Effectiveness of Red Onion Extract (Allium Cepa) on the Elongation of Papillae and Neutrophil Cell in the Gastroesophageal Junction (GEJ) of Sprague Dawley Strain Rats Gastroesophageal Reflux Disease (GERD) Model

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ABSTRACT

Background: Gastroesophageal acid reflux disease (GERD) is a disorder caused by the reflux of stomach acid when the lower esophageal sphincter (LES) relaxes with a prevalence of around 1.03 billion worldwide. One of the supporting examinations carried out to diagnose GERD is a histopathological examination. This study aims to determine the effect of red onion extract on the papillae elongation and neutrophil cells of the gastroesophageal junction in Sprague Dawley rats.

Methods: This experimental study was conducted on Sprague Dawley rats induced with 1 mL of HCl + 1 mL of ascorbic acid, administered into the gastric cardia of the rats, mixed with 1 mL of sodium thiocyanate (NaSCN) and 1.5 mL of sodium nitrite (NaNO₃) to develop a GERD model. The rats were then divided into ten groups with different treatments, including red onion extract (250, 500, 750 mg/kgBW) and PPI (pantoprazole).

Results: It was stated that there was a significant relationship between the effect of red onions and changes in papillae elongation in rats induced by GERD (P-value <0.05). The neutrophil cells showed no significance on the impact of red onion (p-value >0,05). Nor are the papillae elongation and the neutrophil cell changes dose-dependent.

Conclusion: A significant reduction in the histopathological appearance of papillae elongation was seen in the majority of the population. However, no significant results were found for the different doses of shallot extract.

Keywords: Gastroesophageal acid reflux disease, allium cepa, papillae elongation, red onion extract, neutrophil cells

ABSTRAK

Latar belakang: Gastroesophageal acid Reflux Disease (GERD) adalah gangguan akibat refluks asam lambung saat lower esophageal sphincter (LES) relaksasi dengan prevalensi sekitar 1,03 miliar penderita. Salah satu pemeriksaan pendukung yang dilakukan untuk mendiagnosa GERD adalah pemeriksaan histopatologi. Studi ini bertujuan untuk mengetahui efek ekstrak bawang merah pada elongasi papillae dan sel neutrofil gastroesofageal junction tikus sprague dawley.

Metode: Studi eksperimental dilakukan pada hewan coba (tikus strain Sprague Dawley) yang diinduksi dengan HCl 1 mL + asam askorbat 1 mL dimasukkan ke dalam kardia lambung tikus, dicampur dengan natrium tiosianat (NaSCN) 1 mL dan natrium nitrit (NaNO₃) 1,5 mL untuk menghasilkan model GERD. Tikus ini lalu dibagi menjadi sepuluh kelompok dengan perlakuan berbeda-beda, yaitu dengan ekstrak bawang merah (250, 500, 750 mg/kgBB) dan PPI (pantoprazole).

Hasil: Pada hasil penelitian gambaran histopatologi neutrofil menunjukkan hasil yang tidak signifikan (p>0,05), sedangkan terdapat hasil yang signifikan pada gambaran elongasi papillae (p>0,05). Efek ekstrak bawang merah sebagian besar tidak bergantung pada dosis, baik 250,500, maupun 750 mg/kgbb menunjukkan hasil yang signifikan pada pemendekkan elongasi papillae.

Simpulan: Penurunan gambaran histopatologi elongasi papillae terlihat secara signifikan pada sebagian besar populasi. Namun, tidak ditemukan hasil yang signifikan pada perbedaan dosis ekstrak bawang merah.

