Oral Presentation (PCS-12)

Complex Oligosaccharide Detected in the Lingua of *Hystrix javanica* by Lectin Histochemistry

Teguh Budipitojo[1]*, Yosua Kristian Adi[1], Yuda Heru Fibrianto[2], Ariana[1]

[1]Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia;
[2]Department of Physiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.
*Corresponding author's email: budipitojo@ugm.ac.id

Keywords: carbohydrate residue, endemic, *Hystrix javanica*, lectin histochemistry, lingua.

INTRODUCTION

Hystrix javanica or usually called Sunda porcupine is wildlife animal that only found in Java, Bali, Sumbawa, Flores, Lombok, Madura, and Tonahdjampea (Indonesia) [1]. International Union for Conservation of Nature and Natural Resources (IUCN) has classified this Rodentia into Least Concern criteria, that means the species is relatively widespread and abundant [2]. Nevertheless, their population in nature was threatened, since this mammal was considered as pest by some people.

There were anatomical data of Sunda porcupine organ, but it still limited. One of the ways to determine the detail function of the organ can be done by making histological preparations. Lectin histochemistry has been used widely as a probe to detect sugar residues in the organs or tissues because lectin can bind specifically to carbohydrate residues in term of glycoconjugates [3]. Analysis the composition or type of sugar residues in the organs can help to understanding the role of their function.

The aims of this study were to detect and find out the distribution of complex oligosaccharide in the lingua of *Hystrix javanica* using lectin histochemistry method. This data will completes the information that has been obtained before about mucopolysaccharide type of major salivary glands of *Hystrix javanica* [4].

MATERIALS AND METHODS

Three lingua organs of adult Sunda porcupines (*Hystrix javanica*) were obtained from a merchant in Tawangmangu, Central Java, Indonesia. Lingua tissues were fixed in Bouin's solution for 24 hours. The tissues were processed with paraffin method, then cut serially in 5µm thickness. For lectin histochemistry staining, tissue section was deparaffinized in xylene and rehydrated in series of ethanol. After washing in phosphate-buffered saline, the section was incubated in 3% H_2O_2 in methanol for 15 minutes to block endogenous peroxidase activity. Biotin-labeled Phytohaemagglutinin (PHA), Wheat germ agglutinin (WGA), Lens culinaris agglutinin (LCA), and Sophora japonica (SJA) lectin were applied over night at 4^{o}C. The binding of lectin to glycoconjugates were visualized with avidin-biotin-peroxidase complex and chromogen 3,3'-diaminobenzidine (DAB). For counterstain, Harris Hematoxylin was applied for 1 minute. After that, the tissue section was dehydrated in series of ethanol, cleared in xylene, and then the coverslip was mounted with Canada balsam. The result was examined with conventional light microscope, photographs were taken with digital camera, and then analyzed descriptively.

RESULT AND DISCUSSION

Positive reaction of lectin histochemistry with chromogen DAB was shown as brown precipitate within the tissues. The brown color could be seen in lamina epithelialis mucosa, epithelium lining the duct of lingual gland, and nerve. In the anterior part of *Hystrix javanica* lingua, positive reactions of lectin PHA were detected in the stratum basal and spinosum of lamina epithelium mucosa in dorsal lingua (Fig. 1 A) and all stratums of lamina epithelium mucosa in ventral lingua (Fig. 1 B). In the middle part, brown-colored precipitations were visible less in stratum basal and lucidum of dorsal lingua mucosa (Fig. 1 C) and in stratum basal, spinosum, and corneum of ventral lingua mucosa (Fig. 1 D). In the posterior part of *Hystrix javanica* lingua, strong reactions were found in stratum lucidum and less reaction in stratum basal in the dorsal lingua mucosa (Fig. 1 E), whereas very low reaction could be seen in ventral lingua mucosa (Fig. 1 F). In addition, specific reaction of lectin PHA could be detected in epithelium lining the small and large duct of lingual gland but not in the adenomer (Fig. 2). This reaction could be seen also in the nerve along side of *Hystrix javanica* lingua (Fig. 3).

