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KETOPROFEN LYSINE SALT HAS NO GASTROPROTECTIVE EFFECT IN COMPARISON WITH KETOPROFEN IN FEMALE RATS AFTER ETHYL ALCOHOL INTOXICATION

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ABSTRACT

Ketoprofen lysine salt - a new non steroidal antiinflammatory drug (NSAID) is an improved formulation of ketoprofen. The former is believed to have gastroprotective properties, the latter to kill acute pain and increase the risk of gastric mucosa damage. In East Europe binge drinking and taking NSAIDs on the day after is common. The aim of the study was to verify the hypothesis about the gastroprotective effect of ketoprofen lysine salt after exposure to 50% alcohol. The experiment was carried out on 36 female Wistar rats divided into 6 groups of 6:
1. 50% ethanol
2. 0.9% NaCl
3. 0.9% NaCl and ketoprofen
4. 50% ethanol and ketoprofen
5. 0.9% NaCl and ketoprofen lysine salt
6. 50% ethanol and ketoprofen lysine salt
On day 7 animals were sacrificed. Their stomachs were dissected for histopathological examination.
Microscopic examination of stomachs from groups 1, 3,4,5,6 revealed non-specific, high-grade lymphocytic-plasmocytic inflammation of the gastric mucosa.
Conclusions - Ketoprofen lysine salt and ketoprofen damage gastric mucosa in female rats after and without alcohol intoxication. Ketoprofen lysine salt has no gastroprotective effect
Keywords: gastritis; ketoprofen; ketoprofen lysine salt; ethanol.

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INTRODUCTION

Ketoprofen has an inhibitory effect on cyclooxygenase-1 (COX-1) and COX-2, which determines its well known anti-inflammatory effect and increases the risk of gastric mucosa damage [1].

Ketoprofen lysine salt is a new non steroidal antiinflammatory drug (NSAID) competing with ketoprofen on the market. It is a salt unlike vast majority of NSAIDs which are weak acids. Thanks to L-lysine salification it is characterized with an increased solubility and faster gastric absorption if compared with ketoprofen [2]. Cimini et al. conducted an experiment on human gastric epithelial cell line damaged with ethanol exposed to ketoprofen lysine salt. Their results suggested gastroprotective effect of this product [3]. In a former study conducted at the Medical University in Lublin, Poland it was shown on male rat model that ketoprofen lysine salt showed no gastroprotective properties [4]. In the cited study authors chose male animals as in humans alcohol abuse is more often recorded in men.

Data on alcohol consumption in Poland indicate that overall consumption was (in litres of pure alcohol *per capita)* 9.70 l/person in 2021. There are calculations indicating that strong alcohols were 39.2% of consumption in 2021, while beer was 52.5% [5]. Binge and risky drinking is more common in men than women. However toxic effects of ethanol in women develop easier than in men because they have lower activity of alcohol dehydrogenase and lower volume distribution than males as females have more fatty tissue and less muscles than men. The public health experts recommend not drinking alcohol and if one drinks should do so in moderation. It means no more than one alcohol equivalent per day for a non-pregnant woman and no more than two for a man [6]. Alcohol is an addictive neurotoxin. It produces depression of the central nervous system. It is consumed worldwide, especially in Europe. Recommendations on alcohol consumption are controversial. Public health experts support moderate consumption as it has a beneficial cardiovascular effect and lowers the risk of all-cause mortality. At the same time there is evidence that alcohol use is associated with an increased risk of cancer, neurological diseases, or injuries [7,8,9].

