Allantoin improves histopathological evaluations in a rat model of gastritis

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Purpose: Gastritis is found to be one of the most common gastrointestinal diseases worldwide. However, current therapeutic agents cause side effects, interaction, and recurrence. Allantoin has anti-inflammatory and wound healing properties. In this study, the therapeutic effect of allantoin has been assessed on the histopathological indices and gastric mucosal barrier of male rats. *Methods:* Male rats were equally divided into control, ethanol-induced gastritis, and allantoin groups. The therapeutic groups consisted of gastritis plus 12.5 mg/kg allantoin, gastritis plus 25 mg/kg allantoin, and gastritis plus 50 mg/kg allantoin groups. After 5 days of allantoin administration, the rats were sacrificed and a part of their gastric tissue was maintained at −70 °C for prostaglandin E2 (PGE2) and non-protein sulfhydryl (NP-SH) measurements. Another part was stained with hematoxylin and eosin and Masson's trichrome. *Results:* We found that Allantoin increased parietal and mucosal cell counts and mucosal thickness after gastritis induction. In addition, the number of leukocytes and vessels decreased in both of the mucosal and the submucosal layers. Allatoin improved gastric ulcer in all treatment groups. Gastric levels of PGE2 and NP-SH increased after allantoin treatment. *Conclusion:* This study indicated that allantoin had a considerable effect on gastritis treatment, which seems to result from the reinforcement of gastric mucosal barrier.

Keywords: allantoin, gastritis, ethanol, prostaglandin E2, stomach

Introduction

Gastric ulcer disease is identified by inflammation, ulcer, and cell loss in the epithelial layer of the stomach, which can develop to the gastric perforation or even gastric neoplasia (2, 10). In addition, gastric ulcer is one of the most prevalent gastrointestinal disorders throughout the world (2). It has been demonstrated that an imbalance between the protective and risk factors leads to gastritis (20). The risk factors for gastritis include smoking, stress, infection, frequent use of non-steroidal anti-inflammatory drugs, and excessive consumption of alcohol (10, 15, 16). Nowadays, the classic treatment for this disease includes proton pump inhibitors, H2 receptor blockers, and anti-acids. However, some of these drugs have shown a lot of side effects, interaction, and recurrence (15, 19). Therefore, many researchers attempt to find new drugs or approaches for gastritis treatment. Herbal medications are one of the most attractive compounds in this context.

Allantoin is one of the principal compounds in many plants, such as *yam, Nelumbo nucifera* rhizome, sugar beet, and leguminous and is a natural, safe, and non-toxic

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compound (4, 6). The wound healing and tissue regeneration effects of allantoin have already been well known (3, 14). It has also been reported that allantoin decreases IL-4, IL-5, Ig-E levels, and the number of leukocyte cells in the ovalbumin-induced lung inflammation (11). Florentino et al. (8) revealed that allantoin has anti-nociceptive and anti-inflammatory effects on the formalin-induced nociception test. Moreover, it has been shown that allantoin activates imidazoline I1-receptor (I1R) in animal and cell line studies (5, 22). Recent studies have shown that allantoin affects metabolic functions. For example, Chung et al. (5) have stated that allantoin alleviates obesity and hyperlipidemia via I1R activation in mice. In another experiment, allantoin improved cognitive function and neurogenesis in the mice hippocampus (1). Interestingly, da Silva et al. (7) have recently reported the gastroprotective effect of allantoin on gastric ulcer in female mice. Based on these observations, we designed experiments to study the treatment effect of allantoin on ethanol-induced gastritis in male rats.

Material and Methods

Reagents
Allantoin was purchased from Sigma Aldrich Chemical Co. (Lot. MKBB4594V; Sigma Aldrich, Chemie GmbH, Steinheim,Germany) and was dissolved in saline.

