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Andrographolide Attenuates Senna- and Castor Oil-induced Diarrhea in Mice

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SUMMARY. **Andrographolide (AND) is a diterpenoid lactone extracted from *Andrographis paniculata,* a member of family Acanthaceae. AND is a pharmacological component of traditional Chinese medicines, and various AND-derived pharmaceuticals are extensively used clinically to treat infectious diseases of the digestive and respiratory systems. However, the effects of AND on intestinal dynamics have never been reported. In this study, we observed the therapeutic effects of AND on senna- and castor oil-induced diarrhea mouse models and found that AND significantly retarded their small intestinal propulsive motility. The two mouse models exhibited decreased vulnerability to senna- and castor oil-induced diarrhea after administration with 150 mg/kg AND, and their diarrhea indices decreased at all tested doses. AND reduced the large intestine weights per unit of the senna-induced mouse model and the small-intestine weights per unit of the castor oil-induced model. These findings can serve as theoretical basis for the clinical treatment of diarrhea.**

INTRODUCTION

Andrographolide (AND) is a diterpenoid lactone extracted from the traditional medicine *Andrographis paniculata* and is one of the main pharmacological components of this plant (Fig. 1) [1,2]. Modern pharmacological studies have indicated that AND is effective against inflammation, bacteria, viruses, and tumors. AND can also regulate immunity, protect the liver and gall, treat cardiovascular diseases, etc. Accordingly, AND is clinically used to treat viral pneumonia, viral upper respiratory tract infection, bronchitis, tonsillitis, and bacillary dysentery [3-5]. Formulated injections comprising mainly AND are widely used to treat infectious diarrhea with satisfactory results [6]. However, basic studies on the effect of AND on gastrointestinal smooth muscle have not yet been reported. In the current study, the effects of different doses of AND on gastrointestinal smooth muscle movement were investigated by a small-intestine propulsive-motility experiment. The treatment efficacy of AND on senna- and castor oil-induced diarrhea mouse models was then evaluated.

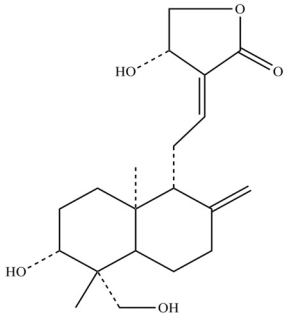

Figure 1. Chemical structure of AND.

MATERIAL AND METHODS

Reagents and chemicals

AND (98.28%) was purchased from Hubei Jianyuan Chemical Co., Ltd (China). Loperamide hydrochloride was obtained from Janssen Pharmaceutical (Xi'an) Ltd (China). Senna was purchased from the traditional Chinese medicine pharmacy of the First Affiliated Hospital of Xinxiang Medical University (Nanjing Zelang Medical Technology Co., Ltd, China). Castor oil was purchased from Hunan Er-kang Pharmaceutical Co., Ltd (China).

KEY WORDS: Andrographolide, Castor oil, Diarrhea, Senna.
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Animals

Seven-week-old BALB/c mice (1:1 sex ratio) weighing 18 to 22 g were provided by the Laboratory Animal Facility in Henan Province (China). The mice were maintained at the Experimental Animal Center of Xinxiang Medical University under specific pathogen-free conditions. The mice were housed in stainless steel cages and kept at controlled temperature (25 ± 2 °C), ambient humidity (50 to 75%), and 12 h light/dark cycle. After being accustomed to feeding for 1 week, the mice were fasted overnight with free access to water immediately before experiment. This study was approved by the Institutional Animal Care and Use Committee of Xinxiang Medical University. All pharmacological experiments were performed in accordance with the National Institutes of Health (NIH) Principles of Laboratory Animal Care (NIH publication #85-23).

