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The Effects of Cayenne Pepper Juice (*Capsicum frutescens L.*) on Decreasing the Number of Goblet Cells in the Small Intestine Mucosa of Rats

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Abstract:

Cayenne pepper contains capsaicin and irritates the mucous of the digestive tract. Goblet cells play a role in mucosal defense because they produce mucin. In previous research, cayenne pepper juice caused gastric mucosal damage and improve at certain doses. This Research is a continuation of previous research, which was to determine the effect of cayenne pepper on the number of goblet cells of the small intestine mucosa of rats and is additional material for the development of therapy for digestive disorders. An experimental study with a post-test only and control group design, used 20 male rats, divided into 1 control (K) and 4 treatment groups (C45, C90, D135, and E180). Cayenne pepper juice was given according to the dose of the group using a gastric probe for 6 days. On the 7th day, the small intestine was taken and processed on histological preparation. Microscopic observations of goblet cells counted on average every 100 epithelia in 20 villi in 15 fields of view and the mean number was tested by One Way ANOVA and Post Hoc test. Analysis showed a significantly different mean number of goblet cells between groups ($p<0.05$), the highest was in the C135 (34.100 ± 1.274), the lowest mean was in the K (12.050 ± 0.462), and there was a decrease in the mean number in the C180 (28.525 ± 2.309). Cayenne pepper juice had effected the highest increase in the number of goblet cells at a dose of 135 mg and decreased at the dose of 180 mg of cayenne pepper in the small intestine of rats.

Keywords: Capsicum frutescens L; Goblet Cells; Small Intestine

Introduction

Cayenne pepper is a food ingredient that is consumed by the entire world population, including in Indonesia.[1] Capsaicin contained in cayenne pepper not only stimulates endorphins that can increase appetite but also acts as an anti-inflammatory agent.[2,3] On the other hand, consumption of capsaicin can cause irritation and inflammation in the stomach with an increase in polymorphonuclear (PMN) inflammatory cells.[4] The effect of capsaicin also occurs in the liver, which is characterized by parenchymatous degeneration.[5] Capsaicin is associated with pain receptors that are sensitive to the sensation of heat in the mouth and throat. A burning sensation also occurs when exposed to the gastric mucosal wall, increasing gastric acid secretion.[6] The food consumed will enter the digestive tract which consists of the mouth, pharynx, esophagus, stomach, and small intestine including the duodenum, jejunum, ileum, large intestine or colon, rectum, and anus.[7] Before the absorption of nutrients by the small intestine occurs, the digestive tract will detect the presence of pathogens that enter with food and carry out a self-defense mechanism to kill pathogens.[8]

Goblet cells are basophil columnar cells that are scattered between the epithelial layers of the digestive tract, conjunctiva, and respiratory tract. In the intestinal mucosa, goblet cells secrete the high molecular weight glycoprotein MUC2.[9] Pathogenic factors and host immune responses determine the number of goblet cells in the epithelium and the production of mucin that will be used to deal with pathogens.[10] In a previous study by Philip Teng, et al in 2013, giving cayenne pepper juice to rats showed an increased gastric mucosal damage effect from doses of 45mg, 90mg to 135mg. This damaging effect decreased at a dose of 180 mg and showed improvement in gastric mucosa. Research on the effect of cayenne pepper on goblet cells of the mucosa of the small intestine of rats is a continuation of previous research and has never been done. This study aims to determine the histology of goblet cells in the small intestine mucosa of rats (Rattus norvegicus) given cayenne pepper (Capsicum frutescens L.) juice. This research is an additional means of information in the development of therapy for the management of digestive disorders

Research Method

The study used a post-test only with a control group design with 20 male rats, body weight 200 grams, 3-4 months old. The number of samples used refers to the calculation of the formula by Wan Nor Arifin and Wan Mohd Zahiruddin11. The mice were divided into 5 groups, namely the control group (K) and the treatment group, namely the C45, C90, C135, and C180 groups. The treatment given was in the form of cayenne pepper juice for 6 days in each group by dosage as seen in the table.1

Table 1. Control and Treatment Group

No.	Group	Treatment
1	K	Standard feed and drink
2	C45	Cayenne Pepper 45 mg, standard feed, and drink
3	C90	Cayenne Pepper 90 mg, standard feed, and drink
4	C135	Cayenne Pepper 135 mg, standard feed, and drink
5	C180	Cayenne Pepper 180 mg, standard feed, and drink

The selection of cayenne pepper was based on a qualitative test of the character of cayenne pepper in a study conducted by Agustina, K., et al. (2021) namely the color of the skin of the young fruit varies between white, yellowish green and light green, while

the color of the skin of the old fruit varies between red, bright red and red old.[12]

Results

The results of microscopic observations of goblet cells for every 100 epithelial cells in 20 villi in 15 fields of view show the average number of goblet cells for each treatment group as shown in table 2.

