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FULL PAPER

The potential role of goat milk in regulating CD4+ and CD8+ T cells in ethanol 80%-induced gastritis: A research using a gastritis rat model

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Gastritis, an inflammation in the stomach, is a result of several factors, including ethanol. In this investigation, rats were used as models in order to examine how goat milk affects the ratio of CD4+ and CD8+ immune cells in the stomachs of rats suffering from gastritis. This study aimed to examine how goat milk affects the ratio of CD4+ and CD8+ T cells in gastritis rat models induced by 80% ethanol. A pre-clinical design was employed in this experimental research. In this study, male Sprague Dawley rats were assigned to five groups. The control group did not receive 80% ethanol induction and no goat milk administration. The Etl 80% group included the rats which were induced by 1 ml of 80% ethanol for three days without goat milk treatment, while the rats in the GM 0.5 group, were daily administered 0.5 ml of goat milk for 30 days, and then induced by 1 ml of 80% ethanol. The fourth group, the 1 ml GM group, included the rats for which 1 ml of goat milk was daily given for 30 days, and then they were induced by 1 ml of 80% ethanol. The GM 1.5 group, the fifth group, included the rats for which 1.5 ml of goat milk was administered for 30 days, and then induced by 1 ml of 80% ethanol. In addition, blood samples were analysed by flow cytometry. The results showed that the groups administered 1.0 ml and 1.5 ml of goat milk per day for 30 days experienced an increase in the ratio of CD4+ and CD8+ T cells in comparison to the control (Etl 80%) group. The immune response in the stomach of gastritis rats induced by 80% ethanol in the goat milk administration is influenced by an increase in the ratio of CD4+ and CD8+ T cells.

KEYWORDS

CD4+; CD8+; 80% ethanol; gastritis; goat milk.

Introduction

The gastric mucosa may become inflamed due to various factors, such as Helicobacter pylori infection, NSAIDs, and alcohol consumption, which lead to Gastritis. Ethanol, especially at high concentrations like 80%, has been shown to cause significant damage to the gastric mucosa. An inflammatory response is triggered by this damage, which worsens the condition of gastritis. Oxidative stress, cell membrane damage, and increased gastric mucosal permeability are among various mechanisms that lead to damage [1-4]. The immune system largely influences the gastritis pathogenesis. T cells, especially CD4+ and

CD8+ T cells, contribute to the gastric inflammatory process. CD4+ T cells function as helper cells that regulate the immune response by stimulating other cells through the cytokines production, while CD8+ T cells function as cytotoxic cells that directly destroy infected or damaged cells [5-7].

Goat milk, on the other hand, has always been acknowledged as a source of beneficial nutrients that have positive effects on health. It has anti-inflammatory properties and can help repair damage to the gastric mucosa. Goat milk contains various bioactive components, including fatty acids, proteins, and minerals, which can modulate the immune response and protect the gastric mucosa from damage [8-11]. However, the effect of goat milk on the ratio of CD4+ and CD8+ T cells in gastritis models induced by ethanol has rarely been studied. Therefore, this study aimed to examine how goat milk influences the ratio of CD4+ and CD8+ T cells in the stomachs of gastritis rat models induced by 80% ethanol. Accordingly, this research is expected to provide new insights into the protective potential of goat milk against gastritis and its impact on the gastric immune response.

Materials and Methods

This research employed male Sprague Dawley rats that aged 12-20 days and weighed 200-250 grams. These rats were obtained from the National Agency of Drug and Food Control and kept in a controlled environment with a 12-hour light/dark cycle and had free access to food and water. Federer's formula was utilized to determine the number of these rats. Therefore, a total of 25 rats were selected and assigned to five groups.

1. Control: No 80% ethanol induction and no goat milk administration.
2. Etl 80%: Induced by 1 mL of 80% ethanol per day for three consecutive days without goat milk treatment.
3. GM 0.5: Administered 0.5 mL of goat milk daily for 30 days, and subsequently induced by 1 mL of 80% ethanol per day for three consecutive days.
4. GM 1.0: Administered 1 mL of goat milk daily for 30 days, and subsequently induced by 1 mL of 80% ethanol per day for three consecutive days.
5. GM 1.5: Administered 1.5 mL of goat milk daily for 30 days, and then induced by 1 mL of 80% ethanol daily for three consecutive days.

