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Gastroprotective effect of Etawa goat's milk through biochemical biomarkers

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Abstract

The prevalence of gastritis in Indonesia in 2020 reached 40.8%. Previous research proved goat milk to be anti-inflammatory in rat models of colitis. Goat milk feeding contributed to intestinal repair and protection with reduced colonic lesion scores in colonic tissue compared to control rats. This study aimed to investigate the gastroprotective effects of Etawa goat's milk in ethanol-induced gastric damage in vivo. In this study, 25 male Sprague-Dawley rats (SD) were divided into 5 groups; normal control group (NOR), ethanol group (EtOH), and three goat milk (GM) groups (given 0.5, 1 mL and 1.5 mL/200 g BW for 30 days intragastrically). After administration of GM for 30 days, all groups except for the normal control group had acute gastroduodenal damage after three days of intragastrical administration of 80% ethanol (5 mL/kg). Myeloperoxidase (MPO), superoxide dismutase (SOD), and histamine biomarkers in plasma were measured. Pretreatment with goat milk significantly protected rats from ethanol-induced gastric damage which aids in the reduction of the MPO and histamine activity and increase SOD activity.

1. Introduction

Gastritis is an inflammatory state that occurs in the lining of the stomach. The World Health Organization (WHO) predicts that the death rate due to gastritis was 40,376 cases in 2005, rising to 43,817 cases in 2010 and then continuing to rise to 47,269 cases in 2015 (WHO, 2015). Gastritis sufferers in Indonesia are 40.8% with a prevalence of 274,396 cases from the population (Mustakim *et al*., 2022; Tiu *et al*, 2022). The equilibrium of aggressive factors and defensive factors is the beginning of gastritis. Ethanol is one of the aggressive factors that can increase the risk of erosion of gastric mucosa (Pratama, 2019).

Induction with ethanol is proven to increase the production of reactive oxygen species (ROS) and reduce antioxidant levels such as superoxide dismutase (SOD). It can damage the gastric mucosa (Widyaningsih and Afdaliah, 2020). The results of histopathological analysis of the gastric wall induced with ethanol 80% inflammation characterized by abnormal gastric mucosal cells, which showed hypertrophy and infiltration of neutrophils into epithelial cells (Usman, 2016).

During damage to the gastric mucosal lining, Myeloperoxidase (MPO) activity is also directly related to the inflammatory process. MPO is produced by activated neutrophils. Increased MPO activity is a sign of significant neutrophil infiltration to the site of gastric mucosal injury (Yang *et al.*, 2018). Therefore, increased

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MPO activity reflects the degree of oxidative damage and inflammatory response in cells (Zhang *et al*., 2015).

Pharmacological treatment therapies used to treat gastritis include proton pump inhibitors (PPIs), H2 receptor antagonists (H2RAs), and antacid groups. However, the use of these drugs causes many clinically significant drug interactions and still has side effects such as headaches, diarrhea, and abdominal pain that can cause pancreatitis and pancytopenia (Dipiro *et al*., 2020). Given this, it is still necessary to develop safer treatment.

One of the natural products that has potential as a gastroprotector is goat's milk (*Cepra aegragus*). According to the Food and Agriculture Organization (FAO), in 2009 Indonesia was ranked 7th in the list of global goat milk production and ranked 1st in the Southeast Asia region (FAO, 2010; Taufik *et al*., 2011). Goat milk has benefits for people with digestive problems, such as gastritis, cachexia, and impaired liver function. Goat milk contains proteins, lipids, carbohydrates, vitamins and minerals (Fernández, 2017). Additional carbohydrates found in goat milk are oligosaccharides, glycopeptides, glycoproteins and small amounts of nucleotides (Zenebe *et al*., 2014).

Oligosaccharides in goat's milk were anti-inflammatory when administered as a pre-treatment model of TBNS colitis in rats (Daddaoua *et al*., 2005). Besides this, goat milk played a role in the repair and protection of the gut after damage caused by Dextran sodium sulfate-induced colitis by showing reduced colonic lesion scores and reduced MPO activity in colonic tissue compared to control rats (Lara-Villoslada *et al*., 2006).

The content of Etawa breed goat's milk has a protein content that is almost comparable to human milk (ASI) and is better than eggs, and goat's milk also has more potential and benefits than cow's milk (Handoko *et al*., 2021). Goat milk can be indicated to treat digestive diseases gastritis, high cholesterol, and osteoporosis in the elderly (Wasiati and Faizal, 2018). Hence, this study evaluates the activity of etawa goat milk as a gastroprotective in rats induced by 80% ethanol with SOD, MPO and histamine biomarkers.

2. Materials and methods

2.1 Etawa goat milk

The main ingredient used in this study was fresh etawa goat milk obtained from Pure Fresh Daily Milk, Ciamis, West Jawa, Indonesia.