Experimental procedures
In this study, 36 male Wistar rats (obtained from Academic Center for Education, Culture & Research, Qom, Iran) weighing 200–250 g were used. The rats were housed in a temperature-controlled room with 12-h light–dark cycles and free access to standard laboratory chow and water. All the procedures followed were in accordance with the Guidelines for Animal Care and Use at the Qom University of Medical Sciences (IR.MUQ.REC.1394.102).

The rats were randomly divided into six equal groups ($N = 6$): the control group, which received 1 ml per oral (p.o) saline by gavage; gastritis group, in which the fasted rats received 100% ethanol (1 ml p.o by gavage, single dose) to induce gastritis (2); and allantoin group, which received 50 mg/kg allantoin (1 ml p.o by gavage) to find out possible toxic effects. In the next groups, rats received allantoin after gastritis induction in different doses. Gastritis plus 12.5 allantoin group (G-A-12.5) received 12.5 mg/kg allantoin [intragavage (i.g.)]. Gastritis plus 25 allantoin group (G-A-25) received 25 mg/kg allantoin (i.g.) (11) and gastritis plus 50 allantoin group (G-A-50) received 50 mg/kg allantoin (i.g.) (11). Allantoin administration was performed for 5 days consecutively (9). On the 6th day, the rats were anesthetized by an intraperitoneal (i.p.) injection of 50 mg/kg of sodium thiopental and sacrificed (17). The abdomen was excised through midline incision and the stomach was removed. A part of the stomach was dissected and fixed in 10% buffered formaldehyde solution. Paraffin blocks were then prepared for histopathological findings. Another part of the stomach was dissected and kept at −70 °C for evaluation of PGE2 and non-protein sulfhydryl (NP-SH) levels.

Histological study
After a minimum of 24 h from fixation, the tissues were dehydrated by putting them in ascending concentrations of ethanol solutions (30%, 50%, 70%, 90%, and 100%), then washed with xylene and embedded in paraffin wax. Afterward, the samples were sectioned with a microtome and stained with hematoxylin and Eosin (H&E) and Masson's trichrome stain, and mounted with Canada balsam. All sections were studied by Olympus light microscope (CX23 LED Microscope, Japan). Photomicrographs of the stomach tissue were

taken in 4×, 10×, and 40× magnification for observation and demonstration of histopatho-logic lesions. Histological features assayed included mucosal thickness and ulcer area and the number of leukocytes, parietal cells, mucosal cells and vessels. For this purpose, five sections of each tissue sample were observed and in each section, four fields were evaluated by ImageJ software (National Institutes of Health, ImageJ 1.49f, USA).

Tissue PGE2 measurement
Weighed samples of tissues (approximately 0.5 g) were homogenized in 2-ml microfuge tubes by an electrical homogenizer (IKA-3420000, Germany). The supernatants were then assayed for PGE2 levels using an immunometric enzyme immunoassay (EIA) kit according to the manufacturer's instruction (ADI-900-001).

NP-SH measurement
The content of gastric NP-SH was measured from supernatant obtained from 100-mg gastric tissue in 1 ml of 0.02 M EDTA, distilled water, and trichloroacetic acid. The supernatants were then mixed with Tris buffer and 5,5-ditiobis (2-nitrobenzoic acid), according to Shin et al.'s method (19). The absorbance was measured at 412 nm and reported as μg/g of the gastric tissue using the standard curve.

Statistical analysis
Data were expressed as mean ± standard error of the mean. Statistical analysis was performed by one-way analysis of variance and Tukey's *post hoc* test using SPSS for Windows version 11.5 (IBM SPSS version 11.5; USA). $p < 0.05$ was considered to be statistically significant.

