PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• BASIC RESEARCH •

Gastroprotective effect and mechanism of amtolmetin guacyl in mice

Yuan-Hai Li, Jun Li, Yan Huang, Xiong-Wen Lü, Yong Jin

Yuan-Hai Li, Jun Li, Yan Huang, Xiong-Wen Lü, Yong Jin, School of Pharmacy, Anhui Medical University, Hefei 230032, Anhui Province, China
Yuan-Hai Li, Department of Anesthesiology, the First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui Province, China
Correspondence to: Professor. Jun Li, School of Pharmacy, Anhui Medical University, Institute of Clinical Pharmacology, Anhui Medical University,Tunxi West Road, Hefei 230032, Anhui Province, China. amuicplj@mail.hf.ah.cn
Telephone: +86-551-5161001 **Fax:** +86-551-5161001
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Abstract

AIM: To investigate the gastroprotective effect and mechanism of amtolmetin guacyl (AMG, MED15) in mice.

METHODS: Male and female Kunming strain mice, weighing 18-22 g, were utilized in the experiment. Normal or ethanol-induced gastric mucosal damage models in mice were successfully established to investigate the gastroprotective effect and mechanism of AMG. In the experiment of gastric mucosal damage after repeated treatment with AMG, the mice were randomly divided into 5 groups: normal group, 3 AMG groups receiving (75, 150 and 300 mg/kg), and tolmetin group receiving 90 mg/kg. The mice were randomly divided into 6 groups as follows: normal group, model group, AMG groups with doses of 75, 150 and 300 mg/kg, respectively, and tolmetin group with a dose of 90 mg/kg in ethanol-induced gastric mucosal damage experiment. The severity of gastric mucosal lesions was scored from 0 to 5. Gastric tissue sections were stained with hematoxylin and eosin (HE) and examined under light microscopy. Also gastric tissue sections were stained with uranyl acetate and lead citrate, and examined under electron microscopy. In addition, nitric oxide (NO) and malondialdehyde (MDA) contents, and nitric oxide synthase (NOS) and superoxide dismutase (SOD) activities in the stomach tissue homogenates were measured by biochemical methods.

RESULTS: Repeated treatment with AMG (75, 150 and 300 mg/kg) for 7 d did not induce any appreciable mucosal damage, and the average score was not significantly different from that of normal mice. In contrast, tolmetin (90 mg/kg) produced significant gastric mucosal lesions compared with the normal group ($P<0.01$). AMG (75, 150 and 300 mg/kg) significantly reduced the severity of gastric lesions induced by ethanol in a dose-dependent manner as compared with the model group ($P<0.05$, AMG 75 and 150 mg/kg *vs* model; $P<0.01$, AMG 300 mg/kg *vs* model). Light and electron microscopy revealed that AMG (150 and 300 mg/kg) induced minimal changes in the surface epithelium layer, without vascular congestion or leucocyte adherence. AMG (75,150 and 300 mg/kg) demonstrated dose-dependent gastroprotective effects on mice in our study. AMG (75, 150 and 300 mg/kg) could significantly increase NO content and NOS level in the stomach homogenates of mice compared with the model group ($P<0.05$, AMG 75 mg/kg and 150 mg/kg groups *vs* model group; $P<0.01$, AMG 300 mg/kg *vs* model group) respectively. Moreover, AMG (150 and 300 mg/kg) not only significantly increased SOD activities but also obviously decreased the MDA content in the stomach homogenates of mice.

CONCLUSION: AMG exerts significant gastroprotective actions on mice and the involved mechanisms may be its antioxidative effect and induction of NO production.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the extensively utilized medicines worldwide with antipyretic, analgesic and anti-inflammatory properties. Besides the direct stimulation, NSAIDs have some other adverse reactions to the gastrointestinal system, such as nausea, vomit, bellyache and even ulcer, perforation. All these effects are considered to be associated with the inhibition of prostaglandin (PG) synthesis in gastrointestinal system, and thereby limiting the clinical application of NSAIDs[1-7].

