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Preventative Effect of Traditional Medicine Anar-5 on the Development of Chronic Gastritis in a Rat Model

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Objective: To study the effect of traditional medicine Anar-5 on the development of chronic gastritis in rat gastric mucosa. **Methods:** Rats were orally administered deoxycholate sodium 1ml/100 gm or a combination of deoxycholate 1 ml/100 gm and Anar-5 at Anar-5 100 mg/kg or 200 mg/kg for 4, 8, and 12 weeks to determine the protective effect of Anar-5. Serum gastrin levels were measured to determine the impact of Anar-5. Gastric specimens were prepared for light microscopy with hematoxylin and eosin and PAS stains. The damage of barrier in mucosa with erosion or ulceration, and the thickness of mucin were examined using light microscopy. The serum levels of gastrin were measured with ELISA method. **Results:** Under light microscopy, the gastric mucosa was diffusely eroded or ulcerated and the thickness of mucin was decreased in rats not treated with Anar-5. The levels of gastrin in serum were significantly lower in chronic atrophic gastritis rats than that in normal rats ($p<0.05$). Doubling the dose of Anar-5 from 100 mg/kg to 200 mg/kg made no difference. **Conclusion:** Treatment with Anar-5 prevented chronic gastritis and gastric atrophy.

Keywords: Mongolian Traditional Medicine, Chronic Gastritis, Anar-5

Introduction

Chronic gastritis is a disease of gastric mucosa with non-specific chronic inflammatory lesions. The basic pathological changes are gastric mucosal epithelial degeneration and inflammatory cell infiltration. The lesions are mostly confined to the superficial mucosa, but sometimes also involves the entire mucosa layer [1, 2]. If allowed to involve the entire mucosa, atrophic gastritis will appear and about 8% of these lesions will become cancer [3]. Digestive tract diseases are the second leading cause of death in Mongolia, and the incidence of chronic atrophic gastritis accounts for 12% of all digestive tract diseases [4]. These data indicate urgent need to develop low cost pharmacologic treatments with minimal side effects. In

addition to pharmaceuticals, herbal drugs might be able to lower the still high morbidity of digestive tract diseases in Mongolia. The search for an appropriate agent was the main reason to conduct this study. The Mongolian traditional medicine Anar-5 is thought to increase the processes of digestion, cold disease, vomiting, and wind of heart. Anar-5 of tastes sour. This is a powerful stimulant of digestive heat and to dispel phlegm cold. In traditional medicine, Anar-5 is used for treatment of phlegm disorder of the stomach and indigestion. It dispels phlegm-cold and improves the stomach heat. Anar-5 preparation consists of five herbs: Punica granatum L., Cinnamomum cassia (L.) J. Presl, Piper longum L, Elettaria cardamomum (L.) Maton Alpinia officinarum Hance [6, 7]. Punica granatum L belonging to Punicacae and possess various activities [8]. Punica granatum L seems to have ulcer cytoprotective effects due to enhanced mucosal resistance and reduction in oxidative mucosal damage possibly via high antioxidant activity [9]. The anti-inflammatory activity of Punica granatum L at gastric level has been evaluated mainly in in vivo studies, and a few in vitro studies that deal with the anti H. pylori activity of Punica granatum L extracts and individual compounds [10]. Cinnamomum cassia (L.) J. Presl has been reported to have anti-inflammatory, anti-oxidant, anti-cancer, anti-fungal, anti-pyretic, antimicrobial, anti-angiogenic and larvicidal activity [11, 12]. Piper longum L extracts have analgesic, ulcer protecting and other diverse therapeutically interesting bioactivities of piperine in animal models that are well known [13-17]. Alpinia officinarum Hance (Zingiberaceae) has long been used as an anti-inflammatory, an analgesic, a stomachic and a carminative in traditional medicine [18]. Unlike conventional medicines, systematic pharmacological data for many herbal and traditional medicine products are not always available. A license to dispense these products as medications has been issued based on their history of medicinal use. However, data on the efficacy and safety of these herbal products are often unavailable. Therefore, we sought to determine the effectiveness of traditional medicine Anar-5 in preventing atrophic gastritis. In the present study, we tested this hypothesis using a rat model of sodium deoxycholate induced atrophic gastritis understanding that chronic atrophic gastritis can be induced in rats by orally administering 20 mmol/L sodium deoxycholate (1 ml/100g) [18, 19].

