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A preliminary histological study, in an experimental murine model, of gastritis induction, compared to the protective effect of a biological preparation.

Un studiu histologic preliminar, într-un model experimental murin, de inducere a gastritei, comparativ cu efectul protector a unui preparat biologic.

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Rezumat

Ulcerul peptic este o afecțiune cronică ce afectează un număr semnificativ de persoane din întreaga lume, estimat la aproximativ 10% din populația globală. Acesta este rezultatul unui dezechilibru între factorii agresivi, precum sucul gastric și aciditatea crescută a acestuia, și mecanismele de apărare ale mucoasei stomacului și intestinului subțire. Aspecte precum expunerea la anumite substanțe sau infecția cu bacterii precum *Helicobacter pylori* (*H. pylori*) sunt considerate factori declanșatori în apariția ulcerului peptic. Printre factorii de risc se numără utilizarea frecventă a medicamentelor antiinflamatoare nesteroidiene (AINS) și nivelurile ridicate de stres. Tratamentele convenționale pentru ulcerul peptic includ inhibitorii pompei de protoni (IPP) și antagoniștii receptorilor de histamină-2 (H2), care sunt eficiente în scăderea secreției de acid gastric. Cu toate acestea, aceste tratamente pot fi asociate cu efecte secundare și pot duce la recurențe sau interacțiuni nedorite cu alte medicamente. În contrast, abordările naturale și terapiile complementare au câștigat popularitate în gestionarea și tratarea ulcerului peptic. Plantele medicinale și compușii activi din acestea au fost recunoscuți pentru proprietățile lor antiinflamatoare și de protecție a mucoasei stomacului. Unele plante precum *Curcuma longa* sau *Angelica keiskei* au fost studiate pentru potențialul lor de a reduce inflamația și de a promova vindecarea ulcerelor, precum și alte preparate naturale cum ar fi lăptișorul de matcă sau propolisul, provenite din apicultură. De exemplu, lăptișorul de matcă este cunoscut pentru proprietățile sale antiinflamatoare și antimicrobiene, care pot contribui la reducerea iritației mucoasei gastrice și la vindecarea ulcerațiilor. *Angelica keiskei*, cu compușii săi activi, a demonstrat capacitatea de a proteja mucoasa și de a reduce producția excesivă de acid gastric. Turmericul, bogat în curcumină, este un alt ingredient studiat pentru efectele sale antiinflamatoare și antioxidante, care pot sprijini vindecarea și reducerea simptomelor ulcerului peptic.

Abstract

Peptic ulcer disease is a chronic condition that affects a significant number of people worldwide, estimated at approximately 10% of the global population. This is the result of an imbalance between aggressive factors, such as gastric juice and its increased acidity, and the defense mechanisms of the lining of the stomach and small intestine. Aspects such as exposure to certain substances or infection with bacteria such as *Helicobacter pylori* (*H. pylori*) are considered triggers in the development of peptic ulcers. Risk factors include frequent use of non-steroidal anti-inflammatory drugs (NSAIDs) and high levels of stress. Conventional treatments for peptic ulcers include proton pump inhibitors (PPIs) and histamine-2 (H2) receptor antagonists, which are effective in decreasing gastric acid secretion. However, these treatments may be associated with side effects and may lead to recurrences or unwanted interactions with other medications. In contrast, natural approaches and complementary therapies have gained popularity in the management and treatment of peptic ulcers. Medicinal plants and their active compounds have been recognized for their anti-inflammatory and protective properties of the stomach lining. Some plants such as *Curcuma longa* or *Angelica keiskei* have been studied for their potential to reduce inflammation and promote ulcer healing, as well as other natural preparations such as royal jelly or propolis, derived from beekeeping. For example, royal Jelly is known for its anti-inflammatory and antimicrobial properties, which can help reduce irritation of the gastric mucosa and heal ulcers. *Angelica keiskei*, with its active compounds, has demonstrated the ability to protect the mucosa and reduce the excessive production of gastric acid. Turmeric, rich in curcumin, is another ingredient studied for its anti-inflammatory and antioxidant effects, which may support healing and reduce peptic ulcer symptoms.