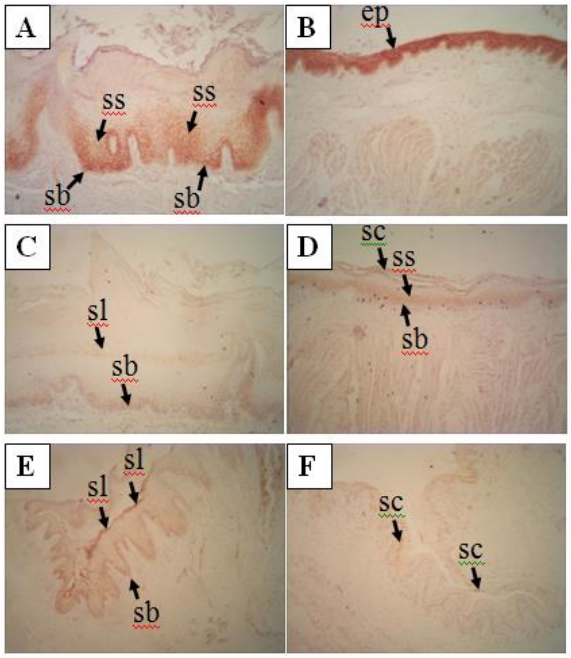

Figure 1. Positive reaction of PHA lectin histochemistry in dorsal and ventral epithelium of *Hystrix javanica* lingua. The reaction in the epithelium (ep) vary among stratum basal (sb), stratum spinosum (ss), stratum lucidum (sl), and stratum corneum (sc) in the anterior (A,B), middle (C,D), and posterior (E,F) part of lingua (PHA Lectin Histochemistry, 125x).

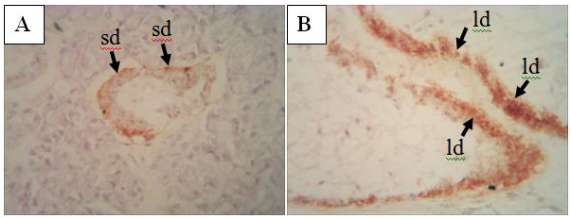

Figure 2. Positive reaction of PHA lectin histochemistry in the epithelium lining the small and large duct of lingual gland. Brown-colored precipitate as positive reaction seen in epithelium lining the small duct (sd) and large duct (ld), but not in adenomer of lingual glands (PHA Lectin Histochemistry, 500x).

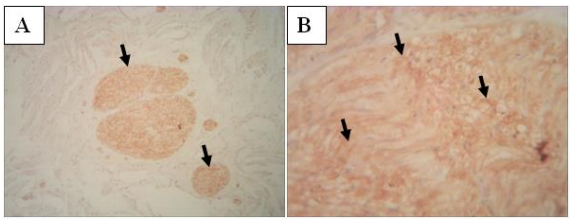

Figure 3. Positif reaction of PHA lectin histochemistry in the nerves alongside the lingua of *Hystrix javanica*. Positive reaction was indicated with brown color (arrow) (PHA Lectin Histochemistry, A 125x, B 500x).

Positif lectin histochemistry reactions of lectin WGA in anterior part of lingua were detected in stratum corneum and spinosum of lamina epithelium mucosa in dorsal lingua (Fig. 4 A) and stratum lucidum of lamina epithelium mucosa in ventral lingua (Fig. 4 B). In the middle part, brown-colored precipitations were visible strong in stratum corneum of dorsal lingua and stratum spinosum of ventral lingua mucosa but less in stratum spinosum of dorsal lingua (Fig. 4 C D). In the posterior part of *Hystrix javanica* lingua, strong reactions were found in stratum corneum of dorsal and ventral lingua but less reaction in stratum spinosum of dorsal lingua mucosa (Fig. 4 E F). Specific reaction of lectin WGA could be detected too in the secret of sublingual gland adenomer and nerve (Fig. 5).

