Majalah Kedokteran Gigi Indonesia
Vol 8 No 3 – December 2022
ISSN 2460-0164 (print), ISSN 2442-2576 (online)
Available online at https://jurnal.ugm.ac.id/mkgi
DOI: http://doi.org/10.22146/majkedgiind.76416

RESEARCH ARTICLE

Effect of 4% and 15% moringa leaf extract gel on gingival wound healing in rats

Eka Pramudita Ramadhany*✉, I Gusti Agung Dyah Ambarawati, Muhammad Rafif Musyaffa*****

*Department of Periodontics, School of Dentistry, Faculty of Medicine, Universitas Udayana, Bali, Indonesia
**Department of Oral Medicine, School of Dentistry, Faculty of Medicine, Universitas Udayana, Bali, Indonesia
***Dentistry Program and Professional Education, Faculty of Medicine, Universitas Udayana, Bali, Indonesia
*Jl P.B. Sudirman, Dangin Puri Klod, Denpasar, Bali, Indonesia; ✉ correspondence: ditaramadhany@unud.ac.id

Submitted: 20th July 2022; Revised: 14th September 2022; Accepted: 22nd November 2022

ABSTRACT

Traumatic dental procedures such as incisions and gingival flaps are the leading causes of injury to the gingival structure. One of natural ingredients that can potentially accelerate the wound healing process is Moringa leaves (*Moringa oleifera*). Moringa leaf has several active compounds, one of which is flavonoids, which can be an anti-inflammatory and antibacterial agent, and increase collagen synthesis. A randomized posttest-only control-group design was used in this study. 48 wistar rats were categorized into four groups: CMC-Na gel, hyaluronic acid, 4% Moringa leaf extract gel, and 15% Moringa leaf extract gel. The samples were then euthanized on days 1, 3, 5, and 7. The two-way ANOVA test described significant differences ($p < 0.05$) for all the components of the observation (neutrophils, fibroblasts, angiogenesis, and epithelial thickness). The lowest mean number of neutrophils, the highest mean number of fibroblasts, and the highest mean of angiogenesis were found in the 15% Moringa extract group on the 7th day. The highest mean epithelial thickness was found in the use of 4% Moringa extract on the 5th day. The microscopic images showed that the treatment group gained more effective wound healing processes than the control group. The microscopic image showed that, in terms of neutrophils, fibroblasts, and angiogenesis, 15% Moringa extract was more effective for wound healing compared to 4% Moringa extract.

Keywords: gingival wound; *Moringa oleifera*; topical gel; wound healing

INTRODUCTION

The function of the gingiva is to protect the underlying structures against the influence of the oral environment. The function of the gingiva as a protective tissue causes it to be susceptible to injury.[1] Wounds can occur as a result of trauma and surgery.[2] Wound healing is divided into several stages, namely hemostasis, inflammation, proliferation, and remodeling.[3] The wound healing process in the oral cavity passes through the same fundamental pathway as that in other regions.[4] However, the abundance of pathogenic and opportunistic bacteria in the oral cavity can trigger acute and chronic infections. Acceleration of the healing process in the oral cavity is highly recommended to reduce discomfort and pain in dental patients.[5] Topical application is used to accelerate the wound healing process. Topical (transdermal) drugs have several advantages, such as eliminating fluctuations in gastrointestinal absorption and providing constant and controlled drug input.[6] One of the topical drug dosage forms that can be applied to the oral cavity is a gel. The advantage of gel preparations is better penetration, which maximizes the local effect and minimizes the systemic effect.[7]

One of natural ingredients that have the potential to accelerate the healing process of gingival wounds are Moringa leaves (*Moringa oleifera*). Moringa leaves have several active ingredients, one of them is flavonoids. Flavonoids function as antibacterial and anti-inflammatory agents, and play a role in increasing collagen synthesis by fibroblasts during a wound healing process.[8,9] Previous research showed that the use of Moringa leaf extract gel was proven to accelerate the wound closure process on the palate of rats.[8] Another study also stated that the

administration of gel preparations from Moringa leaf extract could shorten the bleeding time and increase the collagen density of the incision wound on the gingiva of *Cavia porcellus*.[10] This study aimed to determine the effect of *Moringa oleifera* extract gel on the gingival wound healing process in wistar rats (*Rattus norvegicus*) seen from the number of neutrophils, the number of fibroblasts, angiogenesis, and epithelial thickness.