1. Introduction

Peptic ulcer, a condition involving the formation of lesions in the lining of the stomach or duodenum, is the result of an imbalance between factors affecting the integrity of the mucosa and those that damage it.

This injury is often induced by stomach acid and is characterized by damage to the mucosa, sometimes penetrating even into the deep layers of the stomach, such as the submucosa or muscle layer [58].

Estimates show that between 5 and 10% of the general population faces this condition [1, 13]. However, recent epidemiological data have shown a decreasing trend in the incidence, number of hospital admissions and mortality rate associated with peptic ulcer disease [20].

This decline can be attributed to the introduction of new therapies and improved hygiene, helping to reduce infections with *Helicobacter pylori* (*H. pylori*), a bacterium known for its involvement in peptic ulcers [3].

Traditionally, it was believed that mucosal damage in peptic disease is the consequence of a hypersecretory acidic environment, often associated with dietary factors or stress.

Risk factors include *H. pylori* infection, alcohol and tobacco use, and use of anti-inflammatory drugs (NSAIDs). *H. pylori* infection and NSAID use are considered the main risk factors for both gastric and duodenal ulcers.

However, only a small proportion of people infected with *H. pylori* or using NSAIDs develop peptic ulcers, which highlights the importance of individual sensitivity in mucosal damage [1, 20].

Research has revealed that functional polymorphisms in various cytokine genes, such as interleukin-1-beta (IL-1B), can influence mucosal interleukin 1β production, thereby influencing the association between *H. pylori* and gastroduodenal diseases [20].

Also, the risk of complications associated with peptic ulcers, such as bleeding in the upper gastrointestinal tract, is higher in NSAID and aspirin users, especially when used in combination with anticoagulants [12, 15].

Studies have found that aspirin use and *H. pylori* infection increase the risk of peptic ulcer disease [11].

There is also a subset of idiopathic ulcer cases, which account for about one-fifth of all cases [8].

The precise mechanisms behind the development of idiopathic ulcers remain unclear, but studies suggest the implication of an imbalance between factors contributing to mucosal integrity and those affecting it (such as ischemia, medications, steroids or chemotherapeutic agents, as well as radiation therapy), along with factors such as viruses, histamine, gastric bypass, and metabolic disorders [16].

It appears that psychological stress may also play a role in increasing the incidence of peptic ulcers [13].

Peptic ulcer remains a complex condition, and a full understanding of its developmental mechanisms and factors contributing to its evolution is essential for effective management and treatment of this health problem.

2. Materials and methods

Our study aimed to address an alternative treatment route of gastritis caused by the administration of NSAIDs, the proposed experimental design being carried out on 24 laboratory mice from the BALB/c line (12 males and 12 females).

The mice included in the experiment were divided into 2 large groups, the first batch being the group in which gastritis was induced with aspirin and not treated with any phytotherapeutic preparation, and the second group, which was administered by oral gavage the mixture of biological preparations as described below.

Out of the 2 batches included in the experiment, two slaughters were carried out, in accordance with the methodologies in force [Directive 2010, NRC, 2011].

The first slaughter on day 7 since the initial administration of aspirin and the second slaughter was on day 14.

2.1. Experimental protocol

The induction of gastritis was performed with aspirin from the pharmaceutical company Bayer, at a dose of 20 mg / mouse. 2 plant

extracts (*Curcuma longa* and *Angelica keiskei*), in standardized form, and freeze-dried royal jelly, purchased commercially, were chosen as biological preparations.

The dose was calculated by extrapolating doses from human medicine to veterinary medicine using Löwe's Law [9].

