



1 **Journal of Mathematical and**
2 **Fundamental Sciences**

3 (formerly ITB Journal of Science)

4 *Published by ITB Journal Publisher, the Institute for Research and Community Services*
5 *-ITB,*

6 *Gedung CRCS Institut Teknologi Bandung Lt. 6 & 7, Jalan Ganesha No. 10, Bandung –*
7 *40132, Indonesia*

8

9

10 **ASSIGNMENT OF COPYRIGHT**

11

12

13 Full Title of Paper : **Robusta Coffee Beans (*Coffea canephora*)**
14 **Decrease IL-1 α (Interleukin-1 α) Expression**
15 **and Increase the Number of Fibroblasts in**
16 **Healing Process in Dental Pulp in Wistar Rat**

17

18 Authors (Full Names) : Prof. Dr. drg. I Dewa Ayu Ratna Dewanti, M.Si

19

20

21 With the submission of the paper entitled above and the acceptance for
22 publication, I hereby assign all rights including the copyright in the said
23 paper to the *Journal of Mathematical and Fundamental Sciences*.

24

25

26

27

28

29

30 Prof. Dr. drg. I Dewa Ayu Ratna D, M.Si

Jember,

31 August 4 2018

● Committee ICAMBBE <icambe@gmail.com>
Ke IDewa Dewanti

📧 09/12/17 jam 3:08 PM

Dear,
I Dewa Ayu Ratna Dewanti

We would like to inform you that your abstract entitled **Robusta Coffee Beans (*Coffea canephora*) Decrease IL-1 α (Interleukin-1 α) Expression and Increases the number of Fibroblasts In Dental Pulp of Wistar Rat** is selected for publication in the Journal of Mathematical and Fundamental Sciences (ISSN: 2337-5760, E-ISSN: 2338-5510, Scopus indexed) for Rp 1.750.000 or USD 130. The full-text paper will be reviewed by reviewers from ICAMBBE and the journal. In case proofreading is necessary for the full-text paper, we provide a proofreading service for only Rp 400.000.

For participants that are willing to publish their full-text papers, please inform us by replying this email before 18 September 2017 and prepare the paper according to the guideline (attached in this email) and submit it to the ICAMBBE committee (icambe@gmail.com) by 30 September 2017.

We look forward for a good news from you.

Should you have any question, please do not hesitate to contact us. Thank you very much.

32
33
34
35
36
37
38

Robusta Coffee Beans (*Coffea canephora*) Decrease IL-1 α (Interleukin-1 α) Expression and Increase the Number of Fibroblasts in Healing Process in Dental Pulp in Wistar Rat

39
40
41
42
43
44
45
46
47
48
49
50
51
52

I Dewa Ayu Ratna Dewanti¹. I Dewa Ayu Susilawati². Pujiana Endah Lestari³.
Ristya Widi Endah Yani⁴. Erawati Wulandari⁵. Roedy Budirahardjo⁶. Dyah Setyorini⁷.
Sunlip Wibisono⁸.

^{1,2,3} Department of Biomedical Science Faculty of Dentistry, Jember University,
Kalimantan street 37, Jember 68121

⁴ Department of Public Dental Health Faculty of Dentistry, Jember University,
Kalimantan street 37, Jember 68121

^{6,7} Department of Pedodontia Faculty of Dentistry, Jember University, Kalimantan street
37, Jember 68121

⁸ Department of Statistic, Faculty of Economic and Busines⁸, Jember University,
Kalimantan street 37, Jember 68121

Email: idewadewanti@yahoo.com

53
54
55
56
57
58
59
60
61

Abstract. Fibroblasts and IL-1 α are important components of inflammation and healing process in dental caries. This healing allegedly could be modulated by Robusta coffee . Aim. Analyzed modulation of some concentration of Robusta

62 coffee beans to IL-1 α expression and the number of fibroblasts. We used Wistar
63 Rats were divided into 4 groups and 4 sub groups (day 7, 14, 21) for each group
64 (each 4 tails). Controle group: untreated group. Coffee group 25%: cavities +
65 capping used coffee beans paste 25%. Coffee group 50%: cavities + capping used
66 coffee beans paste 50%, Coffee group 75%: cavities + capping used coffee beans
67 paste 75%. Day 7, 14, 21 rats in serial were sacrificed to make the preparation of
68 the teeth for analysis of the number of fibroblasts with staining with HE and IL-
69 1 α expression by immunohistochemistry. Data were analyzed descriptively and
70 also ANOVA followed by LSD test. The higher the concentration of Robusta
71 coffee beans, the more decrease the expression of IL-1 α , however the more
72 increase the number of fibroblasts. It is suspected that immunomodulatory
73 proteins inhibit IL-1 α . Conclusion. Robusta coffee beans decrease IL-1 α
74 expression and increases the number of fibroblasts in healing process in dental
75 pulp in Wistar rats.