Kata kunci: Penyakit asam lambung, allium cepa., pemanjangan papila, ekstrak bawang merah, sel neutrofil

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a condition caused by the reflux of stomach acid due to the relaxation of the lower oesophageal sphincter (LES).[1] It can be identified microscopically by features such as inflammatory cell infiltration, papillae elongation, and basal cell hyperplasia.[2] GERD is highly prevalent worldwide, with a 2020 systematic review indicating that about 13.98% of the global population, approximately 1.03 billion people, suffer from it.[3] In Indonesia, the prevalence reaches 4.9%.[4] Several risk factors contribute to GERD, including age, high BMI, anxiety disorders, and dietary habits that increase stomach acid production.[1,5] Chronic exposure to acid reflux leads to structural damage in the oesophagus, worsened by oxidative stress and the production of reactive oxygen species (ROS), which further increases inflammation and cellular damage.[2,6,8] The longer the elongation and the more the neutrophil cell in one place indicate more damage to the mucosal lining.[2]

According to the consensus made by the Indonesian Gastroenterological Association, Treatment for GERD focuses on symptom relief, oesophageal healing, prevention of recurrence, quality of life improvement, and avoidance of complications.[13] Pharmacological treatments, particularly Proton Pump Inhibitors (PPIs) like Pantoprazole, are currently the most effective therapy.[7] However, long-term use of PPIs is associated with side effects such as hypomagnesemia, iron deficiency, and kidney disease. Due to these risks, researchers are exploring alternative, natural treatments that may offer similar benefits without adverse effects.[9]

Red onions are one of the plants that are widely used ingredients in Indonesia and are known to have natural properties potential for the treatment of Gastroesophageal acid Reflux Disease (GERD).[10] Red onions contain flavonoids with anti-inflammatory and antioxidant properties, particularly quercetin, which enhances the body's antioxidant capacity by regulating glutathione (GSH) levels. GSH helps neutralize oxidative stress, reducing cellular damage. Animal and cell studies suggest that quercetin stimulates GSH synthesis, providing potential benefits for GERD management.[11,12] Research by Windari T demonstrated that ethanol-extracted onion injections reduced oxidative stress in rats, highlighting the potential of red onion extract as an alternative GERD treatment.[10,11,14] This study aims to see the correlation between the antioxidant property of red onion extract and the reduction of histopathology markers for GERD, in this case, the reduction of papillae elongation and neutrophil cells.

METHODS

The protocol of the research had been ethically accepted by the Research Ethics Committee of the Faculty of Medicine and Health Science, Atma Jaya Catholic University of Indonesia (No.27/01/KEP-FKIKUAJ/2024). The research design was experimentally carried out on experimental animals (Sprague dawley strain rats). Rats were induced with 1 mL HCl + 1 mL ascorbic acid inserted into the rat gastric

cardia, mixed with 1 mL sodium thiocyanate (NaSCN) and 1.5 mL sodium nitrite (NaNO3) to produce a GERD model. These rats were then divided into ten groups with different treatments: red onion extract (250, 500, 750 mg/kgBB) and PPI (pantoprazole). This research took several samples using the Degree of Freedom method, with the following formula;

$$E = (k \times n) - k$$
$$N = k \times n16$$

E: The acceptable range of Degree of Freedom in ANOVA is between 10 - 20, if < 10 then, the number of samples per group needs to be increased; > 20 then, the additional samples do not affect the distribution of the samples.

N: Total number of research samples

K: Number of research groups

N: Number of research samples per research group

This are the following calculation for the number of samples;

E min = (k x n) - k

10 = (10 x n) - 10

10 = 10n - 10

20 = 10n

n = 2

N = k x n = 10 x 2 = 20

E max = (k x n)–k

20 = (10 x n) - 10

20 = 10n-10

30 = 10n

n = 3

N = k x n = 10 x 3 = 30

The total number of samples taken was 32 Rats and The Data collection was carried out from July 2024 to August 2024. Two criteria must be met to proceed with the experiment, the criteria are:

Inclusion criteria:

- The Sprague Dawley rat has to be a male
- The Sprague Dawley age must be minimal 12 weeks

Exclusion criteria:

- The Sprague Dawley died before intervention.
- The Sprague Dawley died during the intervention.

Therefore, the researcher added two additional rats in every group to ensure the number of samples met the number needed for the research based on the degree of freedom equation. After the intervention, histology preparations were made, HE stained and examined using a microscope with 400X magnification in 5 fields of view. The collected data results were then analyzed using the ANOVA correlation test followed by post hoc and Kruskal Wallis tests to calculate the correlation of both variables.