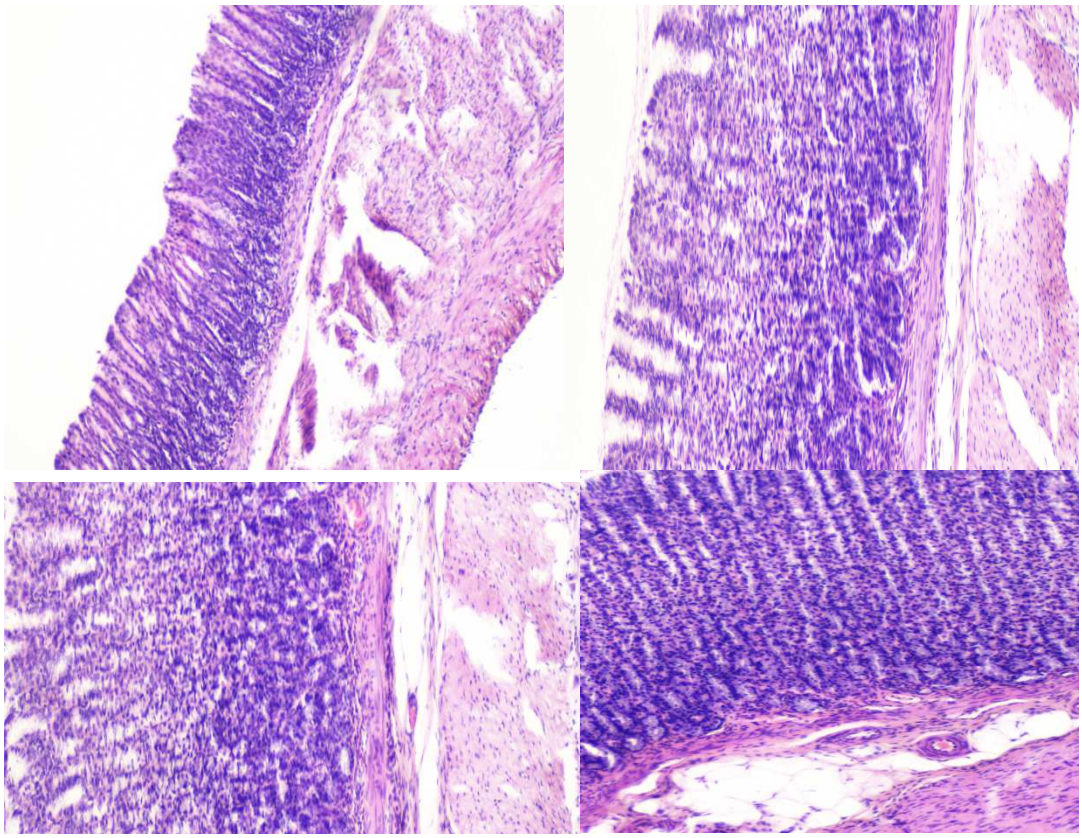

Fig.1 Histopathological examination results from group 1 (after ethanol intoxication).

AIM

The aim of the study was to verify the hypothesis about the gastroprotective effect of ketoprofen lysine salt after exposure to 50% alcohol.

MATERIALS AND METHODS

The investigated products were ketoprofen and ketoprofen lysine salt. Ketoprofen was used in the form of Ketonal solution for injection in ampoules 50ml/mL (Sandoz GmbH, Wien, Austria). Ketoprofen lysine salt was utilized as KETONAL SPRINT available in the form of granules (Sandoz GmbH, Wien, Austria). In order to prepare ketoprofen lysine salt solution 0.9% NaCl was used (B. Braun, Melsungen AG, Hessen, Germany). Ethyl alcohol was purchased from Polmos (Lublin, Poland) as 95% Spirytus. The 50% ethanol solution was prepared with saline. The doses of the investigated products were chosen following previous experiments carried out in our Department [4]. The dose of alcohol was intended to produce the animal model of binge drinking in an adult human. This style of drinking is common in East Europe. On the day after majority of users suffer from hangover and headaches. The doses of ketoprofen and ktoprofen lysine salt used in our experiment are respective to the recommended painkilling doses of these medicines.

The experiment was carried out on 36 randomly selected female Wistar rats, whose weight before the beginning of the experiment was 190-205g. The animals were bred at the Experimental Medicine Centre (EMC) at the Medical University of Lublin, Poland.

