Volume 6, Nomor 2, Juni 2025 **ISSN : 2774-5848 (Online)**
ISSN : 2777-0524 (Cetak)

HISTOPATHOLOGICAL OVERVIEW OF SMALL INTESTINE IN RATS MODEL OF INFLAMMATION INDUCED BY CHILI PEPPER

Sherly Usman[1*], Hanifah Nurusyifa[2]
Department of Histology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta[1], *Medical Faculty and Health Sciences*, Universitas Muhammadiyah Yogyakarta[2]
[*]*Corresponding Author :* sherlyusman@umy.ac.id

ABSTRAK
Cabai rawit memiliki sifat antioksidan namun di sisi lain memicu inflamasi yang mengatur sel makrofag untuk memproduksi sitokin. Tujuan penelitian ini adalah untuk mengetahui gambaran histopatologi usus halus pada tikus model inflamasi yang diinduksi cabai rawit. Penelitian ini merupakan penelitian quasi eksperimental dengan rancangan posttest only control group design. Sebanyak 20 ekor tikus wistar putih dibagi menjadi kelompok kontrol (K) dan 4 kelompok perlakuan (C45, C90, C135, dan C180). Kelompok perlakuan diberikan infusa cabai rawit masing-masing 45 mg, 90 mg, 135 mg, dan 180 mg selama 6 hari. Pada hari ke-7 terminasi, usus halus dibuat preparat histologi pewarnaan HE. Data skoring derajat perdarahan dianalisis dengan menggunakan uji normalitas Saphiro Wilk, uji parametrik One Way Anova dan uji Post Hoc. Hasil penelitian menunjukkan adanya perdarahan epitel pada kelompok perlakuan. Perdarahan paling berat terjadi pada kelompok C135 dan perbaikan terjadi pada kelompok C180. Derajat perdarahan bermakna ($p<0,05$) antara kelompok kontrol dengan kelompok C135 dan C180, kelompok C45 dengan kelompok C135, dan kelompok C45 dengan kelompok C180. Kesimpulan: Gambaran histopatologi mukosa usus halus berupa perdarahan epitel paling berat pada dosis infus cabai rawit 135 mg/hari dengan perbaikan mukosa pada dosis infus 180 mg/hari.

Kata kunci **:** infus cabai rawit, mukosa usus

ABSTRACT
Chili pepper has antioxidants but on the other hand triggers inflammation that regulates macrophage cells to produce cytokines. The purpose of the study was to determine the histopathological description of the small intestine in rats' model of inflammation induced by chili pepper. This was a quasi-experimental research with posttest only control group design. 20 white wistar rats were divided into control group (K) and 4 treatment groups (C45, C90, C135, and C180). The treatment group was given cayenne pepper infusion, respectively 45 mg, 90 mg, 135 mg, and 180 mg for 6 days. On the 7th day of termination, the small intestine was made with HE staining histology preparations. Scoring data on the degree of bleeding were analyzed by using Saphiro Wilk for normality, One Way Anova parametric test and Post Hoc test. The results showed epithelial bleeding in the treatment group. Heaviest bleeding in the C135 group and improvement in the C180 group. The degree of bleeding was significant ($p<0.05$) between the control group and the C135 and C180 groups, the C45 group and the C135 group, and the C45 group and the C180 group. Conclusion: The histopathological appearance of the small intestine mucosa in the form of epithelial bleeding was the most severe at an infusion dose of cayenne pepper 135 mg/day with mucosal repair at an infusion dose of 180 mg/day.

Keywords ***:*** *cayenne pepper infusion, intestinal mucosa*

INTRODUCTION

Chili (Capsicum spp.) is a plant from the family Solanaceae (De, 2003; Izzati, 2018). Chili is a fruit vegetable that is often found in Indonesian cuisine (Arifin, 2010). The dried fruit of chili pepper (Capsicum frutescens L.) has been used as a flavoring agent for centuries and is associated with traditional medicine (Gurnani et al., 2016). Chili is used as a traditional medicine for example to relieve flatulence, as an ointment, headaches, and rheumatism

(Djarwiningsih, 2005). It is estimated that there are 27 types of chili plant species, of which 22 are wild types and 5 other species that have spread throughout the world, namely C. annuum, C. frutescens L, C. baccatum, C. sinense and C. pubescens (Gurung et al., 2015; Izzati, 2018).

