Journal Of Nursing Practice
https://thejnp.org/
ISSN: 2614-3488 (print); 2614-3496 (online) Vol.8 No.1. October 2024. Page.23-32

The Role of Nanopartikel Green Tea in Enhancing Endothelial Cell Migration in HUVEC Culture exposed to EPC-Conditioned Media in Hyperglycemic Conditions

Yuly Peristiowati[1*], Hariyono[2], Zauhani Kusnul[3], Reni Yuli Astutik[4], Nian Afrian Nuari[5]
[1,2] Department Master of Nursing, Universitas STRADA Indonesia, Kediri, Indonesia
[3] STIKes Pamenang, Kediri, Indonesia
[4] Universitas STRADA Indonesia, Kediri, Indonesia
[5] STIKes Karya Husada Kediri, Indonesia
**Corresponding author: yulyperistiowati@strada.ac.id*

ABSTRACT

Backgrounds: The role of endothelial progenitor cells (EPCs) in angiogenesis is impaired in diabetes mellitus. The present study was conducted to observe the effects of nanopartikel green tea on the ability of EPCs exposed to high levels of glucose to release NO and induce endothelial cell migration.
Purpose: This study aims to describe the mechanism of the role of green tea nanoparticles in increasing migration of endothelial cells in HUVEC cultures exposed to EPC-CM. The function of EPCs was assessed by evaluating HUVEC migration after administration of EPC-Conditioned Media. HUVEC migration was assessed by the Boyden chamber assay method.
Method: Green Tea Nano Particles treatment at doses of 30 nM and 50 nM significantly increased endothelial cell migration at high glucose exposure of 22 mM. At a dose of 50 nM, normal glucose exposure increased endothelial cell migration better than high glucose exposure. Administration of high levels of glucose resulted in a decrease in the ability of EPCs to induce HUVEC migration, decrease in NO of EPCs, and decrease in the factor affecting EPC migration, namely SDF-1α and CXCR4.
Results: Administration of 50 μM of nanopartikel green tea could inhibit the decreased ability of EPCs to induce HUVEC migration, and this effect was associated with NO, SDF-1α and CXCR4 concentrations.
Conclusions: Administration of nanopartikel green tea could maintain the ability of EPCs exposed to high levels of glucose to release NO and induce HUVEC migration through an increase in SDF-1α and CXCR4.

Keywords: endothelial progenitor cells (EPCs), HUVEC migration, and EPC-conditioned media, nanopartikel green tea

Received August 10, 2024; Revised September 12, 2024; Accepted October 3, 2024
DOI: https://doi.org/10.30994/jnp.v8i1.626

BACKGROUND

Endothelial dysfunction can occur in patients with type 2 diabetes mellitus, especially when there has been a clinical manifestation of microalbuminuria. Additionally, it can also occur in individuals with insulin resistance such as obese patients or those with a high risk for type 2 diabetes mellitus (impaired glucose tolerance) (Arizmendi-Grijalva et al., 2021; Kondo et al., 2002). The role of endothelial progenitor cells (EPCs) in angiogenesis is impaired in diabetes mellitus(Hu et al., 2018). Endothelial cells line the lumen of all blood vessels and act to connect the blood circulation to vascular smooth muscle cells. In addition to acting as a blood–tissue physical barrier, it facilitates various complex functions of the smooth muscle cells of the blood vessels and cells of the blood compartment. Various studies have shown that endothelial cells play an important role in homeostasis occurring through the integration of various chemical mediators (Taleb Safa & Koohestani, 2024).

Flavonoids from the methanol extract of green tea were capable of protecting endothelial progenitor cells (EPCs) from oxidative damage through the H2O2-induced intracellular ROS suppression mechanism (Hafshah & Simanjuntak, 2020; Widowati et al., 2014). A study of EPC repair using antioxidants performed. Administration of vitamin E can maintain the ability of EPCs exposed to high levels of glucose to release NO and induce HUVEC migration through inhibition of increased superoxide and H2O2 of EPCs (Arizmendi-Grijalva et al., 2021; Islam, 2012).