Figure 1 . Liposomal Turmeric 0.2 ml / mouse (7 mg/mouse)

Figure 2. Royal Jelly - 0.2 ml (0.2 mg/mouse)

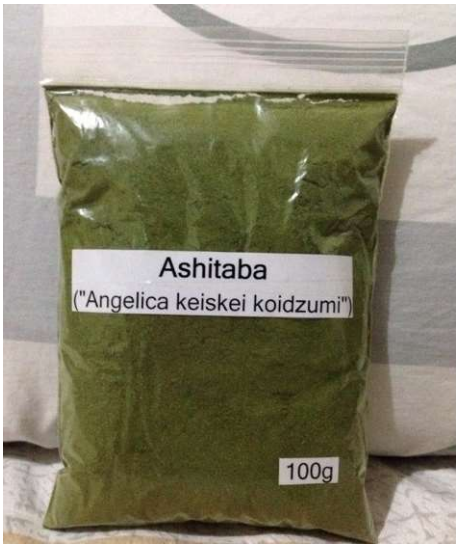


Figure 3 and 4. *Angelica keiskei* - 0.2 ml (2 mg / mouse)

Figure 5. Handling and intragastric administration in BALB/c mice.

From the 3 biological products, a mixture was made and administered for 7 and 14 days, respectively, after induction of gastritis with aspirin. Administration of the mixture was performed by intragastric administration with an *"oral gavage"* probe.

For histological processing, the cytoarchitecture of the stomach (mucosa/submucosa/muscle) was analyzed.

2.2. Histological study

The skin fragments were fixed in ethyl alcohol 80^0 for at least 7 days, after which they were washed, dehydrated and included in paraffin.

The sections thus prepared were processed for histological study by hematoxylin–eosin staining. The histological images were captured using Olympus CX41 microscope software.

3. Results and discussions

Peptic ulcer is the result of a pathological condition in which the biological balance between the defending and aggressing factors of the gastrointestinal tract is disturbed. Among the main aggressive endogenous factors contributing to this disorder are gastric hydrochloric acid, pepsin (a gastric enzyme), reactive free radicals and oxidants, leukotrienes (substances produced by cells of the immune system that can cause inflammation), bile reflux and endothelins (substances that regulate vascular tone). These factors act collectively to affect the integrity of the gastrointestinal mucosa and may contribute to ulceration formation [7].

Defensive factors that protect the gastric mucosa include gastric mucus barrier, bicarbonate, mucosal blood flow, active surface phospholipids, prostaglandins (PG), nitric oxide (NO), and antioxidant performance, both enzymatic and non-enzymatic.

These factors are essential for maintaining the integrity of the mucosa and protecting it against aggression from the gastric environment and other endogenous sources.

Through their synergistic action, these factors contribute to supporting and protecting the gastrointestinal mucosa against lesions and the formation of peptic ulcers [7, 19].

In the present study, the protective action of the combination of biological products between turmeric, *Angelica keiskei* and freeze-dried royal jelly was pursued, compared to the untreated group, which was given aspirin only.

The microscopic examination was performed at the level of the mucosa, submucosa and muscle of the stomach, the organs being harvested on days 7 and 14, post gastritis induction.

From a histological point of view, in the group that received only aspirin and was sacrificed 7 days after administration, the presence of peritubular edema in the mucosa and submucosa was found.

Also, the presence of areas with erosion and inflammatory infiltrate in the mucosa and submucosa.

In the mucous cells of the stomach or the protective mucus layer, the monolayered columnar epithelium shows a slight degeneration, and in areas of mucosa with an apparently normal appearance, a slight edema has been found (Figure 6).

After 14 days, the presence of peritubular edema in the mucosa was again found.

Also, the cyto-architecture of the stomach reveals areas with integral epithelium, areas with apparently normal appearance with slight signs of improvement, but also areas with discontinuous epithelium, accompanied by inflammatory infiltrate (Figure 6).