76 **Keywords:** dental pulp, fibroblasts; IL-1 α ; robusta coffee; wistar rat

77

78 **1 Introduction**

79 Recently dental caries is a risk factor for systemic disease. Dental caries
80 bacteria were found in coronary atherosclerotic plaque specimens in patients
81 who died of a heart attack [1,2]. This shows how important the prevention of
82 dental caries care. 2007 National Health Research (Riskesmas) reported caries
83 prevalence in Indonesia 72.1%, 2009 Household Health Survey (SKRT) showed
84 that Indonesia's dental caries population was 73%. Ministry of Health Republic
85 of Indonesia (Kemenkes RI) in 2009, 89% of Indonesian children under 12
86 years suffered dental caries [3,4]. During this material for treatment of dental
87 caries on the market many cause side effects such as allergies and of course
88 expensive because it still must be imported from abroad. Therefore, it takes a
89 material that can be accepted by the body with minimal side effects, but is
90 immunomodulatory. The solution is to create materials derived from natural
91 materials, one of them from coffee beans.

92 Coffee may protect against periodontal disease [5]. Coffee contains bioactive
93 components such as flavonoids, xanthine, antioxidants, alkaloids. Polyphenols
94 that can serve as anti-inflammatory, antibacterial, platelet aggregation [6,7,8].
95 This has been attributed to the mechanism of one of the proteins in the
96 prevention, inhibition or treatment of disease. The protein content of Robusta
97 and Arabica coffee beans between 10-13%. Coffee has an inhibitory ability to S.
98 mutans, inhibits S. mutans adhesion and whitens teeth, black coffee prevents
99 cavities [9,10,11,12].

100 Caries can be prevented and restored by modulating the immune response of the
101 sufferer. While the dental caries immune response, among others, IL-1 β , IL-1 α ,
102 and TNF- α . Previously our team has proven that Robusta coffee beans can
103 increase the expression of IL-1 β , TNF- α , phagocytosis in vitro to S. mutans and
104 decrease inflammatory cells (monocytes, lymphocytes, neutrophils) in vivo on

105 dental caries [13,14,15]. Robusta's coffee beans were thought to also affect
106 fibroblast cells and IL-1 α expression. Whereas, as-prototypical proinflammatory
107 cytokine, IL-1 drives local and systemic inflammatory after injury and is
108 critically involved in the pathobiology of immune and inflammatory conditions.
109 IL-1 α is a dual-function cytokine, meaning that in addition to functioning as a
110 classical cytokine via cell surface receptor ligation, full-length IL-1 α can also
111 directly regulate gene expression. As the prototypical proinflammatory
112 cytokine, IL-1 drives local and systemic inflammation after injury and is
113 critically involved in the pathobiology of immune and inflammatory conditions.
114 IL-1, IL-6, and b-FGF (basic Fibroblast Growth Factor) may be produced in
115 vivo by residual LECs, causing postoperative inflammation and LEC (cultured
116 lens epithelial cells) proliferation, After cataract surgery [16,17,18]. Fibroblasts
117 are cells that are oval, large and pale, with fine chromatin and a clear core
118 nucleolus, has many cytoplasmic branches irregular. Fibroblasts play an
119 important role in the healing process. As for the various growth factors that
120 strongly support proliferation fibroblasts, such as for a wound healing involving
121 reepithelialisation, granulation tissue formation, inflammatory process, detected
122 interleukin IL-1 [19,20].

123

124 **2. Materials and Methods**

125 2.1 Animal model

126 64 male Wistar Rats weighing 100 -200 gr BW, 2-3 month old were obtained
127 from the Animal House at Biomedic laboratory Faculty of Dentistry Universitas
128 Jember. The rats were kept in separate cages in a well ventilated room at
129 standard experimental conditions. Approval from Gajah Mada University
130 animal ethics committee has been obtained for this project.

