The Effect of Brotowali Stem Extract (Tinospora Crispa) Towards Increasing Number of Lymphocytes in the Healing Process of Traumatic Ulcer on Diabetic Wistar Rat

by Ira Arundina

Submission date: 26-Oct-2018 11:09AM (UTC+0800)
Submission ID: 1027074385
File name: 23.D17_387_Ira_Arundina.pdf (484.82K)
Word count: 4031
Character count: 21011

The Effect of Brotowali Stem Extract (Tinospora Crispa) Towards Increasing Number of Lymphocytes in the Healing Process of Traumatic Ulcer on Diabetic Wistar Rat

Ira Arundina[1]*, *Indeswati Diyatri*[1], Theresia Indah Budhy[2], *Foo Yau Jit*[3]

1. Department of Oral Biology, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.
2. Department of Oral Pathology and Maxillofacial, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.
3. Undergraduate Student, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.

Abstract

One of the common complication of diabetesis delayed wound healing in oral traumatic ulcer. Lymphocyte cell plays an important role in late inflammatory phase during the process of wound healing. Brotowali (Tinospora crispa) contrains flavonoid and terpenoid which can help to control blood glucose levels and accelerate wound healing. Tinospora crispa extract was made with oven-dried method at 50°C. Dry specimens are then made into a powder and macerated with ethanol 80% (1:10 w/v) The experimental animals were divided into 3 groups, control group 1: Normal Wistar Rat with traumatic ulcer, control group 2:DiabeticWistar rat with traumatic ulcer, and treatment group: Diabetic Wistar rat with traumatic ulcer treated with 250 mg/kg Tinospora crispa extract once a day. All sample were euthanized on day 3, 5, and, 7 after traumatic ulcer was made. Then histopathology preparation was made to count the number of lymphocytes. Blood glucose levels measurement was conducted on day 3, 5, 7, and 14 after traumatic ulcer was made. Kruskal-Wallis test showed a significant difference of blood glucose levels between the control group 1, control group 2 and treatment group. One Way Anova test showed a significant difference of number of lymphocytes between control group 1, control group 2 and treatment group. Brotowali stem extract (Tinospora crispa) is proven to affect blood glucose levels on day 3, 5, 7, and 14. It is also proven that Brotowali stem extract affect the number of lymphocytes in healing of traumatic ulcer among diabetic Wistar rats on day 3, 5, and 7.

Experimental article (J Int Dent Med Res 2017; 10(3): pp. 975-980)

Keywords: Diabetes, Traumatic ulcer, Brotowali, Lymphocytes, Wound healing.

Received date: 31 August 2017 **Accept date:** 25 July 2017

Introduction

Diabetes is a type of disease where imbalance of blood glucose levels in a patient's body take place.[1] Type 2 diabetes is the most common diabetes among the patients. Type 2 Diabetes is a metabolic disorder characterized by a rise in blood glucose due to decrease in insulin secretion by pancreatic beta cell function and insulin resistance.[2]

International Diabetes Federation (IDF) reported that the prevalence of diabetes in Indonesia is about 4.8% and more than half of the cases of diabetes (58.8%) had not been diagnosed. IDF also states that approximately 382 million people worldwide suffer from diabetes in 2013 with undiagnosed diabetes category about 46%. It is estimated that prevalence will continue to rise and reach 592 million by 2035.[3]

Oral health complications such as inflammation, traumatic ulcers and pathology of oral mucosa is often associated with diabetes.[4] Oral mucosal lesions such as traumatic ulcers often occur in patients with diabetes. Several studies have shown a prevalence of 80% of diabetic patients had oral mucosal lesions.[5] The prevalence and levels of development of oral mucosal lesions appear to be higher in patients with diabetes. Diabetes can cause vascularization disorder, decreased blood flow, decreased innate immunity, decreased production of growth factors and psychological stress involved in oral mucosal healing among diabetic patients.[6] Prevalence of traumatic ulcer