RESULTS

The results of the descriptive analysis showed that in the papilla elongation variable, the positive control had the highest mean elongation percentage (72.29 ± 3.70 %), while the group given 500 mg red onion extract for 7 days had the lowest mean elongation

Table 1. Descriptive Analysis of Group Papillae Elongation Ratio and Neutrophil Cells

Variabel	Groups	n	Mean	SD	Median (min-max)
Elongasi Papilla	Negative Control	3	46,51	11,33	52,84 (33,42-53,27)
	Positive Control	4	72,29	3,70	72,78 (67,32-76,30)
	PPI 7 Days	3	42,88	14,68	48,31 (26,25-54,08)
	PPI 14 Days	3	49,44	4,76	47,53 (45,93-54,86)
	Red onion 250 mg 7 days	3	47,65	5,40	49,26 (41,63-52,08)
	Red onion 500 mg 7 days	4	41,88	4,05	42,78 (36,21-45,77)
	Red onion 750 mg 7 days	2	54,24	1,21	54,24 (53,38-55,10)
	Red onion 250 mg 14 days	4	49,30	8,89	47,08 (41,24-61,80)
	Red onion 500 mg 14 days	3	46,26	8,83	44,83 (38,24-55,73)
	Red onion 750 mg 14 days	3	45,48	5,52	43,51 (41,21-51,72)
Jumlah Neutrofil	Negative Control	3	0,60	0,40	0,60 (0,20-1,00)
	Positive Control	4	2,20	0,28	2,10 (2,00-2,60)
	PPI 7 Days	3	0,73	0,46	1,00 (0,20-1,00)
	PPI 14 Days	3	0,60	0,20	0,60 (0,40-0,80)
	Red onion 250 mg 7 Days	3	3,53	0,70	3,60 (2,80-4,20)
	Red onion 500 mg 7 Days	4	2,50	1,58	3,00 (0,20-3,80)
	Red onion 750 mg 7 Days	2	2,70	1,27	2,70 (1,80-3,60)
	Red onion 250 mg 14 Days	4	1,40	1,39	1,10 (0,20-3,20)
	Red onion 500 mg 14 Days	3	1,26	0,83	1,00 (0,60-1,60)
	Red onion 750 mg 14 Days	3	1,33	0,12	1,40 (1,20-1,40)

n=Number of Samples ; SD=Standard Deviation

percentage (41 .88±4.05 %). In the variable number of neutrophils the group given 250 mg of red onion for 7 days had the highest mean amount (3.53 ± 0.70), while the negative control group and 14-day PPI had the lowest mean amount.

The results of the comparison test showed that the papilla elongation variable had a significant difference between groups (P<0.05), so further post hoc analysis using LSD was carried out. Meanwhile, in the variable number of neutrophils, no significant differences were found between groups (P>0.05), so post hoc analysis was not carried out.

The results of the comparison test showed that the papilla elongation variable had a significant difference between groups (P<0.05), so further post hoc analysis using LSD was carried out. Meanwhile, in the neutrophil count variable, no significant differences were found between groups (P>0.05), so post hoc analysis was not carried out. Furthermore, in the post hoc analysis of the papilla elongation variable, it can be concluded as follows:

1. The negative control and positive control groups showed significant differences in papilla elongation, indicating that the papilla elongation in the positive control group was significantly greater than that in the negative control group.

2. The positive control group showed a significant difference in papilla elongation (P<0.05) with all treatment groups, with a higher mean elongation value than the other groups. These results indicate that giving PPI for both 7 and 14 days, as well as giving red onion extract of 250, 500, and 750, both 7 and 14 days, had a lower mean elongation compared to the positive control group.

3. No significant differences were found between treatment groups indicating the same effectiveness of shallot extract in reducing papilla elongation.