Chili pepper (Capsicum frutencens L) contains various components such as capsaicin, carotene (some of which have provitamin A activity, flavonoids, tocopherols (vitamin E), and ascorbic acid (vitamin C) (Olatunji & Afolayan, 2018). as an antimicrobial. Based on the research held by Ministry of Health, chili is quite effective for treating stomach pain, heartburn, ulcers, skin irritation and at the same time for stimulants such as stimulating appetite (Setiadi, 2006). Capsaicin contained in chili has various pharmacological effects such as analgesic (Szolcsányi, 2004), anticancer and antimutagenic (Izzati, 2018; Oyagbemi et al., 2010; Szolcsányi, 2004). But on the other hand capsaicin is classified as an irritant that can cause a local burning sensation, erythema, or stinging. In addition, aerosol capsaicin can cause coughing or sneezing (Katritzky et al., 2003).

Capsaicin is a natural protoalkaloids that is the main spicy component in chili peppers, the active compound responsible for the hot taste of chili peppers and is a selective agonist of the vanilloid receptor (TRPV1) (Ilie et al., 2019). Capsaicin has the ability to regulate macrophage cells to produce cytokines that stimulate inflammation, namely TNF alpha and IL 1 (Gurnani et al., 2016). In a previous study by Teng et al., 2013 (Teng et al., 2013) showed signs of acute gastritis in rats induced by cayenne pepper. The study showed a microscopic picture of the stomach of wistar rats given a dose of cayenne pepper as much as 180 mg showed milder signs of acute gastritis than wistar rats given a lower dose of cayenne pepper.

The purpose of the study was to determine the histopathological description of the small intestine in rats' model of inflammation induced by chili pepper.

METHODS

This was a quasi-experimental research with posttest only control group design by using wistar white rats (Rattus norvegicus). The inclusion criteria for the sample were male white rats (Rattus Norvegicus) with an average weight of 200-250 grams, 3-4 months old, healthy and active. The exclusion criteria for this study were if the mice died or showed signs of weakness (inactive) before being given treatment. The drop-out criteria for this study were if the rat died or showed signs of weakness (inactivity) during the research. This study used 20 rats with the division of groups shown in table 1.

Table 1. Treatment Group

No.	Group	Treatment
1	K	-
2	C45	Chili pepper 45 mg/day
3	C90	Chili pepper 90 mg/day
4	C135	Chili pepper 135 mg/day
5	D180	Chili pepper 180 mg/day

Observations using a light microscope with a magnification of 10x and 40x. Preparations were observed in five fields of view then given a score. The scoring criteria based on the scoring criteria adapted from Payne et al., 2019 (Payne et al., 2019).

Data were analyzed by using SPSS 25.0 for Windows. First, the data normality test was carried out using Saphiro Wilk, then followed by the One Way Anova parametric test and then followed by the Post Hoc test.

Table 2. Scoring Degree of Small Intestine Bleeding

Score	Degree of bleeding	Indicator
0	No bleeding	No bleeding
1	Light bleeding	Bleeding less than1/3 of vili
2	Moderate bleeding	Bleeding between 1/3 to 2/3 of vili
3	Heavy bleeding	Bleeding more than 2/3 of vili

RESULTS AND DISCUSSION

During the study, all rats were eligible to be the research sample and there were no drop outs during the study. In observing the small intestine mucosa of each group, the average scoring data was obtained as follows:

Table 3. Mean Scoring Degree of Small Intestine Bleeding in Each Group

Group	Mean
K	1,8 ± 0,574
C45	1,7 ± 0,346
C90	1,6 ± 0,283
C90	2,7 ± 0,258
C180	1,9 ± 0,661

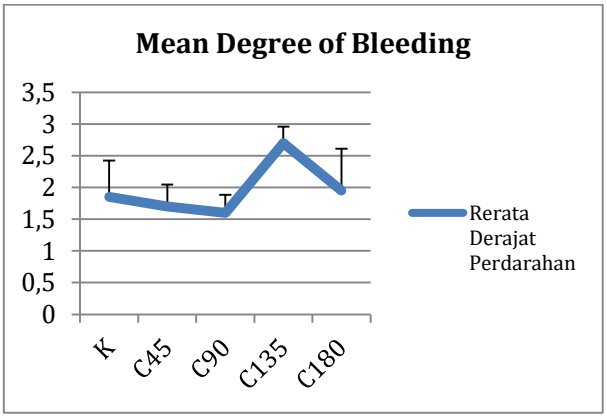

Figure 1. The Average Degree of Bleeding In The Control Group (K) and Each Treatment Group (C45, C90, C135 and C180)

Based on observations of the intestinal mucosa in five visual fields, the C135 group had a mean score of heavy bleeding with a mean of 2.7. In the control group, groups C45, C90, and C180 showed mild to moderate bleeding with a mean bleeding grade of 1.8 in the K group, 1.7 in the C45 group, 1.6 in the C90 group, and 1.9 in the C group. C180. From the results, it showed that the degree of bleeding in the small intestine mucosa was the heaviest in the C135 group, and experienced a decrease in the degree of bleeding in the C180 group.

