0.45c         |
| Yangxuehuoxue                  | 10        | 33.1±8.0               | 1.03±0.29c          |
| Qingrejiedu                    | 9         | 31.0±8.0               | 1.02±0.47c          |
| CBP capsules                   | 10        | 38.2±3.9               | 1.237±0.20c         |

*P<0.05, **P<0.01 vs the model group, *P<0.05, **P<0.01 vs CBP capsules.

Effect on EGF
As shown in Table 1, in terms of the content of EGF in gastric juice, the model group is much lower than the control group (P<0.01), while the groups with large, medium and small WK dosage and the group with CBP capsules, between which there is no dramatic difference, are considerably higher than the model group (P<0.05). In general, groups with partial ingredients are lower than those with entire ingredients (P<0.05) but higher than the model group. The averages of all groups with partial ingredients are very close, with little remarkable difference.

Effect on the content of NO in gastric tissue
As regards the content of NO in gastric tissue, the model group is much lower than the control group (P<0.01), while the groups with large, medium and small WK dosage and the group with CBP capsules are considerably higher than the model group (P<0.05-0.01). The group with large WK dosage and the yangxuehuoxue group are greatly higher than the Jianpiyiqi group, Qingrejiedu group, group with medium and small WK dosage, the group with CBP capsules. See Table 2.

| Table 2 Content of NO in gastric tissue and the content of ET in plasma (mean±SD) |
|---------------------------------|---------------------|------------------|
| Group                          | Specimens | NO (µmol/g) | ET (pg/ml) |
|--------------------------------|-----------|-------------|-----------|
| Control                        | 10        | 0.601±0.081ab | 46.64±21.20ab |
| Model                          | 10        | 0.213±0.049  | 179.96±37.40 |
| WK 20 g/kg                     | 10        | 0.480±0.026c | 81.30±17.20c |
| WK 10 g/kg                     | 10        | 0.390±0.055c | 83.49±25.90c |
| WK 5 g/kg                      | 9         | 0.394±0.026a | 93.87±20.70a |
| Jianpiyiqi                     | 7         | 0.393±0.123c | 130.67±43.66 |
| Yangxuehuoxue                  | 10        | 0.463±0.077c | 115.88±34.09c |
| Qingrejiedu                    | 9         | 0.382±0.082c | 108.22±36.97c |
| CBP capsules                   | 10        | 0.395±0.053c | 91.96±19.06c |

*P<0.05, **P<0.01 vs the model group, *P<0.05, **P<0.01 vs CBP capsules.

Effect on the content of ET in plasma
As shown in Table 2, in regard to the content of ET in plasma, the model group is much higher than the control group (P<0.01), while the groups with herbs and the group with CBP capsules are considerably lower than the model group (P<0.01). For the groups with large, medium and
small WK dosage and the group with CBP capsules, there is no dramatic difference among them. The jianpiyiqi group, yangxuehuoxue group, qingrejiudu group are substantially higher than the groups with entire ingredients and with CBP capsules (P<0.05).

**Effect on SOD and MDA in plasma**

Compared with the control group, the model group is much lower in terms of SOD content (P<0.01), but higher in terms of MDA content (P<0.01). As regards SOD content, the groups with small WK dosage and with CBP capsules are substantially higher than the model group (P<0.05), but much lower than the group with medium WK dosage (P<0.05). The group with large WK dosage is higher than the group with medium WK dosage (P<0.05). In terms of MDA content, the groups with large, medium and small WK dosage and the group with CBP capsules, between which there is no dramatic difference, are hugely lower than the model group (P<0.05-P<0.01). See Table 3.

**Table 3** Content of SOD and MDA in plasma of each group (mean±SD)

| Group     | Specimens | SOD (NU/mL) | MDA (NM/mL) |
|-----------|-----------|-------------|--------------|
| Control   | 10        | 196.6±35.3^a | 15.7±4.8^cd |
| Model     | 10        | 128.6±15.0  | 48.2±4.5     |
| WK 20 g/kg| 10        | 186.4±19.9^b | 21.6±7.4^c   |
| WK 10 g/kg| 10        | 168.2±21.7^b | 32.2±7.3^b   |
| WK 5 g/kg | 9         | 155.6±21.6^a | 34.2±6.2^a   |
| Jianpiyiqi| 7         | 168.0±85.3^a | 34.9±13.8    |
| Yangxuehuoxue| 10      | 165.0±34.0^ab| 35.5±16.7    |
| Qingrejiudu| 9        | 168.2±24.9^a | 42.2±17.6^a  |
| CBP capsules| 10    | 156.3±18.1^a | 30.1±6.1     |

^aP<0.05, ^bP<0.01 vs the model group. ^cP<0.05, ^dP<0.01 vs CBP capsules.