The original source of the herd was Charles River Laboratories (Cologne, Germany). They were 7 weeks old. The experiment was conducted in accordance with European law regulations at EMC. Standard laboratory conditions prevailed in the EMC with a temperature of 21–22 °C, a 12-hour light/dark cycle and a relative air humidity of 55–60%. The animals had free access to sterile water (sterilized with ultraviolet rays) and rodent feed purchased from Altromin International (Lage, Germany). The investigated products wre administered by gavage through a gastric tube. The animals were divided into 6 groups of 6 each:

- 1. 50% ethanol (5mL/kg b.w. on day 1),
- 2. 09% NaCl (5mL/kg b.w. on day 1),
- 3. 09% NaCl (5mL/kg b.w. on day 1) and ketoprofen (8mg/kg b.w. on day 2-6),
- 4. 50% ethanol (5mL/kg b.w. on day 1) and ketoprofen (8mg/kg b.w. on day 2-6),
- 5. 9% NaCl (5mL/kg b.w. on day 1) and ketoprofen lysine salt (12.8mg/kg b.w. on day 2-6),
- 6. 50% ethanol (5mL/kg b.w. on day 1) and lysine salt (12.8mg/kg b.w. on day 2-6).

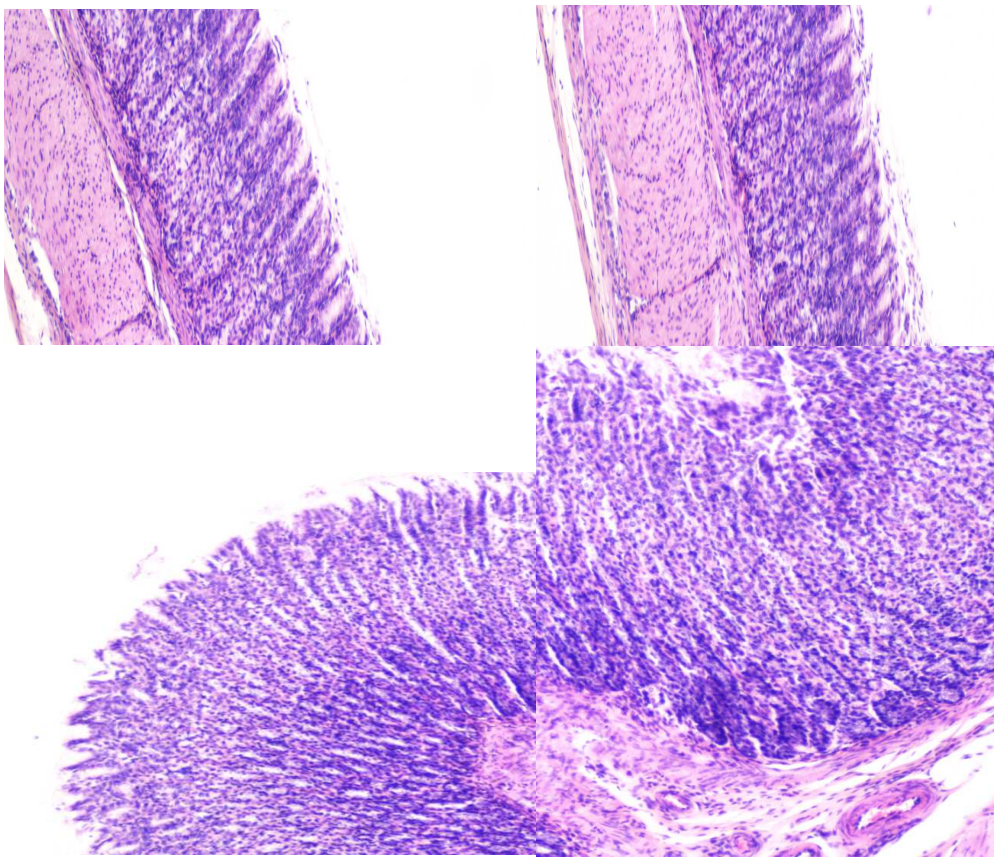

Fig. 2 Histopathological examination results from group 2 (administered 0.9% NaCl).

