Sys Rev Pharm 2020;11(11):182-185
A multifaceted review journal in the field of pharmacy

The differences of T2RS expression as a sensitivity marker of bitter taste on consumed coffee

Intan Nadzirah[1], Ari Triwardhani[2], Hans Lesmana[3], Aqsa Syuhada Okky[4], Hendrik Setia Budi[4], Jenny Sunariani[4]*

[1] Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia.
[2] Department of Orthodontic, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia.
[3] Politeknik Kesehatan Kemenkes, Makassar, Indonesia.
[4] Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia.
*Correspondence Author: jenny-s@fkg.unair.ac.id

ABSTRACT

In the body's protective system against poisonous chemicals, which serves as the primary source of warning signals, bitter taste perception plays a role. (Type II Receptor) induced bitter taste T2Rs (in humans) / t2rs (in animals) mainly expressed on the tongue in taste buds. Circumvallate, foliate, and palatal papillae are found in these receptors. Arabica Civet Coffee is one of the coffees that originated in Java, Indonesia, and is renowned for the civet digestive system (Paradoxurushermaphroditus) process. The goal of this research was to establish the differences in the expressions of t2rs as an indicator of bitter taste sensitivity in coffee-given animal studies. 14 rats separated into two parts were included in this experimental study (seven rats per group). Pellets and aquadest without coffee intake were administered to the control group; pellets, aquadest and coffee intake were administered to the treatment group with a normal coffee dosage of 223 mg / ml / day for 30 days. On day 31, the rats were sacrificed and the data examined by the Mann-Whitney Test. Result of this study showed significant different of t2rs expression in the treatment group (1.49 ± 0.08) than (0.03 ± 0.08) in the control group ($p<0.05$). It can be inferred on the basis of the findings of this analysis that there were higher expressions of t2rs in rats consuming coffee.

Keywords: t2rs expression, sensitivity, bitter taste, arabica civet coffee

Correspondence:
Jenny Sunariani
4 Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia.
*Corresponding author: Jenny Sunariani email-address: jenny-s@fkg.unair.ac.id

INTRODUCTION

Bitter taste is one of five flavors, sweet, salty, sour and umami; taste that was discovered by researchers from Japan.[1] Bitter taste perception has a role in the body's defence mechanism against toxic substances which acts as the main sender of warning signals.[2]Bitter taste has a character in guiding organisms to avoid poisons and harmful substances, especially from humans and animals.[3]

Bitter taste is usually found in coffee, carbonated drinks and dietary supplements.[4] There are various kinds of active chemicals that have bitter taste and result in unpleasant taste in some people, especially in children. Some drugs are made in form of pills or tablets to avoid the unpleasant bitter taste.[5] Type II Receptoror T2R (in humans) / t2r (in animals) is a receptor responsible for bitter taste, which is mostly expressed on type II taste receptor cells in taste buds on the tongue.[6]

The level of sensitivity to a basic sense depends on appetite, body health, and maintaining the balance of energy.[7] In general, individual has higher level of sensitivity towards bitter taste compared to sweet taste, especially in young children.[8] When bitter molecules enter the mouth, the digestive tract can receive signals to work on the molecule. Receptors in the digestive tract will stimulate the production of enzymes that break down food molecules and guard the movements of the digestive tract. This suggests that, by having a high sensitivity of bitter taste can help the digestive tract to work by producing enzymes that help in digestions. Stimulation of bitter taste receptors can facilitate digestion by affecting the flow of blood and smooth muscles of the digestive tract.[9]

Coffee is a beverage that has various health benefits because it has various components of phenols such as phenolic acid and chlorogenic acid which are found in coffee beans. Coffee intake provides a neuroprotective effect that can prevent diseases such as Alzheimer's disease.[10] Arabica Civet Coffee is one of the coffees originating from the Indonesian Islands of Java, Sumatra and Sulawesi and is famous for a process that involves the digestive system of civet (*Paradoxurushermaphroditus*).[11]

This study aims to determine the differences in expression of t2rs as a marker of sensitivity to bitter taste in experimental animals given coffee. Foliate papillae are abundantly found on the tongue compared to the circumvallate papillae. T2rs expressions were highly concentrated in the circumvallate papillae, therefore the right slicing technique was needed in order to examine t2rs expression in rat's tongue.