Nanoparticles consist of macromolecular materials and can be used for therapy as vaccine adjuvant or drug carrier, namely by dissolving, trapping, encapsulate, absorb or attach chemically active ingredients (Hald et al., 2022). The benefits of green tea include anti-inflammatory, antioxidant, anticancer, antiobesity, inhibiting metabolic syndrome, and antitoxin. Making green tea nanoparticles into paste carried out using the wet milling technique. Green tea paste samples were characterized based on particle size with analysis and scanning electron microscope methods. Grade A green tea has granules size 77,014 ± 50,759 nm. Grade B green tea has a particle size of 12,987 ± 7,674 nm. Grade C green tea has a particle size of 4409 ± 5379 nm. Differences in green tea levels determine the structure and size of the particles formed. Thus, the wet milling technique can be an alternative in producing green tea nanoparticles for industrial scale (Peristiowati & Kusnul, 2020).

This research is expected to describe the mechanism of the role of green tea nanoparticles in increasing migration of endothelial cells (HUVEC) in HUVEC cultures exposed to EPC-CM. Endothelial cell migration plays an important role in HUVEC cell repair. This intracellular environment describes the condition of diabetes mellitus patients who experience tissue damage due to hyperglycemia. Hypothesis in this study: Administration of EPC-CM from EPC culture supernatant treated with green tea nanoparticles can increase HUVEC cell migration. The presence of these factors is expected to increase endothelial (HUVEC) cell migration (Bulboaca et al., 2020; Taleb Safa & Koohestani, 2024).

METHOD

Study design

This study was a true in vitro experiment with HUVEC cultures originating from the umbilicus using a post-test only control group design. HUVEC cell culture was carried out by the researchers themselves at the LPPT Laboratory at Gadjah Mada University without using cell lines. Huvec cells were taken from the umbilical cord of newborns with normal labor and HUVEC cells were isolated according to the protocol.

Making Nanopartikel Green Tea preparations

Green tea preparation process, on The first stage of tea is done by hand picking to select young tea leaves, then The withering process is carried out where green tea is placed in a flat container and dried in the sun in a certain place shade to reduce humidity and reduce enzyme activity and minimize the process oxidation. The next process is drying by heating, this process aims reduces the water content in the leaves 3% - 4%. The dried tea leaves are then crushed by using a milling mixer and continuing with the nanoning process using high energy ball milling up to 1-100 nM in size. The ball milling process is a method of reducing size particles of a material to a certain extent using mechanical methods. The green tea powder is then extracted for use the active substance through a maceration method using ethanol-hexane (Shabri et al., 2019).

EPC Culture

Mononuclear cells were isolated from the peripheral blood of healthy subjects. On 7 day, EPC culture was given normal glucose 5 mM (N) with or without administration of Nanopartikel green tea (NGT) isolate as control negative, as positive control given high levels of glucose 22 mM (G) with or without administration of NGT for 24 hours. In the treatment group, (1) EPC exposed to 5 mM glucose was given Nanopartikel green tea 30 nM (NGT30), (2) EPC was exposed to 5 mM glucose and given Nanopartikel green tea 50 nM (NGT50), (3) EPC was exposed to high glucose 22 mM and given Nanopartikel green tea 30 nM (NGT30+G), (4) EPC was exposed to high glucose 22 mM and given Nanopartikel green tea 50 nM (NGT50+G) for 24 hours. The function of EPCs was assessed by evaluating HUVEC migration after administration of EPC-Conditioned Media.

HUVEC Culture Isolation

Normal Primary Human Umbilical Vein Endothelial Cell Morphology 4 Day culture, taken with a Nikon brand inverted microscope with a magnification of 100x shows the characteristics of normal endothelial cells morphologically is the shape of cobblestone endothelial cells with the specific character of cells in the middle looks round and bright flat cell shape with regular and tight spacing between cells, smooth cell surface characterized by the appearance of the nucleus, plasma membrane, cytoplasm, extracellular matrix (ECM) and no apoptotic cells and a primary monolayer.

Boyden chamber assay

The Boyden Chamber migration assay method was used to investigate the enhanced migration of endothelial cells. This approach employs a PVDF membrane (Nucleopore Whatman membrane) in the Boyden Camber kit's lower chamber (Falasca et al., 2011; Tóvári et al., 2014). While the chemo attractant material in this investigation was an EPC supernatant, also known as conditioned-EPC, which was deposited in the lower chamber. Cells in the lower chamber were HUVEC ($5x10^6$) as much as 5 µl in each well. With 24-hour incubation conditioned-EPC which acts as a chemo attractant, will attract HUVEC cells to migrate through the PVDF membrane. HUVEC cells that can migrate well on observing the stained PVDF membrane will appear clean and clear circles. Meanwhile, HUVEC cells that are unable to migrate properly will appear as dark and dirty circles. Through confocal microscopy observation, HUVEC cells were counted which were able to migrate perfectly.