131

132

133 2.2 Robusta coffee beans

134 Robusta coffee beans paste was made by mixing the basic paste extract
135 (Magnesium Carbonat, Calcium Carbonat, Gliserin, TEA (*Triethanolamine*),
136 Propilen glikol, Aquadest).

137

138

139 2.2 Research Groups

140 Animal were divided into 4 groups and 4 sub groups (day 7, 14, 21) for each
141 groups (each 4 tails). Controle group: untreated group, while treatment groups
142 made cavities to perforation as indication for direct pulp capping used Robusta
143 coffee paste. Coffee group 25%: cavities + coffee beans paste 25%. Coffee
144 group 50%: cavities + coffee coffee beans paste 50%, Coffee group 75%:
145 cavities + coffee beans paste 75%. Day 7, 14, 21 rats in serial were sacrificed to
146 make the preparation of the teeth for analysis of the number of fibroblasts with
147 staining with HE and IL-1 α expression by immunohistochemistry.

148

149

150 2.4 Immunohistochemistry methods

151 Mixture done 3 times for deparaffinization use xylol, xylol eliminated with
152 absolute ethanol ranging up to 70%, the last with water, washed with PBS pH
153 7.4. To remove debris by trypsin 0.025%. Mixture flooded 3% H₂O₂ solution
154 for 10 minutes. Washed 2x PBS and blocking process is carried out with 3%
155 BSA for 10 minutes. Reacted with the antibody rat IL-1 α (Dako) was incubated
156 for 24 hours at a temperature of 4^oC in a humidity chamber. Biotiyilized reacted
157 with secondary Ab (Goat anti-rat IL-1 α , Dako) for 1 hour. Washed 3 times with
158 PBS each 5 minutes, then added peroxidase labeled streptavidin and incubated
159 for 1 hour. Washed 3 times with PBS, then treated with DAB (Dako) substrate
160 created a new and incubated for approximately 30 minutes at room temperature
161 with shaken. Washed with distilled water, added Meyer-HE for 10 minutes.
162 Washed with tap water, then with distilled water. Dried preparations, spilled
163 entelan and covered with a coverglass. Parameters are amouts of leucocytes that
164 expressed IL-1 α under light microscope with 400x magnification, which was
165 analyzed 3 fields of view. Data were analyzed by descriptive, ANOVA
166 followed by LSD test.

167

168

169 2.5 HE staining methods

170 Dental tissue inserted in formalin, decalcified, deparanized, paraffin blocked,
171 immersed and washed with PBS. Cutting with microtom 4-6 micron and
172 attached to glass object. Then did staining with Haematoxilin eosin (HE).
173 Assessment and analysis of the fibroblasts was done under light microscope
174 with 400 magnification per 3 viewing fields. Data were analyzed by ANOVA
175 followed by LSD test.

176

177

178 3. Results and Discussion

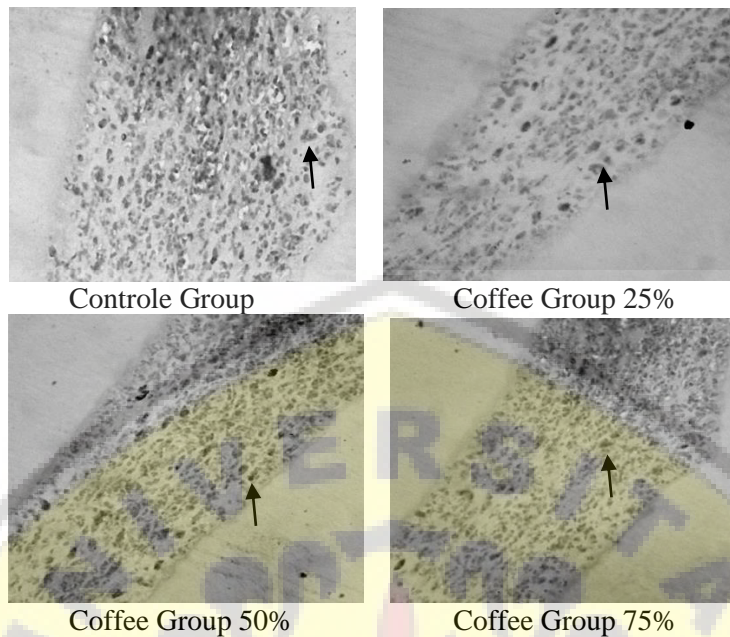
179 3.1 The effect of coffee beans at IL- α expression