MATERIALS AND METHODS

This study was a laboratory experiment with a randomized posttest-only control group design. This research was approved by the research ethics committee of the Faculty of Medicine, Udayana University with clearance number 2303/UN14.2.2.VII.14/LT/2021.The making of Moringa Leaf Extract Gel started by sterilizing, drying, and refining it into powder. The powder was then extracted using a Soxhlet extractor with 95% ethanol as solvent. The process took three cycles per hour. The extract was put into a flash evaporator. Gels containing 4% and 15% Moringa leaf extract were obtained by dissolving 2 g and 7.5 g of Moringa leaf extract in 5 ml of distilled water. A total of 50 g of CMC-Na gel base was added to the mixture gradually until the mixture was homogeneous. Moringa Leaf Extract Gel was prepared in the Laboratory of Phytochemistry and Experimental Animals, Faculty of Mathematics and Natural Sciences, Udayana University.

Fourty-eight (n = 48) male wistar rats (*Rattus norvegicus*) aged 2-3 months whose body weight was 150-200 grams were categorized into four groups: CMC-Na gel, hyaluronic acid, and 4% Moringa oleifera extract gel, and 15% Moringa oleifera extract gel. The research samples were given standard laboratory pellet and tap water *ad libtum*. They were first adapted for seven days under normal laboratory condition with sufficient light and ventilation in the Laboratory of Phytochemistry and Experimental Animals, Faculty of Mathematics and Natural Sciences, Udayana University.

Intramuscular injection was carried out with the administration of anesthetic drugs. The labial gingiva of the rats' mandibular incisor was injured using a 2.5 mm punch biopsy. 0.5 mL of Moringa leaf extract was applied with the help of cotton buds every morning and evening. Hyaluronic Acid (Gingigel) and CMC-Na gel were applied in the control group with the same procedure. Gingival samples were taken using surgical scissors and a scalpel. The tissue was stored in a 10% formalin buffer solution and histological preparations were made. The sacrificed rats were then buried properly. All histological microscopic features were measured on days 1, 3, 5, and 7 and stained with H&E, hematoxylin–eosin in the control and treatment groups. Fibroblasts, neutrophils, and angiogenesis were counted using a binocular light microscope at 400× magnification and Image Raster 3.0 (United States) software. In five fields of view, observations were made on randomly selected as the regions of interest in gingival wound area. The epithelial thickness was measured by microruler with a scale of 1:1,000 at 400x magnification. Observations were made with the help of an Olympus light microscope with an Optilab digital camera. The magnification was 400x in five fields of view. The analysis was carried out using Saphiro-Wilk Test of normality, followed by Levene's test. The statistical test was done with Two-Way ANOVA as a comparative test, followed by post hoc LSD.

RESULTS

The lowest mean number of neutrophils was found in the 15% Moringa leaf extract gel group on the 7th day of euthanasia, while the highest mean number was found in the 2% CMC Na gel group on the 1st day of euthanasia. The lowest mean number of fibroblasts was found in the 2% CMC-Na Gel group on the 1st day of euthanasia, while the highest mean was found in the 15% Moringa leaf group on the 7th day.

The lowest mean number of angiogenesis was found in the 2% CMC Na Gel group on the 1st day of euthanasia, while the highest mean was found in the 15% Moringa leaf group on the 7th day. The lowest mean epithelial thickness was

Majalah Kedokteran Gigi Indonesia. December 2022; 8(3): 192-199
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

found in the 2% CMC Na group on the 3rd day of euthanasia, while the highest mean was found in the 4% Moringa leaf extract group on the 5th day.

The normality and homogeneity test resulted in a p-value of > 0.05 in all the observed cells. It can be concluded that the research data were normal and homogeneous. The two-way ANOVA results indicated a significant difference ($p < 0.05$) in all the observed components (neutrophils, fibroblasts, angiogenesis, and epithelial thickness). The data were then analyzed using the Post-hoc test LSD (Least Significant Different).