Amtolmetin guacyl (AMG) is a novel NSAID, and its chemical name is 2-methoxyphenyl-1-methyl-5-p-methylbenzoyl-pyrrol-2-acetamido acetate, whose metabolites are MED5 (chemical name: 1-methyl-5-p-methylbenzoyl-pyrrol-2-acetamido acetic acid) and tolmetin (TOL)[8-12]. As a novel NSAID, it has been reported that AMG possesses antipyretic, analgesic and anti-inflammatory effects in previous studies[13-20]. At the same time, it can also enhance the NOS activity of gastric mucosae, which facilitates the synthesis and release of NO so as to reduce the gastrointestinal system damage[21-23].

In this study, normal and ethanol-induced gastric mucosal lesion models in mice were established to investigate the gastroprotective effect and associated mechanisms, which could guide the rational use of medicine in clinical practice.

MATERIALS AND METHODS

Materials

Male and female Kunming strain mice, weighing 18-22 g, were purchased from Animal Center of Anhui Medical University. They were housed in plastic boxes, 5 mice in each box. All mice were allowed to take food and tape water *ad libitum*. AMG and TOL were provided by Anhui Kelong Medicine Company. The assay kits of MDA, SOD, NOS and Coomassie brilliant blue reagents were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All experimental protocols described

in this study were approved by the Ethics Review Committee for Animal Experimentation of Anhui Medical University.

Gastric mucosal damage after repeated treatment with AMG[21]

The mice were equally divided into 5 groups randomly: normal group, 3 AMG groups (3 different doses) and TOL group. The mice in AMG groups intragastrically received 75, 150 or 300 mg/kg of AMG a day through an 18-gauge stainless steel animal feeding needle for 7 d prior to the experiment. Similarly, the mice in TOL group intragastrically received 90 mg/kg of TOL a day. In normal group, the mice were only fed with the same volume (0.5 mL/mouse) of carboxymethylcellulose (CMC). On the seventh day, all the mice were sacrificed by cervical dislocation and the stomachs were removed, opened along the great curvature and examined for the macroscopic evaluation of gastric mucosae. The severity of gastric mucosal lesions was examined by an experienced histologist who was unaware of the treatment conditions.

Establishment of ethanol-induced gastric mucosal damage model and drug treatment[21,24]

The mice were randomly divided into six groups as follows: normal group, model group, 3 AMG groups receiving 75, 150 and 300 mg/kg, respectively, and TOL group receiving 90 mg/kg, ten mice in each group. The 5 groups were intragastrically administered AMG, TOL and CMC (model group) once. One hour later, the animals received 500 mL/L ethanol (0.5 mL/mouse) intragastrically except normal group mice. One hour later, the animals were killed and the stomachs were removed. The severity of gastric mucosal lesions and histological assessments were made by an experienced histologist who was unaware of the treatment conditions.

Grading criteria of gastric mucosal damage[21]

The animals were killed, the stomachs were removed, rinsed with 5 mL of saline and immersed in 100 mL/L formalin. They were later opened along the greater curvature, for the macroscopic evaluation of gastric mucosae[21,24]. The severity of lesions was scored from 0 to 5: 0, normal; 0.5, light local reddening; 1, general reddening or small hemorrhage (<1 mm); 2, large hemorrhage (>1 mm); 3, small ulcer (<2 mm); 4, large ulcer (>2 mm); and 5, perforated ulcer. One score was assigned to each lesion. A researcher who was unaware of the treatment conditions gave the scores of gastric mucosal injury.

Microscopic assessment of gastric lesions[21]

Light microscopy At the end of the experiment, the stomach was immediately exposed and a small strip was excised from the glandular portion, 3 mm below and parallel to the limiting ridge, so that the greater curvature was approximately located in the middle of the strip. The tissue samples were fixed in 100 mL/L formalin, 5-μm thick serial sections were taken from each block and stained with hematoxylin-eosin. The image of the sections to be examined was displayed on a color monitor by means of a videocamera attached to the microscope. An experienced histologist who was unaware of the treatment conditions made histological assessments.