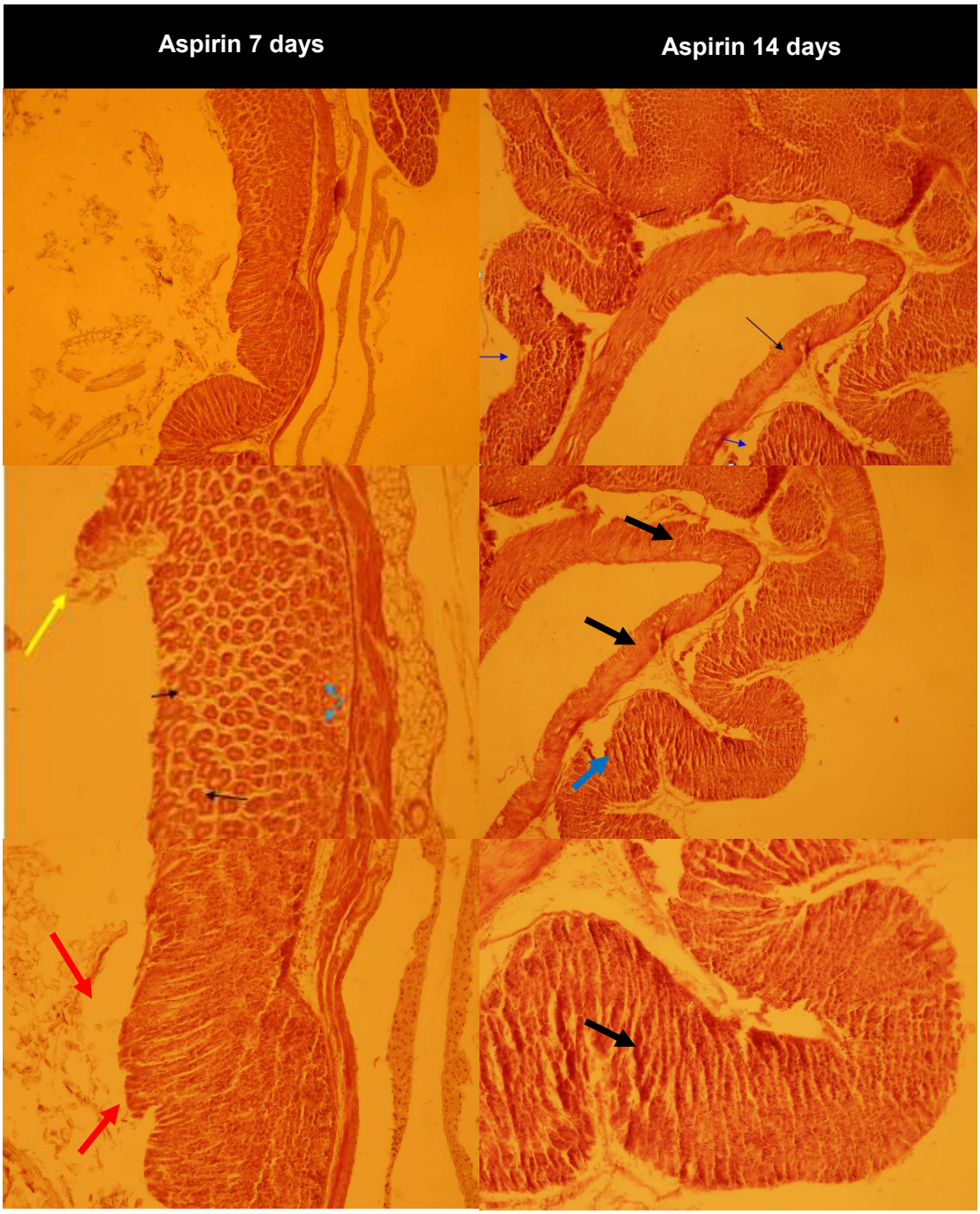
Compared to the batch of mice that received phytotherapeutic treatment, in the group slaughtered at 7 days, a lower inflammatory infiltrate was observed, with normal areas and erosion areas still existing.

An important change was that in the treated group the absence of edema was observed, compared to the untreated group, where peritubular edema was present (Figure 7).

In the slaughtered group after 14 days of treatment, areas of mild erosion are still present, concomitant with normal-looking areas and continuous epithelium. In addition, mucosal cell detachment and lack of inflammatory infiltrate were identified (Figure 7).