Table 1. Descriptive analysis of neutrophils

Group	Day 1		Day 3rd		Day 5th		Day 7th		p - value
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
2% Na CMC gel	47.50	3.536	20.00	7.071	35.00	4.243	30.00	0.000	0.000*
Hyaluronic acid	36.00	1.414	27.50	3.536	12.50	3.536	9.00	2.828	
4% Moringa leaf extract gel	32.50	3.536	21.50	3.536	17.50	3.536	12.50	3.536	
15% Moringa leaf extract gel	25.00	7.071	17.50	3.536	10.00	1.414	5.00	1.414	

*significant ($p < 0.05$)

Table 2. Descriptive analysis of fibroblast

Group	Day 1		Day 3		Day 5		Day 7		p - value
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
2% Na CMC gel	88.00	2.828	107.50	10.607	145.00	7.071	174.00	5.657	0.000*
Hyaluronic acid	95.00	7.071	127.50	3.536	150.00	7.071	190.00	2.828	
4% Moringa leaf extract gel	126.50	2.121	152.00	2.828	163.50	2.121	177.50	3.536	
15% Moringa leaf extract gel	130.00	14.142	152.50	10.607	172.50	10.607	227.50	3.536	

*significant ($p < 0.05$)

Table 3. Descriptive analysis of angiogenesis

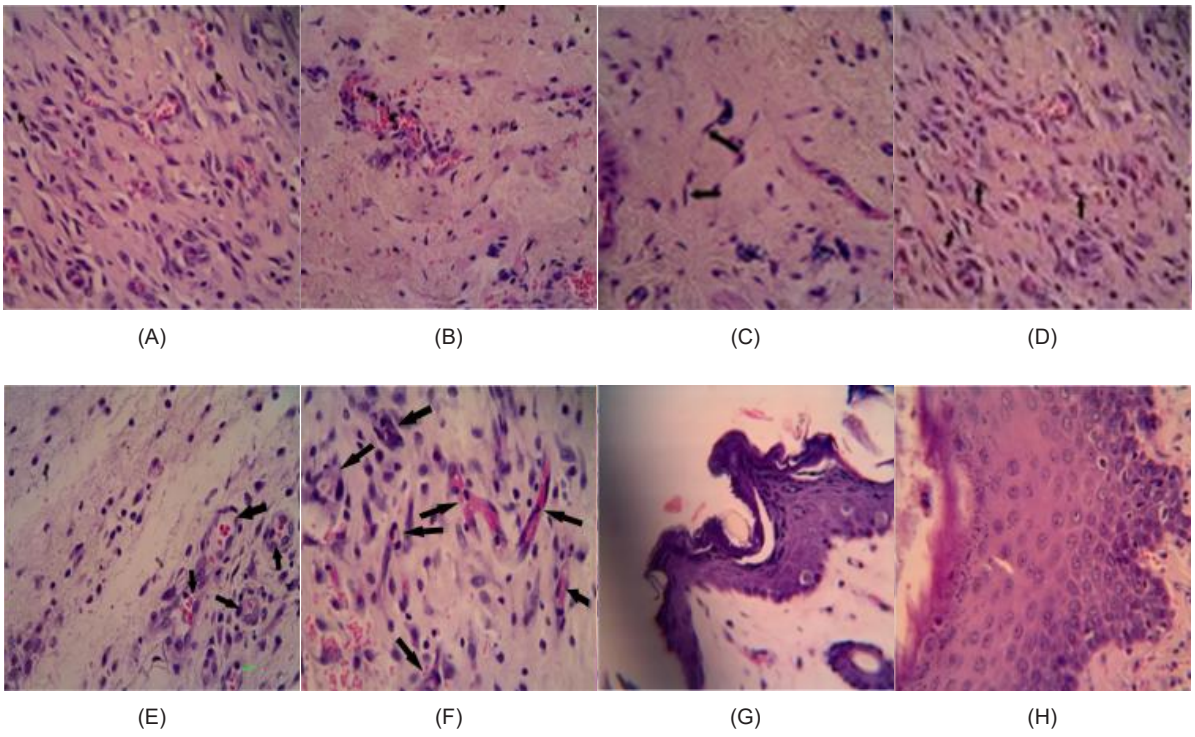
Group	Day 1		Day 3		Day 5		Day 7		p - value
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
2% Na CMC gel	7.50	0.707	8.50	0.707	11.00	1.414	12.50	2.121	0.000*
Hyaluronic acid	14.00	1.414	16.00	2.828	17.00	2.828	19.00	4.243	
4% Moringa leaf extract gel	22.50	3.536	22.50	2.121	22.50	0.707	26.50	7.778	
15% Moringa leaf extract gel	25.00	2.828	30.00	2.828	32.50	0.707	35.50	0.707	

