Evaluation of *Aucklandia lappa* Decne extracts as antiulcer activity in animals

Xian Yang[1], Xue Zhang[4], Shui-Ping Yang[3] , Tao Le[1] and Bin Chen[1]*

[1]College of Life Sciences, Chongqing Normal University, Chongqing, P.R. China
[2]College of Resources and Environment, Southwest University, Chongqing, P.R. China
[3]Chongqing Academy of Chinese Materia Medica, Chongqing, P.R.China

Abstract: *Aucklandialappa* Decne (ALD) is one of the traditional herbs to treat various kinds of disorders including asthma, cough, vomit, diarrhea, hepatitis and cholecystitis. However, its effects on indigestion and particularly antiulcer activity of ethanol extract have not been studied. In the study, the *Aucklandia lappa* Decne extract (ALDE) was investigated to see if it againstgastric injury effects through traditional pathways. Ethyl alcohol and epinephrine hydrochloride were used to induce acute gastric mucous membrane damage in adult SD rats and Kunming mice, respectively. This present study evaluated its effects on peptic ulcer of ALDE treatment in SD rats and Kunming mice. In acute gastric mucous membrane damage induced by ethyl alcohol in rats, the results indicated that three ALDE treatment groups highly significantly decreased the mucosal damage index as compared to the model group. Furthermore, this mucosal damage index of the mid-dose group significantly decreased while the high-range dose group highly significantly decreased, respectively, as compared to the SO group. The ulcer inhibition rate of low-dose, mid-dose and high-dose ALDE treatment groups reached 68.64%, 72.67% and 74.91%, respectively. In acute gastric mucous membrane damage induced by pyloric ligation in rats, the results indicated that three ALDE treatment groups highly significantly decreased the mucosal damage index as compared to the model group. The mucosal damage index of mid-dose group significantly decreased while the high-range dose group highly significantly decreased, respectively as compared to the SO group. The ulcer inhibition rate of low-dise, mid-dose and high-dose ALDE treatment groups reached 68.64%, 72.67% and 74.91%, respectively. In acute gastric mucous membrane damage induced by pyloric ligation in rats, the results indicated that three ALDE treatment groups highly significantly decreased the mucosal damage index of, respectively, as compared to the model group. Furthermore, This mucosal damage index of the mid-range dose group significantly decreased while the high-dose group highly significantly decreased, respectively, as compared to the SO group. The ulcer inhibition rate of low-dose, mid-dose and high-dose ALDE treatment groups reached 68.64%, 72.67% and 74.91%, respectively. Our results indicated that ALDE exhibits a marked effect on peptic ulcer activity in animals, which supports previous results of its use in traditional Chinese medicine.

Keywords: Peptic ulcer; *Aucklandia lappa* Decne; mucosal damage index; ulcer inhibition rate

INTRODUCTION

Pepticulcer (PU) disease is the disease of digestive tract characterized by mucosal damage next to pepsin and gastric acid secretion, which frequently occurs in the stomach and proximal duodenum (Kalyanakrishnan and Robert, 2007). PU disease produced a great effect on morbidity until the last decades of the 20th century, when epidemiological tendency started to point at an impressed fall into its incidence. Two important developments are correlated with the decrease in rates of PU disease: the discovery of valid acid inhibitor, and of *Helicobacter pylori* (*He pylori*). With the discovery of *H pylori* infection, the reasons, pathogeny, and treatment of PU disease have been rewritten. *H. pylori* infection and the use of NSAIDs are the main causes of PU disease (Dikmen *et al*., 2005; Peter and Kenneth, 2009; Xie *et al*.,2010; Joseph *et al*., 2010; Mari¨etta *et al*., 2010). Furthermore, some traditional herbs had very good efficacy suchas the leaves of *Cassia nigricans and Ficus exasperata* and *Synclisia scabrida*, which are commonly used as the treatment of Pudisease (Akah *et al*., 1998). They have been indicated to be valid against PU disease in traditional herb (Schmeda-Hirschmann and Yesilada, 2005). In recent years, many measures have been done to explore new anti-PU drugs from herb resources (Suleyman *et al*., 2001; Odabasoglu *et al*., 2006; Fatih *et al*., 2007).