Gastric mucus acts as a barrier that restricts the exposure of gastric cells to various harmful agents of both exogenous and endogenous origin. Increased mucus production could be a central factor in ulcer healing by protecting damaged tissue from various aggressors, such as some drugs and oxidants. In fact, mucus secretion obviously contributes to improving epithelial recovery after acute injuries, by forming a mucoid cap over the initial reepithelialization.

In addition, the formation of buffering capacity and neutralization of luminal hydrochloric acid are the main physiological functions of gastric mucus [2, 10].



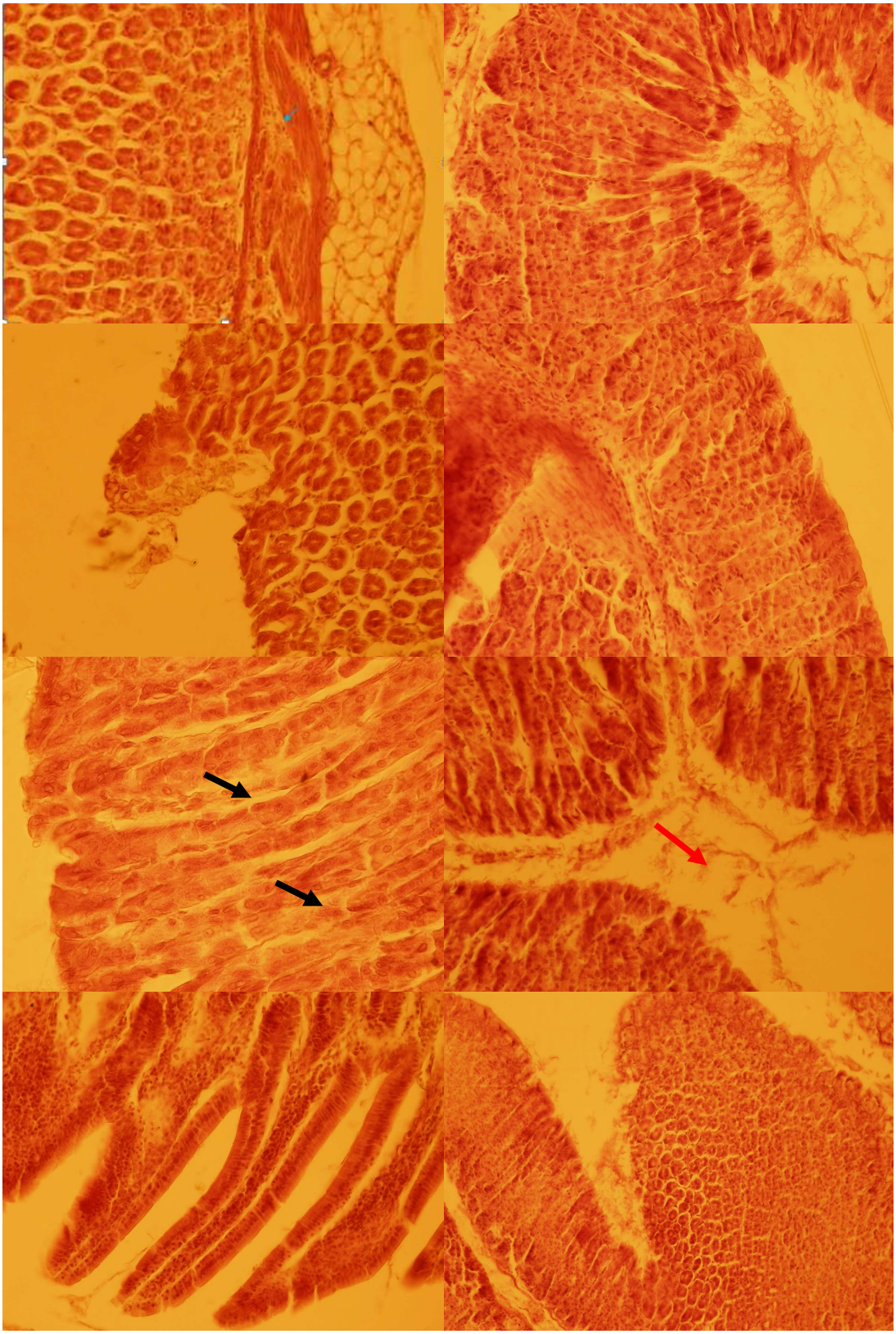
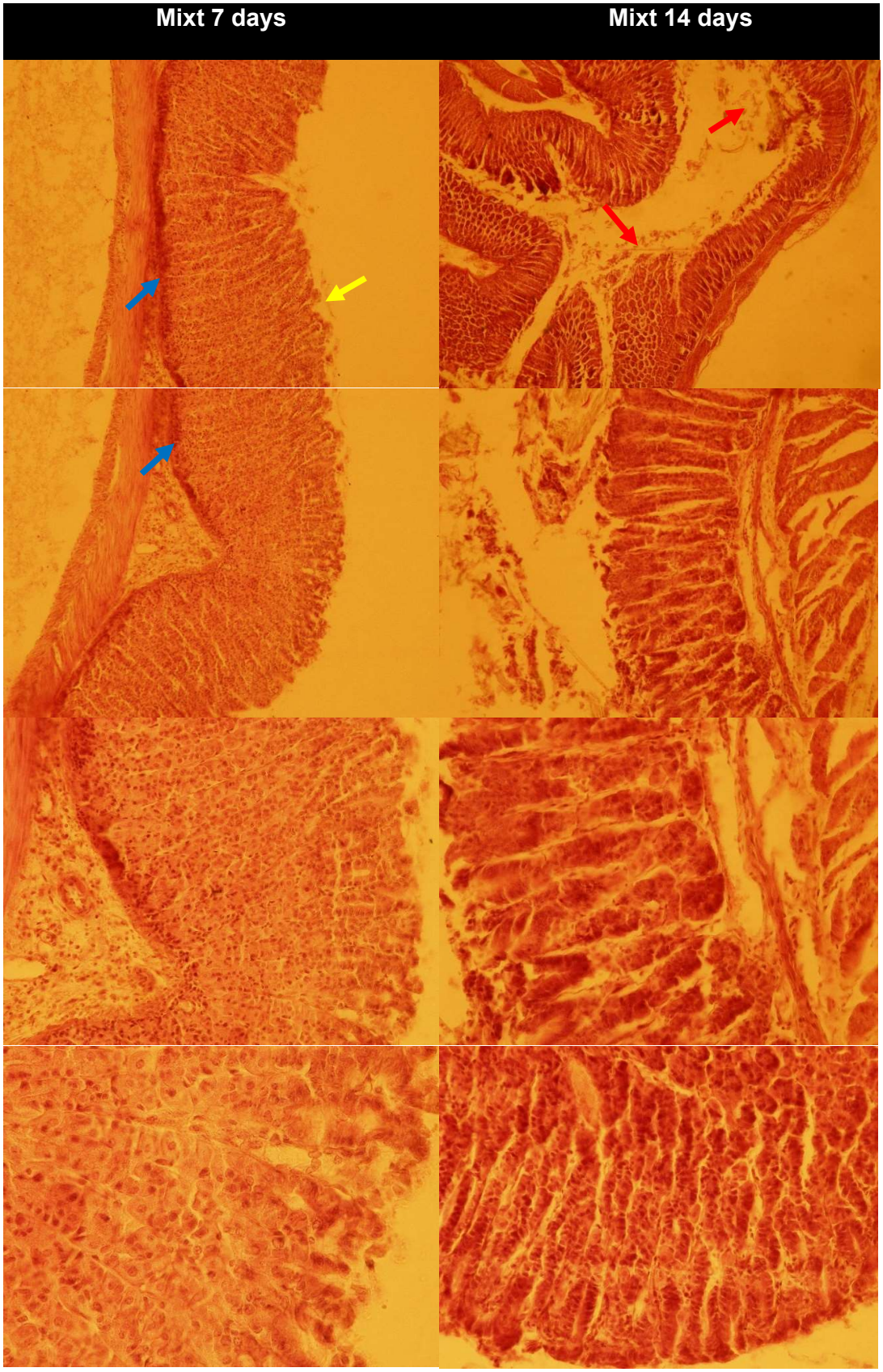