Results

Effect of allantoin on the mucosal thickness
The mucosal layer thickness was measured in millimeter (mm) from the mucosal muscle to the apex of the epithelial cells at a magnification of 40× in four fields from the photomicro-graph by software. For each sample, five sections were examined and their mean was considered as the average thickness of the mucus layer. As shown in Figs 1a and 2, the mucosal thickness significantly decreased in the gastritis group compared to the control group (14.93 ± 1.74 vs. 27.85 ± 1.46, $p < 0.01$). However, allantoin administration increased
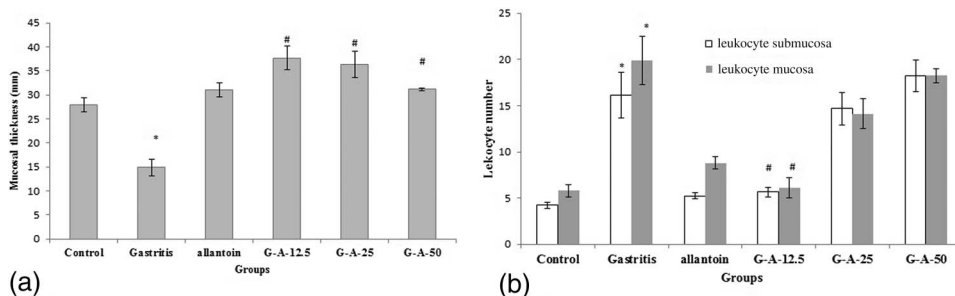

Fig. 1. (a) Mucosal layer thickness of the gastric tissue in different groups. Mean ± SEM, $N = 6$, *$p < 0.01$ compared to control. #$p < 0.001$ compared with gastritis group. (b) Leukocyte numbers of mucosal and submucosal layers in different groups. Mean ± SEM, $N = 6$, *$p < 0.001$ compared to control. #$p < 0.001$ compared to the gastritis group
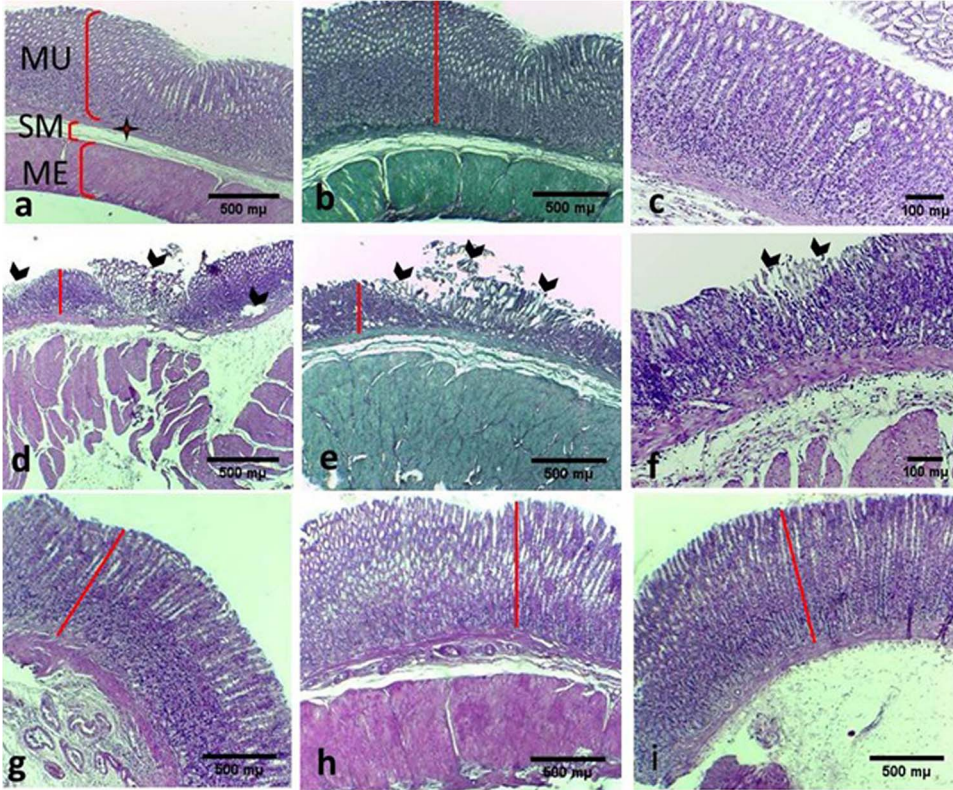
Fig. 2. Histopathological findings of the gastric tissue in different groups. (a–c) The control group: showing normal mucosal layer of the gastric tissue. (d–f) The gastritis group: showing detachment and loss of mucosal layer (head of arrow). (g–i) Treatment groups (12.5, 25, 50 mg allantoin, respectively): showing improvement of ulcers and increase of mucosal layers (b, e: Masson's trichrome, 4×), (a, c, d, f–i: H&E, 4×). MU: mucosal layer; SM: submucosal layer; ME: muscular extern