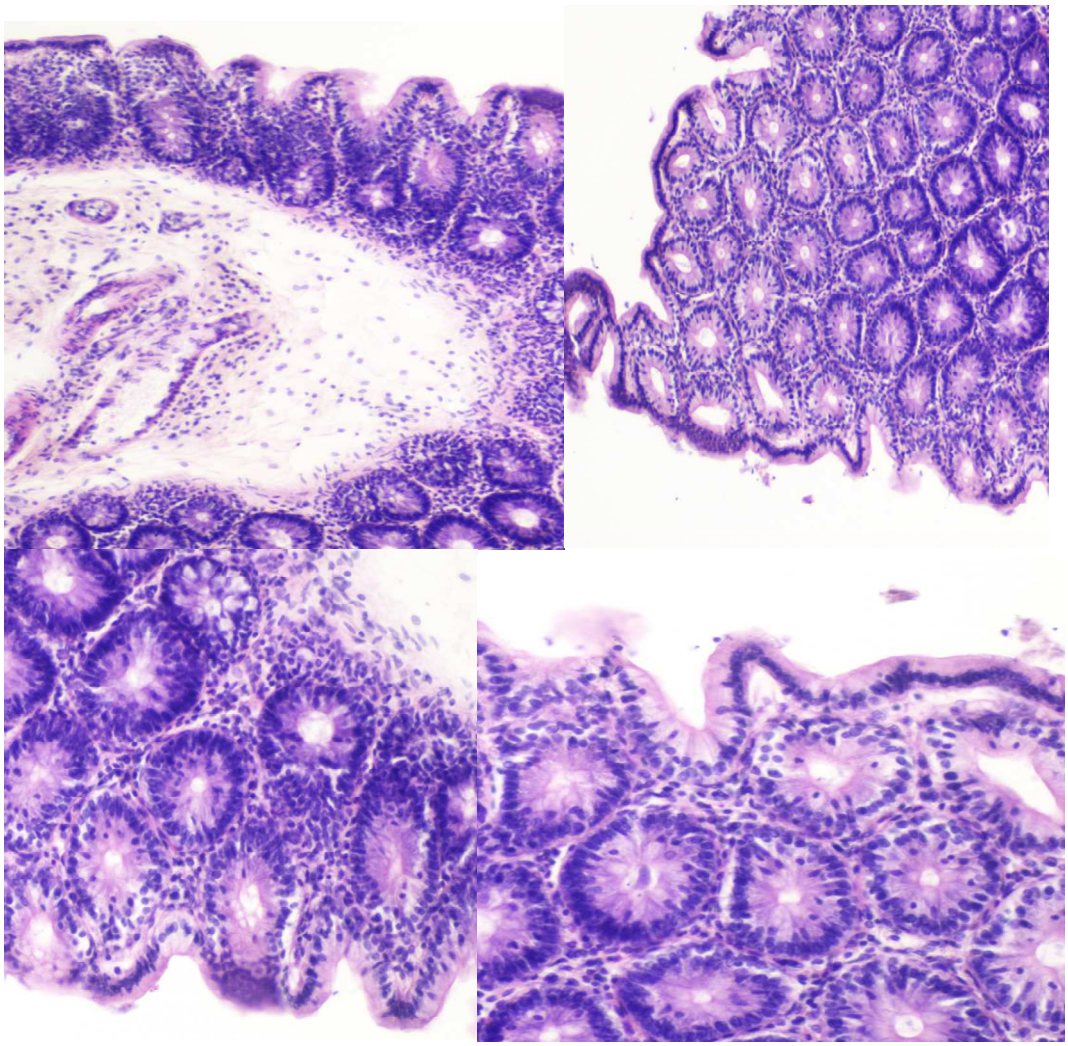

Fig. 3 Histopathological examination results from group 3 (treated with ketoprofen).