MATERIALS AND METHODS

Sample and ethical clearance

Samples were divided into 2 groups: Group K and Group P. The control group (K) was given pellets and aquadest without coffee intake. The treatment group (P) was given pellets, aquadest and coffee intake with a standard dose of 223 mg / ml / day for 30 days and sacrificed on day 31. This research was purely experimental using experimental animals conducted at the Experimental Animal Laboratory - Faculty of Veterinary Medicine, Universitas Airlangga. This study was approved with ethical permission from the Health Ethics Permit Research Committee, Faculty of Dentistry, Universitas Airlangga (Number: 080/HRECC.FODM/VII/2018). The total number of samples was 14 Wistar (*Rattusnorvegicus*) rats, 12 months old.

Preparation of coffee powder

The coffee powder used is coffee powder from pure Arabica Luwak coffee beans that have been dried and smoothed (grinding) in the form of packaging. Coffee powder with a dose of 1.561 mg dissolved in hot water

around 80^{0}C - 85^{0}C as much as 7 ml and stirred then cooled and distributed into 7 doses of coffee drinks each with a volume of 1 ml containing 223 mg of coffee powder.

Hematoxylin Eosin (HE) staining

On the day 31, rats were given anesthesia Ketamine HCl (KTM-100) at a dose of 2 mg / kg (1 mg / lb) of IV weight (intravenously). After that the rat's tongue was taken (posterior tongue; foliate papillae) for making paraffin preparation blocks, deparafinization process, and preparations made for staining Hematoxcylin-Eosin (HE). Staining of HE on rat's tongue tissue aims to determine the location of the foliate papillae.[12]

Immunohistochemistry analysis (IHC)

Immunohistochemistrystaining (IHC) in the tongue tissue of rat containing foliate papillae with TAS2R antibodies. Histology examination and t2rs expression calculation using a light microscopewith 400 times magnification at 5 times the field of view.[13]

Statistical analysis

The data obtained were analyzed using *Statistical Package for the Social Sciences* (SPSS 17) software. Normality test was done using Shapiro-Wilk's Test, and homogenity of samples used Levene'sTest. The Mann-Whitney Test was used to analyze significant differences.

RESULTS

Immunohistochemical staining of t2rs expression in papillae foliata rats

The staining on IHC in foliate papillae of coffee-free rat obtained an immunoreactive (-) result indicating no expression of t2rs in rat taste and IHC staining of foliate papillae in rat fed coffee obtained an immunoreactive (+) result indicates t2rs expression on the taste buds of rat (figure 1).