NO measurement

Nitric oxide levels were detected by the colorimetric method using the Nitric oxide Assay Kit D2NO-100 (BioAssay System, USA). Procedure: Cold centrifugation was used to remove the particles on the EPC culture medium. NO levels were measured using the Bioassay System with colorimetric technique. The analysis of NO levels includes the nitrite test procedure (measures the concentration of endogenous nitrite) and the nitrate reduction

test (measures total nitrite after conversion of nitrate to nitrite). The optical density measurements were taken at 570 nm with a microplate-reader (Csonka et al., 2015; Goshi et al., 2019).

Immunofluorescence

SDF-1α was analyzed using the immunofluorescence method using an antibody SDF-1α (Bioss) labeled as anti-rabbit FITC (Rockland). CXCR4 was analyzed using the immunofluorescence method using the CXCR4 antibody labeled as anti-rabbit Rhodamine (Sigma). The procedure carried out was as follows (Goshi et al., 2019; Sun et al., 2003). Cells were fixed with 2% PFA (paraformaldehyde) for 15 minutes, Washed with PBS pH 7.4 3 x 10 minutes, Discard PBS, replaced with blocking buffer 5 % FBS in PBS for 20 minutes, Wash with PBS 3 x 10 minutes, Incubate with primary antibody SDF -1α (Bioss bs-0783R) in a ratio (1:500) for cells to be detected SDF -1α for 1 hour, Incubate with primary antibody CXCR4 (Bioss bs-1011R) in a ratio (1:500) for cells to be detected CXCR4 for 1 hour, Wash with PBS 3 x 10 minutes, Incubate with secondary antibody anti Rabbit FITC (1:500) for 1 hour for detection of SDF -1α and secondary antibody anti-Rabbit Rhodamine (1:500) for 1 hour for detection of CXCR4. Wash with PBS 3 x 10 minutes. Observe using CLSM.

Data analysis

Data were presented in mean ± SD and differences among treatment groups were analyzed by the one-way ANOVA using SPSS 16.0. Post-Hoc testing was carried out when the results of the ANOVA had significant differences. The *P*-value < 0.05 was considered significantly different.

RESULTS

EPC Culture

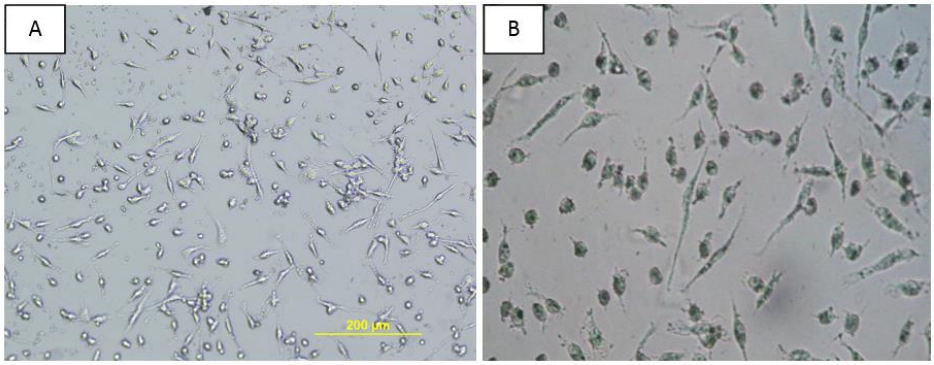

Figure 1. EPC morphology. A. EPC morphology on the 7 day at 200X magnification in medium, B. EPC morphology on the 7 day with 2% Paraformaldehyde fixation at 400X magnification, Shows the image (spindle shape) on the 7 day.

Figure 1 shows the morphology of EPC cells attached to the bottom of the well in an elongated shape with tapered ends (spindle shape) which is a characteristic of EPC morphology. The spindle shape will be seen more clearly on the 7th day of the incubation period. Image A shows the morphology of EPCs on day 7 at 200X magnification in medium, showing the morphology of spindle-shaped EPCs. Meanwhile, picture B shows the morphology of EPC on the 7th day with 2% Paraformaldehyde fixation at 400X magnification. It shows the image (spindle shape) on the 7th day.