Corresponding author:
Ira Arundina
Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga. Jln Mayjend Prof Dr Moestopo no. 47 Surabaya 60132, Indonesia.
E-mail: arundinafkg@yahoo.com

in oral mucosal is quite high and it is about 83.6%.[7] The treatment of traumatic ulcer is basically by eliminating traumatic effect.[8]

Brotowali (Tinospora crispa) from Menispermaceae family are reported to have biological activities such as anti-inflammatory and immunomodulatory.[9] Brotowali contains various chemical compound such as flavonoids, alkaloids, soft resin, starch, glycosides, picroretoside, harsha, bitter substances picroretine, tinocrisposide, berberine, palmatine, columbine, and caoculine.[10] Flavonoids in the Brotowali (Tinospora crispa) stem extract have anti-oxidant properties as well as effective cytotoxic properties.[11]

Lymphocyte plays an important role in wound healing by releasing lymphokines which affect the number of another inflammatory cell. Lymphokines induce the proliferation of fibroblasts with the help of fibroblast activating factor (FAF), which plays a role in wound healing.[12] Lymphokines such as interleukin-2 (IL-2) and transforming growth factor beta (TGF-β) bind to flavonoids which assist the process of lymphocyte proliferation.[13] Based on these thinking, author is encouraged to conduct research on the influence of Brotowali (Tinospora crispa) stem extract at a dose of 250 mg/kg[23] towards decreasing of blood glucose levels on day 3, 5, 7, and 14, as well as increasing number of lymphocytes in the healing process of traumatic ulcer on diabetic Wistar rats on day 3, 5, and 7.

Materials and methods

This type of research is a laboratory experiment. Variable measurement is performed after treatment and sampling is done randomly and control groups are used. Thus, the study design is Randomized Post Test Only Control Group Design. The samples to be used are samples of Wistar rats (Rattus norvegicus). Determination of the minimum sample size is done with Lemeshow formula.

Fresh specimens of Brotowali stem are washed and cut into small pieces and then dried in an oven with a temperature of 50°C. Dry specimens are then made into a powder and macerated with 80% ethanol solution (1:10 w / v) for 24 hours. Then the extract was filtered and separated between the pulp and the filtrate. The pulp is macerated with ethanol. The procedure is repeated until the test results of TLC (Thin Layer Chromatography) and H_2SO_4 do not show anycolour of pink. The pulp and filtrate are collected and evaporated from the first until the last day with a rotary evaporator to remove any excessive solvent. The extract is then stored at -20°C.[11]

Male Wistar rats weighing 150-200g were kept in the same cage with a room temperature of 25 ± 2°C, food pellets are given and distilled water ad libitum for 7 days before the trial begins.[11] Animals were divided into 3 groups which are control group 1, control group 2, and treatment group. Each group contains 8 experimental animals.

Wistar rats in control group 2 and treatment group are diabetes induced through intraperitoneal injection of alloxan (120 mg / kg) which dissolved in 0.05 M citrate buffer of pH 3. Control group 1 was given only sterile saline solution. 3 days after the injection, blood was taken from lateral vein of rats to determine blood glucose levels. Animals declared diabetes when blood glucose levels ≥200 mg/dL after 72 hours' post alloxan induction.[14]

Wistar rats in all treatment group were anesthetized prior to the injection of ketamine (60mg/kg) and xylazine (60mg/kg) intraperitoneal.[15] After anesthesia, each animal is placed on a surgical table in dorsal decubitus position. Then the buccal mucosa is sterilized with a swab moistened with 0.12% chlorhexidine digluconate. After sterilization, the ulceration of buccal mucosa is created by wounding the buccal mucosa by using a scalpel blade no. 15 with a cross-section diameter wound of 8 mm.[16]

Brotowali stem extract at a dose of 250 mg/kg was administered once per day in treatment group with an oral sonde when traumatic ulcer is formed. Extract of brotowali stem is given on day 3, 5, 7, and 14 after the formation of traumatic ulcer.