180

181 Figure 1 and 2 showed that Robusta coffee beans decreased IL-1 α Expression in
182 dental pulp. Cells expressing IL-1 alpha are brown (black arrow). Analysis
183 under a light microscope with 400x magnification. Descriptively, the more days
184 (day 7, 14, 21), so the more decreased IL-1 α Expression in dental pulp. Controle
185 groups IL-1 α Expression the most decreased, because it untreated, while IL-1 α
186 fixed produced physiologically. It is also true with results analyzed ANOVA
187 followed by LSD test, it there were significant differences ($p \leq 0,05$).

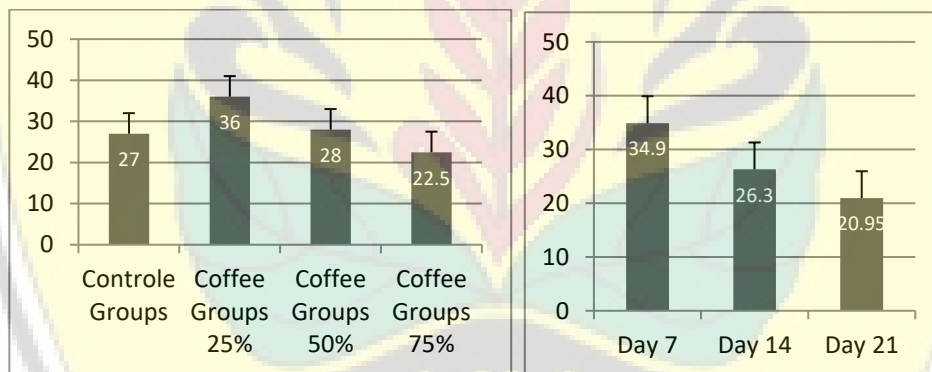
188

189
190



191
192
193
194
195

Figure 1 IL-1 α Expression in dental pulp Wistar Rat (black arrow).



196
197
198
199

Figure 2 Bart chart of IL-1 α Expression in dental pulp Wistar Rat.

200
201
202
203
204
205
206

High protein content or bioactive content in Robusta coffee beans are suspected to be immunogenic or act as immunomodulator, thus allegedly having the ability to improve the immune system. This study proved Robusta coffee beans decreased the expression of IL-1 α either inside the cell or expressed out of the cell. It is suspected that immunomodulatory proteins potentially work inhibit against lymphokines produced by immunocompetent cells such as IL-1 α . Decreasing of IL-1 α was thought to be due to the content of flavonoids,

207 xanthine, chlorogenic acid, alkaloids in Robusta coffee beans. Flavonoids act as
208 anti-inflammatory, analgesic, antioxidant [21]. Some flavonoid compounds
209 may inhibit the release of arachidonic acid and lysosomal enzyme secretion
210 from the membrane by blocking the path of cyclooxygenase and lipoxygenase
211 pathway thus decreasing the levels of prostaglandins and leukotriene
212 (inflammatory mediators), one of inflammatory mediators is IL-1 α [22].