ISSN: 2614-3488 (print); 2614-3496 (online) Vol.8 No.1. October 2024. Page.23-32

HUVEC Culture Isolation

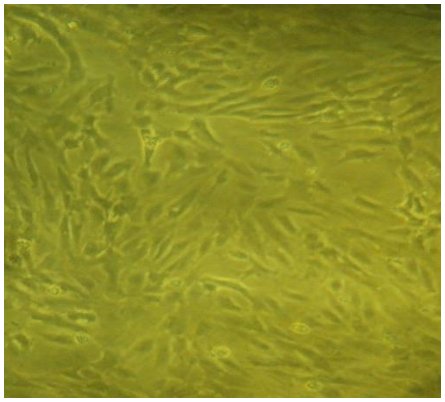

Figure 2. The results of endothelial (HUVEC) cell culture from the 100x-enlarged placenta.

Figure 2 showed that Normal Primary Human Umbilical Vein Endothelial Cell Morphology Day 4 culture, taken with a Nikon brand inverted microscope with a magnification of 100x shows the characteristics of normal endothelial cells morphologically is the shape of cobblestone endothelial cells with the specific character of cells in the middle looks round and bright flat cell shape with regular and tight spacing between cells, smooth cell surface characterized by the appearance of the nucleus, plasma membrane, cytoplasm, extracellular matrix (ECM) and no apoptotic cells and a primary monolayer.

HUVEC cell culture migration assay with chemoattractant EPC culture medium (EPC conditioned-medium) using the Boyden chamber method

As shown in Figure 2, HUVEC cultured cells with EPC-conditioned culture that acts as a chemo attractant using the Boyden Chamber method, the picture shows that the chemo attractant attracts HUVEC cells to migrate through the PVDF membrane. HUVEC cells that can migrate well on the observation of the stained PVDF membrane will appear clean and clear circles. Meanwhile, HUVEC cells that are unable to migrate properly will appear as dark and dirty circles. Through confocal microscopy observation, HUVEC cells were counted which were able to migrate perfectly.

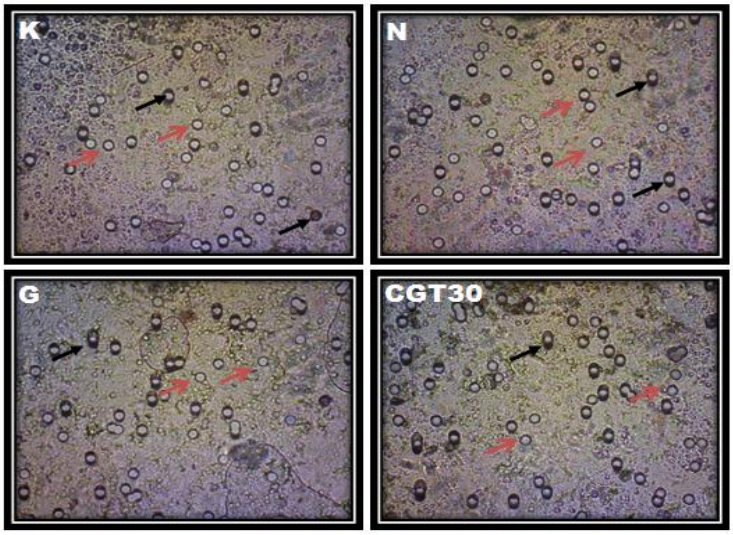

Figure 3. Results of endothelial (HUVEC) cell culture migration assay with chemoattractant EPC culture medium with the Boyden chamber assay method. Endothelial (HUVEC) cells capable of migration through the PVDF membrane and surviving are shown by the red arrows on white (clear) round-shaped image. The endothelial (HUVEC) cells migrating

through the PVDF membrane but undergoing apoptosis are shown by the black arrows on the brownish or blackish round-shaped image. K: (HUVEC exposed to EPC-CM without glucose); N: (HUVEC exposed to EPC-CM with 5 mM of glucose); G: (HUVEC exposed to EPC-CM with 22 mM of glucose). NGT 30 (HUVEC exposed to EPC-CM + 5 mM of glucose + 30 µM of NGT.