Small-intestine propulsive-motility experiment

Forty BALB/c mice were randomly divided into the following groups: normal control, positive control (2 mg/kg loperamide), high-dose AND (150 mg/kg), medium-dose AND (100 mg/kg), and low-dose AND (50 mg/kg) (n = 8). The three AND groups were intragastrically administered with the designated doses, the normal group was intragastrically administered with equal volumes of normal saline, and the positive control group was administered with 2 mg/kg loperamide. The corresponding intragastric administration times were accurately recorded. After 15 min, all mouse groups were intragastrically administered with 5% carbon inks (0.2 mL each) while initiating time counting. After 15 min, the mice were sacrificed by cervical dislocation. The immediately disconnected, full-length, small intestine from pylorus to cecum was then analyzed using the following calculation: *ink propulsion rate (%) = ink movement distance (cm) / full length of small intestine (cm) × 100%.*

Senna-induced diarrhea model

Diarrhea was induced by senna and castor oil in mice as previously described by Li *et al.* [7] and Owolabi *et al.* [8]. Appropriate amounts of dry senna leaves were ground and added with boiling distilled water. After mixing, the product was prepared as an 8% suspension by adding cold distilled water. A total of 48 healthy BALB/c mice were randomly divided into the following groups: normal control, model control, positive control, high-dose AND (150 mg/kg), medium-dose AND (100 mg/kg), and low-dose AND (50 mg/kg) (n = 8). All AND groups were intragastrically administered with the aforementioned doses. The positive control group was administered with 2 mg/kg loperamide. The other groups were intragastrically administered with equal volumes of normal saline. After 30 min, all groups except for the normal control group (treated with identical volumes of normal saline) were intragastrically administered with 300 mg/kg senna. The mice were housed in individual cages with filter papers underneath and observed for 6 h. The filter papers were replaced every 1.5 h.

Castor oil-induced diarrhea model

Healthy BALB/c mice were grouped in a manner similar to that for the senna-induced model. All AND groups were intragastrically treated with specific doses. The positive control group was administered with 2 mg/kg loperamide. The other groups were intragastrically treated with identical volumes of normal saline. After 30 min, all groups, except for the normal control group (administered with the same amount of normal saline), were intragastrically administered with castor oil (0.2 mL each). Similarly, the mice were housed in individual cages with filter papers underneath and continuously observed for 6 h. The filter papers were replaced every 1.5 h.

Diarrhea evaluation indices

The general mental states, behaviors, and defecation changes of diarrhea mice in the two models were observed. The loose stool times, total defecation times, and loose stool grades of all mice within 6 h were examined to calculate the loose stool incidence rates and diarrhea indices.

The loose stool incidence rate was the ratio of the loose stool times of a mouse to its total defecation times. Each granule or pile (for indistinguishable granules) of feces on the filter paper was counted as one defecation time. The following formula was used: *loose stool incidence rate (%) = loose stool times / total defecation times × 100%.*

The loose stool grade represented the degree of loose stool and was graded according to the diameter (cm) of the stain contaminated by loose stools as follows: grade 1, diameter < 1 cm; grade 2, diameter = 1-1.9 cm; grade 3, diameter = 2-3 cm; and grade 4, diameter > 3 cm. The diameters of circular stains were directly measured, whereas those of irregular stains

were determined by dividing the sum of the largest, approximately circular diameters by two. The average loose stool grade of each mouse was acquired by dividing the sum of the grade of each pile by its loose stool times *(average loose stool grade = sum of loose stool grades/loose stool times).*

The diarrhea index was defined as the product of the loose stool incidence rate and the average loose stool grade *(diarrhea index = loose stool incidence rate × average loose stool grade).*

Mouse abdomens were cut open after sacrificing and 6 h after modeling. Small and large intestines were then segmented from pylorus to cecum and from cecum to rectum end, respectively, to calculate the intestine weights per unit length by measuring the corresponding lengths (cm) and weights (mg). The following formula was used: *intestine weight per unit length = intestine weight (mg)/intestine length (cm).*

Statistics

All experimental data are expressed as the mean ± SEM ($\overline{x} \pm s$). Data were analyzed by one-way ANOVA using SPSS (version 16.0). *P* values less than 0.05 were regarded as significant.