The normality test for bleeding is $p>0.05$, so it can be concluded that the data were normally distributed (table 4). The variance test on bleeding got p value = 0.278 ($p>0.05$), it was concluded that the data variance was the same. The results of the One Way Anova test, on bleeding obtained p value = 0.030 ($p <0.05$) which indicates that there is a significant difference in one or more treatment groups. The decision of the hypothesis is to accept H1 and reject H0 which means that there is a significant effect between the dose of cayenne pepper and the degree of bleeding of the small intestine mucosa in the rat model. Post Hoc analysis (table 4) showed that there was a significant difference in the degree of bleeding

($p<0.05$) between the control group and C45 and C90 groups, C90 groups and C135 groups, and C45 groups and C180 groups.

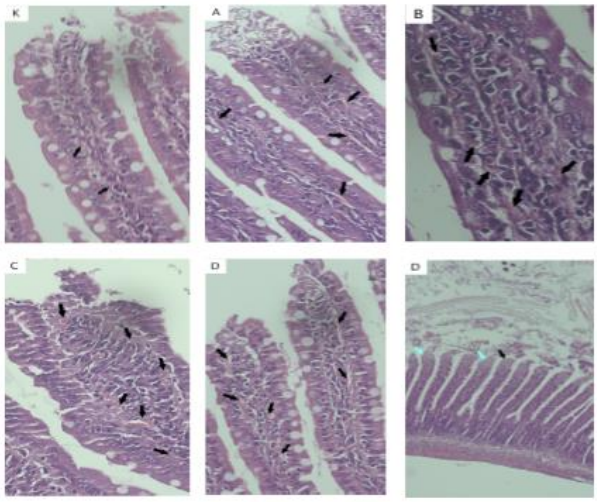

Figure 2. (K) The Control Group Showed Moderate Light Bleeding. (A, B) Groups C45 and C90 Showed Mild To Moderate Bleeding. (C) Group C135 Shows Extensive and Massive Bleeding (Black Arrow) In The Intestinal Villi. (D) Group C180 Showed A Decrease In The Degree of Bleeding, Namely Moderate Bleeding In The Epithelial Mucosal Villi (HE 10x, 40x)

Table 4. Post Hoc Statistical Test Results on Bleeding

Group	K	C45	C90	C135	C180
K	-	0.136	0.768	0.009*	0.019*
C45	0.136	-	0.222	0.175	0.310
C90	0.768	0.222	-	0.016*	0.035*
C135	0.009*	0.175	0.016*	-	0.713
C180	0.019*	0.310	0.035*	0.713	-

The antioxidant activity of chili peppers can also be attributed to ascorbic acid, carotenoids, and capsaicinoids. Capsicum frutescens L has various phytochemicals with well-known antioxidant properties such as carotenoids (Deli et al., 2001; Hervert-Hernández et al., 2010). Antioxidant compounds are compounds that have the ability to inhibit lipid oxidation by free radicals (Pambudi et al., 2014). Besides capsaicin, cayenne pepper also contains flavonoids which are also thought to have antioxidant effects. Flavonoids can act as antioxidants because flavonoids act as free radical scavengers by releasing hydrogen atoms from their hydroxyl groups. Free radicals can bind to the released hydrogen atoms (Pambudi et al., 2014).

In the control group rats that were not induced by the dose of cayenne pepper showed signs of light bleeding. Bleeding in the control group was probably caused by individual factors, for example, the mice used were traumatized so that it could cause bleeding in the small intestine (Hidayati et al., 2018). In addition, it can also be caused by excessive exudation of fluids, electrolytes, and mucus, causing inflammation and damage to the intestinal mucosa resulting in bleeding (Hudayani, 2008). In groups A and B, each dose of chili pepper 45 mg/day and 90 mg/day showed moderate bleeding with signs of inflammation. Inflammation is characterized by the presence of inflammatory cells around the bleeding. In group C, the dose of cayenne pepper induced by 135 mg/day showed moderate to heavy bleeding and the heaviest bleeding compared to the other groups. This shows that this group experienced the most severe inflammatory process than the other groups.