mucosal thickness in all treatment groups (G-A-12.5, allantoin, G-A-25 allantoin, and G-A-50 allantoin groups; 37.66 ± 2.4, 36.4 ± 2.73, 31.16 ± 0.27 vs. 14.93 ± 1.74, $p < 0.001$, respectively).

Effect of allantoin on the number of leukocytes
The obtained findings revealed that the leukocyte numbers in both of the mucosal and the submucosal layers increased in the gastritis group compared to the control group (19.88 ± 2.6 vs. 5.77 ± 0.66, $p < 0.001$ and 16.11 ± 2.46 vs. 4.22 ± 0.32, $p < 0.001$, respectively). However, the leukocyte numbers decreased in the G-A-12.5 allantoin group compared to the gastritis group in both layers (5.66 ± 0.52 vs. 19.88 ± 2.6 and 6.11 ± 0.07 vs. 16.11 ± 2.46, $p < 0.001$, respectively), whereas in other groups, no significant decreases were observed (Fig. 1b).

Effect of allantoin on the ulcer areas
For the ulcer area measurement, the length and thickness of the ulcer in mm^2 were measured at a magnification of 40× with the software and the area of the gastric ulcer was obtained in

each section. Five sections were examined for each sample. Our results showed that ulcer areas significantly increased in the gastritis group compared to the control group (56.52 ± 10.6 vs. $0 \, mm^2$, $p < 0.001$). However, allantoin treatment significantly lowered ulcer areas in the G-A-12.5 allantoin, G-A-25 allantoin, and G-A-50 allantoin groups compared to the control group (24.81 ± 7.48, 10.6 ± 5.35, and 10.24 ± 2.65 vs. 56.52 ± 10.6, $p < 0.001$, respectively; Fig. 3c).

Effect of allantoin on the number of the parietal and mucosal cells
For counting the cells, the average values of the cells at four microscopic fields in each section were considered by the image J software at a magnification of 400× (Fig. 4). The average number of cells in five stomach sections from each sample was considered as the number of cells in that specimen.