RESULTS

Mouse small-intestine propulsive-motility experiment

The ink propulsion rate of the positive control group was significantly lower than that of the normal control group ($P < 0.01$), indicating that loperamide decreased bowel movement and retarded small intestinal propulsion. Compared with the ink propulsion rate of the normal control group, those of the low-, medium-, and high-dose AND groups were lower ($P < 0.05$), indicating that all three AND doses moderately inhibited small intestinal movement (Table 1).

Effects of AND on senna-induced diarrhea mice

Except for the normal control group, all mice experienced aggravated diarrhea symptoms, including more frequent defecations and increased water contents in the feces with elapsed time. The model control group suffered from clearly reduced activities, fatigue, asthenia, low vigor, and watery stools with diminished solid components, indicating that the model was successfully established.

The loose stool incidence rate of the model control group was higher than that of the high-dose AND group ($P < 0.05$). Meanwhile, the diarrhea indices of all AND-administered groups were significantly lower than that of the model control group ($P < 0.01$), suggesting that diarrhea was mitigated by AND regardless of the dose (Table 2).

However, loperamide failed to decrease the intestine weight per unit length significantly compared with that of the model control group. This result may be associated with the suppressed bowel movement caused by the blocked release of acetylcholine and prostaglandin as induced by activated opioid receptor. In contrast to the slightly altered small-intestine weight per unit ($P > 0.05$), that of the large intestine was considerably reduced by AND at all doses ($P < 0.05$) (Fig. 2).

Effects of AND on castor oil-induced diarrhea mice

One hour after intragastric administration of castor oil, all groups except for the normal control group exhibited diarrhea symptoms that deteriorated with prolonged observation, including more frequent defecations, elevated water contents in the feces, and irregularly shaped, loose stools. The model control group exhibited apparently decreased activities and watery stools with decreased solid components in addition to low vigor.

Compared with the loose stool incidence rate of the model control group, only that of the high-dose AND group was significantly lower ($P < 0.05$). By contrast, the diarrhea indexes of all

Groups	Drug dose (mg/kg)	Intestinal length (cm)	Ink propulsive length (cm)	Ink propulsive rate
Normal control group	–	41.8 ± 1.63	35.09 ± 1.30	0.85 ± 0.09
Positive control group	2	41.4 ± 5.56	9.23 ± 0.92	0.23 ± 0.05 **
High-dose AND group	150	38.17 ± 1.15	18.51 ± 1.80	0.48 ± 0.09*
Medium-dose AND group	100	40.73 ± 1.99	23.56 ± 1.68	0.52 ± 0.06 *
Low-dose AND group	50	40.60 ± 1.38	25.21 ± 1.39	0.63 ± 0.08 *

Table 1. Effect of AND on intestinal propulsive motility in mice. *$P < 0.05$, **$P < 0.01$ compared with the normal control group ($n = 8$).

Groups	Drug dose (mg/kg)	6-h loose stool rate	Diarrhea indice			
			1.5h	3h	4.5h	6h
Normal control group	–	0	0	0	0	0
Model control group	–	0.91 ± 0.12	1.37 ± 0.19	2.15 ± 0.24	1.92 ± 0.19	1.85 ± 0.24
Positive control group	2	0.40 ± 0.08**	0.34 ± 0.15**	0.45 ± 0.12**	0.32 ± 0.06**	0.26 ± 0.05**
High-dose AND group	150	0.71 ± 0.17 *	0.63 ± 0.18 **	1.29 ± 0.19 **	0.85 ± 0.12 **	1.04 ± 0.12**
Medium-dose AND group	100	0.88 ± 0.14	0.96 ± 0.19 *	1.43 ± 0.25 **	1.32 ± 0.15 **	1.21 ± 0.23 **
Low-dose AND group	50	0.84 ± 0.21	1.06 ± 0.11 *	1.36 ± 0.14 **	1.08 ± 0.11 **	1.16 ± 0.19 **

Table 2. Effect of AND on folium senna-induced experimental diarrhea mice. *P < 0.05, **P < 0.01 compared with the model control group (n = 8).