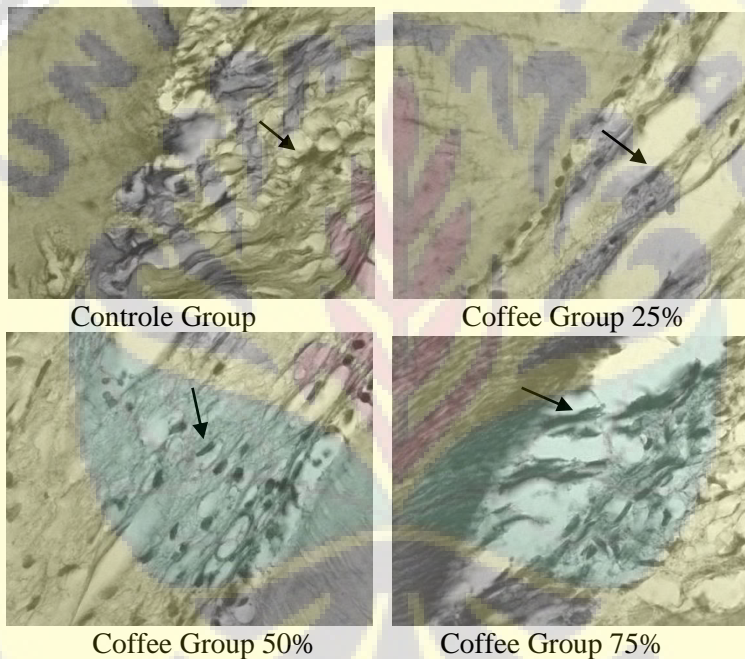
213

214 3.2 The effect of coffee beans at the number of fibroblasts

215 The results of this study indicate that fibroblasts of the control group look at
216 least. In Robusta coffee beans group seen the largest number of fires Coffee
217 Group 25% followed by Coffee Group 50% and Coffee Group 75% (figure 3,4).
218 The longer the day, the more fibroblasts for all groups. Robusta coffee beans
219 increased the number of firoblasts. The higher the concentration, the more
220 increasing the amount of fibroblasts.

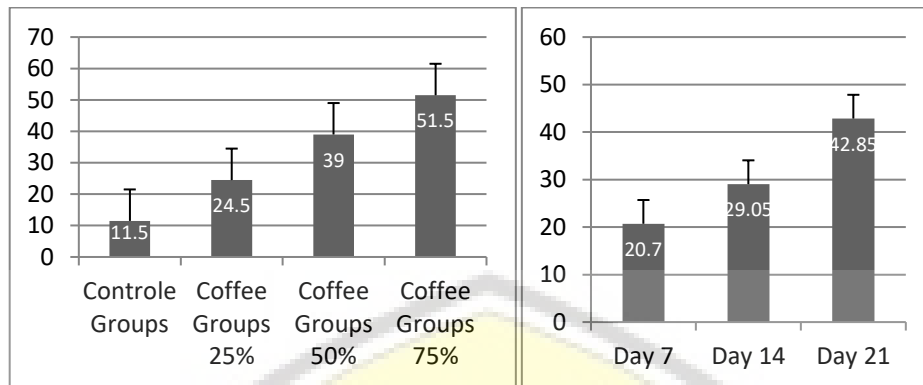
221

222
223



224
225
226
227
228

Figure 3. Fibroblasts in dental pulp in Wistar Rats (black arrow).



229
230

231 Figure 4. Bart chart of the number of Fibroblasts in Dental pulp of Wistar Rat.

232
233

234 Robusta coffee beans as pulp capping material reduced inflammation and speed
235 up the healing process. This caused by IL-1 α that synthesized and released by
236 macrophage will play essential role in all inflammatori process, so if IL- α
237 decreased, it would decreased inflammatory process and increased healing
238 process such as proliferation of fibroblasts [9]. IL-1 α also is made by B cells, T
239 cels, large granular lymphocytes, endothelial cells, astrocytes, muscle cells,
240 keratinocytes and many tips of fibroblasts. IL-1 α may inhibited reparative
241 function of fibroblasts by stimulating their proliferation and synthesis of
242 collagen and TEAM (Tissue Inhibitor of Metalloproteinase) [22].

243

3.3 Supporting data (The number of leukocytes)

244

245 Other data that could support this study is the view of the number of
246 inflammatory cells that decreased from days 7, 14, 21 (we have published).

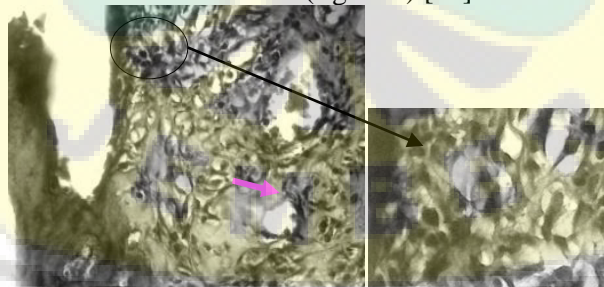
247

248 Where on the 7th day looked neutrophils and many macrophage cells and
249 lymphocytes. Day 14 more domination with macrophage cells and lymphocytes,

248

249 while neutrophils very little. Day 21 there were still macrophages and
lymphocytes, but fibroblasts look more (figure 5) [23]