Blood glucose levels in experimental animals of all treatment groups are checked with a glucometer. Then euthanasia of experimental animals are proceeded with injection of ketamine (180mg/kg) and xylazine (180 mg/kg). Buccal mucosa tissues from all treatment groups are obtained and it is put into formalin solution prior to the processing of preparations.Wistar rats were then buried after obtaining of buccal mucosa tissues.

The number of lymphocytes are

determined through the observation of histopathology preparations stained with H&E under a microscope with a magnification of 400x. The the number of lymphocytes in male Wistar rats are calculated with a graticulae ocular tool which mounted on the lens of a microscope with per field technique conducted in three-way field.[17]

Results

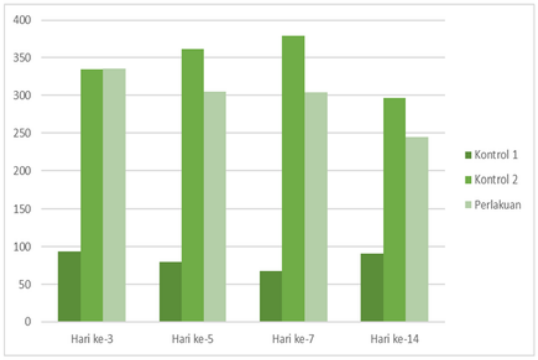

Figure 1. Average blood glucose levels on day 3, 5, 7, and 14.

According to the data, the results of blood glucose levels can be concluded that the average blood glucose levels of Wistar rats on day 3 in control group 1 was 94 mg/dL, control group 2 was 334 mg/dL and treatment group was 335 mg/dL. The average blood glucose levels of Wistar rats on day 5 in control group 1 was 79 mg/dL, control group 2 was 361 mg/dL and treatment group, 304 mg / dL. The average blood glucose levels of Wistarrats on day 7 in control group 1 was 67 mg/dL, control group 2 was 378 mg/dL and treatment group was 304 mg/dL. Lastly, the average blood glucose levels of Wistar rats on day 14 in control group 1 was 90 mg/dL, control group 2 was 296 mg/dL and treatment group only consists 244 mg/dL. From these data, it can be concluded that the lowest average blood glucose levels was control group 1 on day 3, 5, 7, and 14.

Based on the graph, the average blood glucose levels on day 3 had significant differences ($p<0.05$) between blood glucose levels of control group 1, control group 2 and treatment group. On day 5, blood glucose levels of control group 1 and treatment group decreased whereas control group 2 increased. On day 7, blood glucose levelsof control group 1 decreased andcontrol group 2 increased whereas treatment group remained unchange. On the day 14, blood glucose levels of control group 1 increased whereas control group 2 and treatment group decreased.

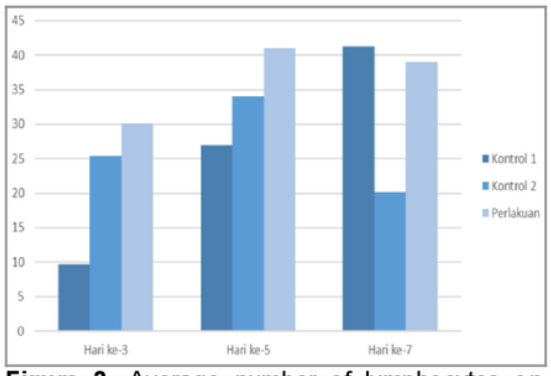

Figure 2. Average number of lymphocytes on day 3, 5, and 7.

From the results of data, the highest average number of lymphocytes in day 3 was the treatment group where Wistarrats were given ulcer wound, induced diabetes and Brotowali stem extract has an average number of 30.10. In control group 2 where Wistar rats were given only ulcer wound and induced diabetes without being given Brotowali stem extract has an average number of 25.34. While in control group 1 where Wistar rats were given only ulcer wound without induced diabetes and Brotowali stem extract has an average number of lymphocytes as much as 9.67.