Transmission electron microscopy After mice were killed, the stomachs were immediately removed, and a strip was excised in the same position as for light microscopy. Small specimens were fixed in 25 g/L glutaraldehyde liquid for 3 h at room temperature and post-fixed in 10 g/L OsO_4 in 0.1 mol/L phosphate buffer (pH 7.2) for 90 min at room temperature. After dehydration in a graded series of acetone and embeded in Araldite resin, semithin sections were cut for orientation. Thin sections (80 nm thick) were cut perpendicularly to the luminal surface, stained with uranyl acetate and lead citrate, and examined with a Zeiss109 electron microscope. An experienced histologist who was unaware of the treatment conditions made histological assessments.

Tissue homogenization

Stomach tissue samples were weighed and homogenized in 9 g/L NaCl for the detection of SOD, MDA, NOS, NO_2^-. Homogenates were centrifuged at 4 000 r/min for 10 min. Aliquots of the supernatants were used for studies. The assayed parameters were expressed as per mg protein, and the protein content of aliquots was determined by the method of Coomassie brilliant blue.

Measurement of MDA, SOD and NOS in stomach homogenates[25]

SOD, MDA and NOS levels in stomach tissue homogenates were assayed by using assay kits (Nanjing Jiancheng Bioengineering Institute).

Examination of NO_2^-

NO_2^- concentration in stomach tissue homogenates was measured as described by Prado *et al*.[26].

Statistical analysis

The data were analyzed by SPSS 10.0 for windows. Results were expressed as mean±SD. Student's *t* test was used for statistical analysis. $P<0.05$ was considered statistically significant.

RESULTS

Repeated treatment with AMG on gastric mucosal damage in normal mice

Repeated treatment with AMG (75, 150 and 300 mg/kg) for 7 d did not induce any appreciable mucosal damage, and the average score was not significantly different from that of normal mice. In contrast, TOL, the prodrug of AMG, intragastrically administered at 90 mg/kg produced significant gastric mucosal lesions compared with the normal group ($P<0.01$) (Figure 1).

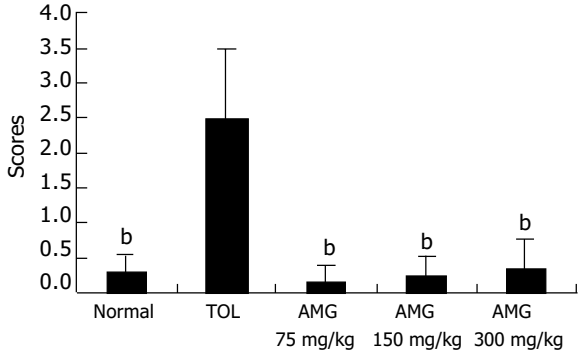

Figure 1 Repeated treatment with AMG on gastric mucosal damage in normal mice (n = 10). $^bP<0.01$ *vs* TOL.

Effect of AMG on ethanol-induced gastric mucosal damage model

As showed in Figure 2, compared with that in the normal group, the average score of mice in the model group significantly increased ($P<0.01$). AMG (75, 150 and 300 mg/kg) significantly reduced the severity of gastric lesions induced by ethanol in a dose-dependent manner as compared with the model group ($P<0.05$, AMG 75 and 150 mg/kg *vs* model; $P<0.01$, AMG 300 mg/kg *vs* model). There was no obvious difference between TOL group and model group (Figure 2).

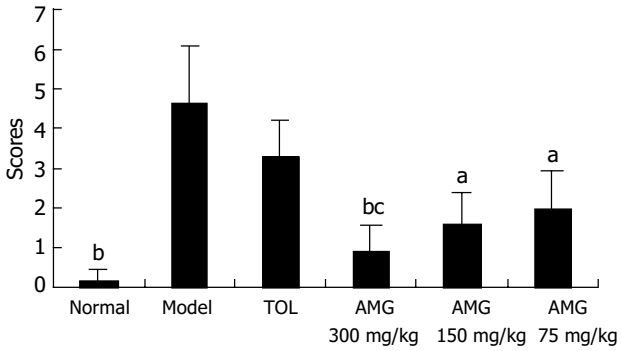

Figure 2 Effect of AMG on ethanol-induced gastric mucosal damage model (n = 10). [a]P<0.05, [b]P<0.01 *vs* model; [c]P<0.05, AMG (300 mg / kg) *vs* TOL.