Table 2. Comparative Analysis of Papilla Elongation between Groups

Variabel	NC	PC	P1	P2	RO1	P
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Elongasi Papilla	46,51±11,33	72,29±3,70	42,88±14,68	49,44±4,76	47,65±5,40	0,001*
	RO2	**RO3**	**RO4**	**RO5**	**RO6**	
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	
	41,88±4,05	54,24±1,21	49,30±8,89	46,26±8,83	45,48±5,52	

*p<0,05; Description : NC=Negative Control PC= Positive Control; P1= PP1 7 days; P2=PPI 14 days; RO1= Red Onion 250 mg 7 days RO2= Red Onion 500 mg 7 days; RO3= Red Onion 750 mg 7 days; RN4= Red Onion 250 14 days; RO5= Red Onion 500 mg 14 days; RO6=Red Onion 750 14 days.

Table 3. Comparative Analysis of Neutrophil Cells between Groups

Variabel	KN	KP	P1	P2	BM1	P
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Jumlah Neutrofil	0,60±0,40	2,20±0,28	0,73±0,46	0,60±0,20	3,53±0,70	0,051
	BM2	**BM3**	**BM4**	**BM5**	**BM6**	
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	
	2,50±1,58	2,70±1,27	1,40±1,39	1,26±0,83	1,33±0,12	

Description : NC=Negative Control PC= Positive Control; P1= PP1 7 days; P2=PPI 14 days; RO1= Red Onion 250 mg 7 days RO2= Red Onion 500 mg 7 days; RO3= Red Onion 750 mg 7 days; RN4= Red Onion 250 14 days; RO5= Red Onion 500 mg 14 days; RO6=Red Onion 750 14 days.

Table 4. Post Hoc Analysis Comparison of Elongstion of Papillae Ratios Between Groups

	Differences in Mean Papillae Elongation	P
NC dan PC	-25,78	0,000*
NC dan P1	3,63	0,575
NC dan P2	-2,93	0,650
NC dan RO1	-1,14	0,859
NC dan RO2	4,62	0,446
NC dan RO3	-7,73	0,289
NC dan RO4	-2,79	0,644
NC dan RO5	0,24	0,970
NC dan RO6	1,03	0,873
PC dan P1	29,42	0,000*
PC dan P2	22,85	0,001*
PC dan RO1	24,64	0,000*
PC dan RO2	30,41	0,000*
PC dan RO3	18,05	0,014*

	Differences in Mean Papillae Elongation	P
PC dan RO4	22,99	0,000*
PC dan RO5	26,03	0,000*
PC dan RO6	26,81	0,000*
P1 dan P2	-6,56	0,314
P1 dan RO1	-4,77	0,461
P1 dan RO2	0,99	0,869
P1 dan RO3	-11,36	0,125
P1 dan RO4	-6,42	0,293
P1 dan RO5	-3,38	0,600
P1 dan RO6	-2,60	0,687
P2 dan RO1	1,78	0,782
P2 dan RO2	7,55	0,218
P2 dan RO3	-4,80	0,507
P2 dan RO4	0,13	0,982
P2 dan RO5	3,17	0,623
P2 dan RO6	3,96	0,541
RO1 dan RO2	5,76	0,343
RO1dan RO3	-6,58	0,365
RO1dan RO4	-1,64	0,785
RO1dan RO5	1,39	0,829
RO1dan RO6	2,17	0,736
RO2dan RO3	-12,35	0,081
RO2 dan RO4	-7,41	0,193
RO2 dan RO5	-4,37	0,470
RO2dan RO6	-3,59	0,553
RO3 dan RO4	4,93	0,473
RO3 dan RO5	7,97	0,275
RO3 dan RO6	8,76	0,232
RO4 dan RO5	3,03	0,615
RO4 dan RO6	3,82	0,528
RO5 dan RO6	-0,78	0,903