**Effect on the content of 6-K-PGF2α in plasma**

In terms of 6-K-PGF2α content, the model group is much lower than the control group (P<0.01), but higher than the groups with large, medium and small WK dosage and the group with CBP capsules (P<0.05). Between the groups with large, medium and small WK dosage and the group with CBP capsules, there is no dramatic difference (P<0.05), but the group with large WK dosage is greatly higher than the groups with medium and small dosage (P<0.05). See Table 4.

**Table 4** Effect of WK on the content of 6-K-PGF2α in plasma in rats (mean±SD)

| Group     | Specimens | 6-K-PGF2α (pg/mL) |
|-----------|-----------|-------------------|
| Control   | 10        | 96.6±43.3^a       |
| Model     | 10        | 59.7±6.3          |
| WK 20 g/kg| 10        | 86.8±17.6^a       |
| WK 10 g/kg| 10        | 77.9±7.0^a        |
| WK 5 g/kg | 9         | 70.0±5.4^a        |
| Jianpiyiqi| 7         | 73.5±12.2^a       |
| Yangxuehuoxue| 10      | 65.1±5.3          |
| Qingrejiudu| 9        | 76.9±14.6^a       |
| CBP capsules| 10    | 93.7±10.7^a       |

^aP<0.05, ^bP<0.01 vs the model group.

**DISCUSSION**

To the present day, major anti-PU medicines aim at fighting against mucosa-attacking factor. Such medicines include H2 receptor antagonism, H+-K+ATP enzyme inhibitor, anticholin drugs, Helicobacter pylori (Hp) counteractant\[16-20\], etc. When the gastric mucosa is normal, the gastric wall can prevent digestion in the stomach by the mucus with hydrogenion’s consistence 3-4 million times higher than that in the blood. However, when in PU, secretion of gastric acid generally stays low in the normal range, while in duodenal ulcer, the secretion is only 1/3 more than the normal level. Furthermore, not all PU are related with HP, which indicates that most ulcerations are caused by the weakening protective ability of mucosa and not by the increasing of the attacking factor. In clinically traditional Chinese herbs have good effect for PU\[16-20\]. The WK decoction derives from Jianzhong Astragalus Root Soup, with some herbs added or removed. This recipe mainly functions for strengthening the spleen and replenishing qi, complemented with other effects, such as invigorating the circulation of and nourishing the blood, and clearing away the heat-evils and expelling superficial evils. In recent years, by virtue of modern approaches, many experts have proved, strengthening the spleen can remove the damage to gastric mucosa in experiments, and can withstand ulcerations by way of consolidating the barrier of gastric mucosa, speeding up the mucosal blood flow, quickening the reproduction of PGI2, and holding back the damage by free radicals. For mucus, perfect structure is the foundation of its protecting function\[21\]. It is revealed by our experiments that, regardless of doses, WK in solid state can shrink ulcerations, build up the mucosa barrier, straighten up the arrangement of glands and better the structure. Additionally, dependent on its dosage, WK decoction also can thicken the mucus on the surface of gastric mucosa, which indicates that it can facilitate mucosa to secrete mucus. EGF of the human body can stimulate the mitosis of cells, improve the proliferation and differentiation of epithelium, and speed up the production of DNA and proteins in the gastric mucosa, all of which are of great importance to regenerate and repair the tissue\[22-26\]. Our experiments show that after modeling the content of EGF of the model group dropped greatly, but that of the groups with large, medium and small WK dosage and with CBP capsules climbed up. Comparatively, large doses of WK exerted more influence on the content of EGF than CBP capsules, which indicates that WK decoction has the function of protecting gastric mucus by enhancing the EGF content and its effect is dependent on dosage. NO protects the stomach by speeding up the circulation of blood in the gastric mucosa. Also, it plays an important role in defending the gastric mucosa as an antioxidant\[26,27\]. Our experiments reveal that the content of NO in the model group is much lower than that in the control group (P<0.01), indicating a decreased content of NO protective factor in the event of gastric ulceration. The content of NO in the groups with large, medium and small WK dosage and with CBP capsules are dramatically higher than that in the model group. Comparatively, large doses of WK exerted more influence on the content of NO than CBP capsules, which indicates that WK decoction can protect the gastric mucosa by enhancing NO content after treatment...
but its effect is dependent on dosage. ET may antagonize the effect of NO, leading to an imbalanced NO/ET. As it may slow down the mucosal blood flow, gastric mucosa may be impaired. Our experiments show that the content of ET in the model group is much higher than that in the control group, indicating increase of ET in the event of gastric ulceration. The content of NO in the groups with large, medium and small WK dosage and with CBP capsules are dramatically lower than that in the model group, which means that WK decoction can lower the content of ET in plasma by slowing down the production and secretion of ET. In general, to lower ET content and enhance the content of NO can keep NO/ET in balance, maintain normal mucosal blood flow and thus protect the gastric mucosa.