Materials and Methods

The study was carried out at the laboratory of pharmacology in Institute of Traditional Medicine and Technology of Mongolia (ITMTM) and Mongolian National University of Medical Science (MNUMS), Institute of Veterinary medicine. Anar-5 herbal medicine was prepared in the traditional medical factory of Institute of Traditional Medicine and Technology of Mongolia. The herbs contained in the Anar-5 were collected and authenticated by the experts at the Department of Botany, Institute of Biology and Mongolian Academy of Sciences. Ten grams of crushed dried Anar-5 plant material was suspended in 250 ml water and boiled until water had evaporated to 100 ml. The prepared compound was then used for pharmacological tests.

1. Establishment of chronic atrophic gastritis in rats

Using an orthogonal design, deoxycholate was orally administered to rats (4 cages, 15 rats/cage). Sixty healthy male wistar rat of weighing between 250-280 gm were purchased from the Experimental Animal Center, Institute of Traditional Medicine and Technology of Mongolia. They were kept under controlled conditions of temperature (20±1 C°) and humidity (about 50-60%), with a 12-hour light/dark cycle, and automatic ventilation (8-15 times every hour). Rats could drink freely, and were fed feedstuff. Animals were divided 4 groups: Group I rats (control group, n=15) received distilled water. Group II rats (n=15) received deoxycholate at dose of 1 ml solution/100 gm body weight. Group III rats (n=15) received Anar-5 100 mg/kg and deoxycholate 1ml/100 gm. Group IV rats (n=15) received Anar-5 200 mg/kg and deoxycholate 1ml/100 gm. The rats were fed these substances daily until euthanized. The study protocol (№14-14/1A) 2014.06.17 was approved by the Ethics Committee of the Mongolian National University of Medical Science (MNUMS).

2. Blood samples

After 4, 8, and 12 weeks 5 rats from each group were anesthetized with ketamine hydrochloride (90 mg/kg, intraperitoneally). A 5 ml blood sample was collected from each rat by cardiac puncture. The serum was separated by centrifugation at 3000 rpm for 15 minutes. The level of serum gastrin was measured by ELISA according following the kit's

instructions (Shanghai MLBIO Biotechnology Co.Ltd).

3. Histology

Gastric tissue specimens were taken larger curvature of the stomach. The specimens were immediately fixed by immersion in 10% buffered formalin and then embedded in paraffin. Paraffin sections (5μm) of the gastric mucosa tissue were cut and stained by hematoxylin and eosin (HE) and periodic acid Schiff (PAS) staining. Healthy rat gastric wall consists of mucosa and submucosa, muscular layer and serosa. The stomach mucosa consists of surface epithelium, lamina propria and muscularis mucosa. The histology of the mucosa of body of stomach consists of straight tubular glands which synthesize and secrete gastric juice. The gastric glands contain a mixed population of cells (surface mucous cell, parietal cells and chief or peptic cell). Histological changes in these tissues were determined using light microscopy.

4. Statistical analysis

Mean ± standard deviation (SD) were calculated for the observed values in each experimental group. Statistical analysis was done by one-way ANOVA followed by tukey post hoc test was performed. Graph Pad Prism-5 software was used for statistical analysis with $p<0.05$ considered statistically significant.

Results

Serum gastrin levels

The serum gastrin levels are reported in Table 1.

After the 12 weeks of treatment the serum gastrin levels of untreated deoxycholate group (Group II, 28.3±0.95) pg/ml were approximately one-third of normal group (Group I, 83.5±1.75) pg/ml ($p<0.05$). After 12 weeks of treatment the serum gastrin levels in both groups treated with Anar-5 and deoxycholate were significantly higher (Group III 82.3±0.39, Group IV 83.2±8.05) than in the untreated deoxycholate group ($p<0.01$) and with numbers available in our study were no different than the normal controls ($p>0.05$). These results show that Anar-5 prevented the fall in gastrin level in rats simultaneously receiving deoxycholate.