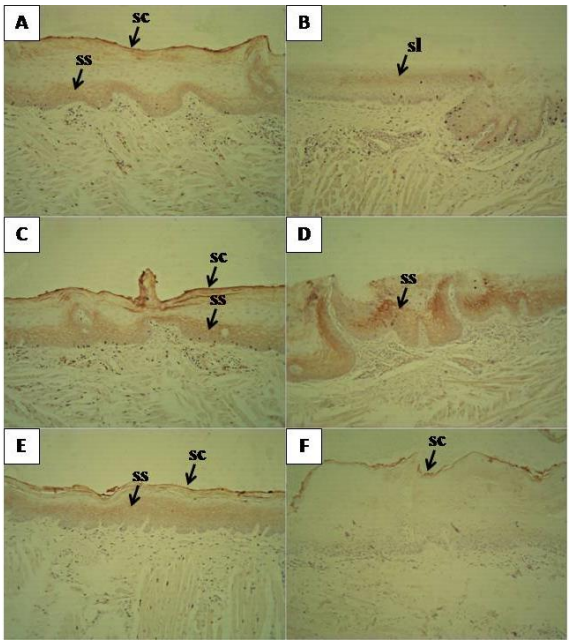

Figure 4. Positive reaction of WGA lectin histochemistry in dorsal and ventral epithelium of *Hystrix javanica* lingua. The reaction in the mucosa epithelium vary among stratum corneum (sc), stratum lucidum (sl), and stratum spinosum (ss) in the anterior (A,B), middle (C,D), and posterior (E,F) part of lingua (WGA Lectin Histochemistry, 125x).

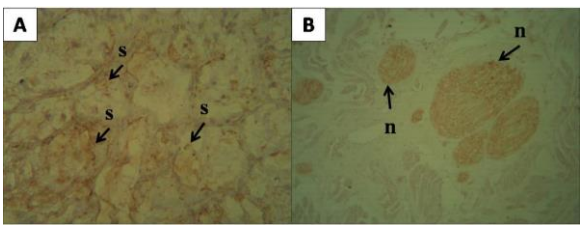

Figure 5. Positif reaction of WGA lectin histochemistry in the secret of sublingual gland adenomer and nerve. Positive reaction was indicated with brown color (arrow) (WGA Lectin Histochemistry, A 500x, B 125x).

In the two other lectin histochemistry, LCA and SJA lectin, there were no positif reaction as indicated by brown precipitation (Fig. 6). From

this result, we can know that there are variation of carbohydrate residues in the mucosa epithelium of *Hystrix javanica* lingua and their distribution.

Lectin histochemistry can be used to determine specific sugar residues or glycoconjugate in the tissues [5], while conventional method of Alcian Blue (AB pH 1 and 2,5)-Periodic Acid Schiff (PAS) only differentiated acid and neutral polysaccharides [6]. Distribution other lectin in the major salivary gland of *Hystrix javanica* has been reported by Budipitojo et al. [4]. Phaseolus vulgaris lectin or phytohaemagglutinin (PHA) is a tetrameric protein that are well-studied lectins of the first group [7]. PHA lectin recognizes and binds specifically to terminal galactose, N-acetylglucosamine, and mannose residues of complex glycans on mammalian glycoproteins [8]. Wheat germ agglutinin (WGA) can binds specifically to N-acetylglucosamine, residue of glucose [9] and N-acetylneuraminic acid [10]. Lectin staining using Sophora japonica (SJA) can used to detect residue of N-acetylgalactosamine [5]. While Lens culinaris agglutinin (LCA) can binds to core-fucosylated alpha-fetoprotein [11]. Variation of positive reaction in the lectin histochemistry in mucosa epithelium also reported in the skin of catfish [5]. Since the skin of fish is continuously contact with water and environmental, the structure of fish skin has been modified to give a protection from acid and infection [12]. The same role apparently occurred in the lingua of *Hystrix javanica*. Oral cavity allowed internal body to contact with environmental material that might carried a harmful substance like bacteria and acid toxic. This condition related to *Hystrix javanica* since they often search their food by digging the soil to find some root of tree or tuber.