Aucklandialappa Decne (ALD, in the family of Saussurea), commonly known as "Yunmuxiang", officially included in the Chinese Pharmacopoeia, has been widely used as traditional herb to treat various kinds of disorders including asthma, cough, vomit, diarrhea, hepatitis and cholecystitis (China Pharmacopoeia Committee, 2010). Severa reports in the current literature contributed to the pharmacological function of some compounds such as soybean oil (SO) and sesquiterpene lactone including costunolide and dehydrocostus lactone from ALD (Li *et al*., 2005;Umpierrez GE. 2012). Although the strengthening stomach effects of *Aucklandia*

**Corresponding author: Bin Chen,* e-mail: bin.chen@cqnu.edu

lappa Decne extract (ALDE) have been widely evaluated, very little is known about their pharmacokinetic characteristics in gastric mucous membrane injury models. Furthermore, its potential pharmacological properties were studied. Therefore, there is necessary to study the effect of ALDE on PU. Soybean oil originated from soybean seed promote powerful moistening the intestines and laxation effects and it was used as the positive control drug in subsequent studies.

MATERIALS AND METHODS

Chemicals and materials

The costunolide and dehydrocostus lactone were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their structures are shown in fig. 1. SO was used as a positive control in the tests and was provided by Chongqing grease Co., LTD (Chongqing, China). All other chemicals used in these studies were analytical grade reagents and purchased from Promega Corporation (Madison, WI, USA).

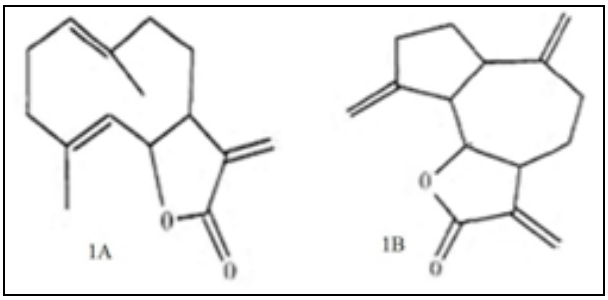

Fig. 1: Chemical structure of Costunolide and Dehydrocostus lactone (A: Costunolide; B: Dehydrocostus lactone)

Plant material and preparation of extract

Aucklandia lappa Decne samples were collected from different locations in Chongqing, China, in October 2009. Professor Chang-hua Wang (Chongqing Academy of Chinese Materia Medica, China) identified the raw medicinal herbs, and the specimens were deposited at the Herbarium of Chongqing Normal University (voucher no. 2013008). ALDE was prepared using traditional methods. Briefly, dried *Aucklandia lappa* Decne powder was extracted by refluxing for 2.0 hours with 10 volumes of 80% ethanol (1:10, w/v). After 2.0h, the solution was filtered using filter paper (Whatman no.4) and the residue was re-extracted two additional times with same volume of 80% ethanol. Finally, the entire filtered solution of mixture was concentrated under reduced pressure and then dried with a spray dryer. The final ALDE product was stored at -20℃. A yield of 35.52% was obtained.

Animals

Adult Kunming mice (18-22g, half male and half female), adult SD (Sprague Dawley) rats (180-220g) were obtained from the Experimental Animal Centre of Chongqing Academy of Chinese Materia Medica. The animals were housed in temperature-controlled rooms with access to water and food ad libitum until they were used. They were fed in a 12-h light/dark cycle. The study was approved by the Animal Research Welfare Committee(certificate no. SCXK-11006), Chongqing Academy of Chinese Materia Medica, Chongqing, China.

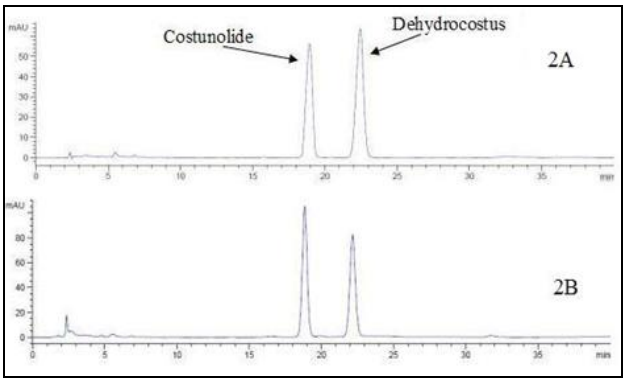

Fig. 2: Representative HPLC chromatograms of (2A) mixed standards, (2B) *Aucklandia lappa* Decne. (Chongqing, China, 2012).