Histopathological analysis

Sections stained with hematoxylin and eosin (H&E) showed mucous neck cells, the slightly pink, fairly large parietal cells and the somewhat darker bluish-violet chief cells (Figure 1). The columnar epithelial cell of superficial layer secretes mucus and this mucin along with the stained a dark purple color with PAS stain.

Histology after 4 weeks While the gastric tissues of the normal controls (Group 1) appeared normal (Figure 1a), the tissues from the untreated deoxycholate group (Group II) contained inflammatory cells that had infiltrated the epithelial cells of gastric mucosa and muscular layer of the stomach (Figure 1b). These findings verified the gastric mucosal damage model. In the Anar-5 treatment group 100 mg/kg (Group III), there was severe infiltration of the muscularis mucosa by eosinophils in lower part of mucosa of the stomach wall by low magnification of light microscopy (Figure 1?). In the rats treated with Anar 200 mg/kg (Group IV), inflammatory cells were observed within parietal and chief cells of the stomach

Table 1. Effect of Anar-5 on serum gastrin levels in rats given deoxycholate.

Group	Gastrin serum level (pg/ml)		
	4 weeks	8 weeks	12 weeks
Normal control	83.5±1.75	83.5±1.75	83.5±1.75
Deoxycholate+vehicle	27.2±0.3[6]#	27.8±0.30#	28.3±0.95#
Deoxycholate+Anar-5 100mg/kg	60.1±3.65**	55.7±4.45*	82.3±0.39**
Deoxycholate+Anar-5 200mg/kg	64±3.4[C*]*	51.9±3.7[5]*	83.2±8.0[E*]*

Data are presented as the mean± SD (n = 15) in each group. #Significantly different from the normal control at $p<0.001$; **Significantly different from the Deoxycholate+vehicle at $p<0.001$; * Significantly different from the Deoxycholate+vehicle at $p<0.01$.

glands but no pathologic changes such us degeneration and necrosis of superficial epithelial cells were seen (Figure 1b). There were also no pathologic changes in the superficial layer of stomach mucosa using the PAS staining method. In the untreated group (Group II) the epithelial cells of the superficial layer of the stomach mucosa were fewer in number and were degenerated by PAS compared to the untreated control group (Group II). These findings show the atrophic changes induced by