Histological evaluations revealed that ethanol administration significantly decreased the number of parietal and mucosal cells compared to the control group (1.55 ± 0.37 vs. 13.66 ± 0.78, $p < 0.001$ and 1.66 ± 0.55 vs. 6.88 ± 0.56, $p < 0.001$ respectively). Meanwhile, the number of parietal cells increased in the G-A-12.5 allantoin, G-A-25 allantoin, and G-A-50 allantoin groups compared to the gastritis group (14.22 ± 1.1, 9.66 ± 1.17, and 9.44 ± 0.74 vs. 1.55 ± 0.37, $p < 0.001$, respectively). Similarly, allantoin administration increased the mucosal cells in the G-A-12.5 allantoin, G-A-25 allantoin, and G-A-50 allantoin groups compared to the gastritis group (10.11 ± 1.79, 9.11 ± 0.71, and 6.8 ± 0.59 vs. 1.66 ± 0.55, $p < 0.001$, respectively; Figs 3a and 5).
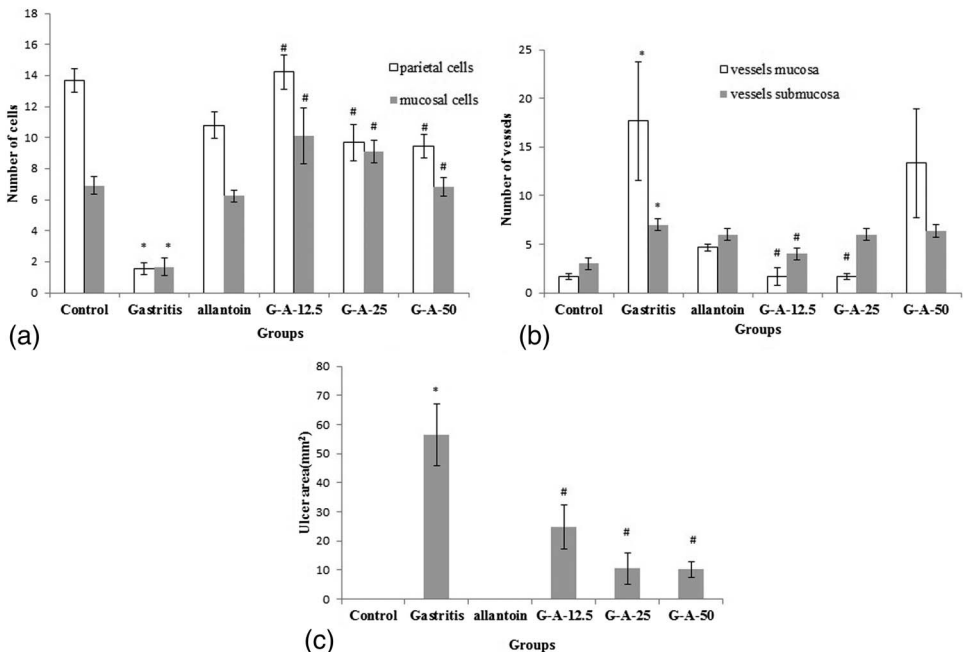