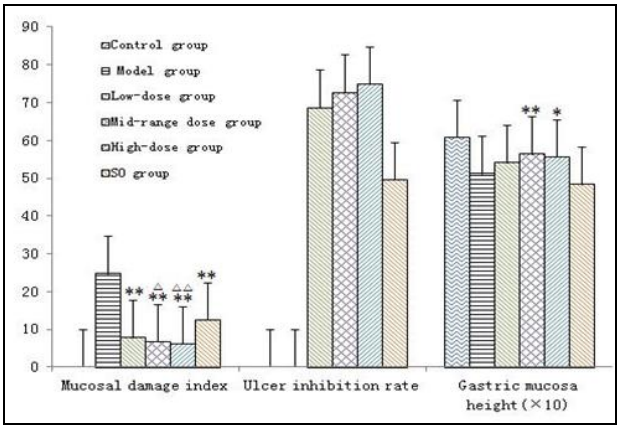

Fig. 3: Effects of ALDE on acute gastric mucous membrane damage induced by ethyl alcohol in each group (n=10). The asterisks denote significance levels when compared with the model group:*p <0.05;**p <0.01. The asterisks denote significance levels when compared with the SO group:$^{\triangle}$p <0.05;$^{\triangle\triangle}$p <0.01.

Dosage regimen design

These studies followed Chinese Pharmacopoeia recommendations (2010 edition) for the effective and safe dosage of ALDE and SO. The low-dose received ALDE as follows: SD rats received 0.5g/kg, whereas Kunming mice received 0.75g/kg. The mid-range dose received ALDE as follows: SD rats: 1.0g/kg; Kunming mice: 1.5g/kg. Finally, the high-dose received ALDE as follows: SD rats: 1.5g/kg; Kunming mice: 2.25g/kg. The dose received SO as follows: SD rats received 0.75g/kg, whereas Kunming mice received 1.75g/kg. All doses were administered intragastrically (i.g.) 3 times per day.

Chemical analysis of active compositions by HPLC

The HPLC analyses were performed using a Agillent1200 HPLC system (USA) equipped with a dual pump, an auto sampler, and a Waters Acquity TM BEH C_{18} analytical column (250mm×4.6mm×5μm) in this study (Xian Y *et al*., 2014). The mobile phase was initially composed of 65% methyl alcohol and 35% solution. The mobile phase flow rate was 1.0ml/min, and the injection volume was 10μL. The wavelength was 225nm.

A standard stock solutions of each of the 2 compounds (costunolide and dehydrocostus lactone) were directly prepared in methanol. The standard solutions containing the 2 compounds were prepared and diluted with methanol to appropriate concentrations for establishment of calibration curves. The standard stock solutions were all prepared in dark brown calibrated flasks and stored at 4℃. The concentrations of the costunolide and dehydrocostus lactonesolutions were 0.1012mg/ml and 0.1028mg/ml, respectively. The linearity of the responses was determined for six concentrations.

The sample preparation step in an analytical method was described as follow: 0.30g drying ALD powder were accurately weighed and transferred to calibrated, amber flasks and extracted with 50ml of methanol in an ultrasonic bath for 30 minutes. Additional methanol was added after sonication to compensate for any lost volume, and the resulting solution was filtered through a 0.45μm membrane filter prior to HPLC injection.

Model test research on gastric injury

Effects of ALDE on acute gastric mucous membrane damage induced by ethyl alcoholin rats

Sixty adult male SD rats (half male and half female) were randomly and equallydivided into a control group, a model group, a SO group or one of three ALDE treatment groups (n=10 for each group). The control group received an equivalent volume of saline (i.g) while the other four groups received treatment for 7 days as outlined in Section "Dosage regimen design". The rats were housed in a temperature controlled room with access to water but were subjected to fasting conditions for 24 hours before the final administration. Meanwhile, The SD rats were fasting water on the final administration. One hour after the final administration, the control group was given an equivalent volume of saline alone, and the remaining five groups were treated with ethyl alcohol (1.0ml/rat, i.g). After the next 1hour, rats were sacrificed, pyloris were ligatured, cardiacs were ligatured after the stomach contents were sent out by the cardia. The stomach was taken out and fixedin 10% formaldehyde at least10 minutes, stomach wall was cut open along greater curvature of stomach, the gastric mucosa was observed. According to the lesion severity score: Local redness was 1 point, spotting or erosion was 2 point, linear erosion was 3 points, their total number was as ulcer index, the ulcer index and the inhibiting rate was determined according to traditional method. Ulcer inhibition rate=(Model group ulcer index-Experimental group ulcer index)/ Model group ulcer index×100%. Resulting pathomorphological changes in gastric mucosa were studied with conventional hematoxylin-eosin (HE) staining of samples (Salim SA *et al*., 2012) While observing the height of the gastric mucosa, gastric mucosal height for each experiment were randomly selected five sites for height measurement, whichever is the mean height as the experimental rat gastric mucosa.