Blood samples were taken from either the retro-orbital sinus or the heart if the retro-orbital sinus was not available. Behind the eye is where the retro-orbital sinus is located. Thus, it was important to collect blood carefully to avoid scratching the cornea. On the other hand, a micro-hematocrit was used to collect blood from the orbital sinus. The micro-hematocrit and vacutainer tube were prepared, and the micro-hematocrit was inserted into either the orbital sinus or medial canthus of the eye, directed towards the optic fossa under the eyeball. The micro-hematocrit was then rotated until blood was released. The blood was collected into a vacutainer tube containing an anticoagulant. Blood from the heart was collected using a 3 ml syringe, and the blood was then transferred to a vacutainer tube. Figure 1 displays the materials and methods.

Results

The Effects of Goat Milk on the Ratio of CD4+ Cells

A total of 25 rats were assigned to five groups, each containing five rats. The flow cytometry method was employed to measure CD4+ levels. Each sample was measured twice and the average was calculated. Figure 2 depicts the results of the CD4+ measurements for each rat.

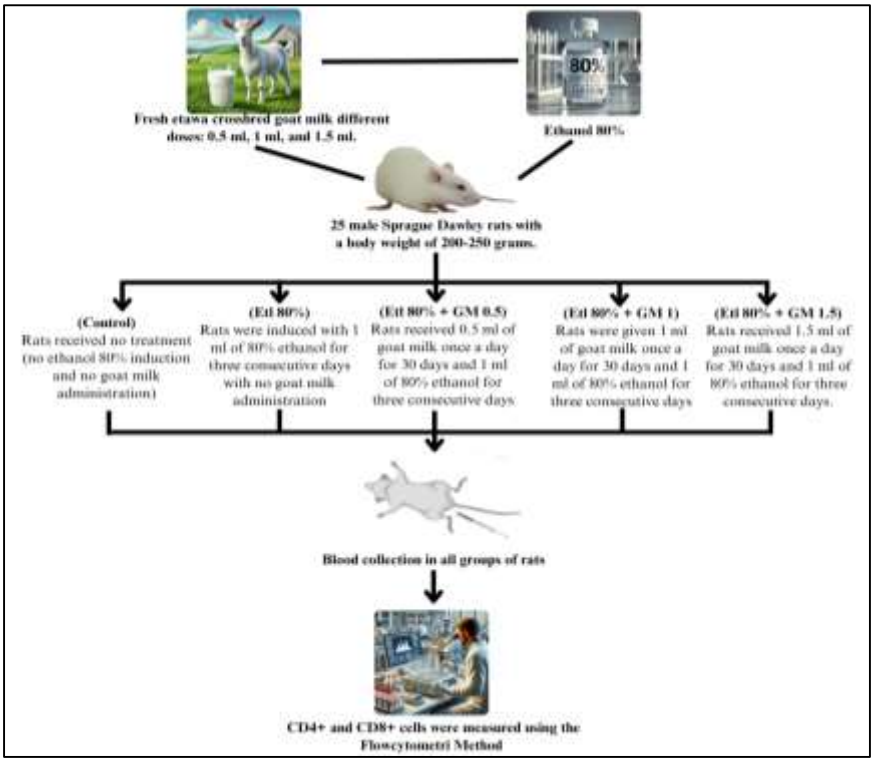

FIGURE 1 Materials and methods The experimental protocols used in this research were approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Indonesia, under protocol number KET-1019/UN2.F1/ETIK/PPM.00.02/2022, and the research was conducted in accordance with ethical guidelines for animal use.