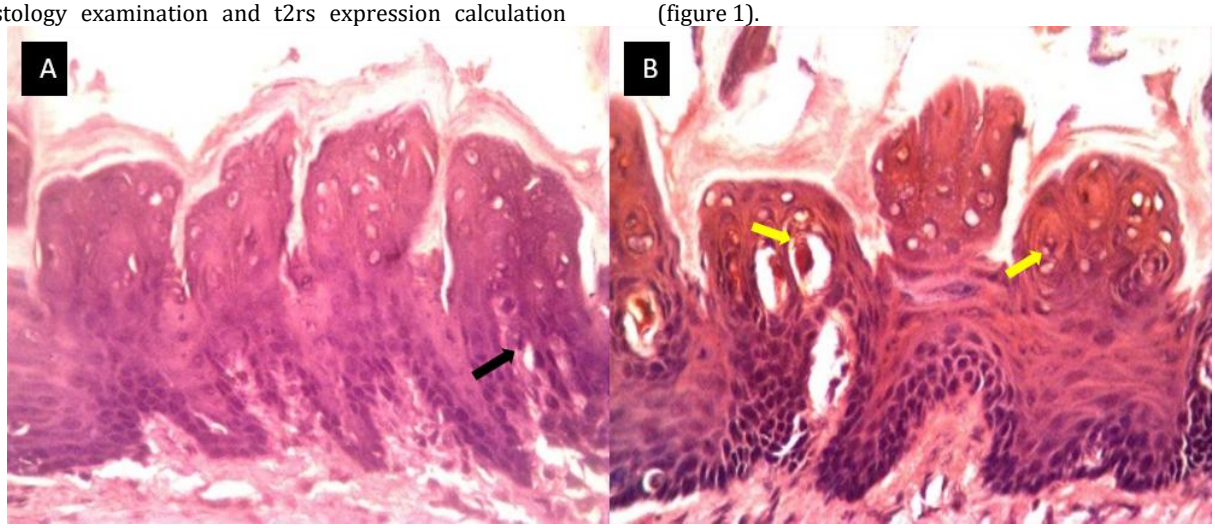

Figure 1: t2rs expressions on foliate papillae with immunohistochemical staining magnification 400x. Control group without tr2s expression on black arrow (A). Treatment group show t2rs expression on yellow arrow (B).

The highest expression of t2rs show in the treatment group (1.49 ± 0.08), and (0.03 ± 0.08) in the control group. This result indicates that the expression level of t2rs increased with the treatment group. The normality test in all study sample groups using Shapiro-Wilk's Test. The control group was not normally distributed (p<0.05), then the next test used a non-parametric test. The treatment group showed a normal distribution (p>0.05). The homogeneity test using Levene's Test obtained a significance value of 0.008 (p>0.05), which means that all t2rs expression data in this study are not homogeneous.

The differences of t2rs expression between rats coffee induced group and without coffee

The different test used is the Mann-Whitney Test. It is said to be significantly different if the value of p<0.05. The results of Mann-Whitney Test obtained a significance level 0.005 (p<0.05) which showed that there were significant differences between the control and treatment groups (Table 1).

Table 1: The differences of T2Rs expression between rats coffee induced group and without coffee

Groups	n	T2Rs Expression $\bar{X}$ ± SD	p value
Control (K)	7	0.03 ± 0.08	0.005 (p<0.05)*
Treatment (P)	7	1.49 ± 0.86	

Note: Data are presented as mean ±standard deviation (n = 7 per group). * Significant different

DISCUSSION

This study aimed to determine the differences in expression of t2rs as a marker of sensitivity to bitter taste in rats consuming coffee. The subject of this study used Wistar (*Rattusnorvegicus*) rats with male sex and around 12 months old. Wistar rats were chosen as the subject of this study because in rats there were more (18%) α-gustducin which was an important mediator for bitter and sweet taste transduction compared to mice (14.6%) and rats had more (64,200µm^3) average taste volume (average volume of taste bud) compared to mice (42,000µm^3) .[14.15] Rats were given adaptation for one week before treatment with the aim of maintaining the body's condition to remain constant in different environmental conditions.[16]

In this study, the selected papillae were foliate papillae. This was because the bitter taste receptors (t2rs) were found in the foliate papillae.[17] In this study Arabica Civet Coffee was chosen as a bitter taste molecule because Arabica Civet Coffee had better quality compared to robusta coffee,[18] although the caffeine content in Arabica Civet Coffee beans was lower than (0.94-1.59%) robusta coffee (2.2% -2.8%).[13,19]

The sample of 14 rats were divided into 2 groups, namely the control group (Group I) given standard food in the form of pellets without the intake of Arabica Luwak Coffee and treatment groups (Group II) who were given standard food in the form of pellets and given Arabica Luwak coffee with a dose of 223 mg / ml / day. This aimed to prove the difference in expression of t2rs on the tongue of rats that consume coffee.