Effects of ALDE on acute gastric mucous membrane damage induced by pyloric ligation in rats

Sixty adult SD rats (half male and half female) were randomly and equally divided into a control group, a model group, a SO group or one of three ALDE treatment groups (n=10 for each group). The control group was given an equivalent volume of saline alone, and the remaining five groups received treatmentas specified in Section "Dosage regimen design" for 7 days. The rats were housed in a temperature controlled room with access to water but were subjected to fasting conditions for 48 hours before the final administration. Meanwhile, The SD rats were fasting water on the final administration. Two hour after the final administration, the rats were anaesthetised by urethane (1.0g/kg) and carried out pylorus ligation. Water and diet were prohibited. The rats were sacrificed by cervical spine dislocation after 18 hours in pyloric ligation rats, stomach wall was cut open along greater curvature of stomach, the gastric mucosa was observed. According to the lesion severity score: The mottled erosion was 1 point, the length of the erosion which was <1mmwas used 2 point, the length of the erosion which was 1~2mm the length of the erosion which was 2~4mm was used 4 point, was used 3point, the length of the erosion which was＞4mm was used 5 point (If erosion width was >2mm, its score × 2). The Statisticaltotal number was calculated. Ulcer inhibition rate as specified. Finally, recovered gallbladders were fixed in 10% neutral formalin. Resulting pathomorphological changes in gallbladder histology were studied with conventional hematoxylin-eosin (HE) staining of samples.

Effects of ALDE on abnormal intestinal propulsion in mice induced by adrenaline

Sixty adult Kunming mice were randomly and equally divided into a control group, a model group, a SO group or one of three ALDE treatment groups (n=10 for each group). The control group received an equivalent volume of saline (i.g) while the other four groups received treatment for 5 days as outlined in Section "Dosage regimen design". The rats were housed in a temperature controlled room with access to water but were subjected to fasting conditions for 16 hours before the final administration.30min after the final administration, All groups except the control group were injected epinephrin

Hydrochloride(0.5mg/kg,0.1ml/10g) with subcutaneous injection. After the next 15min, All groups were gavaged with carbon powder (5% injectable suspension, 0.1ml/10g). The mice were sacrificed by cervical spine dislocation after 20min,the intestine was selected from pyloric orifice to ileocecal junction, the length of the intestine and the carbon powder movement was determined. The carbon powder movement ratio was calculated as: the length of carbon powder movement (cm)/the length of intestine (cm) × 100%.

STATISTICAL ANALYSIS

The data obtained were analysed using the Graph Pad software program and expressed as the mean ± standard error of the mean (SEM). Statistically significant differences between the treatment groups were analyzed by ANOVA followed by the Newman-Keuls test. A result of probability was considered to be statistically significant ($p<0.05$).

RESULTS

Preparation of ALD extract and quantitative analysis of the main compounds

Calibration curve linearity was detected by using standard solutions. The linear regression equations established by plotting peak area (Y) comparatived with concentration (X) for each compound are showed in table 1. All calibration curves indicated good linearity ($r^2 \geqslant 0.999$) within the concentration ranges tested.

The limits of quantification (LOQ) and detection (LOD) were stipulated as the concentrations of a compound with signal-to-noise ratios (S/N) of 10:1 and 3:1, respectively. 2compounds were measured by serial dilution of a standard solution by using the decided HPLC parameters. The LOQs and the LODs for the compounds were found to be less than 0.273μg/L and 0.091μg/L for all 2 compounds under the described HPLC parameters (table 1).

The recoveries of the methods, six portions of costunolide and dehydrocostus lactone, were spiked with the mixed standard solution, respectively. The samples were disposed as Section Chemical analysis of active compositions by HPLC, and the results are showed in table 1. The recoveries of costunolide and dehydrocostus lactone were 99.16% and 99.57%, with RSDs of 0.73% and 0.99%, respectively, which is well within acceptable limits (see table 1).