The average number of lymphocytes on day 5 increased in each group and the highest number of lymphocytes is the treatment group. The treatment group where Wistar rats were given ulcer wound, induced diabetes and Brotowali stem extract has an average number of lymphocytes as much as 41.05. In control group 2 where Wistar rats were given only ulcer wound and induced diabetes without being given Brotowali stem extract has an average number of lymphocytes as much as 34.05. While in control group 1 where Wistar rats were given only ulcer wound without induced diabetes and Brotowali stem extract has an average number of lymphocytes as much as 26.92.

The average number of lymphocytes on day 7 increased and decreased simultaneously. Control group 1 and treatment group increased

whereas control group 2 decreased in number of lymphocytes. Control group 1 has the highest average number of lymphocytes. Control group 1 was given ulcer wound only has an average number of lymphocytes as much as 41.29. In treatment group was given ulcer wound, induced diabetes and Brotowali stem extract has an average number of lymphocytes as much as 39.00. While in control group 2 where Wistar rats were given only ulcer wound and induced diabetes without Brotowali stem extract has an average number of lymphocytes as much as 20.1.

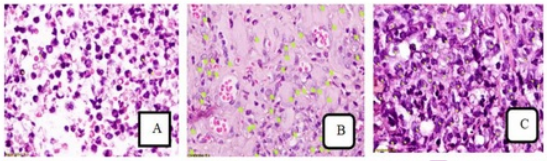

Figure 3. Microscopic appearance of lymphocytes (green arrows) on day 3. (A) Control group 1, (B) Control group 2 and (C) Treatment group on day 3 with HE staining and 400x magnification.

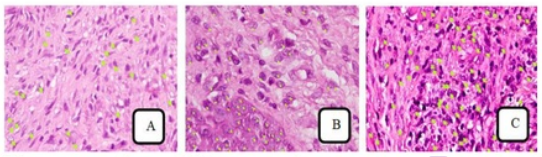

Figure 4. Microscopic appearance of lymphocytes (green arrows) on day 5. (A) Control group 1, (B) Control group 2 and (C) Treatment group on day 5 with HE staining and 400x magnification.

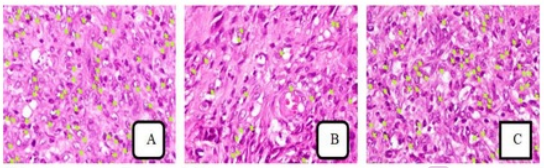

Figure 5. Microscopic appearance of lymphocytes (green arrows) on day 7. (A) Control group 1, (B) Control group 2 and (C) Treatment group on day 7 with HE staining and 400x magnification.

Discussion

Based on the data, the results of the average blood glucose levels on day 3 showed no difference between control group 2 and treatment group because blood glucose levels of diabetes Wistar rats was still high. On day 5, blood glucose levels in treatment group decreased whereas control group 2 increased slightly. On day 7 and 14, blood glucose levels of control group 2 and treatment group decreased. The average blood glucose levels in treatment group always decreased on day 5, 7, and 14, and always lower than the levels of blood glucose of control group 2. This is because control group 2 was not given brotowali stem extract whereas treatment group was given brotowali stem extract. Based on research, it is shown that the bioactivities of Brotowali stem extract (Tinospora crispa) can lower blood glucose levels by 10% on the day 14 after the administration of Brotowali stem extract at a dose of 250 mg/kg.[11] The average blood glucose levels in control group 1 remained unchange. This happened because Wistar rats are experiencing stress and this may lead to changes in blood glucose levels.

Based on the results, some blood glucose levels which were not significant because the duration of administration of Brotowali stem extract was not long enough. Brotowali can lower blood glucose levels because of the content, terpenoids which contain borapetoside A which increases phosphorylation of insulin receptor and protein kinase B as well as increasing the expression of GLUT2 in the liver. This leads to increase glucose utilization in peripheral tissues and reduced gluconeogenesis in the liver and thus it loweredblood glucose levels.[18]According to Sharma[19] studies, significant results required a duration of 60 days. In this study, the time between manufacture of extracts and treatment time is about one month. Thus, this may lead to the unstability of Brotowali stem extract.