In this study, rats were given a standard dose of coffee intake of 223 mg / ml per day.[13] Safe daily coffee intake of rats was estimated at around 100 mg / kg body weightto 367 mg / kg.[20] It was clear that coffee dosing (223 mg / ml / day) in this study not exceeding 367 mg / kg. If rats were given at a higher dose level for 30 days, there would be a histopathological picture of erosive chronic gastritis.[21]

The time for coffee intake in rat was around 9:20 in the morning until 11:30 in the morning. This was because at that time the cortisol in the body was low.[15] Rats are nocturnal animals; animals that are active at night, not during the day. This is related to cortisol levels in rats that are low at noon than at night.[12] If rats are given caffeine intake when the cortisol levels are high, blood pressure will increase and can cause cardiovascular disorders.[22]

This study used a standard coffee dose with the aim to determine the expression of bitter (t2rs) taste receptors on the tongue of rats that consume coffee, and this study was different from previous studies which discussed more about gastric histopathological images of rats given coffee with various levels of coffee doses.[13]

Based on the results of the study, there was an expression of t2rs in the foliate papillae of rats that consumed coffee. This proves that bitter (t2rs) taste receptors are expressed in the rat taste bud, namely in the circumvalate papillae and foliate papillae.[18,23] Cell receptor (Type II) is a sensory cells that are responsible for sweet, bitter, and umamiwhich tranduscted by G protein coupled receptor.[24] The mechanism of bitter taste begins with the presence of tastan; the coffee molecule binds to the bitter taste receptor on the apical cell type II taste cell and activates protein G and phospholipase C-β2 (PLC-β2). Then, it produces the production of InsP3 (IP3) and diacylglycerol (DAG). IP3 binds to the InsP3 type 3 receptor (IP3R3), triggering the release of Ca^{2+} from the endoplasmic reticulum (ER). Increased cytoplasmic Ca^{2+} concentration activates transient receptor potential cation channer subfamily M member 5 (TRPM5), which depolarizes the membrane, triggering the potential for action of Na^{+} which activates the calcium homeostasis modulator (CALHM) channel to release ATP. ATP binds to $P_2X_{2\ /\ 3}$ receptors on afferent nerves to transmit primary gustatory cortex and the perception of bitter taste is produced.[25]

The results of this study have answered the objectives and problems of this study, that there is an expression of t2rs in rats that consume coffee compared to rats that do not consume coffee.

CONCLUSION

Based on the results of this study, it can be concluded that consume coffee could increase the expression of t2rs in foliate papillae rats compared to rats that did not consume coffee.