Microscopic assessment of AMG on ethanol-induced gastric lesion

Light microscopy As shown in Figure 3 for evaluation by light microscopy, the structure of gastric mucosae was normal in normal group. The cells were well stained by HE and there was no edema or exfoliation. In contrast, a visible hemorrhagic area was found in model group. In mice treated with AMG (300 mg/kg), the epithelial and parietal cells were slightly edematous with few epithelial cells exfoliated. In the group treated with AMG (150 mg/kg), severe edema was found with inflammatory cell infiltration. In the group administered AMG (75 mg/kg), edema was more severe in epithelial and parietal cells than that in AMG (300 mg/kg) group and many epithelial cells were deciduous. Conversely, in the TOL group, together with inflammatory cell infiltration, a hemorrhagic area was observed (Figure 3A).

Transmission electron microscopy As shown in Figure 4, gastric mucosal microvilli in normal group were well arranged with no defection and shorting, while those in the model and TOL groups were mostly deciduous, broken and defective. The microvilli were almost integrated and well arranged with no defection in mice treated with AMG (300 mg/kg). In mice administered AMG (150 mg/kg), the lesions were much smaller, while the microvilli were short and small and in a bad order with obvious defection in mice treated with AMG (75 mg/kg) (Figure 3B).

Effects of AMG on NOS level and NO content in ethanol-induced stomach homogenates

Compared with those in the normal group, the NO content and NOS level were significantly decreased in the model group (P<0.01). AMG (75, 150 and 300 mg/kg) could significantly increase the decreased NO content and NOS level compared with the model group (P<0.05, AMG 75 mg/kg and 150 mg/kg groups *vs* model group; P<0.01, AMG 300 mg/kg *vs* model group) respectively. The NO content and NOS level in TOL group where not evidently different from those in the model group (Table 1).

Table 1 Influence of AMG on NO and NOS contents in stomach homogenates in ethanol-induced gastric mucosal damage models (n = 10, mean±SD)

Group	n	Dose (mg / kg)	NO_2^- (μmol / mgprot)	NOS (U / mgprot)
Normal	10	-	60.57±22.11[b]	4.22±0.92[b]
Model	10	-	33.08±12.85	2.41±0.56
TOL	10	90	46.46±12.69	3.27±0.68
AMG	10	300	70.37±27.97[b]	5.25±1.62[b]
	10	150	58.65±17.11[a]	4.12±1.02[a]
	10	75	30.98±11.97	3.47±1.17[a]

[a]P<0.05, [b]P<0.01, *vs* Model.

Effects of AMG on SOD activity and MDA content in ethanol-induced stomach homogenates

Results are shown in Table 2. Compared with that in the normal group, the SOD activity in the model group was significantly lower, but the MDA content in the model group was increased (P<0.01). However, compared with model group, AMG (150 and 300 mg/kg) could significantly increase the SOD activity while evidently reduce the MDA level in stomach homogenates (P<0.01, Table 2).

Table 2 Influence of AMG on SOD, MDA contents in stomach homogenates in ethanol-induced gastric mucosal damage models (n =10, mean±SD)

Group	n	Dose (mg / kg)	SOD (U / mgprot)	MDA (nmol / mgprot)
Normal	10	-	702.61±144.77[b]	2.85±1.32[b]
Model	10	-	551.94±125.94	4.18±1.19
TOL	10	90	526.14±110.80	5.07±2.09
AMG	10	300	624.97±117.19[a]	3.13±1.35[a]
	10	150	612.06±101.90[a]	3.46±1.21[a]
	10	75	606.13±65.81	3.57±1.05

[a]P<0.05, [b]P<0.01, *vs* Model.