Administration of Brotowali stem extract method plays an important role in blood glucose levels of *Wistar* rats. Previous research stated that Brotowali stem extract may be administrate through two types of method, namely orally[11] or intraperitoneal.[20] Both methods have advantages and disadvantages. Oral method is easier to administrate than intraperitoneal method but the use of oral sonde might cause residue extract on oral sonde. Intraperitoneal method is more accurate than oral method however its drawback ishard to administrate on animals and it will die if syringe needle enters peritoneal cavity or chest[21]

Observationof lymphocytes cell count were also done related to healing process of

traumatic ulcer. Healing process of traumatic ulcer may be interrupted on diabetic patient because of the dysfunction in inflammatory response, angiogenesis disorders, and increasing lymphocytes apoptosis.[6] Wound healing process can be observed histologically by counting the number of lymphocytes to determine cell proliferation. Based on research data, the average number of lymphocytes on day 3, 5, and 7 showed treatment group and followed by control group 2are higher than control group 1. This is because Wistar rats in treatment group were given Brotowali stem extract whereas control group 1 and control group 2 were not given any Brotowali stem extract. Brotowali stem extract contains flavonoids which act as antioxidants and play a role in declining of Reactive Oxygen Species (ROS) where cellular response against bacteria and macromolecular damage that occurs from traumatic ulcer wound.[22] Thus, the average number of lymphocytes in treatment group is higher than control group 1 and control group 2. The average number of lymphocytes began to increase on day 3 because day 3 is the phase of inflammation and lymphocytes will initiate to maintain immunology on wound sites.[23] Lymphocytes play an important role in immune system and the number of lymphocytes will increase in inflammatory phase until it peaks on day 3. On day 7, the number of lymphocytes start to decrease.[24] However, the results showed that the average number of lymphocytes peak on day 5 and began to decline on the day 7. This happened because acute inflammatory process is slow and thus on day 5, lymphocytes which are late inflammatory cells start to infiltrate the wound sites. The average number of lymphocytes on day 7 decreased and it indicatesthat antigen is no longer presence, the inflammatory phase is over and the wound began to enter proliferation phase.[25] Control group 1 on day 7 showed the average number of lymphocytes was higher than control group 2 and treatment group altogether. This is because Wistar rats might be stressed and thus period of wound healing is longer. The results showed that treatment group is the most effective group in increasing number of lymphocytes in the process of wound healing (traumatic ulcer).

Brotowali lowers blood glucose levels because of the content of terpenoids in plants containing borapetoside A. Borapetoside A increasesphosphorylation of insulin receptor and protein kinase B as well as increasing the expression of GLUT2 in the liver. It also leads to increase of glucose utilization in peripheral tissues and reduced gluconeogenesis in the liver so that it can lower blood glucose levels.[26] Other contents in Brotowali stem extract are luteolin and quercetin. Luteolin and quercetin are able to trap superoxide radicals in human body. Luteolin and quercetin also have antioxidant effects that can causedeclining in Reactive Oxygen Species (ROS) whichled to apoptosis of lymphocytes decrease.[27]

Flavonoids of Brotowali act as an antioxidant and it can stimulate the production of nitric oxide which led to dilation of blood vessels and potentially as a free radical scavenger. Quercetin is a flavonoid compound that is potential as an antioxidant that trap free radicals so that cells which have been damaged by free radicals get a chance to regenerate.[1]

Conclusions

Based on the results, it can be concluded that Brotowali stem extract (Tinospora crispa) at a dose of 250 mg/kg for 14 days can lower blood glucose levels in diabetic Wistar rats. It can also increase the number of lymphocytes in day 3, 5, and 7 in the healing process of traumatic ulcer on oral mucosal of diabetic Wistar rats.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