CONCLUSION

Carbohydrate residues in the lingua mucosa of *Hystrix javanica* can be detected with lectin histochemistry using PHA and WGA but not with LCA and SJA lectin. Specific residues of carbohydrate in the organ can be used as indicator of their function.

ACKNOWLEDGMENTS (optional)

This research was supported by a research grant from Directorate Research and Community Service, Directorate General of Higher Education, The Ministry of Research, Technology, and Higher Education, Republic of Indonesia with grant number: 1829/UN1/DITLIT/DIT-LIT/LT/2018.

REFERENCES

[1] Woods C.A., Kilpatrick C.W. 2005. Infraorder Hystricognathi. *In*: Wilson, D.E. and Reeder, D.M. (eds), Mammal Species of the World, pp. 1538-1599. The Johns Hopkins University Press. Baltimore (USA).

[2] Aplin K. 2016. *Hystrix javanica*. The IUCN Red List of Threatened Species 2016: e.T10752A22231749. http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T10752A22231749.en

[3] Alroy, J., Ucci, A.A., and Pereira, M.E.A. (1988). Lectin histochemistry. An update. In: advances in immunohistochemistry. Raven Press; New York (USA). 93-131p.

[4] Budipitojo T, Ariana, Fibrianto Y.H. 2017. Structure and Mucopolysaccaride Type of Major Salivary Glands of the Sunda Porcupines (*Hystrix javanica*). *J. Vet. Sci. Res*. 2(3): 000139.

[5] Al-Banaw A, Kenngott R, Al-Hassan J.M., Mehana N, Sinowatz, F. 2010. Histochemical Analysis of Glycoconjugates in the Skin of a Catfish (*Arius tenuispinis*, day). *Anat. Histol. Embryol* 39: 42-50.

[6] Budipitojo T, Fibrianto Y.H., Mulyani G.T. 2016. Phytobenzoars-like Structure Covers the Mucosal Surface of Sunda Porcupines (*Hystrix javanica*) Stomach. *Asian J. Anim. Vet. Adv.* 11: 709-716.

[7] Nagae M, Soga K, Morita-Matsumoto K, Hanashima S, Ikeda A, Yamamoto K, Yamaguchi Y. [2014]. Phytohemagglutinin from *Phaseolus vulgaris* (PHA-E) displays a novel glycan recognition mode using a common legume lectin fold. *Glycobiology* 24: 368-378.

[8] Summers C, Forrest J, Norval M, Sharp J.M. 2002. The potenitally insecticidal Narcissus pseudonarcissus lectin demonstrates age-related mitogenecity. *FEMS immunology and medical microbiology* 33(1): 47-9.

[9] Park C, Ahn M, Kim J, Kim S, Moon C. 2015. Histological and lectin histochemical studies on the olfactory mucosae of the Korean roe deer, Capreoluspygargus. *Tissue Cell* 47(2): 221-227.

[10] Bogoeva V, Radeva M, Atanasova L, Stoitsova S, Boteva R. 2004. Fluorescence analysis of hormone binding activities of wheat germ agglutinin. *Biochem Biophys Acta* 1698(2): 213-218.

[11] Tateno H, Nakamura-Tsuruta S, Hirabayashi J. 2009. Comparative analysis of core-fucose-binding lectins from Lens culinaris and Pisum sativum using frontal affinity chromatography. *Glycobiology* 19(5): 527-536.

[12] Pinky S, Mitta S, Mittal A.K. 2008. Glycoproteins in the epithelium of lips and associated structures of a hill stream fish Garra lamta (Cyprinidae, Cypriniformes): a histochemical investigation. *Anat. Histol. Embryol.* 37: 101-113.