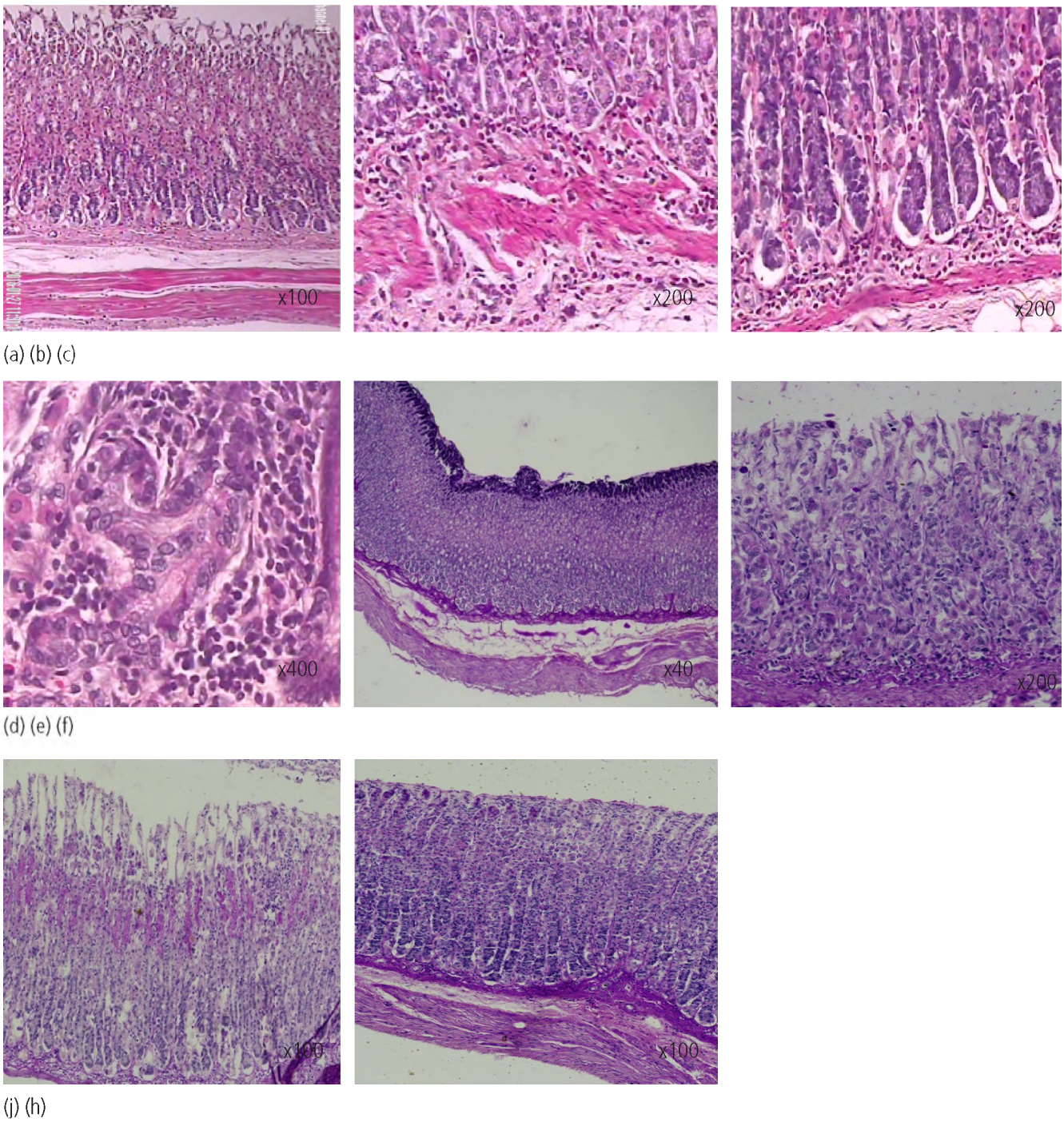

Figure 1. Histological examination of stomach 4 weeks after treatment. (a) Normal gastric mucosa with gastric glands and layers of regularly arranged gastric epithelial cells stained with H&E, (b) untreated group, (c) Anar-5 treatment group 100 mg/kg, (d) Anar-5 200 mg/kg treatment group, (e) normal group stained with PAS, (f) untreated group, (j) Anar-5 100 mg/kg treatment group, (h) Anar-5 200 mg/kg treatment group.

deoxycholate. Stomach mucosal epithelial cells were decreased and PAS positive cells were fewer in Anar-5 (100 mg/kg) group (Group III) compared to Group IV. In Anar-5 (200 mg/kg) group (Group IV), there was degeneration of the superficial epithelial cells of the stomach mucosa after 4 weeks of the experiment.

Histology after 8 weeks

There were also inflammatory cell infiltrating the muscular cells of muscular layer of the cardia, near esophagus and hyperemia was observed in the glandular cells of the antrum mucosa in the 8 weeks after the experiment (Figure 2). There were no significant changes detected in straight columnar epithelium of the mucosa. Hyperemia and congestion were observed between mucosa and gland in the Anar-5 (100 mg/kg) treatment group.

Histology after 12 weeks

In the Anar-5 200 mg/kg treatment group at 12 weeks, all layers of stomach fundus were normal and hyperemia and congestion were observed in isthmus, neck mucous and base of glands (Figure 3). Loose connective tissue and blood vessels were abundant in submucosa. Inflammatory cell infiltration was observed in the lower part of glands. The mucosa muscularis of the antrum showed hronic gastritis. While the chief and parietal cells were normal, eosinophils were observed at higher magnification using light microscopy.