Fig. 3. (a) Number of cells in different groups. Mean $\pm$ SEM, $N = 6$, *$p < 0.001$ compared to the control group. #$p < 0.001$ compared to the gastritis group. (b) Number of vessels of mucosal and submucosal layers in different groups. Mean $\pm$ SEM, $N = 6$, *$p < 0.05$ compared to control. #$p < 0.05$ compared to the gastritis group. (c) Ulcer areas of the gastric tissue in different groups. Mean $\pm$ SEM, $N = 6$, *$p < 0.001$ compared to control. #$p < 0.001$ compared to the gastritis group
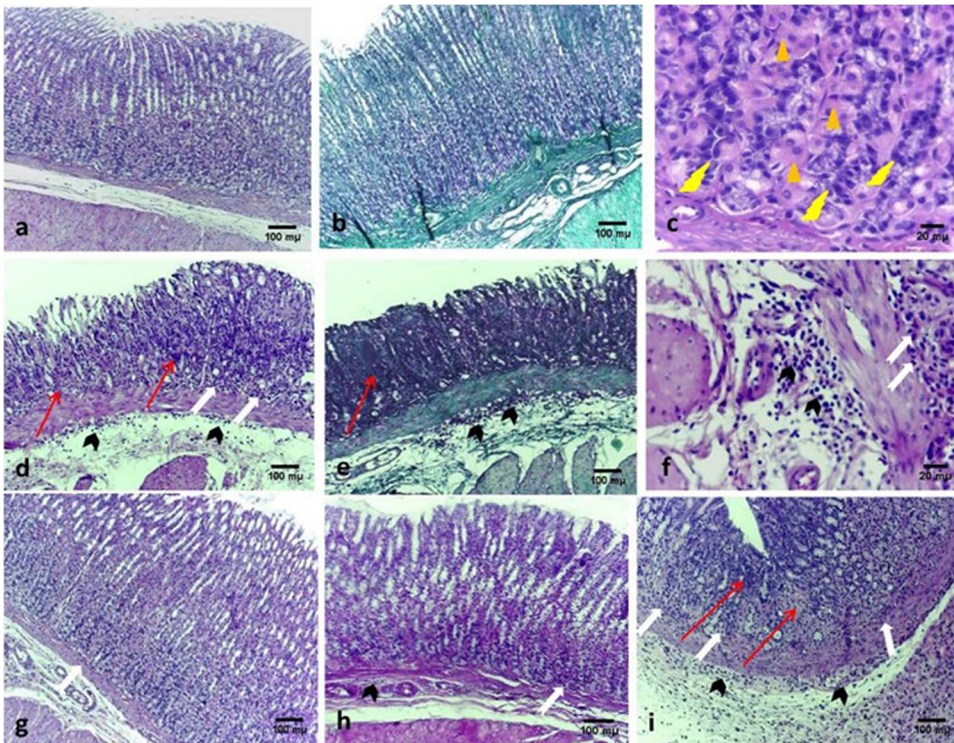
Fig. 4. Histopathological findings of the inflammatory cells in the mucosal (white arrow) and submucosal layers (head of black arrow) of different groups. Parietal cells (triangle), chief cells (wavy arrow). (a–c) The control group. (d–f) The gastritis group: showing granulated tissue and fibrosis (thin arrow) and inflammatory cells. (g–i) G-A-12.5, G-A-25, G-A-50 groups, respectively; less inflammation in G-A-12.5 group (b, e: Masson's trichrome, 10×; a, c, d, f–i: H&E, 4×; c, f: H&E, 40×)