250



251

Day 7

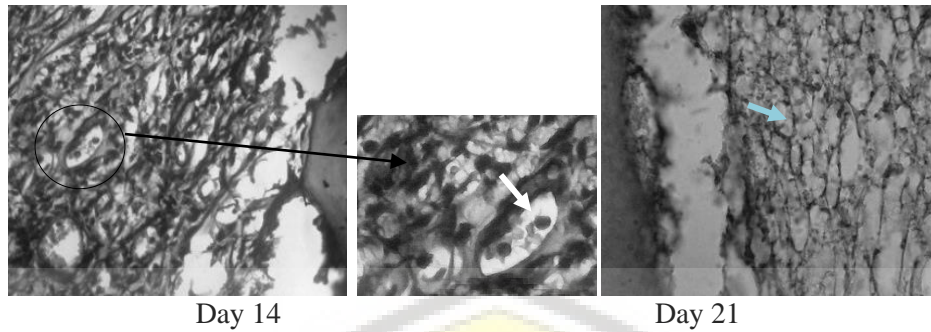


Figure 5. Inflammatory cells in dental pulp in Wistar rats in Coffee groups. Neutrophiles (pink arrow), monocytes (white arrow), fibroblasts (blue arrow). Analysis under a light microscope with 400x magnification

252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285

Conclusion

Robusta coffee beans paste as pulp capping material increased healing process in dental pulp in Wistar rat.

Acknowledgements

A big thank you to Research University of Jember which gives an opportunity to obtain research grants and RISTEK DIKTI which has provided funding for this study.

References

- [1] Lockhart B. Peter DDS, Michael T Brennan, DDS, MHS, Martin Thornhill, MBBS, BDS, Ph.D, Bryan S. Michalowicz, DDS, MS, Jenene Noll, RN, BSN, Farah K. Bahrani-Mougeot, Ph.D and Howell C. Sasser, PhD., Poor oral hygienen as a risk factor for infective endocarditis-related bacteremia, J. Am Dent Assoc,140(10), pp. 1238-1244, 2009 Oct.
- [2] Nomura R, Nakano Knemoto H, Fujita K, Inagaki S, Takahashi T, Taniguchi K, Takeda M, Yoshioka, Isolation and characterization of Streptococcus mutans in heart valve and dental palque specimens from a

- 286 patient with infective endocarditis, *J. Med Microbiol*, 55(8), pp. 1135-40,
287 2006 Aug.
- 288 [3] Sondang P, Harmada T. Towards healthy teeth and mouth, prevention and
289 maintenance, 1st ed Medan: USU Press, 1-34, 2008.
- 290 [4] Hermawati G, Hidayanti L, Korneliani K. Impact of consumption of
291 cariogenic food on the severity of dental caries in pre-school children,
292 Available from URL: <http://journal.unsil.ac.id/> [serialonline] 2009 [cited
293 April 2013].
- 294 [5] Nathan Ng, Elizabeth Krall Kaye, and Raul I. Garcia. Coffee Consumption
295 and Periodontal Disease in Males. *Journal of Periodontology*, 85 (8), pp.
296 1042-1049, August 2014.
- 297 [6] Scalbert Augustin and Gary Williamson. Dietary Intake and Bioavailability
298 of Polyphenols, *J. Nutr*, 130(8), pp. 2073S-2085S, August 1, 2000.
- 299 [7] Coralie J. Dupas, Agnès C. Marsset-Baglieri, Claire S. Ordonaud, Fabrice
300 M. G. Ducept, Marie-Noëlle Maillard, Coffee Antioxidant Properties:
301 Effects of Milk Addition and Processing Conditions, *Issue Journal of Food*
302 *Science*, 71(Issue 3), pp. S253–S258, April 2006.
- 303 [8] Natella F, Nardini M, Belevi F, Pignatelli P, Di Santo S, Ghiselli A, Violi F,
304 Scaccini C. Effect of coffee drinking on platelets: inhibition of aggregation
305 and phenols incorporation. *Br J Nutr*. 2008 Dec;100(6):1276-82, (Epub
306 2008 Apr 28), doi: 10.1017/S0007114508981459.
- 307 [9] Ted Kallmyer B.A., M.Ed., James Foster. Caffeine Informer Staff. 19 Good
308 Health Reasons to Drink Coffee. Email: press@caffeineinformer.com.
309 <https://www.caffeineinformer.com/about> (Published August 31, 2016).
310 Accessed January 23, 2017
- 311 [10] Le Vu. Coffee Can Decrease Tooth Decay, created by Web. Dental,
312 <http://www.webdental.com> (On May 2, 2013 at 2:00am). Accessed January
313 23, 2017
- 314 [11] Namboodiripad P., K. Srividya: Can Coffee Prevent Caries? - An In-Vitro
315 Study. *The Internet Journal of Dental Science*, 7(2), 2009. DOI:
316 10.5580/101c.
- 317 [12] PC Anila Namboodiripad and Sumathi Kori. Can coffee prevent caries? *J*
318 *Conserv Dent*; 12(1), pp. 17–21, Jan-Mar 2009.
- 319 [13] Dewanti I Dewa Ayu Ratna. Potential of coffee seeds as immunomodulator
320 to prevent and overcome dental caries. 9th International Dental Federation
321 - Indonesia Dental Association Joint Meeting Yogyakarta - Indonesia,
322 November 16th – 17th, 2013
- 323 [14] Lestari Pujiana Endah, I Dewa Ayu Ratna Dewanti, Roedy Budirahardjo.
324 Robusta Coffee Beans Increase Levels of TNF- α as A Response to
325 *Streptococcus mutans*. 1st International Conference on Medicine and Health
326 Sciences, Aug 31st – Sep 1st 2016.

