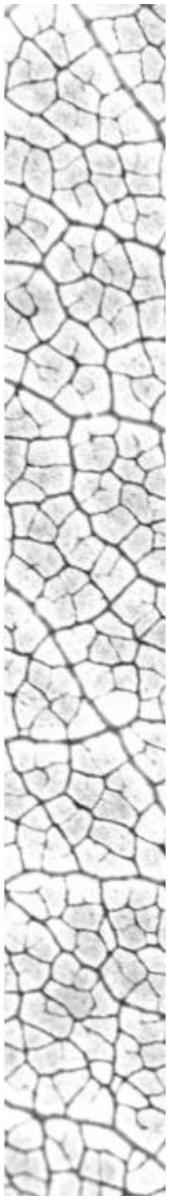

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The effect of green tea extract to angiogenesis in bone regeneration

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Abstract

Background: Polyphenols contained in tea have anti-angiogenesis effects. Epigallocatechin-3-gallate (EGCC) extracted from green tea is a strong inhibitor of neutrophil-mediated angiogenesis, both in vitro and in vivo. Some studies have shown that oral or IP injection of ECGC could be a strong angiogenesis inhibition. **Purpose:** This study aims to determine the effect of green tea extract on angiogenesis in bone fracture healing. **Method:** The study utilized the Randomized Control Group Post Test-Only Design research. In the first draft, the sample was divided into several groups, i.e., K1 as a control group, and P1, P2, P3, and P4 as treatment groups. For the first draft, the experimental animals were terminated on the 10th day. The termination was undertaken on the 14th day for the second draft. This study utilized the Anova test to analyze the data. **Results:** The recommended dose for administering the green tea extract was 20 mg (p=0.447), then there would be a significant reduction (p=0.034) of cell numbers that represented VEGF if the dose was 25 mg or more. A significant reduction of blood vessel numbers (p=0.009) was indicated after administering 25 mg or more. **Conclusion:** The cross-sectional area of blood vessels decreases when transferring 20 mg of the extract, while the blood vessels and cells number that represented VEGF decrease when administering 25 mg of the extract. The blood vessels contained in the callus also decrease after injecting 25 mg of green tea extract.

Keywords: anti-angiogenesis, green tea, EGCG, fracture

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INTRODUCTION

Green tea contains polyphenols compounds, such as 30% of Flavonols known as catechin. It is effective as antioxidants, modulation of carcinogen metabolism, tumor growth inhibitor, cell proliferation, and cell cycle arrest, apoptosis inductor, invasion and metastasis inhibitor, and angiogenesis inhibitor. Epigallocatechin-3-gallate is the most abundant polyphenols compounds in green tea, with 67% in total (Fassina, et al. 2004., Kim, et al. 2000).

Polyphenols found in tea have antiangiogenic effects. 80-90 µM of EGCG will reduce the production of vascular endothelial growth factor (VEGF) in breast cancer (Sartippour, et al. 2002), 5-10µM inhibits VEGF-2 receptor activity (Lamy, Gingras, & Béliveau, (2002), 5-50µM inhibits VEGF receptor expression (Kojima-Yuasa, et al. 2003)., and nanomolar concentrates inhibits protein Bcl-2. Bcl-2 proteins have a critical role in the process of angiogenesis (Leone, et al. 2003). Green tea or EGCG at concentrations of 10-100µM provides a significant anti-angiogenesis effect in vivo. EGCG and green tea are strong inhibitors of neutrophil-mediated angiogenesis, both in vitro and in vivo (Donà, et al. 2003; Tabeekh, et al, 2017).

Giving green tea or EGCG orally has a strong anti-angiogenic effect (Fassina, et al. 2004). The in vitro effect of EGCG in concentrates of 50 µM inhibits the expression of PDGF-induced VEGF mRNA, PDGF-induced activation of Erk-1/2, and Active-1/2 (Park, eet al. 2006). Injecting 5mg/kg and 50mg/kg of EGCG in rats inhibits the dose-dependent angiogenesis (Xu, et al. 2009). EGCG is excreted through bile while the other polyphenols are excreted through urine. At a concentrate up to 70mg/kg, EGCG does not cause side effects. Injecting a 10-fold dose of EGCG on humans does not cause any side effects (Kim, et al. 2000, Chow, et al. 2001). Green tea is a strong anti-angiogenic; therefore, this research will prove the effect of green tea extract levels in fracture healing angiogenesis.

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METHOD

This study utilized the Randomized Control Group Post Test-Only Design research. This study utilizes two research designs in this study, i.e., green tea effect on cell numbers that represented VEGF on callus and green tea effect on the blood vessels cross-sectional number and area on the callus.