Bleeding that occurs can cause erosion of the intestinal mucosal epithelium to necrosis. Congestion can also be found because the inflammatory process triggers the release of inflammatory mediators that cause vasodilation and increased capillary wall permeability. Capsaicin is thought to have an indirect effect on the inflammatory process (Rains & Bryson, 1995). Capsaicin has the ability to regulate macrophage cells to produce cytokines that stimulate inflammation, namely TNF alpha and IL-I (Gurnani et al., 2016). In group D, the dose of chili pepper induced 180 mg/day showed moderate bleeding. In this group, the picture of the intestinal mucosa has improved in the intestinal villi. Similar results were also obtained in the research of Teng., et all in 2013 (Teng et al., 2013) which stated that the gastric mucosa induced by chili pepper juice at a dose of 180 mg/day showed a better picture of the gastric mucosa than other lower doses, namely a dose of 45 mg/day, 90 mg/day, 135 mg/day and 180 mg/day.

The significant repair of villous epithelium compared to group C was caused by repeated exposure, causing a desensitizing effect of capsaicin so that it could reduce the effects of the first exposure. The mechanism underlying capsaicin-induced desensitization is not well understood. One possibility is depletion of the neuropeptide Substance P and CGRP from type C nerve fibers leading to desensitization of nociceptors. Capsaicin is known to trigger the release of this peptide from the primary afferent terminal (Ilie et al., 2019). TRPV1 activation causes an influx of Ca2+ which triggers the release of substance P which functions as a neurotransmitter and neuromodulator (Izzati, 2018). There are two types of desensitization resulting from capsaicin application. The first is pharmacological desensitization, in which repeated or prolonged application of capsaicin can lead to a gradual reduction of the subsequent response to capsaicin. The second is functional desensitization, where capsaicin can reduce nerve sensitivity to various noxious stimuli such as heat, pressure, chemical irritants, endogenous or exogenous agents (Ilie et al., 2019). Although initially stimulating neuropeptide release, capsaicin has a long-term inhibitory effect on the efferent function of sensory neurons that may underlie its analgesic and anti-inflammatory action. The functional desensitization that occurs with increasing capsaicin concentrations is thought to be the basis of the analgesic and anti-inflammatory effects of capsaicin (Ilie et al., 2019).

CONCLUSION

From the results of the study, it can be concluded that there is a change in the histopathological picture of the small intestine in the form of bleeding from the small intestine of white rats induced by a dose of cayenne pepper. Bleeding in the C45 group with a dose of chili pepper 135 mg/day showed signs of the heaviest bleeding compared to the other groups, while the C180 group with a dose of 180 mg/day showed signs of improvement in the epithelial mucosa. Chili pepper has the effect of bleeding the small intestine mucosa with different doses. At a dose of 180 mg / day there is a mucosal repair effect due to a decrease in the degree of bleeding. Therefore, it is necessary to do further research on the effect of cayenne pepper on the histopathological picture of the small intestine with an additional dose of more than 180 mg/day. The scoring system using subjective scoring is still possible to cause bias in the results of this study, so it is necessary to use a more effective method in further research.

ACKNOWLEDGMENT

The researcher would like to express his gratitude for the support, inspiration and assistance to all parties in helping the researcher complete this research, including the participants who were willing to participate in the research until it was completed.

REFERENCES

Arifin, I. (2010). Pengaruh cara dan lama penyimpanan terhadap mutu cabai rawit *(Capsicum frutencens L var. Cengek)*. UIN Maulana Malik Ibrahim.

De, A. K. (2003). *The genus Capsicum*. CRC Press.

Deli, J., Molnár, P., Matus, Z., & Tóth, G. (2001). *Carotenoid Composition in the Fruits of Red Paprika (Capsicumannuum var. lycopersiciforme rubrum) during Ripening; Biosynthesis of Carotenoids in Red Paprika. Journal of Agricultural and Food Chemistry*, *49*(3), 1517–1523. https://doi.org/https://doi.org/10.1021/jf000958d

Djarwiningsih, T. (2005). *Capsicum spp. (Chilli): origin, distribution, and its economical value. Biodiversiitas*, *6*(4), 292–296.

Gurnani, N., Gupta, M., Shrivastava, R., Mehta, D., & Mehta, B. K. (2016). *Effect of extraction methods on yield, phytochemical constituents, antibacterial and antifungal activity of Capsicum frutescens L. Indian Journal of Natural Products and Resources*, *7*(1), 32–39.