The established method was subsequently applied to the simultaneous determination of the costunolide and dehydrocostus lactonein the ALD. The contents of costunolide and dehydrocostus lactone were respectively 1.836±0.376% and 2.581±0.249% HPLC chromatograms of the standards and ALD sample are shown in figs. 2A and 2B, respectively.

Effects of ALDE on acute gastric mucous membrane damage induced by ethyl alcohol in rats

SD rats in the model group that received ethyl alcohol exhibited changes in mucosal damage index (MDI) and ulcer inhibition rate as compared to animals in the control group (fig. 3). The mucosal damage index of three ALDE treatment groups highly significantly decreased to 7.78±4.92, 6.78 ± 3.27 and 6.22 ± 2.91 ($P<0.01$), respectively, as compared to the model group. Furthermore, the mucosal damage index of the mid-range dose group significantly decreased ($P<0.05$) while the high-dose group highly significantly decreased, respectively, as compared to the SO group ($P<0.01$, fig. 3). On the other hand, the ulcer inhibition rate of low-dose, mid-dose and high-dose ALDE treatment groups reached 68.64%, 72.67% and 74.91%, respectively.

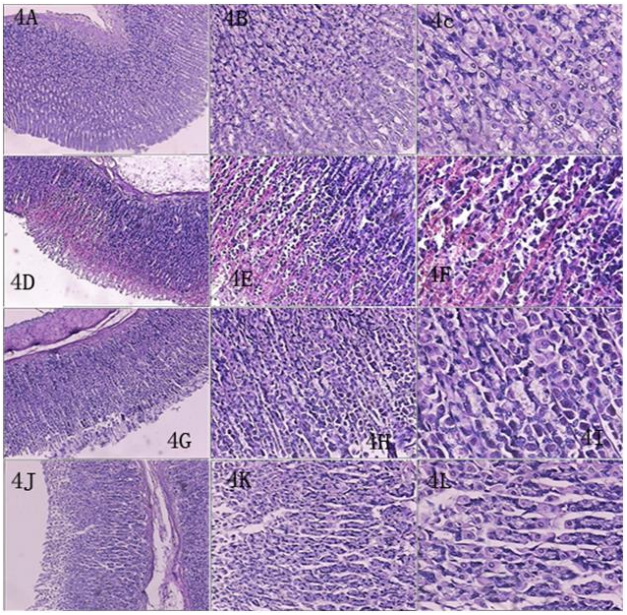

Fig. 4: Pathological changes of ethyl alcohol induced acute gastric mucous membrane damage in SD rats.

As demonstrated in fig. 4, as compared to the control group [fig. 4A (HE X40), fig. 4B (HE X100) and fig. 4C (HE X2000)], pathological changes in the gastric mucosatissue were mainly generated degeneration, necrosis, abscission, the lesion area ooze blood orbleedingin the model group [fig. 4D (HE X40), fig. 4E (HE X100) and fig. 4F(HE X2000)]. However, as compared tothe control group, the gastric mucosa tissue structure of three ALDE treatment groups had gradually different degree of recovery, among the mid-dose and high-dose ALDE treatment group sreturned to normal [Mid-dose ALDE treatment groups: fig. 4G (HE X40), fig. 4H (HE X100) and fig.4I (HE X2000); High-dose ALDE treatment groups: fig. 4J (HE X40), fig.4K (HE X100) and fig. 4L (HE X2000, respectively].

Histopathological results indicate that the mid- dose group highly significantly increased the height of gastric mucosa (fig. 3, P<0.01), the high-dose group significantly increased the height of the gastric mucosa (fig. 3, P<0.05). The height of gastric mucosa of the low-dose group exhibited an increasing trend while SO group had a decreasing trendas compared to the model group; however, these changes were not statistically significant (fig. 3, P>0.05).