In the Anar 5 100 mg/kg treatment group at 12 weeks, the structures of columnar epithelial and glandular cells were not changed but eosinophils were observed in lamina propria and between mucosa muscularis in the mucosa. Chief or zimogen cells, which secrete pepsinogen, were stained darkly

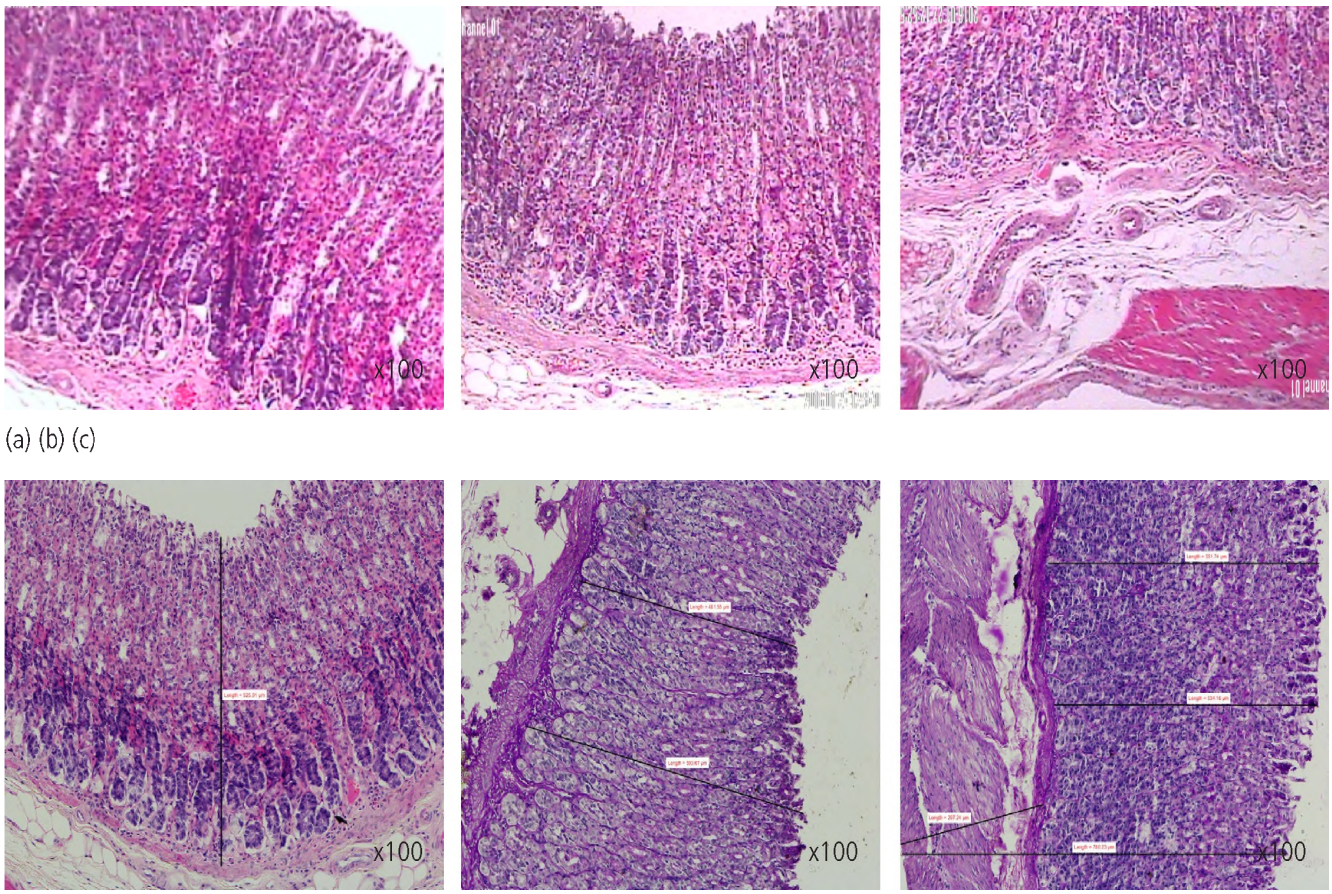

Figure 2. Histological examination of stomach 8 weeks after treatment. (a) untreated group stained with H&E, (b) Anar-5 100 mg/kg treatment group, (c) Anar-5 200 mg/kg treatment group, (d) untreated group stained with PAS, (e) Anar-5 100 mg/kg treatment group, (f) Anar-5 200mg/kg treatment group.