Figure 6. Histological section of the stomach, with changes of peritubular edema ➡ ,Erosion zone ➡, Inflammatory infiltrate ➡, Degenerated mucous cells ➡ (Staining H.E. ob. 4×, 10×, 20×, 40×).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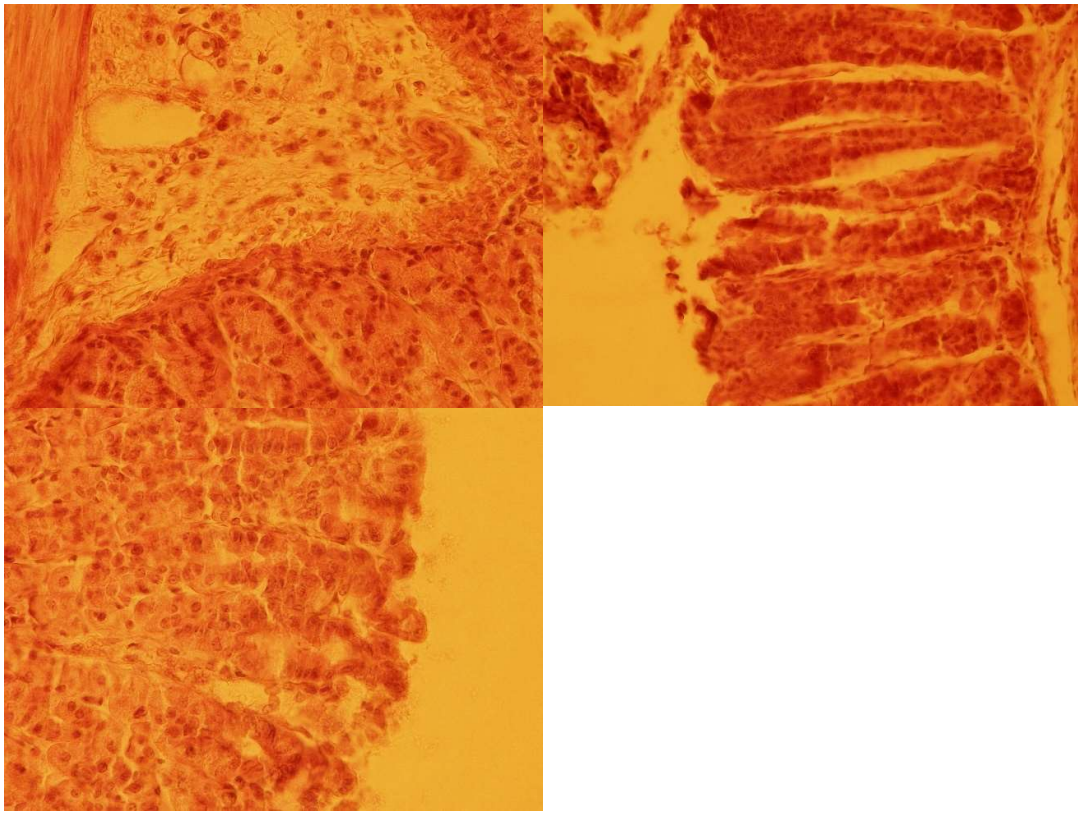

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According to several scientific articles, peptic ulcers induced by NSAIDs are the most serious complication of any synthetic drug therapy [4, 21].

It is widely accepted that NSAID-induced ulceration is mediated by suppressing the cyclooxygenase-dependent pathway (COX) and subsequently blocking prostaglandin (PG) synthesis.

Various aspects, such as interactions between leukocytes and endothelium, neutrophil infiltration, cytokine imbalance and oxidative stress of the mucosa, contribute to the pathogenesis of gastric mucosal lesions initiated by anti-inflammatories.