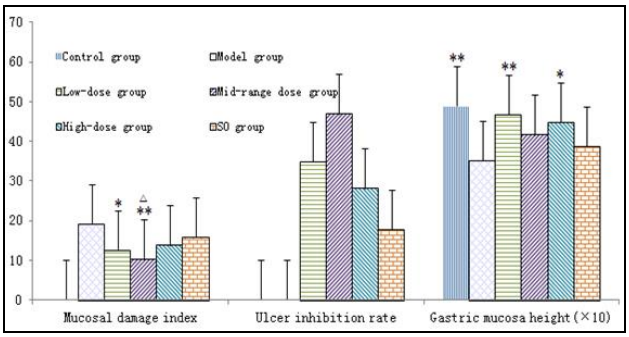

Fig. 5: Effects of ALDE on acute gastric mucous membrane damage induced by pyloric ligation in each group (n=10). The asterisks denote significance levels when compared with the model group: *p <0.05; **p <0.01. The asterisks denote significance levels when compared with the SO group: $^{\triangle}$p <0.05;$^{\triangle\triangle}$p <0.01.

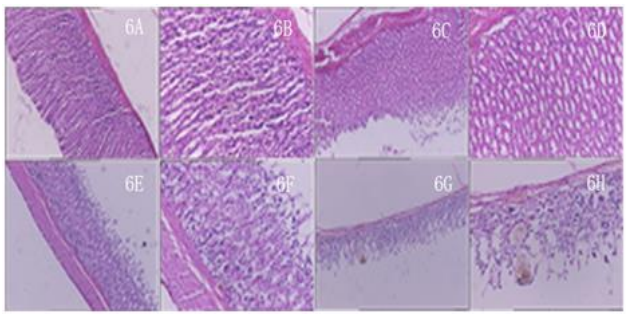

Fig. 6: Pathological changes of pyloric ligation induced acute gastric mucous membrane damage in SD rats.

Effects of ALDE on acute gastric mucous membrane damage induced bypyloric ligation in rats

SD rats in the model group that induced by pyloric ligation exhibited changes in mucosal damage index (MDI) and ulcer inhibition rate as compared to animals in the control group (fig. 5). The mucosal damage index of the low-range dose group significantly decreased to 12.50±6.29(P<0.05), the mid-range dose group highly significantly decreased to 10.20 ± 5.85 (P<0.01), respectively, as compared to the model group. Levels of high-range dose group exhibited decreasing trend while its changes was not statistically significant (P>0.05, fig. 5). Furthermore, The mucosal damage index of the mid-range dose group significantly decreased (P<0.05) as compared to the SO group (fig. 5). The low- and high-dose exhibited decreasing trend while their changes were not statistically significant (P>0.05, fig. 5). On the other hand, the ulcer inhibition rate of low-, mid- and high-dose ALDE treatment groups reached 34.90%, 46.88% and 28.13%, respectively.

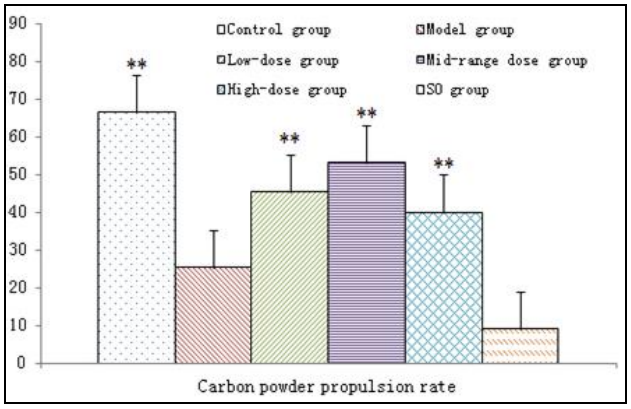

Fig. 7: Effects of ALDE on abnormal intestinal propulsion in mice induced by adrenaline in each group (n=10). The asterisks denote significance levels when compared with the model group:*p <0.05;**p <0.01.