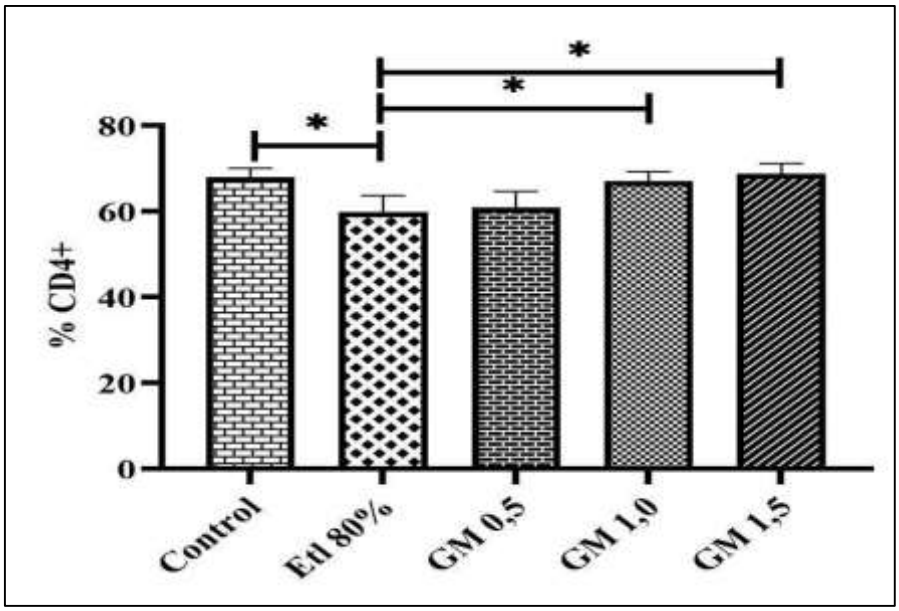

FIGURE 2 The ratio of CD4+ cells to total lymphocytes in the whole blood of gastritis rat models. The data were processed using GraphPad, and then normality and ANOVA tests were then conducted
(*Note*: Control = no 80% ethanol induction and no goat milk administration; Etl 80% = induced by 80% ethanol; GM 0.5 = administered 0.5 ml of goat milk and subsequently induced by 1 mL of 80% ethanol; GM 1.0 = administered 1.0 mL of goat milk and subsequently induced by 1 mL of 80% ethanol; and GM 1.5 = administered 1.5 mL of goat milk and subsequently induced by 1 mL of 80% ethanol).

Upon comparison between the Etl 80% group and the control group and the Etl 80% group and the GM 0.5 group, respectively, the results showed a p-value of 0.0011 and 0.9257. In addition, the results revealed a p-value of 0.0036 and 0.0005 when comparing the Etl 80% group with the GM 1.0 group and the Etl 80% group with the GM 1.5 group, consecutively. These findings indicate that the administration of 80% ethanol led to a reduction in CD4+ cells compared to the control group. Meanwhile, 80% ethanol administration increased CD4+ cells in both the GM 1.0 and GM 1.5 groups, with statistically significant differences compared to the control group.

The Effects of Goat Milk on the ratio of CD8+ Cells

In the same vein, samples of 25 rats were assigned to five groups, each with five rats. CD8+ measurements were also carried out using the flow cytometry method. Each sample was measured twice, and the average was calculated. Figure 3 demonstrates the results of the CD8+ measurements for each rat.