Effect of allantoin on the number of vessels

For counting lamina propria and submucosa vessels, similar to the cells counting, 100× magnification was used. As noted in Fig. 3b, gastritis markedly increased the number of vessels in the mucosal layer compared to the control group (17.66 ± 6.06 vs. 1.66 ± 0.33, $p = 0.01$). However, the number of vessels significantly decreased in the G-A-12.5 allantoin and G-A-25 allantoin groups compared to the gastritis group (1.66 ± 0.88 and 1.66 ± 0.33 vs. 17.66 ± 6.06, $p = 0.01$). However, the vessel numbers increased in the G-A-50 allantoin group.

Our findings also showed a significant increase in the gastritis group compared to the control group in the vessel number of the submucosal layer (7 ± 0.57 vs. 3 ± 0.57, $p = 0.004$), but allantoin administration significantly decreased it only in the G-A-12.5 allantoin group compared to the gastritis group (4 ± 0.57 vs. 7 ± 0.57, $p = 0.03$).

Effect of allantoin on PGE2 levels

This study also revealed that gastric PGE2 levels significantly decreased in the gastritis group compared to the control group (666.32 ± 56.46 vs. $1,533.25 \pm 16.41$, $p < 0.001$). However, PGE2 levels showed a significant increase in the treatment groups (G-A-12.5,
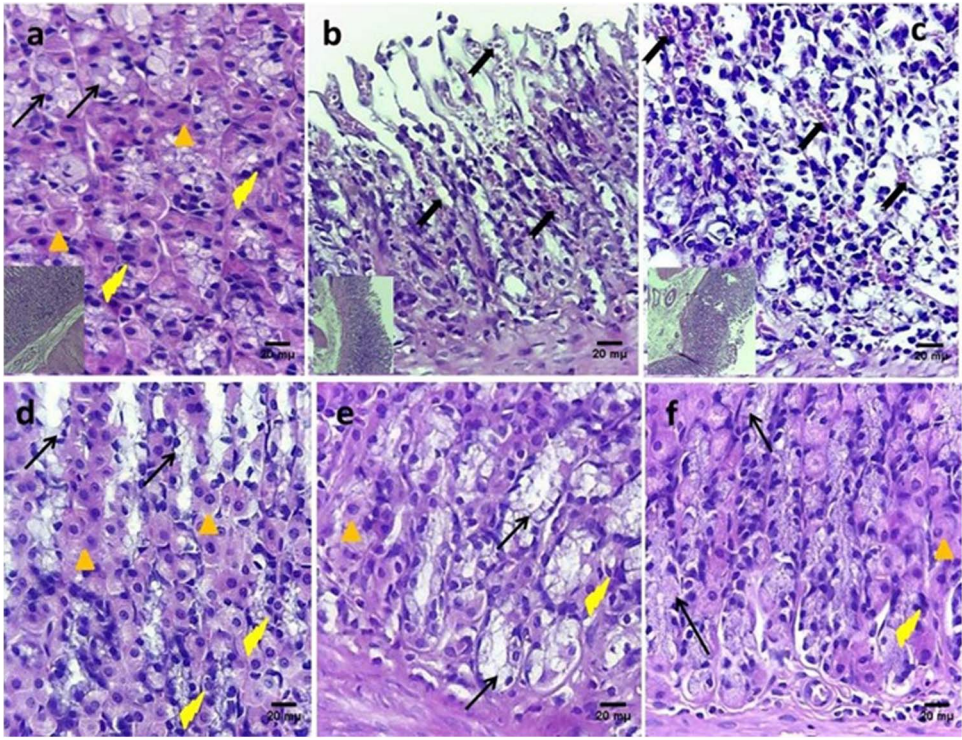
Fig. 5. Histopathological findings of the gastric tissues in different groups. Parietal cells (triangle), chief cells (wavy arrow), mucosal cells (thin arrow), and blood infiltration in submucosal layer (thick arrow). (a) The control group. (b, c) the gastritis group: showing the mess of the tissue and loss of gland and cell numbers. (d–f) G-A-12.5, G-A-25, G-A-50 groups; increase of glands and cell numbers, respectively (a–f: H&E, 40×)
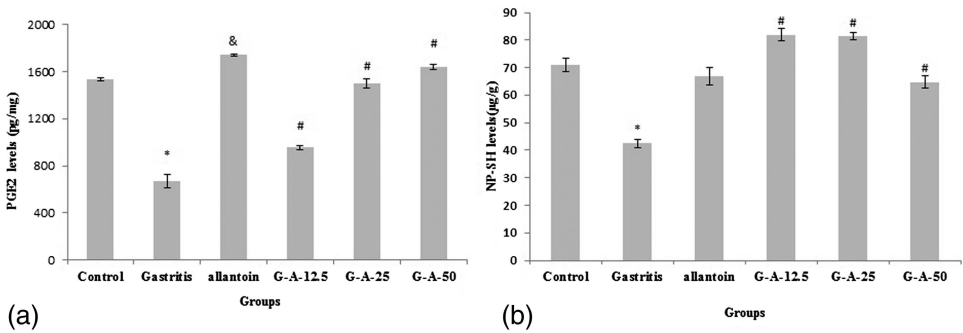

Fig. 6. (a) PGE2 levels of the gastric tissue in different groups. Mean ± SEM, $N = 6$, *$p < 0.001$ compared to control. [#]$p < 0.001$ compared to the gastritis group. (b) NP-SH levels of the gastric tissue in different groups. Mean ± SEM, $N = 6$, *$p < 0.001$ compared to control. [#]$p < 0.001$ compared to the gastritis group

allantoin, G-A-25 allantoin, and G-A-50 allantoin groups) compared to the gastritis group (956.12 ± 17.83, $1,499.62 \pm 42.25$, and $1,638.95 \pm 21.31$ vs. 666.32 ± 56.46 pg/mg, $p < 0.001$, respectively; Fig. 6a).

Effects of allantoin on the gastric NP-SH levels
Our results showed that gastric contents of mucosal NP-SH significantly decreased in the gastritis group compared to the control group (42.46 ± 1.48 vs. 71.0275 ± 2.34, $p < 0.001$). However, allantoin treatment in the G-A-12.5 allantoin, G-A-25 allantoin, and G-A-50 allantoin groups significantly increased NP-SH levels compared to the gastritis group (81.8875 ± 2.14, 81.3125 ± 1.26, and 64.745 ± 2.15 vs. 42.46 ± 1.48, $p < 0.001$, respectively; Fig. 6b).