*significant ($p < 0.05$)

Table 4. Descriptive analysis of epithelial thickness

Group	Day 1		Day 3		Day 5		Day 7		p - value
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
2% Na CMC gel	23.000	6.3640	15.400	0.1414	15.600	1.6971	31.450	0.4950	0.000*
Hyaluronic acid	19.150	8.4146	37.150	4.0305	41.400	7.2125	37.200	17.1120	
4% Moringa leaf extract gel	22.700	10.8894	29.250	9.9702	58.600	5.3740	37.300	7.7782	
15% Moringa leaf extract gel	25.900	12.5865	24.800	5.3740	26.050	8.5560	32.850	3.4648	

*significant ($p < 0.05$)



(A) (B) (C) (D)

(E) (F) (G) (H)

Figure 1. Histological microscopic feature of: neutrophil (A) 15% Moringa leaf extract gel group on the 7th day (B) 2% CMC Na gel group on the 1st day; fibroblast (C) 2% CMC-Na Gel group on the 1st day (D) 15% Moringa leaf group on the 7th day; angiogenesis (E) 2% CMC Na Gel group on the 1st day (F) 15% Moringa leaf group on the 7th day; epithelial thickness (G) 2% CMC Na group on the 3rd day (H) 4% Moringa leaf extract group on the 5th day of euthanasia**.** All of histological microscopic feature stained with H&E, hematoxylin–eosin, and 400× magnification (indicated by black arrow).

Table 5. *Post hoc* neutrophil test of 4% Moringa leaf extract gel group

Day	1	3	5	7
1		0.011*	0.0001*	0.000*
3			0.309	0.031
5				0.208
7				

Table 6. *Post hoc* neutrophil test of 15% Moringa leaf extract gel group

Day	1	3	5	7
1		0.066	0.001*	0.000*
3			0.066	0.005*
5				0.208
7				

Majalah Kedokteran Gigi Indonesia. December 2022; 8(3): 192-199
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

Table 7. *Post hoc* fibroblast test of 4% Moringa leaf extract gel group

Day	1	3	5	7
1		0.002*	0.000*	0.000*
3			0.121	0.002*
5				0.064
7				

Table 8. *Post hoc* fibroblast test of 15% Moringa leaf extract gel group

Day	1	3	5	7
1		0.006*	0.000*	0.000*
3			0.012*	0.000*
5				0.000*
7				

Table 9. *Post hoc* angiogenesis test for 4% Moringa leaf extract gel group

Day	1	3	5	7
1		1.000	1.000	0.193
3			1.000	0.193
5				0.193
7				

*significant($p < 0.05$)

Table 10. *Post hoc* angiogenesis test of 15% Moringa leaf extract gel group

Day	1	3	5	7
1		0.109	0.021*	0.003*
3			0.408	0.080*
5				0.323
7				

Table 11. *Post hoc* test of the epithelial thickness of 4% Moringa leaf extract gel group

Day	1	3	5	7
1		0.432	0.000*	0.091
3			0.002*	0.336
5				0.018*
7				

Table 12. *Post hoc* test of the epithelial thickness of 15% Moringa leaf extract gel group

Day	1	3	5	7
1		0.894	0.985	0.405
3			0.880	0.336
5				0.415
7				

DISCUSSION

The descriptive analysis of neutrophils showed that the total mean was lower in the treatment group (4% and 15% Moringa leaf gel) than the control group (2% Na CMC gel and hyaluronic acid). The observations of fibroblasts, angiogenesis, and epithelial thickness showed a higher total mean in the 4% and 15% Moringa leaf gel groups than the 2% Na and hyaluronic acid CMC gel groups. The two-way ANOVA test indicated significant differences in all the observed wound healing factors (neutrophils, fibroblasts, angiogenesis, and epithelial thickness).