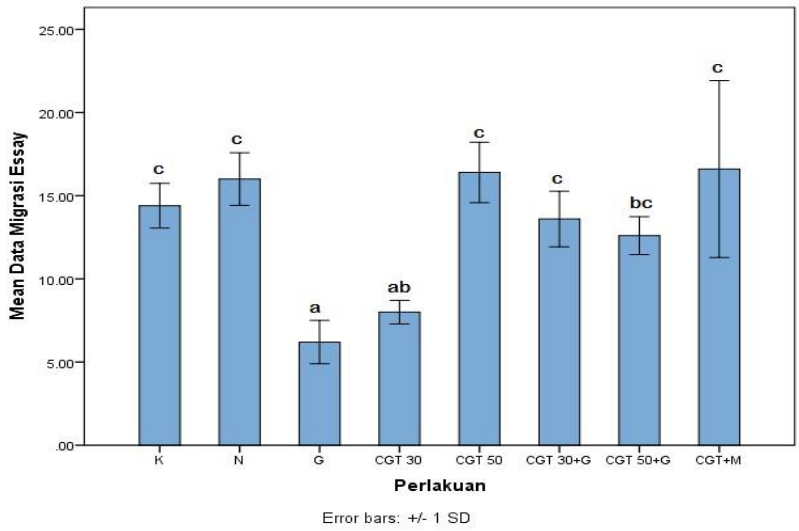

Figure 4. Chart of the results of endothelial (HUVEC) cell culture migration assay with chemoattractant EPC culture medium (EPC conditioned-medium) using the Boyden chamber assay method. K: (Control); N: group treated with D - (+) - 5 mM of glucose; G: group treated with D - (+) - 22 mM of glucose; NGT30 (group D - (+) - 5 mM of glucose and 30 µM of NGT; NGT30 + G: group treated with D - (+) - 22 mM of glucose and 30 µM of NGT; NGT50: group treated with D - (+) - 5 mM of glucose and 50 µM of NGT; NGT50 + G: group treated with D - (+) - 22 mM of Glucose and 50 µM of NGT.

As shown by the bar chart of the average Essay Migration Data, only the NGT30-treated group is significantly different from the treatment groups K and N control. The treatment groups NGT30 + G and NGT50 + G differed significantly with a *p*-value of 0.000 < 0.05 from the control group G. Based on the significant differences (*p*-value of 0.002 < 0.05) among the treatment groups, the treatment with NGT30 is the most effective treatment since it produces a significantly different migration assay from the control group K.

DISCUSSION

Migration Assay of HUVEC culture added with EPC culture supernatant using the Boyden Chamber method

The Boyden chamber method is a method used to determine migration of cells drawn or bound by chemoattractant. This method uses the PVDF membrane (Nucleopore Whatman membrane) placed on the lower chamber of the Boyden chamber kit (Falasca et al., 2011). The chemoattractant used in the present study was the EPC supernatant, also called EPC-conditioned, placed on the lower chamber. The cells on the lower chamber were HUVEC (5 x 10^6) of 5 µl at each well. Incubated for 24 hours, the EPC-conditioned medium acted as the chemoattractant, attracting the HUVEC cells to migrate through the PVDF membrane. The HUVEC cells capable of migrating properly on the observation of the stained PVDF membrane would appear as clear and obvious spheres. Meanwhile, the HUVEC cells unable

to migrate properly would appear as dark and dirty spheres. The HUVEC cells capable of migrating perfectly were calculated by means of confocal microscopy observation.
Results of statistical tests showed a significant difference (P <0.05) for the HUVEC group (G) exposed to EPC- conditioned medium with high levels of glucose from the HUVEC control group (N) exposed to EPC-conditioned medium with low levels of glucose. The four treatment groups NGT30, NGT30 + G, NGT50, and NGT50 + G had a significant difference (P <0.05) from the HUVEC group (G) exposed to EPC-conditioned medium with high levels of glucose and the Huvec control group (N) exposed to EPC-conditioned medium with low levels of glucose.

The EPC culture model with high glucose conditions was used to simulate clinical hyperglycemia in the evaluation of EPC function *in vitro*. Glucose concentration in normal media was 4.5 to 5 mM, which was proportional to blood glucose levels of 80 to 89 mg/dl, and glucose concentration of 22 mM in the media used in the present study was in accordance with blood glucose levels of 400 mg/dL that can occur in patients with uncontrolled DM (Chen et al., 2007; Kondo et al., 2002; Zhang et al., 2008). Although 24-hour glucose exposure is not suitable to EPC pathology in patients with chronic diabetes mellitus, this *in vitro* model is acceptable with the aim of studying the mechanism of EPC dysfunction in diabetic conditions (Arizmendi-Grijalva et al., 2021; Bulboaca et al., 2020).