Discussion

The beneficial effects of allantoin on inflammation, oxidative activities, and wound healing have been well known (8, 9). In this study, we showed that allantoin improved gastritis, mucosal thickness, and cell numbers after ethanol-induced gastritis, decreased leukocyte, and vessel numbers and increased the PGE2 and NP-SH levels in the gastric tissue of male rats.

Long-term alcohol consumption is considered to be a risk factor for peptic ulcer, and ethanol administration experimentally induces gastritis, mucosal layer hyperemia, mucosal cell loss, and leukocyte infiltration in rats (3, 14, 18). Our results showed that ethanol decreased the mucosal layer thickness, parietal, and mucosal cell numbers and NP-SH levels, whereas allantoin treatment increased mucosal thickness, parietal, and mucosal cell counts and NP-SH levels. Gastric mucosal barrier and bicarbonate rich mucus are the first defense line against ulceration and it seems that allantoin treatment could increase mucosal cell number, mucus secretion, and mucosal thickness and cause gastritis improvement. NP-SHs help to maintain mucosal integrity and free radical scavenging. Increased NP-SH levels after allantoin treatment probably provide mucosal thickness increment and gastritis healing. These findings are also consistent with several reports, which demonstrated that silymarin and extract of glutinous rice increased NP-SH levels and mucus contents and had gastroprotective effect on the gastric ulcer (19, 20). The increased number of leukocyte cells is considered to be a sign of inflammation in the tissue. Our findings revealed that in both mucosal and submucosal layers, leukocyte cells increased in the gastritis group and allantoin administration led to decrement of the leukocyte cell number. Vessel numbers also increased after ethanol use, whereas allantoin decreased the number of vessels. It seems that increasing leukocyte and vessel numbers result from inflammation and hyperemia induced by gastritis in the stomach (12). In this context, it has been reported that allantoin decreased IL4, IL5, and inflammatory cell infiltration in asthma-induced rats (11). Furthermore, da Silva et al. (7) have recently shown that allantoin decreases TNFα and IL1β in gastritis induced in female mice. Our results are in agreement with these documents and demonstrate anti-inflammatory effects of allantoin in the ethanol-induced gastritis in male rats. However, in this study, leukocyte cell and vessel numbers increased in G-A-50 allantoin compared to other treatment groups, which may be due to the high dose of allantoin. In total, 12.5 mg/kg allantoin dose presented better results. Initially, we selected 50 and 25 mg doses according to the study of Lee et al. (11). Afterward, because of the positive results obtained, it was decided to use lower doses (12.5 mg). This is an important point in the study showing fewer side effects at lower doses.

One of the prostaglandins in the gastric mucosa is PGE2. PGE2 enhances mucus secretion, inhibits gastric acid secretion, and improves gastric ulcer (9, 13). As it has been shown in many studies, our results also indicated that ethanol consumption led to PGE2

decrease, and allantoin administration in gastritis groups was observed to increase PGE2 levels in the gastric tissue. Previously, it has been documented that allantoin increased the activity of COXs production pathways (21, 23). Therefore, it is probable that increased COX1 and COX2 synthesis will cause increased PGE2 secretion in the stomach tissue. It seems that allantoin increases mucosal cells, followed by increased mucus secretion and NP-SH levels. NP-SH compounds and PGE2 help to increase antioxidant defense, to keep on mucus barrier integrity and thereby to improve gastritis.

Conclusions

This study revealed that allantoin had positive effects on the histopathological indices and could promote and keep the gastric mucosal barrier in ethanol induced gastritis. Moreover, it was found that 12.5-mg allantoin showed the best results after 5 days of consequent treatment. This caused better results with the lower doses as shown. Therefore, allantoin can help to decrease side effects of the agents and drug interactions.

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