On day 7 all animals were decapitated with a guillotine. We used no anaesthetic in order to prevent possible interaction. Stomachs were dissected. The tissue material was fixed for 24 hours in 10% formalin with pH 7.2, and then, within 24 hours, stomachs were transferred through increasing concentrations of alcohol solutions, acetone and xylene, to paraffin blocks in a tissue processor. The 4µm thick tissue sections were made on a microtome and placed on glass slides. After staining with haematoxylin and eosin (HE) histopathological evaluation of the preparations was done under a light microscope (Nikon ECLIPSE Ci-L, Tokyo, Japan) at 10x and 20x objective magnification. For the assessment of gastric mucosa damage Li's scale was used [10]. The damage stores were classified as follows:

- 0 - normal mucosa
- 1 - oedema and or vacuolisation but minimal changes in crypt architecture,
- 2- epithelial disruption,
- 3 - erosion extending to the muscularis mucosa.

Due to limited finances no immunochemical examination was performed.

The data were analysed using IBM SPSS Statistics v.25 software. Comparisons between the analysed groups were made using the Kruskal-Wallis test.

If statistically significant differences were detected, a post hoc analysis was performed using Dunn's test (to indicate statistically significantly different groups: study group *vs* control group). The significance level was $p < 0.05$.

The project was approved by the Local Ethical Committee for Animal Experiments in Lublin (70/2021 issued on 8 NOV 2021).