This study employed a simple random method, and the first design utilized male mice as the experimental unit. There were five experimental groups, consisted of seven mice. The thirty five of the mice femur bone was being fractured after the anesthesia process, and this process was conducted by bending both thumbs on the femur medial side. After that, immobilized the femoral fracture with a spica cast. *Ad libitum* pure water was given to K1, 15mg of green tea extract (0.5ml per tube) for P1, 20mg of green tea extract (0.5ml per tube) for P2, 25mg of green tea extract (0.5ml per tube) for P3, and 30mg of green tea extract (0.5ml per tube) for P4. 60-100 mg/kg of pentobarbital was injected to the mice on the 10th day in a slight midline lateral area between the xypoid process and the pubis. Then the sample was taken in the callus area.

There were five experimental groups in the second design, consisted of seven mice. The 35 mice femur bone was being fractured after the anesthesia process. The breaking process was conducted by bending both thumbs on the femur medial side. After that, immobilized the femoral fracture with a spica cast. *Ad libitum* pure water was given to K1, 15mg of green tea extract (0.5ml per tube) for P1, 20mg of green tea extract (0.5ml per tube) for P2, 25mg of green tea extract (0.5 ml per tube) for P3, and 30mg of green tea extract (0.5ml per tube) for P4. 60-100 mg/kg of pentobarbital was injected to the mice on the 14th day in a slight midline lateral area between the xypoid process and the pubis. After the callus was collected, the sample was carried out for the HE staining procedure. These study variables are numerical data, so the Anova test was carried out to determine the concentrations of green tea extract on the blood vessels cross-sectional number and area in the callus.

RESULTS

The cell nucleus that could absorbed color was the one that represented VEGF, while the cell nucleus that could not absorbed color was the cell nucleus that did not represent VEGF. Based on the analysis of **Table 1**, there was a significant difference in the cell numbers that represented VEGF, especially in the group given green tea extract compared to the control group. The number of cells represented VEGF was significantly different in administering 25 mg and 30 mg of green tea extract compared to the control group. The number of cells representing VEGF did not differ significantly at doses of 15 mg and 20 mg compared with the control group. At the doses of 25 mg and 30 mg, the number of cells presenting VEGF also did not differ significantly.

Table 1. The Test Results of Multiple Comparison Cells Represented VEGF

I) Mice Group	J) Mice Group	Sig.
Pure water	Green tea extract 15 mg	1.000
	Green tea extract 20 mg	, 447
	Green tea extract 25 mg	, 034
	Green tea extract 30 mg	, 048
Green tea extract 15 mg	Pure water	1.000
	Green tea extract 20 mg	, 310
	Green tea extract 25 mg	, 008
	Green tea extract 30 mg	, 012
Green tea extract 20 mg	Pure water	, 447
	Green tea extract 15 mg	, 310
	Green tea extract 25 mg	, 012
	Green tea extract 30 mg	, 040
Green tea extract 25 mg	Pure water	, 034
	Green tea extract 15 mg	, 008
	Green tea extract 20 mg	, 012
	Green tea extract 30 mg	, 755
Green tea extract 30 mg	Pure water	, 048
	Green tea extract 15 mg	, 012
	Green tea extract 20 mg	, 040
	Green tea extract 25 mg	, 755

VEGF: Vascular Endothelial Growth Factor

Table 2. Multiple Comparison of Vascular Average Number in Callus

I) Mice Group	J) Mice Group	Sig.
Pure water	Green tea extract 15 mg	, 361
	Green tea extract 20 mg	, 091
	Green tea extract 25 mg	, 009
	Green tea extract 30 mg	, 019
Green tea extract 15 mg	Pure water	, 361
	Green tea extract 20 mg	, 411
	Green tea extract 25 mg	, 066
	Green tea extract 30 mg	, 122
Green tea extract 20 mg	Pure water	, 091
	Green tea extract 15 mg	, 411
	Green tea extract 25 mg	, 281
	Green tea extract 30 mg	, 447
Green tea extract 25 mg	Pure water	, 009
	Green tea extract 15 mg	, 066
	Green tea extract 20 mg	, 281
	Green tea extract 30 mg	, 743
Green tea extract 30 mg	Pure water	, 019
	Green tea extract 15 mg	, 122
	Green tea extract 20 mg	, 447
	Green tea extract 25 mg	, 743

Based on the analysis of the number of blood vessels, there was a significant difference found in the treatment group compared to the control group. The number of blood vessels was significantly different in administering 25 mg and 30 mg green tea doses compared to the control groups. However, there was no significant difference in administering 25 mg and 30 mg of green tea in the group.