The observation of the neutrophils in the 4% Moringa leaf group showed a significant difference on the 1st day compared to the 3rd, 5th, and 7th day. This significant difference was caused by the active compounds in the Moringa leaf extract gel. Flavonoids play a role in accelerating wound healing. In the inflammatory phase, flavonoids help the wound healing process by inhibiting the activity of the COX-2 enzyme.[11] The observation of the fibroblasts in the 4% Moringa leaf group showed significant differences on the 1st day compared to the 3rd, 5th, and 7th days and the 3rd compared to the 7th day. This significant difference was caused by the bioactive compounds in the gel, one of which is flavonoids. Flavonoids have antibacterial and anti-inflammatory properties, which cause the proteins in the bacteria around the wound to become denatured so that the bacterial cells break down and make the proper wound-healing process.[8,11,12] The results of the observation of the epithelial thickness in the 4% Moringa leaf extract gel group showed a significant difference on the 1st euthanasia day compared to the 5th euthanasia day, the 3rd euthanasia day compared to the

5th euthanasia day, and the 5th euthanasia day compared to the 7th euthanasia day. It is because Moringa leaf extract contains flavonoids. The flavonoids in Moringa leaf extract were proven to increase the expression of TGF-ß1, CD68, and VEGF. Increased VEGF stimulates endothelial cell proliferation in wound healing mechanisms. Saponin compounds in leaf extract activate the functions of VEGF, TGF-ß, FGF, and epidermal growth factor (EGF).[13,14]

The results of the observation of the neutrophils in the 15% Moringa leaf group showed a significant difference on the 1st euthanasia day compared to the 5th and 7th euthanasia day, and the 3rd euthanasia day compared to the 7th euthanasia day. This significant difference was caused by several bioactive compounds in the Moringa leaf extract gel, which have anti-inflammatory properties, including flavonoids and alkaloids.[11,15] The observation of the fibroblasts in the 15% Moringa leaf extract gel group showed a significant difference between all the euthanasia days. This significant difference was due to several bioactive compounds in Moringa leaf extract, one of which is flavonoids. Flavonoids in Moringa leaf extract can accelerate the wound healing process by increasing collagen synthesis, decreasing the number of macrophages, and increasing the number of fibroblasts.[11,16] This is directly proportional to research conducted by Poernomo and Setiawan, 2019, showing that 15% Moringa leaf extract gel can shorten bleeding time and increase collagen density. The increase in collagen density occurs because fibroblasts play a role in the formation of collagen fibers. In a normal wound healing process, collagen provides tissue integrity and strength in the injured area.[16,17,18]

The observation of the angiogenesis in the 15% Moringa leaf extract gel group found a significant difference on the 1st euthanasia day compared to the 5th and 7th euthanasia day and the 3rd euthanasia day compared to the 7th euthanasia day. This is because flavonoids can stimulate angiogenesis during the wound healing process.[11] On the other hand, the post hoc analysis of the groups using 4% and 15% Moringa leaf extract gel showed no significant differences, presumably because of some confounding factors that could interfere with the wound healing process.[19]

However, this study has some limitations. This study involved a limited number of samples and a limited variation of extract concentrations in the gel. There is still a possibility that other concentrations might have a significant impact on healing process. It is recommended for further research to use other variations of extract concentrations in the gel and more samples. In addition, the application of the extract with other gel bases in the healing process of gingival wounds is still needed to identify its impact on wound healing. It is also necessary to carry out a quantitative phytochemical testing to determine which compounds play an important role in the healing process.

CONCLUSION

The study results concluded that 15% Moringa leaf extract gel had a better anti-inflammatory effect in reducing the number of neutrophils than 4% Moringa leaf gel, especially on the 3rd day. In addition, 15% Moringa leaf extract gel was also better in increasing the number of fibroblasts compared to 4% Moringa leaf gel, especially on the 7th day of wound healing. The study results also concluded that 15% moringa leaf extract gel was better in increasing angiogenesis than 4% Moringa leaf gel, especially on the 7th day of wound healing. However, 4% moringa leaf gel was better in increasing epithelial thickness than 15% Moringa leaf gel, primarily on the 5th day of wound healing.

ACKNOWLEDGMENT

This study was carried out with the help of Udayana University PNPB research program with letter number B/4969-5/UN14.2.2.VII.1/PT.01.03/2021. The authors express gratitude to the Pharmacy Laboratory and Histology Laboratory of Udayana University for the completion of this research.

CONFILCT OF INTEREST

There is no potential conflict of interest in this study.