Activation of adhesion molecules, including cell adhesion molecules (CAM) and intercellular adhesion molecule (ICAM-1), is associated with infiltration of neutrophils into the gastric mucosa.

These complex processes are part of the mechanisms by which NSAIDs can induce lesions of the gastrointestinal mucosa, which can lead to ulceration and serious complications for patients.

Relevant scientific articles highlight the magnitude and complexity of these adverse effects associated with the use of NSAIDs, in clinical treatment [16, 20].

In other studies conducted, as well as the current study, aspirin has been shown to be an excellent gastritis-inducing agent, confirming the validity of the use of this molecule in experimental gastritis induction studies on laboratory animals.

Also, the association between phytotherapeutic preparations, *Curcuma longa* and *Angelica keiskei*, the latter being less studied, plus royal jelly, is already scientifically proven to have a role in improving inflammation of the stomach mucosa, in studies performed on laboratory animals.

The study has shown that their administration can improve histological lesions induced after administration. Specifically, the main mechanism of NSAID-associated lesions in the gastroduodenal mucosa involves

systemic inhibition of cyclooxygenase-1 (COX-1), which is responsible for prostaglandin synthesis.

It is associated with a number of adverse effects, including reduced mucosal blood flow, diminished mucus and bicarbonate secretion, and inhibition of cell proliferation.

Concomitant administration of exogenous prostaglandins and use of selective NSAIDs for cyclooxygenase-2 (COX-2) have been associated with a reduction in mucosal damage and the risk of ulcers.

Therefore, therapeutic strategies aimed at simultaneous administration of these compounds may diminish the negative effects of NSAIDs on the gastrointestinal mucosa.

These selective approaches could represent a new direction in effective disease management, minimizing side effects associated with the use of NSAIDs in clinical treatments [5].

However, the different physicochemical properties of NSAIDs cause differences in their toxicity [6].

Nonsteroidal anti-inflammatory drugs disrupt phospholipids in the mucosa and lead to the decoupling of oxidative mitochondria, phosphorylation, thus initiating mucosal damage.

NSAIDs, when exposed to an acidic environment, such as gastric juice with a low pH around 2, experience a chemical change.

They become protonated, that is, they bind to a proton at the atomic level.

This process allows them to more easily pass through lipid membranes to enter epithelial cells lining the gastrointestinal mucosa.

There, the environment is less acidic, with a pH of about 7.4.

In this more alkaline environment, NSAIDs change chemically and release hydrogen ions (H+), making them less able to efficiently cross lipid membranes.

As a result, they remain stuck inside epithelial cells, accumulating in them. This accumulation can trigger changes in normal cellular functioning.

For example, it can affect the process of oxidative phosphorylation, which is essential for energy production in our cells.

By reducing mitochondrial activity, NSAIDs can compromise the ability of cells to generate energy, causing a decrease in the overall level of energy available for optimal body function.

In addition, nonsteroidal anti-inflammatory drugs can increase the permeability of epithelial cells, which can lead to easier penetration of harmful substances or loss of beneficial substances, disrupting the integrity and normal functioning of the gastrointestinal mucosa.

Thus, these NSAID-induced changes can contribute to mucosal damage and ulceration in the gastrointestinal tract [17].

4. Conclusions

- Our study reconfirmed aspirin's effectiveness in inducing gastritis in murine models.
- Signs of improvement in the cytoarchitecture of the stomach were found in both the treated and untreated groups on day 14 of slaughter.
- Signs of improvement in the cytoarchitecture of the stomach were found in both the treated and untreated groups on day 14 of slaughter.
- Taking aspirin, along with certain phytochemical constituents, can reduce side effects associated with long-term medication and reduce overall expenses for peptic ulcer treatments and inappropriate NSAIDs.

5. Recommendations

- We recommend conducting new studies by investigating different plant extracts that may lead to the discovery of new safe drugs with gastroprotective activity.
- Always use plants with high antioxidant activity, especially in treatments used in peptic ulcers.
- Formulation of new medicinal pharmaceutical forms, including herbal active principles.

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