Based on the analysis of the blood cross-sectional area, a significant difference was found in the blood storage area in the treatment group compared to the control group at a dose of 15 mg. A significant difference was also found after administering 20 mg to 30 mg of green tea dose compared to the control group. However, the number of blood vessels did not differ significantly after administering 20 mg and 30 mg green tea dose.

Table 3. The Average values of blood vessels cross-sectional area in callus

I) Mice Group	J) Mice Group	Sig.
Pure water	Green tea extract 15 mg	, 365
	Green tea extract 20 mg	, 003
	Green tea extract 25 mg	, 002
	Green tea extract 30 mg	, 001
Green tea extract 15 mg	Pure water	, 365
	Green tea extract 20 mg	, 247
	Green tea extract 25 mg	, 142
	Green tea extract 30 mg	, 082
Green tea extract 20 mg	Pure water	, 003
	Green tea extract 15 mg	, 247
	Green tea extract 25 mg	, 316
	Green tea extract 30 mg	, 015
Green tea extract 25 mg	Pure water	, 002
	Green tea extract 15 mg	, 142
	Green tea extract 20 mg	, 316
	Green tea extract 30 mg	, 296
Green tea extract 30 mg	Pure water	, 001
	Green tea extract 15 mg	, 082
	Green tea extract 20 mg	, 015
	Green tea extract 25 mg	, 296

There was also no significant difference after administering 25 mg and 30 mg green tea dose.

DISCUSSION

The oral administration of green tea or EGCG inhibited angiogenesis (Fassina, et al. 2004). Based on the results of this study, it was found that administering the green tea extract starting from 20 mg would induce a significant reduction of blood vessel cross-sectional area. Administering 25 mg of green tea ectract or more would also induce a significant reduction in the number of cells represented VEGF. VEGF played a critical role in neo-angiogenesis and bone regeneration in the endochondral process (Street, et al. 2002., Gerber, et al. 1999). Exogenous VEGF could enhance fracture healing. Osteoblasts were associated with an increased number of VEGF. Therefore, osteoblasts were referred to as primary regulators in fracture healing (Deckers, et al. 2002, Yeh, & Lee, 1999).

EGCG inhibited the tyrosine phosphorylation process in the platelet derived growth factor receptor (PDGF-R). It had been proven that EGCG also inhibited the activation of extracellular signal-regulated or ERK-1/2. It was induced by the barriers in the PDGF receptor phosphorylation process so that the downstream kinase was disturbed. EGCG could also inhibit ERK-1/2 directly (Park, et al. 2006). For the next process, there was found a significant decrease in the number of blood vessels starting from 25 mg of green tea extract. Some polyphenols contained in green tea such as flavanols, flavandiol, flavonol, phenolic acid, and 70% of catechin could also be an antioxidant and angiogenesis inhibitor, those were: 67% of epigallocatehchin-3-gallate (EGCG), epigallocatehchin (EGC), epicatechin gallate (ECG), epicatechin (EC), gallocatechin, and catechin (Fassina, et al. 2004., Kim, et al. 2000., Bokuchava, Skobeleva, & Sanderson, 1980).

Fracture healing in mice also went through the inflammatory phase, the soft callus on the 7th day, the hard callus on the 14th day, and the remodeling phase. VEGF was expressed by hypertrophic chodrocyte on the 10th day after fracturing in mice. VEGF played a role in the convertion of soft callus to hard callus through angiogenesis, cartilage resorption, and ossification processes in mice growth plates. On the 7th day, vascularization exposed the importance of VEGF in the angiogenesis process (Street, et al. 2002).).

The results of this study were in accordance with some previous studies where administering 1.25% of green tea (4.69 mg/ml) containing 708 µg/ml of EGCG orally, produced 0.1-0.3 µM plasma EGCG levels and equivalent to human plasma EGCG levels after drinking two or three cups of green tea (Wang, et al. 1992., Leong, Mathur, & Greene, 2008). Administering green tea extract at these doses proved to be able to inhibit neovascularization in corneas, which given 160 mg of VEGF (Wang, et al. 2013, Yeh,, & Lee, 1999). The fluid requirement of mice was 1 ml/10 g/day body weight, so the fluid needed for mice with body weight 30 g/day was 3 ml. From these data, we could compute that the administration of green tea 1.25% (4.69 mg/ml) *ad libitum* was equivalent to 15 mg of single dose green tea extract.

CONCLUSION

Administering 20 mg of green tea extract is the recommended dose and will induce a significant reduction of blood vessel cross-sectional area Administering 25 mg of green tea extract will induce a significant reduction of blood vessel numbers and cell numbers represented VEGF. The blood vessels contained in the callus will also decrease after injecting 25 mg of green tea extract.