To observe EPC function in high glucose conditions in angiogenesis, the migratory ability of mature endothelial cells was evaluated after the administration of EPC culture supernatant previously exposed to high levels of glucose for 24 hours. The supernatant derived from the relevant EPC was used to observe the role of EPC regarding the function of mature endothelial cells since EPC releases various mediators including NO which then dissolve in the EPC culture supernatant. This was demonstrated by the measurement of NO stable metabolites, namely nitrite and nitrate, in the EPC supernatant.[3] In the present study, 7-day EPC culture (late EPCs) was used since late EPCs were found to be capable of producing more NO than early EPCs. This can occur since late EPCs express more VEGFR-2 than early EPCs (Csonka et al., 2015; Griendling, 2007; Sulistyo et al., 2017).

The present study demonstrated that 24-h high glucose exposure could significantly inhibit the ability of EPCs to induce endothelial cell migration, and this was associated with a decrease in NO of EPCs. It has been widely demonstrated that NO synthesized by eNOS plays an important role as a regulator of endothelial cell migration and angiogenesis. NO is known to positively regulate the VEGF/VEGFR-2 signaling pathway. Activated VEGFR-2 can then trigger a VE-cadherin phosphorylation cascade on tyrosine residues mediated by Src kinase. VE-cadherin sequestration in the endosome further impairs focal adhesion factors, triggers loss of contact among cells, increases vascular permeability, and triggers endothelial cell migration. Activation of VEGFR-2 also triggers actin polymerization and turnover of focal adhesion factors (vinculin, paxillin, and talin) which contribute to the formation of stress fibers. It is these stress fibers that allow contraction of endothelial cells and make endothelial cells capable of migration (Griendling, 2007; Meng et al., 2019; Widowati et al., 2014). In the course of endothelial cell migration, VEGF also activates the VEGFR-2/PI3K/Akt-PKB/eNOS signaling pathway; subsequently, the activated eNOS synthesizes NO. NO can then modulate angiogenesis through induction of endothelial cell surface expansion associated with vasodilation, which allows a better endothelium response to angiogenic and pro-migratory agents (Peristiowati et al., 2015; Sun et al., 2003). Inhibition of NO production is known to interfere with the role of VEGF/VEGFR2 in endothelial cell migration.

The administration of nanopartikel green teaat a dose of 50 nM in EPC culture exposed to high levels of glucose was able to significantly increase endothelial (HUVEC) cell migration ($P<0.05$) relative to the high glucose treated group and control group. This indicates that nano partikel green tea with polyphenol content can increase endothelial cell migration through increased NO production, increased concentration intensity of SDF-1α and CXCR4 receptors in hyperglycemic conditions. Endothelial cell migration also increased significantly ($P <0.05$) in the group treated with nanopartikel green teaat a dose of 30Mm relative to group (G), but comparable to control (N) ($P>0.05$). This explains that the administration of nanopartikel green teaat a dose of 30 mM was unable to significantly increase endothelial cell migration relative to the control group (N) in hyperglycemic conditions.

The use of antioxidant green tea catechin can inhibit the response to ROS formation as shown by decreased levels of superoxide dismutase (SOD), reduce oxidation stress in diabetes mellitus conditions and increase expression of SDF-1α in circulation. SDF-1α bound to CXCR4 receptors on the cell surface generates signals that can increase mobilization of bone marrow and affect EPC homing in injured cells (Leone et al., 2009; Peristiowati & Kusnul, 2020; Shabri et al., 2019; Wright et al., 2002).

The bonds of SDF-1α and CXCR4 result in increased interaction of Ca2+ levels in cytoplasm and phosphorylation of PI3 kinase and other protein kinases such as Akt, MEK/ERK and Janus Kinase (JAK)-2 (Cells et al., 2003; Fu et al., 2017; Qin et al., 2021). Activation of protein kinase Akt leads to upregulation of eNOS activity, thus increasing eNOS expression and phosphorylation. eNOS is an enzyme that catalyzes the production of nitric oxide (NO). NO is a signaling molecule for vascular protection and vascular repair.

CONCLUSION

In this phase-3 study, a conclusion can be drawn that the administration of Noano partikel Green Tea (NGT) at a dose of 30 μM can increase endothelial cell migration in HUVEC culture treated with EPC-CM with 5 mM of glucose. Further studies are recommended to use nanopartikel green teato increase EPC migration/homing in diabetes mellitus conditions using better methods. Furthermore, it is important to administer EPC supernatant to HUVEC culture, to add SOD to EPC culture to prevent NO interaction with superoxide so that it can extend the half-life of NO of EPCs and to use NO of EPCs directly to improve HUVEC function.