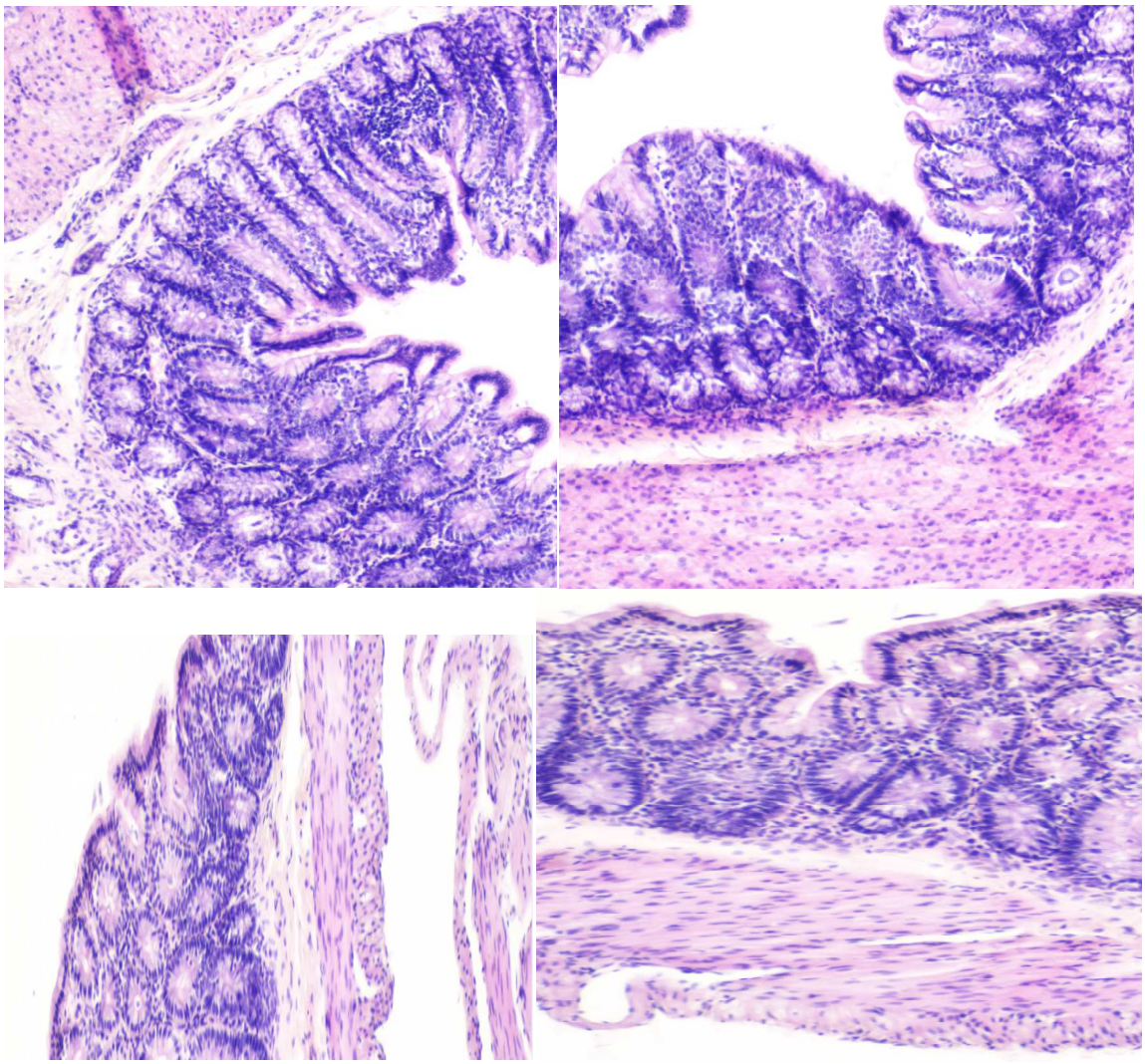

Fig. 4 Histopathological examination results from group 4 (treated with ketoprofen after intoxication with ethanol).

RESULTS

Macroscopic assessment revealed redness of gastric mucosa in all animals from group 1,3,4,5,6. Microscopic examination of stomachs from group 2 revealed normal structure of the gastric mucosa without obvious pathological changes and without signs of inflammation. Damage score equal 0 (Fig.1,2,3,4,5,6).

Microscopic examination of stomachs from groups 1,3,4,5,6 revealed significant thinning of the gastric mucosa. Within the gastric mucosa, between the clusters of glandular tissue of the glands of the stomach wall, numerous inflammatory cell infiltrates are visible, consisting mainly of lymphocytes and plasma cells. The described inflammatory infiltrates cover the gastric mucosa, with no signs of infiltration of the submucosa or muscle membrane. It was a non-specific, high-grade lymphocytic-plasmocytic inflammation of the gastric mucosa. There was no difference in the severity of pathological changes between groups 1,3,4,5, and 6. Damage score was equal 1 (grade 2) in groups 1,3,4,5 and 6 ($p<0.05$ *vs* group 2) [10].

DISCUSSION

Our study conducted on female rats did not confirm gastroprotective properties of ketoprofen lysine salt as expected by authors conducting experiment on cell lines [3]. In 2015 Cimini et al. compared gastro-protective activity of ketoprofen lysine salt and ketoprofen using a model of gastric mucosa injury on a cell line. In their experiment gastric mucosa cells treated with ethanol and ketoprofen were severely damaged and tissue integrity damage was evident, while the cells exposed to ethanol an ketoprofen lysine salt remained preserved [3]. Lysine was supposed to reduce the negative effect of the formula on gastric mucosa. There are several drugs with added lysine. For example lysine clonixinate is an NSAID. It was compared with paracetamol/codeine (500 mg + 30 mg)in postoperative pain and showed to produce more painkilling effect and less side effects than the comparator[11]. Ketoprofen lysine salt falls into the group of medicinal drugs combined with lysine [12].

In 2021 Kuczyńska et al. tried to confirm the gastroprotective efefct of ketoprofen lysine salt in an animal model. She chose male rats and administered

ketoprofen or ketoprofen lysine salt after ethanol intoxication by gavage. We reproduced her experiment but on female rats. In her experiment histopathologic stomach examination revealed no pathology in the group exposed to saline only, in the group exposed to ethanol only and in the males exposed to ketoprofen and saline. She fund infiltration with lymphocytes, plasmocytes and eosinophils in gastric mucosa of male rats exposed to ethanol and ketoprofen as well as both groups exposed to ketoprofen lysine salt (with saline and with ethanol)[4]. In our experiment the effect was exacerbated showing grade 2 gastric mucosa damage in all female rats exposed to ketoprofen as well as ketoprofen lysine salt suggesting that females are more vulnerable to NSAIDs induced gastric mucosa damage than males.