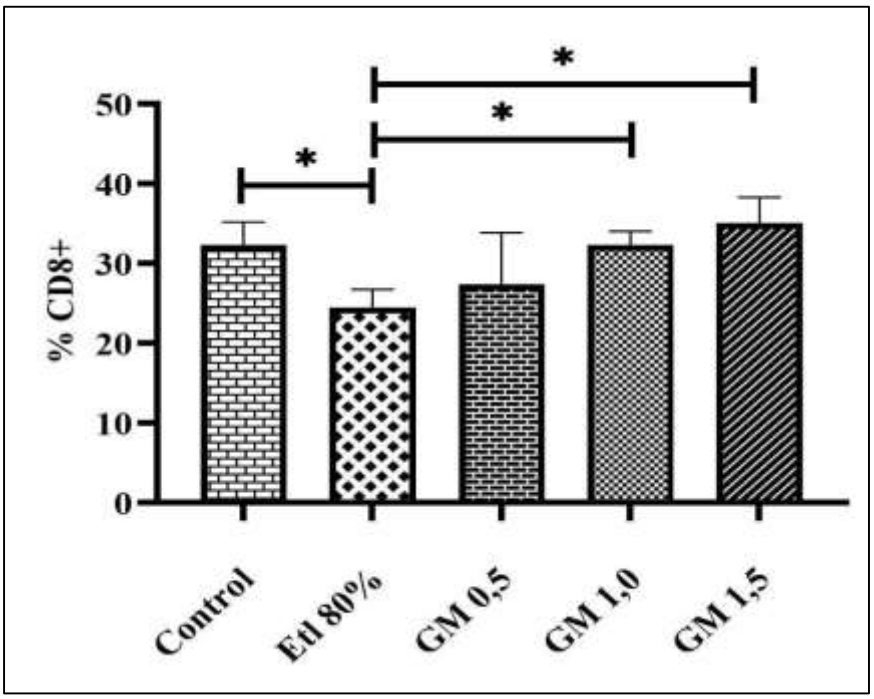

FIGURE 3 The ratio of CD8+ cells to total lymphocytes in the whole blood of gastritis rat models. The data were processed using GraphPad, and then normality and ANOVA tests were conducted
(*Note*: Control = no 80% ethanol induction and no goat milk administration; Etl 80% = induced by 80% ethanol; GM 0.5 = administered 0.5 mL of goat milk and subsequently induced by 1 mL of 80% ethanol; GM 1.0 = administered 1.0 mL of goat milk and subsequently induced by 1 mL of 80% ethanol; and GM 1.5 = administered 1.5 mL of goat milk and subsequently induced by 1 ml of 80% ethanol).

The results revealed a p-value of 0.0124 and 0.5691 when comparing the Etl 80% group with the control group and the Etl 80% group with the GM 0.5 group, respectively. The results also revealed a p-value of 0.0119 and 0.0008 when comparing the Etl 80% group with the GM 1.0 group and the Etl 80% group with the GM 1.5 group, consecutively. These results indicate that the administration of 80% ethanol reduced CD8+ cells comapred to the control group. Meanwhile, 80% ethanol administration increased CD8+ cells in both the GM 1.0 and GM 1.5 groups, with statistically significant differences compared to the control group.

Discussion

The role of CD4+ and CD8+ cells in gastritis

CD4+ and CD8+ cells are sub-populations of T lymphocytes that are crucial in the immune response. CD4+ cells, or T-helper cells, function to regulate the immune response through the activation of B cells, macrophages, and CD8+ cells. Meanwhile, CD8+ cells, or cytotoxic T cells, are responsible for attacking and killing infected or damaged cells [12-14].

The potential mechanism of goat milk in immune modulation

Goat milk contains various bioactive components, including short- and medium-chain fatty acids, vitamins, minerals, growth factors, and bioactive peptides, which have anti-inflammatory and immunomodulatory properties. Fatty acids such as caprylic and capric acids are known to have anti-inflammatory and antimicrobial activities, helping to repair ethanol-induced damage to the gastric mucosa [8,15-16].

Furthermore, goat milk is rich in selenium and zinc, both of which are crucial for immune function. The presence of selenium in antioxidant enzymes, such as glutathione peroxidase, is also crucial for protecting cells from oxidative damage. Zinc, on the other hand, is required for enzymatic activities involved in the differentiation and proliferation of T cells, including CD4+ and CD8+ cells. The combination of these factors contributes to the protective effects observed in this investigation [17-19].