REFERENCES

Bokuchava, M. A., Skobeleva, N. I., & Sanderson, G. W. (1980). The biochemistry and technology of tea manufacture. Critical Reviews in Food Science & Nutrition, 12(4), 303-370.

Chow, H. S., Cai, Y., Alberts, D. S., Hakim, I., Dorr, R., Shahi, F.,... & Hara, Y. (2001). Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiology and Prevention Biomarkers, 10(1), 53-58.

Deckers, M. M., Van Bezooijen, R. L., Van Der Horst, G., Hoogendam, J., van Der Bent, C., Papapoulos, S. E., & Löwik, C. W. (2002). Bone morphogenetic proteins stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A. Endocrinology, 143(4), 1545-1553.

Donà, M., Dell'Aica, I., Calabrese, F., Benelli, R., Morini, M., Albini, A., & Garbisa, S. (2003). Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. The Journal of Immunology, 170(8), 4335-4341.

Fassina, G., Vene, R., Morini, M., Minghelli, S., Benelli, R., Noonan, D. M., & Albini, A. (2004). Mechanisms of inhibition of tumor angiogenesis and vascular tumor growth by epigallocatechin-3-gallate. Clinical Cancer Research, 10(14), 4865-4873.

Gerber, H. P., Vu, T. H., Ryan, A. M., Kowalski, J., Werb, Z., & Ferrara, N. (1999). VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nature medicine, 5(6), 623-628.

Kim, S., Lee, M. J., Hong, J., Li, C., Smith, T. J., Yang, G. Y.,... & Yang, C. S. (2000). Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. Nutrition and cancer, 37(1), 41-48.

Kojima-Yuasa, A., Hua, J. J., Kennedy, D. O., & Matsui-Yuasa, I. (2003). Green tea extract inhibits angiogenesis of human umbilical vein endothelial cells through reduction of expression of VEGF receptors. Life Sciences, 73(10), 1299-1313.

Lamy, S., Gingras, D., & Béliveau, R. (2002). Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. Cancer research, 62(2), 381-385.

Leone, M., Zhai, D., Sareth, S., Kitada, S., Reed, J. C., & Pellecchia, M. (2003). Cancer prevention by tea polyphenols is linked to their direct inhibition of antiapoptotic Bcl-2-family proteins. Cancer Research, 63(23), 8118-8121.

Leong, H., Mathur, P. S., & Greene, G. L. (2008). Inhibition of mammary tumorigenesis in the C3 (1)/SV40 mouse model by green tea. Breast cancer research and treatment, 107(3), 359-369.

Park, J. S., Kim, M. H., Chang, H. J., Kim, K. M., Kim, S. M., Shin, B. A.,... & Jung, Y. D. (2006). Epigallocatechin-3-gallate inhibits the PDGF-induced VEGF expression in human vascular smooth muscle cells via blocking PDGF receptor and Erk-1/2. International journal of oncology, 29(5), 1247-1252.

Sartippour, M. R., Shao, Z. M., Heber, D., Beatty, P., Zhang, L., Liu, C.,... & Brooks, M. N. (2002). Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. The Journal of nutrition, 132(8), 2307-2311.

Street, J., Bao, M., deGuzman, L., Bunting, S., Peale, F. V., Ferrara, N.,... & van Bruggen, N. (2002). Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proceedings of the National Academy of Sciences, 99(15), 9656-9661.

Tabeekh, M. A. S. A., Maktoof, A. R., & Mohsen, H. A. M. A. (2017). Solutions or alternatives to protect livestock, especially buffalo from the risk of drought in Basra Governorate. Canadian Journal of Agriculture and Crops, 2(2), 84-89.

Wang, S., Li, Y., Zhao, J., Zhang, J., & Huang, Y. (2013). Mesenchymal stem cells ameliorate podocyte injury and proteinuria in a type 1 diabetic nephropathy rat model. Biology of Blood and Marrow Transplantation, 19(4), 538-546.

Wang, Z. Y., Huang, M. T., Ferraro, T., Wong, C. Q., Lou, Y. R., Reuhl, K.,... & Conney, A. H. (1992). Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-O-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. Cancer Research, 52(5), 1162-1170.

Xu, H., Lui, W. T., Chu, C. Y., Ng, P. S., Wang, C. C., & Rogers, M. S. (2009). Anti-angiogenic effects of green tea catechin on an experimental endometriosis mouse model. Human reproduction, 24(3), 608-618.

Yeh, L. C. C., & Lee, J. C. (1999). Osteogenic protein-1 increases gene expression of vascular endothelial growth factor in primary cultures of fetal rat calvaria cells. Molecular and cellular endocrinology, 153(1-2), 113-124.