2.2 Preliminary test for making gastritis model rat

Rat were divided into several groups with each group consisting of 4 rats. Rat groups were fed orally with 20%, 40%, 60%, and 80% ethanol (0.5 and 1 mL/200 g body weight) to induce the acute ulcer, while the normal group received water only. After 3 hrs of treatment, surgery was performed on one rat per group. After surgery, the stomach was isolated to determine the score for gastric surface damage. Inflammatory cell infiltration: 0 if normal, 1 if gastric mucosal damage is 1-2 mm, 3 if gastric mucosal damage is 3-4 mm, 4 if gastric mucosal damage is 5-6 mm, 5 if gastric mucosal damage is 6 mm.

2.3 Experimental animal treatment

A total of twenty-five male Sprague-Dawley rats (SD) were divided into 5 groups; normal control group (NOR), ethanol group (EtOH), three goat milk (GM) groups (given 0.5, 1 mL, and 1.5 mL/200 g BW for 30 days). After intragastric administration for 30 days, the acute gastroduodenal injury was induced by 80% ethanol (5 mL/kg, for 3 days intragastrically) in all groups except the normal control group. On the 34th day, blood samples were taken to assess the activity of MPO, SOD, and histamine biomarkers. All animal experiments were approved by the Committee Ethics Commission of the Muhammadiyah University Prof. Dr. Hamka (certificate number 02/23.05/02495).

2.4 Examination of Myeloperoxidase, superoxide dismutase, and histamine levels

Examination of SOD, MPO, and histamine levels was carried out using the Elisa Rats Kit from Bioassay Technology Laboratory according to the manufacturer's instructions.

2.5 Data analysis

Data was analyzed using a one-way analysis of variance (ANOVA) with Bonferroni's multiple testing. All data are presented as mean ± standard error of the mean (SEM). All results with $p < 0.05$ were considered statistically significant.

3. Results

According to earlier studies providing 80% ethanol for 3 days can cause gastric mucosal lesions in experimental rats (Kim *et al*., 2012; Usman, 2016).

After induction of 80% ethanol on the 3rd day, the animals fasted for 3 hrs to avoid vomiting and regurgitation that could occur during or after anesthesia. This also helps to minimize contamination of food or materials that are in the digestive tract which can affect research results (Savenije *et al*., 2010). The animals were anesthetized using 0.1 mL of ketamine and 0.05 mL of

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xylazine i.p. and blood samples were taken in sinus orbital (Li *et al*., 2018). Samples were collected in EDTA tubes and then samples were centrifuged at 3000 rpm for 20 minutes to obtain plasma.

Plasma can measure MPO levels because MPO can concentrate in polymorphonuclear leukocytes and neutrophil activation leads to excessive radical release. When neutrophils are activated and migrate to sites of inflammation, MPO will be released into the extracellular space and can be detected in plasma. Therefore, measuring MPO activity is a marker of neutrophil infiltration and activation, which can be used to assess the degree of inflammation in various diseases (Li *et al*., 2018).

The activity of myeloperoxidase (MPO) was investigated for a biochemical marker of neutrophil infiltration, after ethanol induction. As shown in Figure 1 The MPO activity increased significantly after ethanol treatment, suggesting that this triggers infiltrates neutrophils. On the other hand, pretreatment goat milk decreased MPO activity and significantly attenuated in dose 1.5 mL.

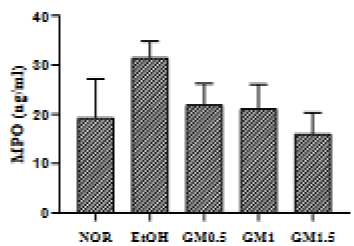

Figure 1. Effect of Etawa goat milk on MPO. Data are presented as mean±SEM. * $p<0.05$.

The levels of histamine were also dramatically increased in rats with acute gastric injury induced by ethanol (Figure 2). Interestingly, pretreatment with goat milk successfully reduced histamine levels. There was a significant difference in the ethanol group with the 0.5 mL, 1 mL and 1.5 of Etawa goat's milk groups. The greatest decrease was indicated by a dose of 1.5 mL. These findings suggest that goat milk has physiological effects that are gastroprotective and help to reduce inflammation and neutrophil infiltration.

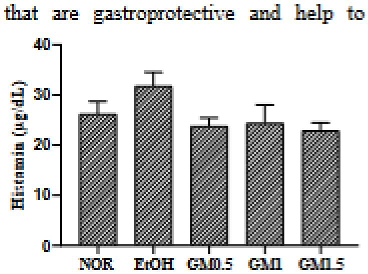

Figure 2. Effect of Etawa goat milk on histamine. Data are presented as mean±SEM. * $p<0.05$.