DISCUSSION

Gastrointestinal system lesions are resulted from the major adverse reaction of NSAIDs, which limits the wide clinical application of NSAIDs. Though the mechanism of the lesion remains unclear, some documents have proposed that these effects are related with the inhibition of PGs releasing from the mucosal epithelial cells[1,3,27]. It is known that vascular damage is considered to be the earliest process of gastric mucosal ulcer. Vasodilators, such as PGs and NO, play an important role in gastroprotection. At present ,NO, gastroprotective NSAIDs, specific cyclooxygenase-2 (COX-2) inhibitors as well as COX and 5-lipoxygenase (5-LOX) double inhibitors are used in reducing the adverse reactions of NSAIDs[28,29].

It has been demonstrated that AMG has gastroprotective properties[21-23]. Tubaro *et al.*[30] found that repeated treatment with AMG did not induce gastric mucosa damage. Another animal experiment indicated that AMG (50-300 mg/kg ig) did not induce stomach lesions in rats, while its metabolite TOL (15-60 mg/kg ig) did in a dose-dependent manner[21]. Light and electron microscopic assessment suggested that AMG only caused very slight epithelial cell changes without vascular congestion and WBC adherence.

In our study, the 7-d treatment with AMG (75, 150 and 300 mg/kg ig) did not induce any appreciable mucosal damage, and the score was not different from that of normal group, while TOL (90 mg/kg ig) produced severe gastric mucosal lesions compared with normal group. Compared to its metabolite TOL, AMG had obvious gastroprotective effects. In ethanol-induced gastric mucosal damage model, the scores of AMG (75, 150 and 300 mg/kg ig) obviously decreased in a dose-dependent fashion. Compared to that in TOL group, the scores in AMG (300 mg/kg) group were greatly decreased. Furthermore, the scores had no obvious difference between TOL and model groups. Microscopic assessment of ethanol-induced gastric lesions showed that the degree of gastric damages in AMG (300 mg/kg) group was smaller than that in TOL and model groups, indicating the effect of AMG on gastric mucosa. Ultrastructural studies suggested that the microvilli were almost integrated and well arranged with no defection in mice treated with AMG (300 mg/kg), but those in the model and TOL groups were mostly deciduous, broken and defective.