References

1. Utomo OM, Azam M, Anggraini DN. Pengaruh Senam Terhadap Kadar Gula Darah Penderita Diabetes. Unnes Journal of Public Health. 2012; 1(1): 37-40.
2. Trisnawati SK, Setyorogo S. Faktor Risiko Kejadian Diabetes Melitus Tipe II Di Puskesmas Kecamatan Cengkareng Jakarta Barat Tahun 2012. Jurnal Ilmiah Kesehatan. 2013; 5(1): 6-11.
3. da Rocha FJ, Ogurtsova K, Linnenkamp U, Guariguata L, Seuring T, Zhang P, Cavan D, Makaroff LE. IDF Diabetes Atlas estimates of 2014 global health expenditures on diabetes. Diabetes Research and Clinical Practice. 2016; 117: 48-54.
4. Al-Maweri SAA, Ismail NM, Ismail AR, Al-Ghashm A. Prevalence of Oral Mucosal Lesions in Patients with Type 2 Diabetes Attending Hospital Universiti Sains Malaysia. Malays J Med Sci. 2013; 20(4): 39-46.
5. Silva MFA, Pereira JV, Godoy GP, Barbosa KGN, Bento PM, Gomes DQC. Prevalence of oral mucosal lesions among patients with diabetes mellitus types 1 and 2. An Bras Dermatol. 2015 ; 90(1): 49-53.

6. Al-Maskari AY., Al-Maskari MY, Al-Sudairy S. Oral Manifestations and Complications of Diabetes Mellitus- A Review. SQU Medical Journal. 2011; 11(2): 180-186.
7. Mendrofa AN, Karsini I, Mulawarmanti D. Ekstrak Daun Mangrove (A. marina) Mempercepat Kesembuhan Ulkus Traumatikus. Dentofasial. 2015; 14(1): 11-14.
8. Hidayat S, Hanum F, A.K. Al. Efectivitas Daya Hambat dan Daya Bunuh Bakteri Ulkus Traumatikus pada Mukosa Mulut dengan Berbagai Konsentrasi Propolis (Trigona sp.). Medali Jurnal. 2014; 2(1): 79-84.
9. Ahmad W, Jantan I, Bukhari SNA. Tinospora crispa (L.) Hook. f. & Thomson: A Review of Its Ethnobotanical, Phytochemical and Pharmacological Aspects. Frontiers in Pharmacology. 2016; 7(59): 1-19.
10. Malik MM. The Potential of Brotowali Stem Extract (Tinospora crispa) as Analternative Antimalarial Drug. J Majority. 2015; 4(5): 45-49.
11. Talubmook C, Buddhakala N. Bioactivities of Extracts from Tinospora crispa Stems, Annona squamosal Leaves, Musa sapientum Flowers, and Piper sarmentosum Leaves in Diabetic Rats. International Journal of Advancements in Research & Technology. 2013; 2(6): 144-149.
12. Broughton G, Rohrich RJ. Wounds and Scars. Selected Readings in Plastic Surgery. 2005; 10(7): 1-54.
13. Wrenshall L., Cerra F., Carlson A. Perlecan: A major IL-2 binding proteoglycan in murine spleen. J. Immunol. 2007; 86:192- 199.
14. Purwanto B., dan Paulus L. Model Hewan Coba untuk Penelitian Diabetes. Surabaya: PT Revka Petra Media. 2014: 9-11.
15. Brizeno LAC, Assreuy AMS, Alves APNN. Delayed healing of oral mucosa in a diabetic rat model: Implication of TNF-α, IL-1β and FGF-2. Life Sciences. 2016 ; 155: 36-47.
16. Cavalcante, GM, de Paula RDS, de Souza LP, Sousa FB, Mota MRL, Alves APNN. Experimental Model of Traumatic Ulcer in the Cheek Mucosa of Rats. Acta Cir. Bra. 2011; 26(3): 227- 243.
17. Pradita AU, Agung PD, Catur AR, Ali T. Periodontal Dressing-containing Green Tea Epigallocathecin gallate Increases Lymphocytes Number in Gingival Artificial Wound Model. Journal of Dentistry Indonesia. 2013; 20(3) : 68-72.
18. Ruan CT, Lam SH, Lee SS, Su MJ. Hypoglycemic action of borapetoside A from the plant Tinospora crispa in mice. Phytomedicine. 2013; 20: 667- 675.
19. S Sharma, R. Heetal, A. Galib, Pradeep, KP. "*Antidiabetic claims of Tinospora cordifolia (Willd.) Miers: critical appraisal and role in therapy*". Asian Pacific Journal of Tropical Biomedicine. 2015; 5(1) : 68-78.
20. Arcueno RO, Jinger LB, Retumban, Joycelyn EE, Jonathan JGG. Wound healing potential of Tinospora crispa (Willd.) Miers [Menispermaceae] stem on diabetic mice. Journal of Medicinal Plants Studies. 2015; 3 (2) :106-109.
21. Mirjana P, Jasmin F, Vera RN, Cena D, Lidija P, Aneta M, Natasa TS, Biljana D. The effects of NBF gingival gel in the treatment of recurrent oral ulcer : case report. Journal of International Dental and Medical Research. 2016; 9 (1) : 81-85.
22. Mohammed AIC, Manish G, Dinesh CK. Antimicrobial Activity of Tinospora crispa Root Extracts. IJRAP. 2012; 3(3): 417-419.
23. Bellanti J. Immunology: Volume 6 of Reticuloendothelial system, a comprehensive treatise. USA: Springer Science & Business Media. 2013: 240-242.
24. Arisa I, Nurdiana D, Dewi INP. Ekstrak haruan (Channa striata) secara efektik menurunkan jumlah limfosit fase inflamasi dalam penyembuhan luka. Dentofasial. 2014; 13 (3) : 176-181.
25. Guyton AC., Hall JE. Buku Ajar Fisiologi Kedokteran. Edisi 11. Jakarta: EGC. 2008 : 455-463.
26. Ruan CT, Lam SH, Lee SS, Su MJ. Hypoglycemic action of borapetoside A from the plant Tinospora crispa in mice. Phytomedicine. 2013; 20: 667-675.
27. Otton R, Soriano FG, Verlengia R and Curi R. Diabetes induces apoptosis in lymphoctyes. Journal of Endocrinology. 2004; 182 : 145-156.