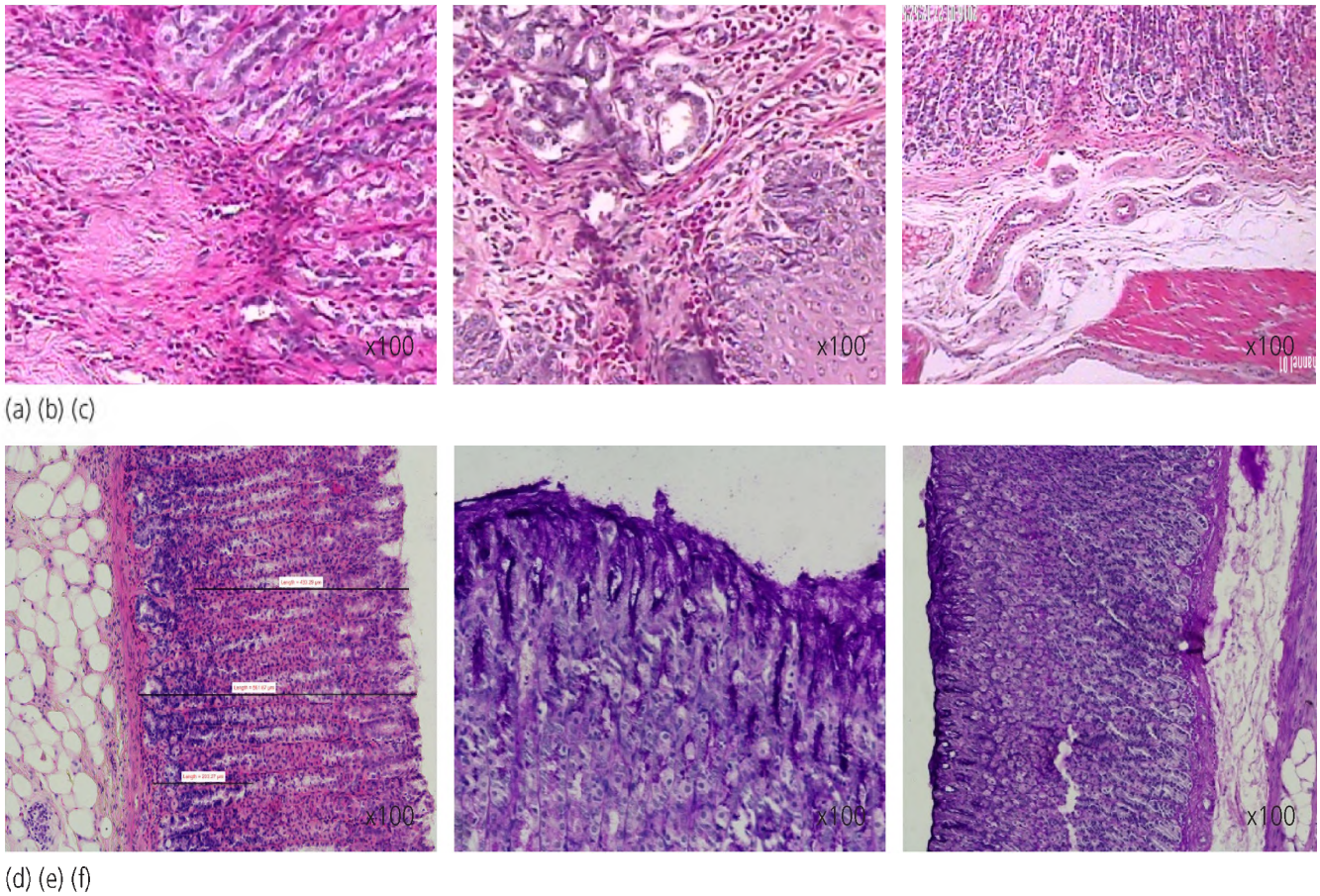

Figure 3. Histological examination of stomach 12 weeks after treatment. (a) untreated group stained with H&E, (b) Anar-5 100 mg/kg treatment group, (c) Anar-5 200 mg/kg treatment group, (d) untreated group stained with PAS, (e) Anar-5 100 mg/kg treatment group, (f) Anar-5 200mg/kg treatment group.

with basophilic dye. The parietal cells, which were larger than chief cells, had their cytoplasm stained well by eosinophilic dye and were observed in stomach wall at higher magnification. There was no desquamation of the mucous columnar cell. The glands of stomach antrum mucosa were slightly thinned and hemorrhagic changes were around the neck of the glands. The secretory epithelial cells of the mucosa were degenerated and PAS staining of cells was reduced in the superficial layer of mucosa at 8 weeks to 12 weeks of the experiment. These changes indicate that atrophy had developed in the superficial layer of stomach mucosa due to the influence of deoxycholate.