Table 2. Average Number of Goblet Cells for Each Treatment Group

Group	Goblet Cell Count
Control (K)	12,050 ± 0,462[a]
Cayenne pepper 45 mg (C45)	21,475 ± 1,543[b]
Cayenne pepper 90 mg (C90)	31,175 ± 0,363[cd]
Cayenne pepper 135 mg (C135)	34,100 ± 1,274[d]
Cayenne pepper 180 mg (C180)	28,525 ± 2,309[c]

Notes: The average number of goblet cells in the control group (K) has the lowest mean number of goblet cells. The C135 group had the highest mean amount and then decreased in the C180 mg group.

Table 2. shows that the control group (K) had an average goblet cell count of 12,050 ± 0,462 which showed the lowest mean number of goblet cells compared to other groups. In the treatment group, the C45 group shows the lowest mean number of goblet cells with an average goblet cell count of 21,475 ± 1,543. Different from the treatment C135 group, those who were given cayenne pepper juice at a dose of 135 mg had the highest mean number of goblet cells compared to the other groups with an average goblet cell count of 34,100 ± 1,274. The mean number of goblet cells increased in line with the increase in the number of doses of cayenne pepper juice from a dose of 45 mg to a dose of 135 mg then the average number of goblet cells decreased, namely in the C180 mg group (figure 1).

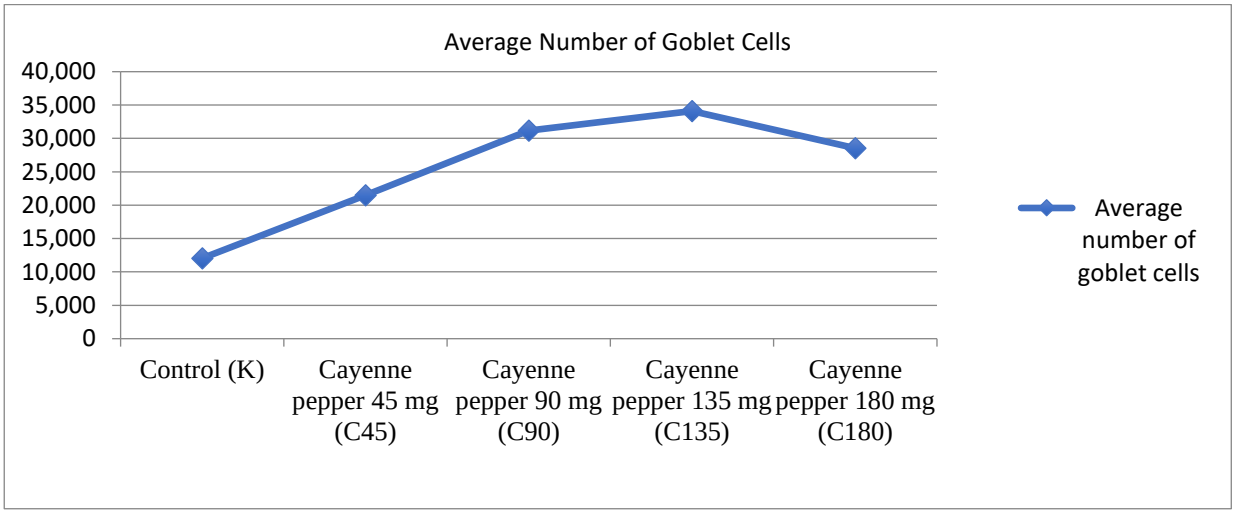

Figure 1. The average number of goblet cells increased significantly from the 45mg dose to the highest dose at 135 mg then decreased at a dose of 180 mg

The normality test of the data using the Shapiro-Wilk test showed that the data were normally distributed (p>0.05). Data analysis using the One-Way ANOVA test showed that the mean data on the number of goblet cells had a significant difference between groups (p<0.05) (table.2). Duncan's Post Hoc test showed that the mean number of goblet cells in the control group (K) and C45 were significantly different from the other groups, the C90 group was significantly different from the K and C45 groups, the C135 group was significantly different from the control group (K), C45, and C180, and the C180 group was significantly different from the control group (K), C45, and C135 (table.1).

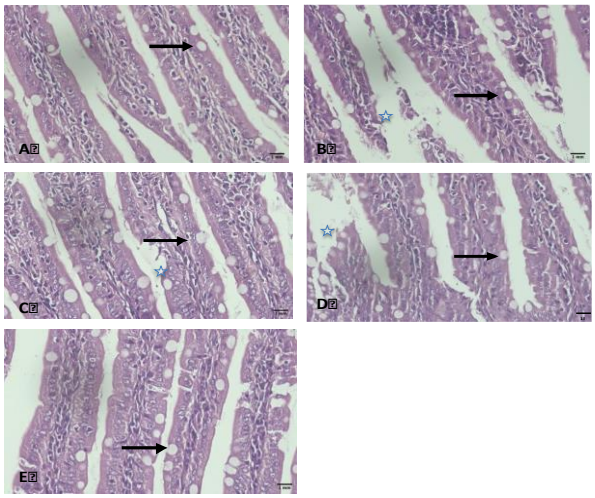

Figure 2. Histology of the small intestine of rats (Rattus norvegicus), arrows indicate goblet cells in groups, star indicate erosion in epithelium mucosa; control (A), the treatment group with cayenne pepper juice at a dose of 45 mg (B), the treatment group with cayenne pepper juice at a dose of 90 mg (C), the treatment group with cayenne pepper juice at a dose of 135 mg (D), the treatment group with cayenne pepper juice at a dose of 180 mg (E). (HE, 400x).