Acknowledgement: Ministry of Research, Technology and Higher Education through competitive grant programs

REFERENCES

Arizmendi-Grijalva, A., Martínez-Higuera, A. A., Soto-Guzmán, J. A., Martínez-Soto, J. M., Rodríguez-León, E., Rodríguez-Beas, C., López-Soto, L. F., Alvarez-Cirerol, F. J., Garcia-Flores, N., Cortés-Reynosa, P., Pérez-Salazar, E., & Iñiguez-Palomares, R. (2021). Effect on Human Vascular Endothelial Cells of Au Nanoparticles Synthesized fromVitex mollis. ACS Omega, 6(38), 24338–24350. https://doi.org/10.1021/acsomega.1c01506.

Bulboaca, A. E., Boarescu, P. M., Porfire, A. S., Dogaru, G., Barbalata, C., Valeanu, M., Munteanu, C., Râjnoveanu, R. M., Nicula, C. A., & Stanescu, I. C. (2020). The effect of nano-epigallocatechin-gallate on oxidative stress and matrix metalloproteinases in experimental diabetes mellitus. Antioxidants, 9(2), 1–15. https://doi.org/10.3390/antiox9020172.

Cells, E. P., Szmitko, P. E., Fedak, P. W. M., Weisel, R. D., Stewart, D. J., Kutryk, M. J. B., & Verma, S. (2003). Brief Review : From Bench to Bedside. 3093–3100. https://doi.org/10.1161/01.CIR.0000074242.66719.4A.

Chen, Y., Lin, S., Lin, F., Wu, T., & Tsao, C. (2007). High Glucose Impairs Early and Late Endothelial Progenitor Cells by Modifying Nitric Oxide – Related but Not Oxidative Stress – Mediated Mechanisms. Diabetes, 56(June), 1559–1568.

Csonka, C., Páli, T., Bencsik, P., Görbe, A., Ferdinandy, P., & Csont, T. (2015). Measurement of NO in biological samples. British Journal of Pharmacology, 172(6), 1620–1632. https://doi.org/10.1111/bph.12832.

Falasca, M., Raimondi, C., & Maffucci, T. (2011). Boyden Chamber. Methods in Molecular Biology, 769(January 2015), 86–95. https://doi.org/10.1007/978-1-61779-207-6.

Fu, Q. Y., Li, Q. S., Lin, X. M., Qiao, R. Y., Yang, R., Li, X. M., Dong, Z. B., Xiang, L. P., Zheng, X. Q., Lu, J. L., Yuan, C. B., Ye, J. H., & Liang, Y. R. (2017). Antidiabetic effects of tea. Molecules, 22(5), 1–19. https://doi.org/10.3390/molecules22050849.

Goshi, E., Zhou, G., & He, Q. (2019). Nitric oxide detection methods in vitro and in vivo. Medical Gas Research, 9(4), 192–207. https://doi.org/10.4103/2045-9912.273957.

Griendling, K. K. (2007). Endothelial Cell Migration During Angiogenesis. 782–794. https://doi.org/10.1161/01.RES.0000259593.07661.1e.

Hafshah, & Simanjuntak, K. (2020). Efektivitas Ekstrak Teh Hijau (Camellia sinensis L.) terhadap Penurunan Kadar Glukosa Darah Puasa Tikus Wistar (Rattus norvegicus) yang diInduksi Aloksan. Jurnal Sehat Mandiri, 15(1), 86–97. https://doi.org/10.33761/jsm.v15i1.194.

Hald, C., Kulkarni, J. A., Witzigmann, D., Lind, M., Petersson, K., & Simonsen, J. B. (2022). The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Advanced Drug Delivery Reviews, 188(1–18).

Hu, L., Dai, S.-C., Luan, X., Chen, J., & Cannavicci, A. (2018). Dysfunction and Therapeutic Potential of Endothelial Progenitor Cells in Diabetes Mellitus. Journal of Clinical Medicine Research, 10(10), 752–757. https://doi.org/10.14740/jocmr3581w.

Islam, M. A. (2012). Cardiovascular effects of green tea catechins: Progress and promise. Recent Patents on Cardiovascular Drug Discovery, 7(2), 88–99. https://doi.org/10.2174/157489012801227292.

Kondo, T., Ohta, T., Igura, K., Hara, Y., & Kaji, K. (2002). Tea catechins inhibit angiogenesis in vitro, measured by human endothelial cell growth, migration and tube formation, through inhibition of VEGF receptor binding. Cancer Letters, 180(2), 139–144. https://doi.org/10.1016/S0304-3835(02)00007-1.