Mucous epithelial cells of stomach mucosa were regenerating in the base and tip of the mucous glands in increasing numbers in the Anar 5 group (100 mg/kg) in at 4 weeks, 8 weeks to 12 weeks of the experiment. It can be seen in specimens stained with PAS that mucous secreting cells were regenerated at 8 weeks (Figure 2d-f) and the mucous secreting cells were increasing in the 12 weeks of the experiment in the Anar 5 (100 mg/kg) group of the rats (Figure 3d-f).

Discussion

We selected the doses of 100, 200 mg/kg for this study [22]. The median lethal dose for Anar-5 in mice was determined as 2.2 g/kg during the acute toxicity studies carried out by Prozorovskii et al [21]. These doses of Anar-5 used in our study were 11-22 times lower than those. In the present study, a rat model of chronic gastritis was successfully established by the orally administration of deoxycholate. The results of our study showed Anar-5 had significant protective effect on chronic atrophic gastritis CAG in rats. Compared with the control group, the histological abnormalities using light microscopy were

significantly less in groups receiving Anar-5. Gastrin is a peptide hormone released by neuroendocrine G cells located mainly in the gastric antrum. Gastrin stimulates gastric acid secretion and gastric mucosal cell growth. Studies by Wang et al. in 2006 demonstrated that the levels of gastrin and the number of G cells were decreased in rat model of chronic atrophic gastritis. Moreover, low levels of serum gastrin have been detected in patients with multifocal atrophic gastritis by Rembiasz K et al .[25]. Studies by Redlak et al. in 2003 and Yanyan et al. 2016 indicate that deoxycholate has induces both necrosis and apoptosis of gastric mucosal cells [26, 27]. Many researchers have used deoxycholate to establish chronic gastritis animal models [28, 29]. Therefore we used sodium deoxycholate was used in the present study. In traditional Mongolian medicine, it has been suggested that Anar-5 can treat chronic atrophic gastritis by improving pathological changes. The average gastrin levels in large and small dose Anar-5 treatment groups were increased compared to the control group and were indistinguishable from normal controls. There was no significant difference in gastrin levels between high dose Anar-5 group and low dose Anar-5 group, indicating that at the doses studied the effect of Anar-5 has little relation with its dosage. The present study suggested that CAG in rats was related to the damage gastric mucosa barrier and the misbalance of cell proliferation and apoptosis. Since CAG has been considered a precancerous disease, it is essential to establish a stable, economic, and effective experimental animal model of CAG for further study of gastritis [18]. Deoxycholate sodium administration could simulate the damage by bile refluxes and the hyperammonia induced by helicobacter pylori infection. This study successfully established atrophic gastritis, with the histopathological finding diagnostic of antrum-dependent atrophic gastritis. It is thought that when bile refluxes into the stomach in humans, the bile salt may damage the lipoprotein in the mucosa [26]. Chronic atrophic gastritis in rats is related to the damage of the gastric mucosa barrier and further injury to glandular tissues in the deep layer by long term stimulation with inflammatory factors, while the gastric mucosa incompletely regenerates [26, 27]. Many natural substances such as 1'S-1'-acetoxychavicol acetate and related phenylpropanoids, ellagic acid, tannin have shown gastroprotective effects [8, 9, 28, 29]. Punica granatum L is the main ingredient in Anar-5. The pharmacological research of Anar-5 to date has been performed only on experimental animals and it is necessary to conduct future human clinical trials to see if these results apply to humans. In addition, it is important to study the Anar-5 effects on an acute gastritis model, anti-inflammatory induced gastritis in experimantal animals, to determine if Anar-5 therapeutic in treating these conditions.

Conclusion

Treatment with preparation from Anar-5 protected against the development chronic gastritis and gastric atrophy.

Conflict of Interest

The authors have declared no conflict of interest.

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