Discussion

This study reported that administration of cayenne pepper juice starting at a dose of 45 mg to 135 mg showed an increase in the mean number of goblet cells. The C45 group show the lowest mean number of goblet cells with an average goblet cell count of 21,475 ± 1,543 and the C135 group had the highest mean number of goblet cells compared to the other groups with an average goblet cell count of 34,100 ± 1,274. The difference in the mean number between the control group (K) and the treatment group also showed a significant difference. A study conducted by Ermund, A., et al. (2013) showed that the total number of goblet cells in the small intestinal mucosal epithelium of rats was 11,000-12,000.[13] This indicates that the administration of cayenne pepper juice in this research causes inflammation in the small intestine epithelium. Inflammation can trigger goblet cells to produce mucin. This is by following research by Philip Teng et.al., (2013) which states that capsaicin in cayenne pepper triggers irritation of the digestive tract wall. [4]

This inflammation is caused because the capsaicin in cayenne pepper as a pro-inflammatory agent is detected by the Transient Receptor Potential Vanilloid receptor subtype 1 (TRPV1) which is found in primary afferent nerve membranes for pain and heat sensations. TRVP1 is located along the gastric gland mucosa, around the blood vessels of the submucosal layer, myenteric plexus, and smooth muscle layer. The gastrointestinal mucosal defense mechanism is carried out by TRVP1 when inflammation triggers hypersensitivity in afferent tissue.[14] Inflammation triggers small intestinal barrier agents, namely goblet cells to increase mucin production.[15,16]

In this study, the inflammatory process in the treatment group was indicated by the histology of the small intestine mucosa in the form of erosions to mucosal ulcers (figure 2).

Erosion has eroded the surface of the mucosal epithelium triggered by an inflammatory. Mucous ulcers are a disconnection of the epithelial mucosa so that it becomes incomplete mucosa.[17] This erosion will affect the increase in the number of goblet cells.

Under normal conditions, goblet cells homeostasis by producing mucin to influence pathogens and suppress inflammation.[14] The presence of secretions from pathogens detected by the Toll-Like Receptor (TLR) will activate the immune system in a self-defense mechanism.[18] Mucin produced by goblet cells serves to coat the epithelial cells. The released H+ ions activate TRVP1 in afferent nerve fibers and release calcitonin gene-related peptide (CGRP) to relax submucosal arteriolar smooth muscle. This trigger increased blood flow and stimulates mucin secretion by goblet cells on epithelium.[19]

The role of the immune system in increasing the number of goblet cells and production of mucin is influenced by Toll-Like Receptors (TLRs) located on the cell surface or on intracellular endosomes which are then activated by pathogen-associated molecular patterns (PAMPs). Toll Like Receptor 5 (TLR5) present in goblet cells in the small intestine functions as a detection organelle to stimulate MUC2 mucin secretion which is then used to remove pathogens from crypts. Proinflammatory substances such as IL-1, IL-6, and TNF- can increase MUC2 mucin production due to the mediation of type 2 T-helper cell cytokines that induce goblet cell hyperplasia (Th2).[20]

In this study, the C180 group who was given a dose of 180 mg of cayenne pepper showed a decrease in the mean number of goblet cells in the small intestine. This could be due to the effect of repairing the small intestine mucosa. Decreased mucosal inflammation causes a decrease in the activity of goblet cells in producing mucin. This is in line with the results of research conducted by Philip Teng et.al., 2013 that the effect of cayenne pepper on the gastric mucosa showed a mucosal repair effect at a dose of 180 mg of cayenne pepper juice. This mucosal repair can be caused by the content of other cayenne peppers, namely flavonoids and vitamin C which act as anti-inflammatory, antibacterial, and antioxidant.[21,22] Flavonoids can inhibit the accumulation of leukocytes thereby inhibiting the inflammatory process.[23,24] This study is in line with research by Philip Teng et.al., 2013 which is suspected that the anti-inflammatory effect of cayenne pepper only appears at a certain dose, namely at a dose of 180 mg according to the results of this study.

Conclusions

The administration of cayenne pepper (Capsicum frutescens L.) juice had an effect in increasing the number of goblet cells in the small intestine of rats (Rattus norvegicus) starting at doses of 45 mg, 90 mg, to 135 mg. The decrease in the number of goblet cells was seen in the administration of cayenne pepper juice at a dose of 180 mg. The results of the study on the effect of cayenne pepper juice in the repair of inflamed small intestinal mucosal epithelium can be a means of additional information in the development of treatment therapies for digestive disorders.

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