When stimulated by chemicals or in shortage of blood, the gastric and intestinal mucosa will produce tremendous free radicals, which impair mucosa. Through experimental results, we can see that the model group is much higher than the control group (P<0.01) in terms of MDA content but much lower than the latter in terms of SOD content (P<0.01). These data indicate that oxygen-derived free radicals will take part in ulceration and meanwhile oxygen-derived free radical scavengers that have protective function decrease. The groups with large, medium and small WK dosage and with CBP capsules are considerably lower than the model group in terms of MDA content but higher than the latter in terms of SOD content. Furthermore, the groups with medium and small WK dosage are higher than the group with CBP capsules in terms of SOD content (P<0.05), which shows that WK has better effect in increasing SOD contents, removing oxygen-derived free radicals and protecting the gastric mucosa. Its effect is dependent on dosage.

PGI2, can restrain the secretion of gastric acid, enhance the mucosal blood flow and protect the cells and TXB2. However, it has a short half-life period. Within two minutes, it can hydrolyze into stable 6-K-PGF2α. Our experiments show that the content of 6-K-PGF2α in the model group is much lower than that in the control group (P<0.01), indicating that in case of ulceration protective factor 6-K-PGF2α will decrease. But, the groups with large, medium and small WK dosage and with CBP capsules are considerably higher than the model group (P<0.01), which indicates that WK can protect the gastric mucosa and further repair ulcerations by increasing 6-K-PGF2α.

The WK decoction derives from huangqijianzhong decoction[31], with some herbs added or removed. However, huangqijianzhong decoction is mainly used for restoring qi by virtue of warm herbs. In consideration of the pathogenesis of ulcer diseases, such as deficiency of vital energy, blood stasis and tremendous heat toxins[32], we add some herbs for activating blood circulation to dissipate blood stasis[33], such as xuanwu (rhizomacorydalis) and sanqi (pseudoginseng), some herbs dahuang (rhubarb) and pungongying (dandelion)[34] for clearing away the heat-evils and toxic materials. This recipe mainly functions for strengthening the spleen and replenishing qi, complemented with other effects, such as invigorating the circulation of and nourishing the blood, and clearing away the heat-evils. These experiments show that all the groups with partial ingredients have favorable effect of curing gastric ulcer for rats. These ingredients can increase contents of PG12, EGF, SOD and NO, thicken the mucus on the gastric mucosa, and decrease the impairing factor MDA and ET. Through comparison, we find out that partial ingredients do not have as good effect as the entirety. In some cases, they show some dramatic statistical difference, while in other cases although there is no such difference, the group with entire ingredients has better figures. Therefore, the entire ingredients have better comprehensive effect in curing ulcerations. All medical herbs have their own substantial foundation supporting their activity and effect in treatment. Since the medicine has so many ingredients that may produce many complicated chemical reactions, its treatment effect is not equal to the aggregate effects of each ingredient. As for its effective substantial foundation, it is to be explored in further researches.

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