In addition, the effects of goat milk on the immune system are generally attributed to the presence of bioactive components in milk, such as casein, hormones, whey, and growth factors [19,20]. Milk proteins or peptides including lactoferrin exhibit the biological properties such as protein assimilation, antibacterial activity, and the regulation of physiological functions. Milk also contains hormones such as prolactin and its analogues, which serve not only as immunomodulators, but also in tissue and organ development. The results of the present research demonstrate that goat milk administration can affect the ratio of CD4+ and CD8+ cells in the stomachs of gastritis rat models. An increased ratio of CD4+ and CD8+ cells can be explained by several mechanisms believed to involve the interaction of bioactive components in goat milk and the immune system [8,21-22].

The antioxidant and anti-inflammatory activities of goat milk

Goat milk is rich in antioxidant compounds, such as selenium and vitamin E, which are essential in reducing oxidative stress in gastric tissue. Oxidative stress often triggers excessive activation of CD8+ cells, known as cytotoxic T cells. The excessive activation of these cells can exacerbate tissue damage through the release of pro-inflammatory cytokines and toxic molecules, such as granzymes and perforin. By reducing oxidative stress, goat milk can reduce the excessive activation of CD8+ cells, ultimately decreasing their proportion [9, 23-24].

Moreover, goat milk also contains short- and medium-chain fatty acids, which have anti-inflammatory properties. For instance, caprylic acid and capric acid can inhibit the production of pro-inflammatory cytokines, such as TNF-α and IL-6, which usually trigger increased CD8+ cell activity. The reduced levels of pro-inflammatory cytokines can reduce the infiltration of CD8+ cells into the areas affected by inflammation, such as the gastric mucosa during gastritis [9,15,25].

The modulation of the immune response by fatty acids and bioactive peptides

Goat milk contains fatty acids that are essential, such as linoleic acid and linolenic acid, and they are well-known for their immunomodulatory effects. These fatty acids

can influence the function of T-helper cells (CD4+), promoting the differentiation of T-helper cells toward the antiinflammatory Th2 subset rather than the proinflammatory Th1 subset. Differentiation towards Th2 can increase the production of anti-inflammatory cytokines, such as IL-4 and IL-10, supporting an increase in the ratio of CD4+ cells and reducing inflammation. Furthermore, goat milk contains bioactive peptides that function as immunomodulating agents. These peptides can interact with specific receptors on T cells, directing the proliferation and activation of CD4+ cells. By increasing the ratio of CD4+ cells, goat milk can restore disturbed immune balance in inflammatory conditions, such as gastritis [8,26].

The effects of gut microbiota

Gut microbiota is essential in regulating immune responses, including balancing CD4+ and CD8+ cells. Goat milk has been reported with prebiotic effects, modulating gut microbiota composition by promoting the proliferation of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*. These changes in the gut microbiota can influence systemic immune responses, including in the stomach, by increasing immune regulation that supports CD4+ cell proliferation and reducing the cytotoxic activity of CD8+ cells [2,27].

Immunomodulation via macronutrients and micronutrients

Goat milk is rich in essential nutrients, such as amino acids, vitamins, and minerals, which support immune cell function. For instance, arginine, an essential amino acid found in goat milk, has been proven to enhance CD4+ cell activity by stimulating T cell proliferation and differentiation. Zinc, also present in goat milk, is necessary for the maturation and function of T cells, including CD4+ cells. By increasing the availability of these nutrients, goat milk can directly support a healthy balance between CD4+ and CD8+ cells [17,21,28].

Conclusion

To sum up, the results revealed that the immune response in the stomachs of gastritis rats induced by 80% ethanol is positively affected by goat milk administration. Therefore, it can be mentioned that goat milk administration to rat models suffering from gastritis induced by 80% ethanol can increase the ratio of CD4+ and CD8+ cells in their stomachs.

Ethical Issues

The experimental protocols used in this research were approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Indonesia, under protocol number KET-1019/UN2.F1/ETIK/PPM.00.02/2022 and the research was conducted in accordance with ethical guidelines for animal use.

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Authors' contributions

Farihatun A. designed and conducted the study, analysed, interpreted data, prepared, and revised the manuscript; Kusmardi K., Estuningtyas A., and Salleh M.N., reviewed the manuscript and provided critical input.

Conflict of Interest

The authors declared no conflict of interest was existed in this article.

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