The Effect of Brotowali Stem Extract (Tinospora Crispa) Towards Increasing Number of Lymphocytes in the Healing Process of Traumatic Ulcer on Diabetic Wistar Rat

ORIGINALITY REPORT

19%	17%	12%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Liao, Dan. "Therapeutic effect of Zhuang herb Hanyitai on duck hepatitis B", World Chinese Journal of Digestology, 2015. Publication	1%
2	digilib.stikeskusumahusada.ac.id Internet Source	1%
3	Submitted to University of Iowa Student Paper	1%
4	www.uniklinik-duesseldorf.de Internet Source	1%
5	www.ffhdj.com Internet Source	1%
6	Submitted to Universiti Teknologi MARA Student Paper	1%
7	journal.unair.ac.id Internet Source	1%

Bruno A. L. A. Mariz, Ciro D. Soares, Thayná M.

8	L. Morais, Felipe P. Fonseca, Maria G. F. de Carvalho, Jacks Jorge. "Expression of FGF-2/FGFR-1 in normal mucosa, salivary gland, preneoplastic, and neoplastic lesions of the oral cavity", Journal of Oral Pathology & Medicine, 2018 Publication	1%
9	Barker, G.A.. "Effect of oxolinic acid on bacterial flora and hatching success rate of rainbow trout, Oncorhynchus mykiss, eggs", Aquaculture, 19901215 Publication	1%
10	www.dovepress.com Internet Source	1%
11	iaast.org Internet Source	1%
12	repository.maranatha.edu Internet Source	1%
13	Submitted to University of Alabama at Birmingham Student Paper	1%
14	worldwidescience.org Internet Source	1%
15	journal.itb.ac.id Internet Source	<1%