The damaging effect of ethanol on gastric mucosa is attributed to the increase of reactive oxygen species, which plays a key role in the increase of lipid peroxidation products, including malonyldialdehyde and 4-hydroxy-2-nonenal [13].

CONCLUSIONS

1. Ketoprofen lysine salt and ketoprofen damage gastric mucosa in female rats after and without alcohol intoxication.
2. Ketoprofen lysine salt has no gastroprotective effect

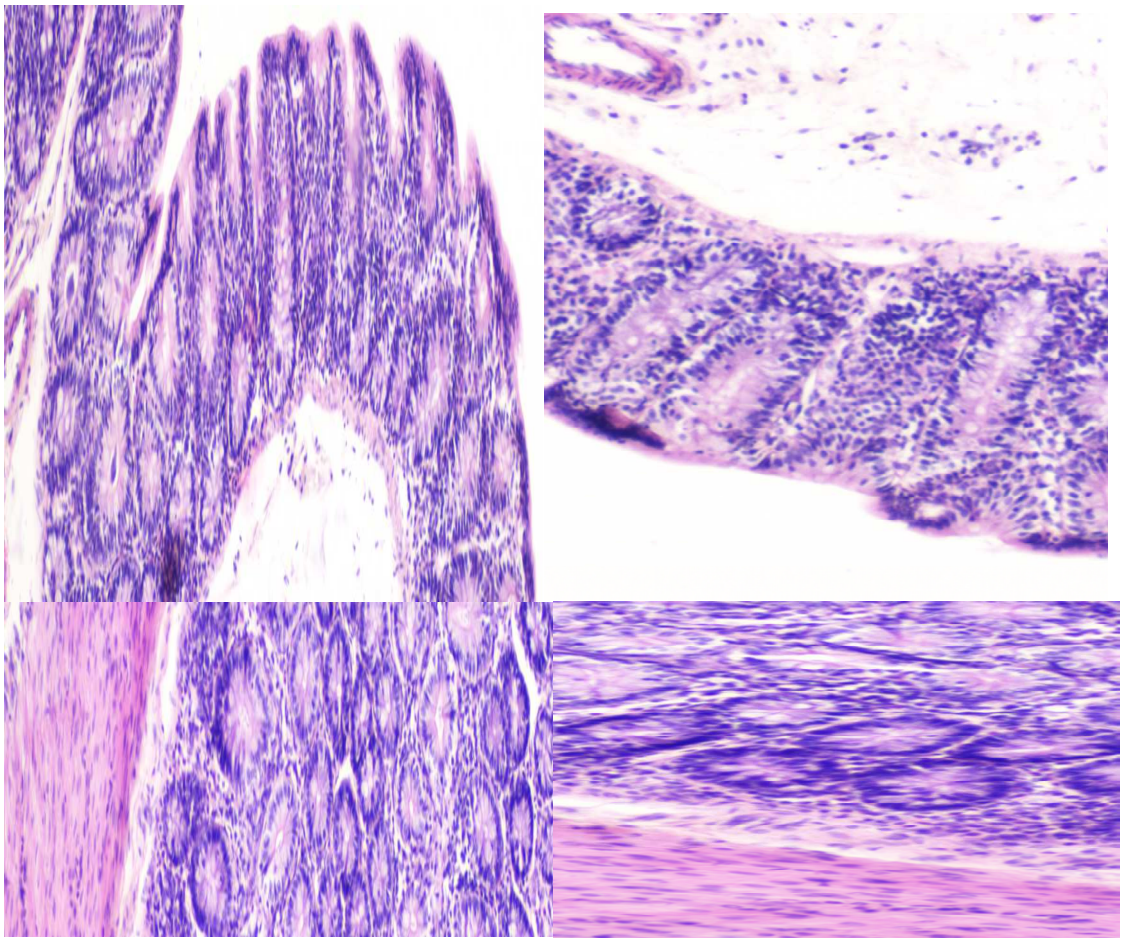

Fig. 5 Histopathological examination results from group 5 (treated with ketoprofen lysine salt).