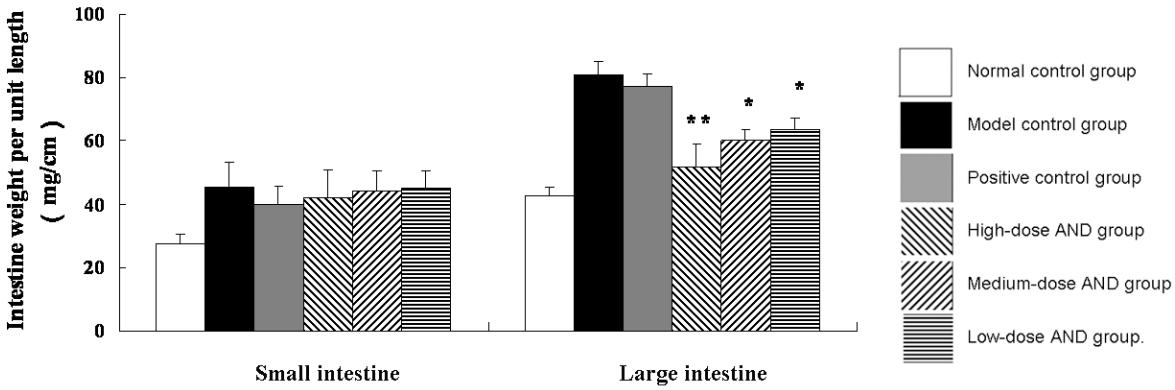

Figure 2. The intestine weight per unit length of mice with folium senna-induced experimental diarrhea. *p < 0.05, **p < 0.01, significant difference compared with model control group (n = 8).

AND-administered groups were dramatically decreased compared with that of the model control group (P < 0.05) (Table 3).

Furthermore, loperamide barely affected the intestine weight per unit of diarrhea mice. However, the small-intestine weights per unit of all AND-administered groups were lower than that of the model control group (P < 0.05), and only the high-dose AND group had a lower large intestine weight per unit (P < 0.05) (Fig. 3).

DISCUSSION

Diarrhea pathologically stems from intestinal mucosal inflammation, edema, and hyperkinesia because of infections or gastrointestinal disorders. Diarrhea manifests as loose stool and increased defecation frequency [9]. In the present study, the effects of AND on bowel movement were evaluated by calculating the ink propulsion rate based on a small-intestine propulsive-motility experiment. The results showed that low-, medium-, and high-dose AND significantly decreased the ink propulsion rates and hindered bowel movement, suggesting that AND terminated diarrhea by restraining bowel movement in a dose-dependent manner. Meanwhile, AND may have less side effects (such as constipation) than loperamide because of the milder functioning protocol.

Groups	Drug dose (mg/kg)	6-h loose stool rate	Diarrhea indice			
			1.5h	3h	4.5h	6h
Normal control group		0	0	0	0	0
Model control group	—	0.96 ± 0.08	1.42 ± 0.21	2.11 ± 0.23	1.83 ± 0.12	1.81 ± 0.25
Positive control group	2	0.37 ± 0.06**	0.44 ± 0.13 **	0.55 ± 0.11 **	0.57 ± 0.09 **	0.41 ± 0.04 **
High-dose AND group	150	0.75 ± 0.11 *	0.44 ± 0.28 **	1.23 ± 0.19 **	0.80 ± 0.24 **	1.33 ± 0.18 *
Medium-dose AND group	100	0.94 ± 0.09	0.99 ± 0.21 **	1.53 ± 0.22 *	1.33 ± 0.17 *	1.25 ± 0.23 *
Low-dose AND group	50	0.89 ± 0.12	1.15 ± 0.14 *	1.34 ± 0.16 *	1.01 ± 0.22 *	1.16 ± 0.26 *

Table 3. Effect of AND on castor oil-induced experimental diarrhea in mice. *P < 0.05, ** P < 0.01 compared with the model control group (n = 8).