SOD examination showed a decrease in SOD levels in ethanol-induced rats, whereas in the goat's milk treatment group, there was a significant increase in SOD levels at all three doses (Figure 3). The largest increase in SOD was in the 0.5 mL dose group, but when compared to normal controls, administration of goat's milk did not significantly increase SOD levels.

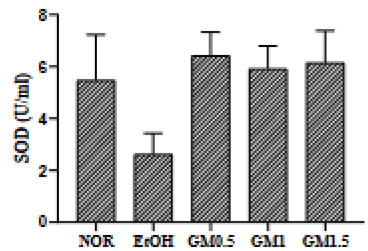

Figure 3. Effect of Etawa goat milk on SOD. Data are presented as mean±SEM. * $p<0.05$.

4. Discussion

The study material used was Etawa crossbreed goat milk obtained from the farm Pure Fresh Dairy Ciamis, West Java, Indonesia. In this farm, the animals were given the same feed, corn trees aged 85-90 days which are chopped and then fermented and concentrated, so that the livestock have the same nutrition. In previous studies, studies on the feeding of dairy animals that were given corn in fermented concentrates showed increased and improved milk production (Christi, 2019). Feed made by fermentation can produce good physical quality and high palatability compared to feed that is not fermented (Nadhifah *et al*., 2012).

In this study, 80% ethanol induction was carried out for 3 days aiming to obtain optimal conditions that could cause damage to the gastric mucosa. Other research proved rat strain Sprague Dawley induced with 100% ethanol can cause severe gastric mucosal damage compared to induction using indomethacin and hydrochloric acid. Meanwhile, induction using 80% ethanol showed neutrophil infiltration into the epithelial cells, indicating inflammation in the rat stomach (Usman, 2016).

Ethanol causes damage to gastric mucosa through the production of free radical products as metabolic products. One of these free radical products is reactive oxygen species (ROS). The accumulation of ROS production results in the failure of the endogenous antioxidant system in the body (Yang *et al.*, 2018). This study showed that, in comparison to the normal control group, the ethanol group had lower SOD levels.

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Ethanol has a strong and fast penetration ability into the gastric mucosal layer, which triggers damage to the epithelium (Usman, 2016). It was also demonstrated by increased MPO activity, which can be associated with increased neutrophil and infiltration rates H_2O_2 damaged gastric tissue. The results of this study also showed a significant increase in MPO activity in the ethanol-induced rat group. MPO is an enzyme released by neutrophils that is activated in response to infection or injury (Khan *et al*., 2018).

Ethanol-induced gastritis involves the accumulation of neutrophils and increased MPO activity in the gastric mucosa indicating oxidative injury. MPO is secreted at sites of inflammation by activated monocytes and neutrophils. MPO generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) capable of modifying DNA and proteins. MPO can bind to formyl peptide receptor-1 (FPR1), which can trigger neutrophil function during inflammation in the gastric mucosa (Hussein *et al*., 2016). The accumulation of ROS production results in the failure of endogenous antioxidant systems in the body such as SOD and catalase (Yang *et al.,* 2018). Besides that, increased ROS can react with mast cells which will release histamine and stimulate parietal cells to secrete HCl (stomach acid) levels. The direct contact of ethanol with the stomach results in metabolic and functional changes in the gastric mucosa (Pratama, 2019).

Elevated histamine levels indicate damage and are associated with inflammatory processes, gastric acid secretion, and impaired immune function (Thangam *et al*., 2018). The release of histamine causes dilation of blood vessels (Desty, 2021). H $receptors_2$-histamine, which is present in cells and tissues such as gastric parietal cells, has a role in increasing permeability and peripheral vasodilation causing inflammation and can cause gastritis (Yuniastuti, 2014). The results of this study also showed that ethanol-induced rats can cause damage to the stomach, which is characterized by an increase in MPO and histamine biomarkers, as well as a decrease in SOD levels.

The mechanism for reducing this biomarker is probably due to the composition of Etawa goat milk. Several studies have shown that the composition of fat, protein, carbohydrates, selenium minerals, and vitamin C from goat milk has pharmacological activity. Previous studies have shown that goat's milk has a different composition from cow's milk, including higher levels of fat, protein and minerals. Goat milk also has properties that are more easily digested by the human body and contain less lactose, so it is often considered an alternative for people who are lactose intolerant (Adriani *et al.,* 2014). Goat milk fat contains fat globules that act as anti-inflammatories. The content of short-chain fatty acids in goat's milk, such as butyric acid, has been known to have an anti-inflammatory effect by increasing the integrity of the gastric mucosa (Zenebe *et al*., 2014). The use of goat's milk fat as a source of nutrition can provide benefits for gastritis sufferers. In this study, giving Etawa goat milk reduced MPO, and histamine levels compared to ethanol-induced controls.