Leone, A. M., Valgimigli, M., Giannico, M. B., Zaccone, V., Perfetti, M., Amario, D. D., Rebuzzi, A. G., & Crea, F. (2009). From bone marrow to the arterial wall : the ongoing tale of endothelial progenitor cells. 890–899. https://doi.org/10.1093/eurheartj/ehp078.

Meng, J. M., Cao, S. Y., Wei, X. L., Gan, R. Y., Wang, Y. F., Cai, S. X., Xu, X. Y., Zhang, P. Z., & Li, H. Bin. (2019). Effects and mechanisms of tea for the prevention and management of diabetes mellitus and diabetic complications: An updated review. Antioxidants, 8(6), 1–25. https://doi.org/10.3390/antiox8060170.

Peristiowati, Y., Indasah, I., & Ratnawati, R. (2015). The effects of catechin isolated from green tea GMB-4 on NADPH and nitric oxide levels in endothelial cells exposed to high glucose. Journal of Intercultural Ethnopharmacology, 4(2), 114. https://doi.org/10.5455/jice.20141224104135.

Peristiowati, Y., & Kusnul, Z. (2020). Synthesis and characterization of green tea paste

nanoparticles based on wet milling. Journal of Advanced Pharmaceutical Technology and Research, 11(2), 86–88. https://doi.org/10.4103/japtr.JAPTR_148_19.

Qin, H., Zhao, X., Hu, Y. J., Wang, S., Ma, Y., He, S., Shen, K., Wan, H., Cui, Z., & Yu, B. (2021). Inhibition of SDF-1/CXCR4 Axis to Alleviate Abnormal Bone Formation and Angiogenesis Could Improve the Subchondral Bone Microenvironment in Osteoarthritis. BioMed Research International, 2021, 1–13. https://doi.org/10.1155/2021/8852574.

Shabri, S., Rohdiana, D. D., Maulana, H., Haryanto, S., Prawira-Atmaja, M. I., Mauludin, R., Insanu, M., & Perdana, A. W. (2019). Karakteristik nanopartikel ekstrak teh hijau dengan metode nano milling dan nano spray. Jurnal Sains Teh Dan Kina, 21(2), 74–84. https://doi.org/10.22302/pptk.jur.jptk.v21i2.146.

Sulistyo, H., Kurniawan, D. W., & Rujito, L. (2017). Biochemical and histopathological effects of green tea nanoparticles in ironized mouse model. Research in Pharmaceutical Sciences, 12(2), 99–106. https://doi.org/10.4103/1735-5362.202448.

Sun, J., Zhang, X., Broderick, M., Fein, H., Instruments, W. P., & International, S. (2003). Measurement of Nitric Oxide Production in Biological Systems by Using Griess Reaction Assay. Sensors, 3, 276–284. http://www.mdpi.net/sensors.

Taleb Safa, M. A., & Koohestani, H. (2024). Green synthesis of silver nanoparticles with green tea extract from silver recycling of radiographic films. Results in Engineering, 21(January), 101808. https://doi.org/10.1016/j.rineng.2024.101808.

Tóvári, J., Futosi, K., Bartal, A., Tátrai, E., Gacs, A., Kenessey, I., & Paku, S. (2014). Boyden chamber-based method for characterizing the distribution of adhesions and cytoskeletal structure in HT1080 fibrosarcoma cells. Cell Adhesion and Migration, 8(5), 509–516. https://doi.org/10.4161/cam.28734.

Widowati, W., Widyanto, R. M., Husin, W., Ratnawati, H., Laksmitawati, D. R., Setiawan, B., Nugrahenny, D., & Bachtiar, I. (2014). Green tea extract protects endothelial progenitor cells from oxidative insult through reduction of intracellular reactive oxygen species activity. Iranian Journal of Basic Medical Sciences, 17(9), 702–709.

Wright, D. E., Bowman, E. P., Wagers, A. J., Butcher, E. C., & Weissman, I. L. (2002). Hematopoietic Stem Cells Are Uniquely Selective in Their Migratory Response to Chemokines. 195(9). https://doi.org/10.1084/jem.20011284.

Zhang, M., Lv, X., Li, J., Xu, Z., & Chen, L. (2008). The Characterization of High-Fat Diet and Multiple Low-Dose Streptozotocin Induced Type 2 Diabetes Rat Model. 2008. https://doi.org/10.1155/2008/704045.