16	acikerisim.selcuk.edu.tr:8080 Internet Source	<1%
17	repository.phb.ac.id Internet Source	<1%
18	www.esciencecentral.org Internet Source	<1%
19	id.portalgaruda.org Internet Source	<1%
20	www.springer.com Internet Source	<1%
21	Submitted to Universiti Sains Malaysia Student Paper	<1%
22	Hendrijantini, Nike, Utari Kresnoadi, Sherman Salim, Bambang Agustono, Endang Retnowati, Iwan Syahrial, Pungky Mulawardhana, Manggala Pasca Wardhana, Coen Pramono, and Fedik Abdul Rantam. "Study Biocompatibility and Osteogenic Differentiation Potential of Human Umbilical Cord Mesenchymal Stem Cells (hUCMSCs) with Gelatin Solvent", Journal of Biomedical Science and Engineering, 2015. Publication	<1%
23	Submitted to Delaware County Community College Student Paper	<1%

24	repository.uinjkt.ac.id Internet Source	<1%
25	unhas.ac.id Internet Source	<1%
26	www.icsoffice.org Internet Source	<1%
27	www.pjbs.org Internet Source	<1%
28	www.florajournal.com Internet Source	<1%
29	balimedicaljournal.org Internet Source	<1%
30	onlinelibrary.wiley.com Internet Source	<1%
31	www.jbpe.org Internet Source	<1%
32	Jan Broomfield, Barbara Dodd. "Is speech and language therapy effective for children with primary speech and language impairment? Report of a randomized control trial", International Journal of Language & Communication Disorders, 2011 Publication	<1%
33	www.veterinaryworld.org	

Internet Source

<1%

---

34 Daniel S. Scheller. "The Effects of Neighborhood Democracy on Cooperation: A Laboratory Study", Journal of Urban Affairs, 2016

Publication

<1%

---

35 www.stuartxchange.com

Internet Source

<1%

---

36 Luiz André C. Brizeno, Ana Maria S. Assreuy, Ana Paula N.N. Alves, Fabrício B. Sousa et al. "Delayed healing of oral mucosa in a diabetic rat model: Implication of TNF-α, IL-1β and FGF-2", Life Sciences, 2016

Publication

<1%

---

37 Brizeno, Luiz André C., Ana Maria S. Assreuy, Ana Paula N.N. Alves, Fabrício B. Sousa, Paulo Goberlânio de B. Silva, Suzana Cantanhede O. Machado de Sousa, Nelise A.S. Lascane, Janaína Serra-Azul M. Evangelista, and Mário Rogério L. Mota. "Delayed healing of oral mucosa in a diabetic rat model: Implication of TNF-α, IL-1β and FGF-2", Life Sciences, 2016.

Publication

<1%

---

38 Wang, Ya-Dong, Jin-Dao Wu, Zhong-Li Jiang, Yu-bang Wang, Xue-Hao Wang, Chao Liu, and Ming-Qing Tong. "Comparative Proteome

<1%

Analysis of Neural Retinas from Type 2 Diabetic Rats by Two-dimensional Electrophoresis", Current Eye Research, 2007.
Publication

---

39 Hafizur, Rahman M. Hameed, Abdul Shukrana, Mishkat Raza, Sayed Ali Chishti, Sidra Kabir, Nurul Siddi. "Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance in vivo and by stimulatin", Phytomedicine: International Journal of Phytotherapy & Phytopharmacology, Feb 15 2015 Issue <1%
Publication

---

40 "Smaller Reporting Company Definition.", Federal Register <1%
Publication

---

41 Tengstrand, B., E. Larsson, L. Klareskog, and I. Hafström. "Randomized withdrawal of long-term prednisolone treatment in rheumatoid arthritis: effects on inflammation and bone mineral density", Scandinavian Journal of Rheumatology, 2007. <1%
Publication

Exclude quotes	On	Exclude matches	< 5 words
Exclude bibliography	Off		

The Effect of Brotowali Stem Extract (Tinospora Crispa) Towards Increasing Number of Lymphocytes in the Healing Process of Traumatic Ulcer on Diabetic Wistar Rat

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6