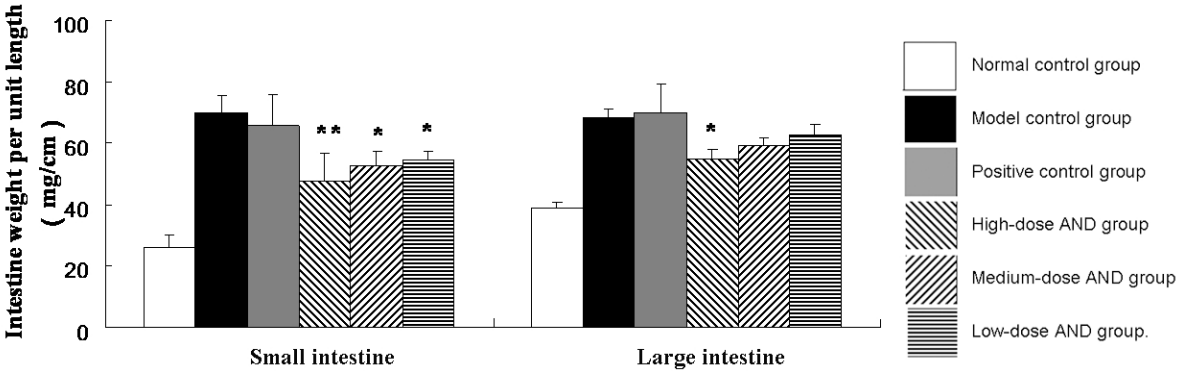

Figure 3. Intestine weight per unit length of mice with castor oil-induced experimental diarrhea. Low-dose AND group. $^{*}p < 0.05$, $^{**}p < 0.01$, significant difference compared with model control group ($n = 8$).

Moreover, the antidiarrheal effects of AND were evaluated using a diarrhea index that objectively and comprehensively quantified the degree of loose stool for possible comparison. After modeling with senna or castor oil, all mice successively experienced diarrhea symptoms and sluggishness. Compared with medium- and low-dose AND ($P > 0.05$), high-dose AND clearly reduced the diarrhea incidence rate and senna-induced diarrhea index ($P < 0.05$). Similarly, only high-dose AND reduced the castor oil-induced loose stool incidence rate ($P < 0.05$). Nevertheless, all AND groups had clearly lower diarrhea indices. These results suggested that AND had antidiarrhea activity in both cases.

Senna and castor oil both induced diarrhea by stimulation but through intrinsically different action sites and mechanisms [8,10]. The anthraquinone derivatives in senna were highly stimulating and cathartic. In particular, the decomposition of sennosides A and B after absorption in the small intestine and their subsequent release in the blood resulted in the excited pelvic ganglion and diarrhea through large-intestinal contractions [11]. Nevertheless, after oral administration and subsequent hydrolysis in the small intestine, castor oil transformed into sodium castor oil, which stimulated the active secretion of the small intestine, minimized intestinal absorption, facilitated bowel movement, and eventually resulted in diarrhea [12]. In the present study, AND partially shielded the mouse intestines from stimulation by senna and castor oil. Xing *et al.* [13] also found that AND maintains the integrity of the intestinal mucosa intact by blocking the secretion of intestinal epithelial cells and reducing intestinal permeability, which may contribute to the alleviated intestinal exudation and edema as well as the lower intestine weight per unit length.

CONCLUSIONS

AND effectively relieved senna- and castor oil-induced mouse diarrhea possibly by inhibiting intestinal propulsive motility and secretion. The results of this study can serve as theoretical basis for the clinical treatment of diarrhea using AND formulations. However, thorough mechanism-related studies must be conducted.

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