- 327 [15] Dewanti I Dewa Ayu Ratna Dewanti. Robusta Coffee Beans Decrease of
328 Inflammation in Dental Caries, 1st International Conference on Medicine
329 and Health Sciences Aug 31st – Sep 1st 2016
- 330 [16] Nelson C Di Palo & DmitryM Shayakhmetov. Interlekin 1 α and the
331 inflammatory process. *Nature Immunology*, 17, pp. 906-913, 2016.
- 332 [17] Dinarello and Ron N. Apte Idan Cohen, Elena Voronov, Malka R. White,
333 Charles A, Peleg Rider, Yaron Carmi, Ofer Guttman, Alex Braiman. IL-
334 1alpha and IL-beta Recruit Different Myeloid.ol.1102048,
335 <http://www.jimmunol.org/content/early/2011/09/16/jimmun>, *J Immunol*
336 published online 19 September 2011. Accessed January 2, 2017
- 337 [18] M I Suwara, N J Green, L A Borthwick, J Mann, K D Mayer-Barber,
338 L Barron, P A Corris, S N Farrow, T A Wynn, A J Fisher and D A Mann.
339 IL-1 α released from damaged epithelial cells is sufficient and essential to
340 trigger inflammatory responses in human lung fibroblasts *Open. Mucosal*
341 *Immunology*, 7, 2014, pp. 684–693(published online 30 October 2013)
342 doi:10.1038/mi.2013.87
- 343 [19] Okihiro Nishi, MD, Kayo Nishi, MD, Yasukazu Ohmoto, PhD. Synthesis
344 of interleukin-1, interleukin-6, and basic fibroblast growth factor by human
345 cataract lens epithelial cells, *Journal of Cataract & Refractive Surgery*,
346 22(suppl. 1), pp. 852-858, doi: [http://dx.doi.org/10.1016.S0886-](http://dx.doi.org/10.1016.S0886-3350(96)80174-x)
347 3350(96)80174-x, copyright by Elsevier, 2017. Accessed January 2, 2017
- 348 [20] Luger, T.A., Schwarz, T.The role of cytokines and neuroendocrine
349 hormones in cutaneous immunity and inflammation. *Allergi J*, 50, pp. 292-
350 302, 2000.
- 351 [21] Mulato Sri, Edy Suharyanto, Coffee, steeping, and Health, *Coffee and*
352 *Cocoa Research Center Indonesia*, Jember, 2015.
- 353 [22] Sabir Ardo, Response of Rat Dental Pulp to Propolis as Direct Pulp
354 Material, *Indonesia Dental Journal*, Special Edition KPPIKG XIV, pp. 57-
355 61, 2006.
- 356 [23] Dewanti I Dewa AR, I Dewa A Susilawati, Pujiana EL, Roedy BR.
357 Robusta Coffee Beans Decrease of Inflammation in Dental Caries.
358 *Proceeding, 1st International Conference on Medicine and Health Sciences*
359 *Aug 31st – Sep 1st 2016*. ISBN: 978-602-74798-8-3.p:173-176
360
361
362