*p<0,05; Description : NC=Negative Control PC= Positive Control;
P1= PP1 7 days; P2=PPI 14 days; RO1= Red Onion 250 mg 7 days
RO2= Red Onion 500 mg 7 days; RO3= Red Onion 750 mg 7 days;
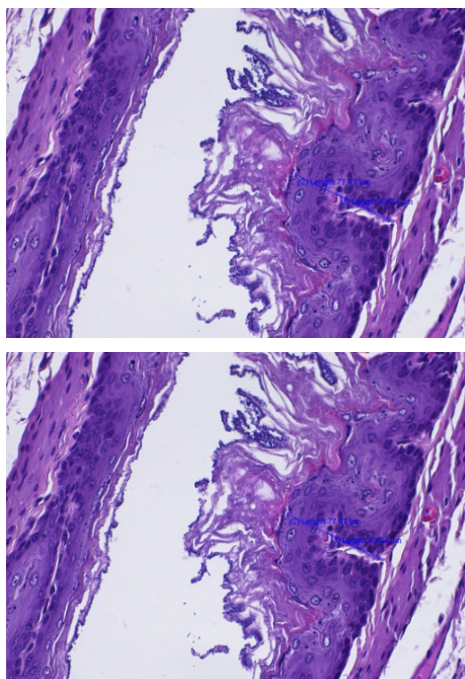RN4= Red Onion 250 14 days; RO5= Red Onion 500 mg 14 days;
RO6=Red Onion 750 14 days.

Figure 1. Papillae elongation dan neutrophil cells of the Gastroesophageal Junction (GEJ) of rats (400x)

DISCUSSION

The histological changes in GERD include intercell epithelial dilatation, basal hyperplasia, papillae elongation, and inflammatory cells. The death of surface cells caused by acid is believed to trigger the proliferation of basal progenitor cells, leading to papillae elongation and drawing granulocytes to the area.[18] Acid reflux triggers the increase in cytokine and creates an imbalance of the redox system, which increases oxidative stress.[19]

In this study, there was a significant relationship between the effect of red onions and changes in papillae elongation in rats induced by GERD (P-value <0.05). Changes in papillae elongation align with research conducted by Windari T., which states a significant relationship between red onion extract and reduced oxidative stress.[10] This study also used three doses of red onion extract, namely 250mg/kg bb, 500 mg/kg bb, and 750 mg/kg bb. The doses are then converted into the appropriate experimental animal dose. The

different doses are not in line with research conducted by Windari T., who stated that there was a significant relationship (P-value <0.05) between the doses used of Red onion extract.[10] According to research conducted by Kavita Thakur et al. Elongation of the papillae is one of the most sensitive and significant histological features for seeing changes in patients with GERD. It shows that more than 60% of patients with GERD have this structural change.[15]

Apart from that, a test was carried out on neutrophil cells in this study. However, the results showed no significant results in the test according to the Kruskal Wallis test (P-value >0.05). The results of this study are considered inconsistent with research conducted by Windari T, who stated that there was a significant relationship between the decrease in neutrophil cells in rats given red onion extract and the group not given the red onion extract.[10] This study also had different results from research conducted by Narges Marefati et al., who stated that red onion extract with ethanol could reduce inflammatory cells significantly.[11] Furthermore, the effect of red onion extract on neutrophil count did not yield significant results, likely because of their nonspecific involvement in GERD pathophysiology.[20]

In addition, according to Takubo et al., histologic changes usually associated with reflux do not always appear in a small proportion of biopsy samples examined. Although such histologic changes may appear, they are not typical in patients with GERD. The results showed that these changes were seen individually in 23% to 71% of patients with GERD, but no single typical histologic change was found in all patients (100%).[17]

CONCLUSION

This research shows that red onion extract can significantly reduce papilla elongation in rats induced by GERD, with effectiveness comparable to Proton Pump Inhibitor (PPI) drugs. However, variations in the dose of red onion extract (250 mg/kg bb, 500 mg/kg bb, and 750 mg/kg bb) did not provide a significant difference in the histopathological picture, in contrast to the results of previous studies, which showed a dose effect. In addition, the impact of red onion extract on the number of neutrophils did not show significant results, possibly due to the acute nature of neutrophils and their nonspecific role in GERD pathophysiology. The researchers suggest that to help pathologists identify this set of histologic changes, endoscopists need to perform targeted biopsies from multiple sites with different endoscopic findings.

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