Gurung, S., Short, D. P. G., Hu, X., Sandoya, G. V., Hayes, R. J., & Subbarao, K. V. (2015). *Screening of Wild and Cultivated Capsicum Germplasm Reveals New Sources of Verticillium Wilt Resistance. APS Publications*, *99*(10). https://doi.org/https://doi.org/10.1094/PDIS-01-15-0113-RE

Hervert-Hernández, D., Sáyago-Ayerdi, S. G., & Goñi, I. (2010). *Bioactive compounds of four hot pepper varieties (Capsicum annuum L.), antioxidant capacity, and intestinal bioaccessibility. Journal of Agricultural and Food Chemistry*, *58*(6), 3399–3406. https://doi.org/10.1021/jf904220w.

Hidayati, E., Berata, I. K., Samsuri, Merdana, I. M., & Sudimartini, L. M. (2018). Gambaran Histopatologi Limpa Tikus Putih yang Diberi Deksametason dan Vitamin E. *Buletin Veteriner Udayana*, *10*(1), 18–25. https://doi.org/10.24843/bulvet.2018.v10.i01.p03

Hudayani, M. (2008). Efek Antidiare Ekstrak Etanol Rimpang Kunyit (Curcuma Domestica Val.) Pada Mencit Jantan Galur Swiss Webster. Universitas Muhammadiyah Surakarta.

Ilie, M. A., Caruntu, C., Tampa, M., Georgescu, S.-R., Matei, C., Negrei, C., Ion, R.-M., Constantin, C., Neagu, M., & Boda, D. (2019). *Capsaicin: Physicochemical properties, cutaneous reactions and potential applications in painful and inflammatory conditions. Experimental and Therapeutic Medic*, *18*(2), 916–925. https://doi.org/10.3892/etm.2019.7513

Izzati, F. N. (2018). *Capsaicinoids dari Capsicum spp. dan Penggunaanya Sebagai Riot Control Agent. BioTrends*, *9*(2).

Katritzky, A. R., Xu, Y.-J., Vakulenko, A. V, Wilcox, A. L., & Bley, K. R. (2003). *Model compounds of caged capsaicin: design, synthesis, and photoreactivity. The Journal of Organic Chemistry*, *68*(23), 9100–9104. https://doi.org/10.1021/jo034616t.

Olatunji, T. L., & Afolayan, A. J. (2018). *The suitability of chili pepper (Capsicum annuum L.) for alleviating human micronutrient dietary deficiencies: A review*. *Food Science and Nutrition*, *6*(8), 2239–2251. https://doi.org/10.1002/fsn3.790

Oyagbemi, A., Saba, A., & Azeez, O. (2010). *Capsaicin: A novel chemopreventive molecule and its underlying molecular mechanisms of action. Indian Journal of Cancer*, *47*(1), 53–58. https://doi.org/10.4103/0019-509X.58860

Pambudi, A., Syaefudin, Noriko, N., Azhari, R., & Azura, P. R. (2014). Identifikasi Bioaktif Golongan Flavonoid Tanaman Anting-Anting (*Acalypha indica L.*). *Jurnal Al-Azhar Indonesia (Seri Sains Dan Teknologi)*, *2*(3). https://doi.org/http://dx.doi.org/10.36722/sst.v2i3.139

Payne, S. C., Furness, J. B., Burns, O., Sedo, A., Hyakumura, T., Shepherd, R. K., & Fallon, J. B. (2019). *Anti-inflammatory Effects of Abdominal Vagus Nerve Stimulation on*

Experimental Intestinal Inflammation. *Frontiers in Neuroscience*. https://doi.org/https://doi.org/10.3389/fnins.2019.00418

Rains, C., & Bryson, H. M. (1995). *Topical capsaicin*. *Drugs & Aging*, *7*(4), 317–328.

Setiadi, Y. (2006). Cabai Rawit Jenis dan Budaya. Penebar Swadaya.

Szolcsányi, J. (2004). *Forty years in capsaicin research for sensory pharmacology and physiology*. *Neuropeptides*, *38*(6), 377–384. https://doi.org/10.1016/j.npep.2004.07.005

Teng, P., Kairupan, C., & Loho, L. (2013). Gambaran Histopatologi Lambung Tikus Wistar Yang Diberi Cabe Rawit (*Capsicum Frutescens*). *Jurnal E-Biomedik*, *1*(3), 1109–1113. https://doi.org/https://doi.org/10.35790/ebm.v1i3.3268