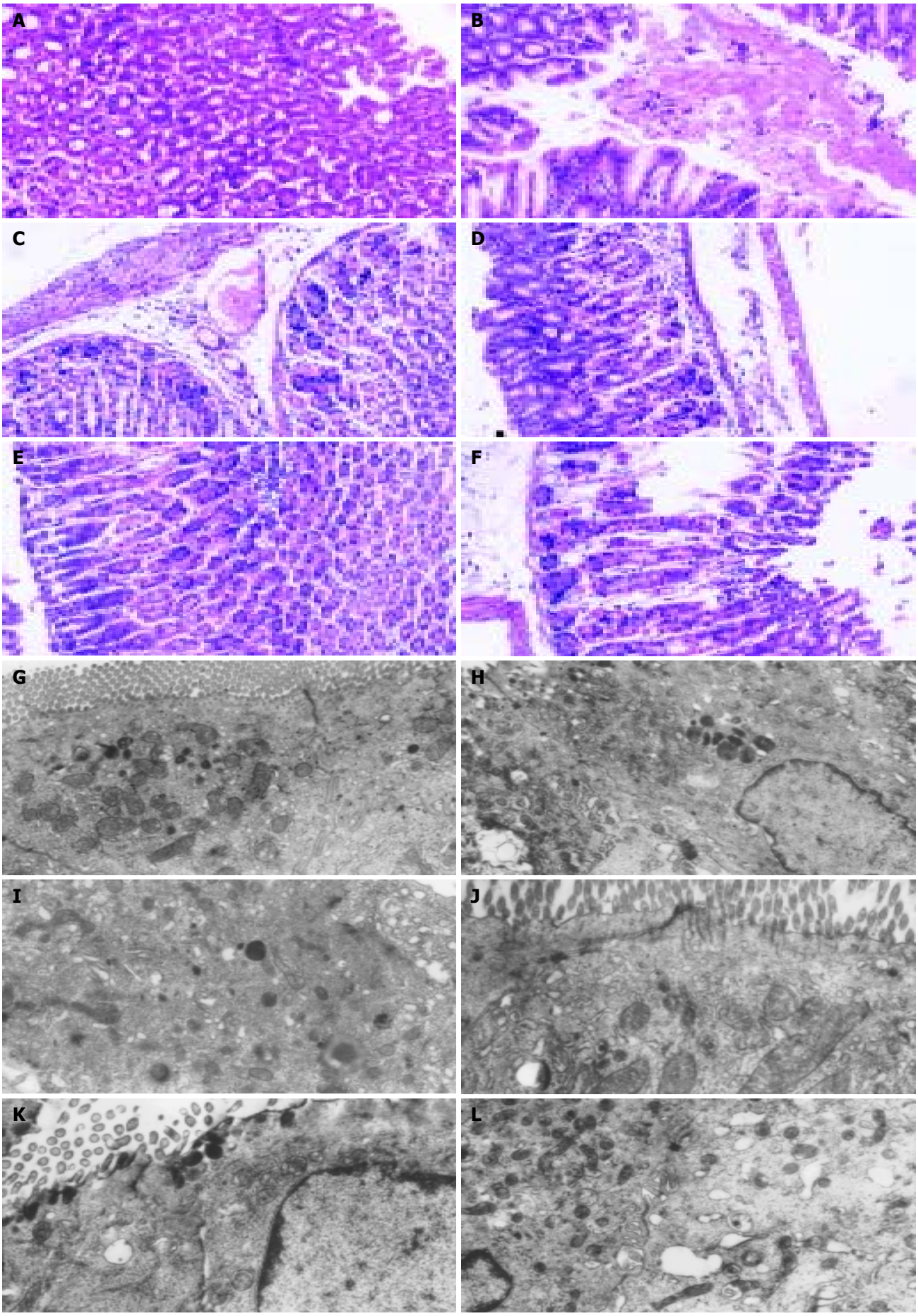

Figure 3 Light and electronic microscopy assessments of ethanol-induced gastric lesion. A: light microsocopy assessment of ethanol-induced gastric lesion (*100). A: normal group, B: model group, C: ToL group, D: AMG (300 mg / kg) group E: AMG (150 mg / kg) group, F: AMG (75 mg / kg) group. B: Electronic microscopy assessment of ethanol-induced gastric lesion (×6 000) G: normal group, H: model group, I: TOL group, J: AMG (300 mg / kg) group, K: AMG (150 mg / kg) group, L: AMG (75 mg / kg) group.

Tubaro *et al.*[30] found that AMG could strongly inhibit the hydrochloric acid (HCl) excretion caused by histamine so as to exert gastroprotective effects. Compared with acetylsalicylic acid, AMG (50 mg/kg ig) caused no appreciable change in basal potential difference (PD) values in gastric mucosae. AMG (100 mg/kg ig) alleviated ethanol-induced damages, increased NOS activity and stimulated NO release, and thereby showing its gastric protective effect. A research showed that the nonspecific NOS inhibitor (L-NAME 10 mg/kg sc) could reverse the effect of AMG[21]. Therefore, we concluded that the protective effect of AMG might be involved in NO release from gastric epithelial cells. In our study, AMG (75, 150 and 300 mg/kg) could significantly increased the NO content and NOS level in ethanol-induced gastric mucosal damage model group. These results suggested that the gastroprotective effect of AMG might be associated with promotion of NOS activity and induction of NO release, which is in agreement with previous reports[21,31].

Moreover, it has been reported that ethanol is capable of generating oxygen radicals, inhibiting glutathione synthesis, producing glutathione loss from tissues, increasing MDA levels and impairing antioxidative defense systems in experimental animals. Our study also showed that AMG (150 and 300 mg/kg) could sharply decrease the increased MDA level while enhance the decreased SOD activity in the models of mice, suggesting that the gastroprotective effect of AMG could at least partly contribute to the antioxidative action, which has not been reported before.

In summary, AMG has significant gastroprotective effects in mice, and its mechanism may be associated with its antioxidative effect and promotion of NO release.

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