REFERENCES

1. Michael G. N, Takei H, R. Klokkevold P, Carranza F. Newman and carranza's clinical periodontology. Elsevier Health Sciences; 2018. 182-219.
2. Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, Nusgart M, Cartwright D. An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds. Value Health. 2018; 21(1): 27-32. doi: 10.1016/j.jval.2017.07.007
3. Singh S, Young A, McNaught CE. The physiology of wound healing. Surgery (Oxford). 2017; 35(9): 473-477. doi: 10.1016/j.mpsur.2017.06.004
4. DesJardins-Park HE, Mascharak S, Chinta MS, Wan DC, Longaker MT. The spectrum of scarring in craniofacial wound repair. Frontiers in physiology. 2019; 10(322): 1-14. doi:10.3389/fphys.2019.00322
5. Habiboallah G, Mahdi Z, Majid Z, Nasroallah S, Taghavi AM, Forouzanfar A, Arjmand N. Enhancement of gingival wound healing by local application of silver nanoparticles periodontal dressing following surgery: a histological assessment in animal model. Modern Research in Inflammation. 2014; 3(3): 129. doi:10.4236/mri.2014.33016
6. Durga KN, Bhuvaneswari P, Hemalatha B, Padmalatha K. A review on transdermal drug delivery system. Asian Journal of Pharmacy and Technology. 2022; 12(2): 159-166. doi: 10.52711/2231-5713.2022.00027
7. Sailaja AK. An Overall review on topical preparation gel. J Curr Pharma Res. 2014; 4(2): 1138–1143. doi: 10.24018/10.24018/iijmps.2018.v1i1.22
8. Amaliya A, Muhaimina RK, Susanto A, Sutjiatmo AB. Histological assessment of palatal donor site wound healing after application of moringa oleifera lamarck leaf extract in rats. European journal of dentistry. 2019; 13(2): 248-254. doi: 10.1055/s-0039-1695065
9. Azevedo ÍM, Araújo-Filho I, Teixeira MMA, Moreira MDF de C, Medeiros AC. Wound healing of diabetic rats treated with moringaoleifera extract. Acta cirúrgica brasileira. 2018; 33(9): 799-805. doi:10.1590/s0102-865020180090000008
10. Poernomo H, Setiawan. The effect of moringa leaf (moringa oleifera) gel on the bleeding time and collagen density of gingival incision wound healingin marmot (cavia porcellus). 2019; 15(1): 34-39. doi: 10.46862/interdental.v15i1.342
11. Carvalho MTB, Araújo-Filho HG, Barreto AS, Quintans-Júnior LJ, Quintans JSS, Barreto RSS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phytomedicine. 2021; 90: 153636. doi: 10.1016/j.phymed.2021.153636
12. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochemistry Reviews. 2018; 18: 241–272. doi: 10.1007/s11101-018-9591-z
13. Gopalakrishnan A, Ram M, Kumawat S, Tandan SK, Kumar D. Quercetin accelerated cutaneous wound healing in rats by increasing levels of VEGF and TGF-β1. Growth Factors. 2016; 38(2):187-195. doi: 10.1080/08977194.2020.1822830
14. Pang Y, Zhang Y, Huang L, Xu L, Wang K, Wang D, Guan L, Zhang Y, Yu F, Chen Z, Xie X. Effects and mechanisms of total flavonoids from Blumea balsamifera (L.) DC. on skin wound in rats. Int J Mol Sci. 2017; 18(12): 1–12. doi: 10.3390/ijms18122766
15. Bai R, Yao C, Zhong Z, Ge J, Bai Z, Ye X, Xie T, Xie Y. Discovery of natural anti-inflammatory alkaloids: Potential leads for the drug discovery for the treatment of inflammation. Eur J Med Chem. 2021; 213: 113165. doi: 10.1016/j.ejmech.2021.113165
16. Kim YA, Tarahovsky YS, Gaidin SG, Yagolnik EA, Muzafarov EN. Flavonoids determine the rate of fibrillogenesis and structure of collagen type I fibrils in vitro. Int J Biol Macromol. 2017; 104: 631–637. doi: 10.1016/j.ijbiomac.2017.06.070

17. Lakra R, Kiran MS, Korrapati PS. Collagen scaffold reinforced with furfural for wound healing application. Materials Letters. 2022; 315: 131956.
doi: 10.1016/j.matlet.2022.131956
18. Smith PC, Martínez C, Martínez J, McCulloch CA. Role of fibroblast populations in periodontal wound healing and tissue remodeling. Frontiers in physiology. 2019; 10: 270. doi: 10.3389/fphys.2019.00270
19. Suckow MA, Hankenson FC, Wilson RP, Foley PL. The laboratory rat. Academic Press. 2019. 429.