REFERENCES

1. Ninomiya K. Science of umami taste : adaptation to gastronomic culture. Flavour 2015;4(13). doi:10.1186/2044-7248-4-13.
2. Jaggupilli A, Howard R, Upadhyaya J, Bhullar RP, Chelikani P. Bitter taste receptors: novel insights into the biochemistry and pharmacology. Int J Biochem Cell Biol 2016;77:184-96. doi:10.1016/j.biocel.2016.03.005.
3. Lu P, Zhang CH, Lifshitz LM ZhuGe R. Extraoral bitter taste receptors in health and disease. J Gen Physiol 2017;149:181-97. doi:10.1085/jgp.201611637.
4. Selvamuthukumaran M, Pathak YV. Flavor development for functional foods and nutraceuticals. London: Boca Raton-CRC-Press; 2020:183.
5. Julie AM, Kristi MR, Phoebe SM, Danielle RR. Children's perceptions about medicines : individual differences and taste. BMC Pediatr 2015;15:130. doi:10.1186/s12887-015-0447-z.
6. Shaik FA, Singh N, Arakawa M, Duan K, Bhullar R, Chelikani P. Bitter taste receptors : extraoral roles in pathophysiology. Int J Biochem Cell Biol 2016;77:197–204. doi:10.1016/j.biocel.2016.03.011.
7. Nakanishi Y, Inoue Y, Ito Y, Nethery V. Exercise intensity differentially impacts sensitivity thresholds to specific tastes. Biol Exerc 2015;11:69–80. doi:10.4127/jbe.2015.0087.
8. Mennella JA, Bobowski NK. The sweetness and bitterness of childhood: insights from basic research on taste preferences. Physiol Behav 2015;152(Pt B):502-7. doi:10.1016/j.physbeh.2015.05.015.
9. Xie C, Wang X, Young RL, Horowitz M, Rayner CK, Wu T. Role of intestinal bitter sensing in enteroendocrine hormone secretion and metabolic control. Front Endocrinol 2018;9:576. doi:10.3389/fendo.2018.00576.
10. Wierzejska R. Can coffee consumption lower the risk of alzheimer's disease and parkinson's disease? A literature review. Arch Med Sci 2017;13(3):507-14. doi:10.5114/aoms.2016.63599.
11. Juniati T, Rubiyo R. Physical quality and flavor of arabica coffee beans fermented by probiotic microbes from civet digestive system. J Tanaman Industri Penyegar 2016;3(2),61-70. doi:10.21082/jtidp.v3n2.2016.p61-70. (bahasa)
12. Karolina GH, Joanna KN, Karolina B, Krzysztof M, Tomasz N, Klaudia M, et al. Biological aspect of the tongue morphology of wild-captive WWCPS rats - histological, histochemical and ultrastructural study. Anat Sci Int 2018;93(4):514-32. doi:10.1007/s12565-018-0445-y.
13. Eratilla V, Uysal I, Deveci E, Güneş RF, Eratilla E, Yildiz I, et al. Effects of nicotine on rat tongue mucosa : histopathological and immunohistochemical analyses. Anal Quant Cytopathol Histpathol 2016;38(1):17-22.
14. Ma H, Yang R, Thomas SM, Kinnamon JC. Qualitative and quantitative differences between taste buds of the rat and mouse. BMC Neuroscience 2007;8:1-13. doi:10.1186/1471-2202-8-5.
15. Jui-Chin Hsu, Ippei Watari, Yukiha Funaki, Satoshi Kokai, Takashi Ono. Unilateral nasal obstruction induces degeneration of fungiform and circumvallate papillae in rats. J Formosan Med Assoc 2018;117(3):220-6. doi:10.1016/j.jfma.2017.04.013.
16. Parsons Michael H, Sarno Ronald J, Deutsch Michael A. Jump-starting urban rat research: conspecific pheromones recruit wild rats into a behavioral and

pathogen-monitoring assay. Front Ecol Evol 2015;3:146. doi:10.3389/fevo.2015.00146.
17. Bachmanov AA, Bosak NP, Lin C, Matsumoto I, Ohmoto M, Reed DR, et al. Genetics of taste receptors. Curr Pharm Des 2014;20(16):2669-83. doi:10.2174/13816128113199990566.
18. Roper SD, Chaudhari N. Taste buds: cells, signals and synapses. Nat Rev Neurosci 2017;18(8):485-97. doi:10.1038/nrn.2017.68.
19. Folmer B. The Craft and Science of Coffee. USA: Academic Press; 2016:50-167.
20. Hall JE. Guyton and hall textbook of medical physiology. Philadelphia: Elsevier Health Sciences; 2016.
21. Adamson RH. The acute lethal dose 50 (LD50) of caffeine in albino rats. Regul Toxicol Pharmacol 2016;80:274-6. doi.org/10.1016/j.yrtph.2016.07.011.
22. Marta S, Anna L. The effect of diet components on the level of cortisol. Eur Food Res Technol 2016;242:2001-9. doi:10.1007/s00217-016-2772-3.
23. Calvo SS, Egan JM. The endocrinology of taste receptors. Nat Rev Endocrinol 2015;11(4):213-27. doi:10.1038/nrendo.2015.7.
24. Hartley IE, Liem DG, Keast R. Umami as an 'alimentary' taste: a new perspective on taste classification. Nutrients 2019;11(1):182. doi:10.3390/nu11010182.
25. Ma Z, Tanis JE, Taruno A, Foskett JK. Calcium homeostasis modulator (CALHM) ion channels. Pflugers Arch 2016;468:395-403. doi: 10.1007/s00424-015-1757-6.