As demonstrated in fig. 6, pathological changes in the gastric mucosa tissue were mainly generated degeneration, necrosis, abscission, the lesion area ooze blood or bleeding. If the gastric mucosa tissue didn't generate obvious pathological changes with normal structure which is denoted by"-"(fig. 6A and Fig. 6B) However, the gastric mucosa tissue generated small amount of degeneration, necrosis and exfoliated epithelial cells, and the height of gastric mucosa exhibited unconspicuous downtrend which is denoted by"+"(fig. 6C and fig. 6D). Further, the gastric mucosa tissue generated a small part of degeneration, necrosis and exfoliated epithelial cells, and the height of gastric mucosa exhibited obvious downtrend, the partblood vessel within mucous layer and blood cells with in blood vessel produced degeneration, with containing typical for hemosiderin deposition which is denoted by"++"(fig.6E and fig.6F). At last, the gastric mucosa tissue generated most degeneration, necrosis and exfoliated epithelial cells, and the height of gastric mucosa exhibited significantly downtrend, the blood vessel with in mucous layer and blood cells with in blood vessel produced degeneration and disappearance, with containing typical for hemosiderin deposition which is denoted by"+++"(fig.6G and fig. 6H). As demonstrated in table 2, The number of SD with "-", "+", "++" and "+++" were 2, 1, 1 and 6, respectively, in the model group. The results revealed that pyloric ligation successfully induced acute gastric mucous membrane damage in this model. The low- and high-dose group exhibited significantly (P<0.05) as compared to the model group. However, mid-dose group was not statistically significant (P>0.05, table 2).

Table 1: Linearity, LOD, LOQ, recovery and recovery of 2 control compositions

Compound	Linear range (μg/ml)	Linear equation	Regression r^2 (n=5)	LOD (μg/ml)	LOQ (μg/mL)	Recovery	
						Average (%)	RSD (%)
Costunolide	0.021-10.8	Y=9613.3X+3997.3	r=0.9998	0.030	0.010	99.16	0.73
Ligustilide	0.081-24.30	Y=7865.8X+2965	r=0.9999	0.091	0.273	99.57	0.99

Table 2: Pathological pattern of ALDE on acute gastric mucous membrane damage induced by pyloric ligation

Group	Animal number	Pathological pattern				P value As compared to the model group
		-	+	++	+++	
Model group	10	2	1	1	6	-
Control group	10	10	0	0	0	P<0.01
Low-dose group	10	6	2	2	0	P<0.05
Mid-dose group	10	5	0	2	3	P>0.05
High-dose group	10	5	1	2	2	P<0.05

Note: Effects of ALDE on acute gastric mucous membrane damage pattern

Effects of ALDE on abnormal intestinal propulsion in miceinduced by adrenaline

As demonstrated in fig. 5, the carbon powder propulsion rate of the model group highly significantly decreased to25.35±6.71as compared to the control group (66.45±16.48). the carbon powder propulsion rate of three ALDE treatment groups highly significantly increased to 45.20±18.03, 53.10±7.42 and 39.99±10.81 (P<0.01), respectively, as compared to the model group. The SO group exhibited an increasing trend while the change was not statistically significant (fig.5, P>0.05).

CONCLUSION

Our results indicated that ALDE had a marked effects onantiulcer activity in animals, which supports former claims of its use in traditional herb. In acute gastric mucous membrane damage induced by ethyl alcohol in rats, the results indicated that three ALDE treatment groups highly significantly decreased the mucosal damage indexas compared to the model group. Furthermore, the mucosal damage index of the mid-range dose group significantly decreased while the high-dose group highly significantly decreased, respectively, as compared tothe SO group. The ulcer inhibition rate of low-, mid- and high-dose ALDE treatment groups reached 68.64%, 72.67% and 74.91%, respectively. In acute gastric mucous membrane damage induced by pyloric ligation in rats, the results indicated that three ALDE treatment groups highly significantly decreased the mucosal damage indexas compared to the model group. Furthermore, the mucosal damage index of the mid-range dose group significantly decreased while the high-dose group highly significantly decreased, respectively, as compared to the SO group. The ulcer inhibition rate of low-, mid- and high-dose ALDE treatment groups reached 68.64%, 72.67% and 74.91%, respectively.

DISCUSSION

The ethanol, which produces severe gastric hemorrhagic lesions, is a frequently used ulcerogenic agent and when given by gavage to rats. The mechanism of ethanol-induced gastric lesions is manifolded, which includes the depletion of gastric mucus content, damaged mucosal blood fow or mucosa cell injury. Moreover, ethanol-induced gastric mucosadamage is bounded up with overproduction of free radicals, they lead to an increased lipid peroxidation (Kahraman *et al*., 2003; Fatih*et al*., 2007).

Pyloric ligation is one of the important factorsin mucosal damage because it interfers with gastric mucosal resistance. Moreover, pyloric ligation lead to an increase in calcium level which is known to excite free radical generation. This increase in calcium and free radicals is demonstateto have induced tissue injury and PU (Shaija and Arunachalam, 2009).

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