The content of goat's milk also contains oligosaccharides as a source of carbohydrates which have an anti-inflammatory role in inflammation and oligosaccharides in goat's milk are good sources of oligosaccharides which are the same as breast milk (Sousa *et al.,* 2019). Oligosaccharides have an effect by stimulating the growth of bifidobacteria in the digestive tract which protects against pathogens enteric and selectively by stimulating the growth of good microbes in the digestive tract. Administration of goat's milk containing oligosaccharides (0.5 g/kg body weight/day) has protection from inflammation that occurs in the colon induced by TBNS (Daddaoua *et al*., 2005). This oligosaccharide has good benefits as an anti-inflammatory, by reducing histamine levels (Nurliyani, 2013) or reducing MPO levels (Lara-Villoslada *et al*., 2006). Prebiotic bacteria, such as *Bifidobacterium* and *Lactobacillus* use oligosaccharides as a food source. Oligosaccharides can affect the immune system and stabilize the immune response. This can reduce the release of proinflammatory cytokines and inhibit excessive inflammatory reactions that can involve MPO (Park, 2009).

Goat milk contains bioactive peptides, lactoferrin, and protein whey as well as casein. Goat milk contains specific proteins as bioactive peptides which are released from milk proteins by enzymatic proteolysis and are anti-inflammatory (Chatterton *et al*., 2013). Bioactive peptides in goat's milk can play a role in the regulation of the digestive system and reduce gastric mucosal damage caused by ethanol. By improving the integrity of the mucosal layer, bioactive peptides can help prevent the absorption of harmful substances that can trigger inflammation (Singh *et al*., 2021), to prevent increased production of inflammatory mediators such as MPO and histamine. Lactoferrin can help protect gastric mucosal cells from the effects of ethanol. MPO is an enzyme that contributes to the production of free radicals and other reactive oxygen species, which cause oxidative stress and cell damage. Lactoferrin works by inhibiting free radical chain reactions caused by ethanol so that lactoferrin can inhibit MPO activity (Alkaisy *et al*., 2023). The main proteins in milk are casein and protein whey where in fractionated casein α-, β- and k-casein and protein whey including α-lactabumin, β-lactabumin

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with a concentration in goat's milk of 17.7 mg/mL, whereas in cow's milk, it is 11.74 mg/mL (Susanti *et al.*, 2016).

Protein whey is an amino acid that contains sulfur, such as cysteine and methionine and can improve the function of the immune system through intracellular conversion to glutathione. Glutathione has an important role in the stability of cell membranes and has cell protection that is affected by free radicals such as the induction of 80% ethanol which increases the production of free radicals ROS (Wong and Watson, 1995). This was also proven by the results of the study which showed an increase in SOD levels in the group given etawa goat's milk compared to ethanol control.

Protein whey contained in goat's milk can reduce the expression of cytokines associated with an increase in histamine cytokines (T_H2) which results in mast cell degranulation and inflammation caused by histamine. In anti-inflammatory, bioactive peptides such as Lactoferrin which is a milk glycoprotein and is derived from whey have a role in stimulating the mucosal immune system and can inhibit the production of inflammatory cytokines in the release of histamine levels from mast cells. Based on in-vivo studies that have been carried out from milk-derived peptides anti-inflammatory properties show anti-inflammatory bioactivity in intestinal enterocolitis models (Chatterton *et al*. 2013).

Moreover, other studies prove the presence of selenium and vitamin C in etawa goat milk which can act as antioxidants. Selenium is thought to increase the glutathione sulfhydryl group (GSH). GSH has an important role in maintaining the integrity of the cell wall by removing peroxidation in cells (Yuniastuti, 2014). Besides, vitamin C can neutralize oxidative stress (Caritá *et al*., 2020), and works by donating one or two H atoms to bind to superoxide radicals, forming hydrogen peroxide and ascorbate radicals (Rahmadi and Bohari, 2018). According to previous studies, it was explained that vitamin C can suppress oxidative stress by increasing the activity of SOD and IL-4 immunologically.

Another important component in goat's milk is bioorganic sodium the highest source of bioorganic sodium in goat's milk (Getaneh *et al*., 2016). Digestion in the abdomen stores a lot of sodium and digestive disorders occur due to a lack of bioorganic sodium. Mineral deficiency inhibits the production of enzymes needed in the stomach, causing inflammation or inflammation of the stomach. Giving 80% ethanol to animal studies can reduce organic sodium in the body. In this case, sodium content in goat's milk for digestion with inflammatory disorders can have a good effect.

4. Conclusion

These studies indicate that etawa goat milk exerts gastro-protective activities in the reduction of the MPO and histamine activity and increases SOD activity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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