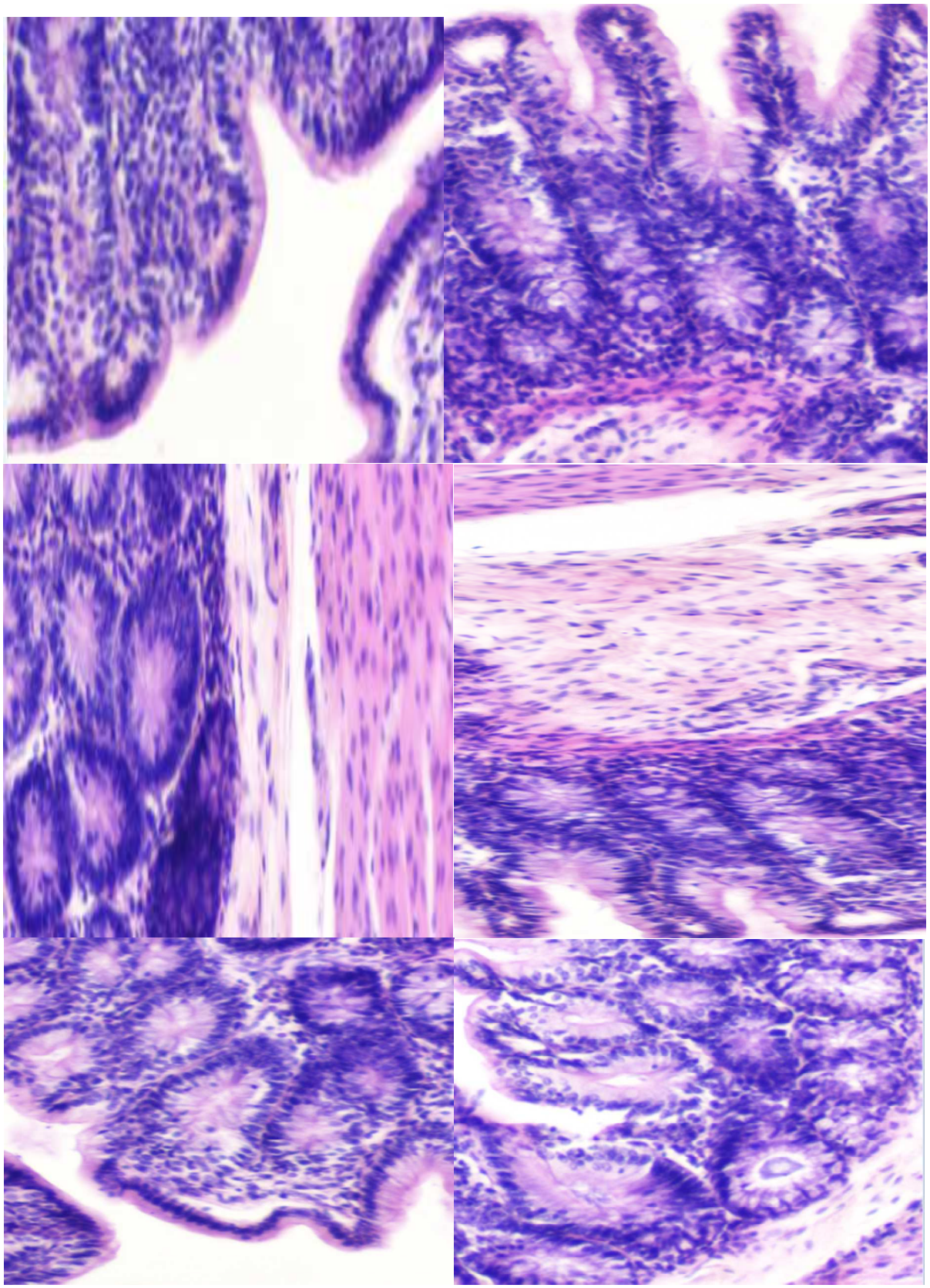

Fig. 6 Histopathological examination results from group 6 (treated with ketoprofen lysine salt after intoxication with ethanol).

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