Coffee Diterpene Derivatives as Anti-angiogenesis Agents

Marzieh Moeenfard[1], Alice Cortez[2,3], Vera Machado[2,3], Pedro Coelho[2,3,4], Raquel Soares[2,3], Arminda Alves[1], Nuno Borges[5], Alejandro Santos[3,5]

[1] LEPABE, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; [2] Department of Biochemistry, Faculty of Medicine, University of Porto, Al. Prof. Hernâni Monteiro, 4200-319, Porto, Portugal; [3] I3S—Instituto de Investigação e Inovação em Saúde, Porto, Portugal ; [4] Ciências Químicas e Biomoléculas, Escola Superior de Tecnologias da Saúde do Porto, Instituto Politécnico do Porto, Portugal ; [5] Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Introduction

Studying the biological attraction of coffee diterpene has gain substantial attention, in these decades. Several studies have exhibited a strong association between coffee-specific diterpenes, cafestol and kahweol, and reduced risk for certain types of cancer (1). According to the literature reviews, free cafestol and kahweol revealed anti-angiogenic properties in vitro and ex vivo models (2,3,4). Nevertheless, free diterpenes are found in small quantities (0.7-3.5%) in green and roasted beans (5). However, the anti-angiogenic properties of diterpene esters, which compose up to 98% of coffee diterpenes, remain unclear so far. Angiogenesis plays a crucial role in the growth and metastasis of tumors (3,4). In addition, angiogenesis is a complex process that has a vital role in tumor growth. Therefore, multi-target compounds are of interest due to their ability to prevent different angiogenic steps and may be introduced in the clinic (2). Therefore, the present paper aimed to characterize and compare the effect of two compounds present in coffee on angiogenesis in an ´*in vitro*´ model. The chemical structure of the compounds analyzed in the present study is shown in Figure 1.

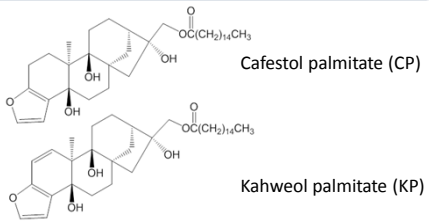

Figure 1. Chemical structure of compounds analyzed

Experimental

Cell viability (MTS assay)

- ✓ Treating HMVECs (1×10^5 cells/mL; 96 well plate) with CP and KP at concentration of 50, 70 and 100 µM prepared in RMPI with 2% FBS.
- ✓ Assessing the cell viability in comparison to untreated control cells using MTS colorimetric assay at 490 nm.

Cell proliferation (BrdU assay)

- ✓ Treating HMVECs (1×10^5 cells/mL) with CP and KP (50 µM) and BrdU labeling solution (5-bromo-2´-deoxyuridine).
- ✓ Detection by ELISA Kit using anti-BrdU specific antibodies.
- ✓ Measuring the absorbance at 450 and 690 nm against the background control (blank).

Cell migration (Injury assay)

- ✓ Seeding the HMVECs (1×10^5 cells/mL) in 24 well plate.
- ✓ Scrapping a single wound in the center of each well.
- ✓ Treating with CP and KP at concentration of 50 µM.
- ✓ Taking photo immediately after wounding and after a 24 h incubation.

Cell apoptosis (TUNEL assay)

- ✓ Seeding the HMVECs (6×10^4 cells/mL) in 24 well plate and treating with CP and KP at concentration of 50 µM.
- ✓ Performing the TUNEL assay using In Situ Cell Death Detection Kit.
- ✓ Evaluation of TUNEL-stained nuclei in relation to every DAPI-stained nuclei.

Capillary-like structures formation (Matrigel assay)

- ✓ Polimerization of matrigel in 96-well plate.
- ✓ Seeding the treated HMVECs with CP and KP (50 µM) on matrigel-coated plate.
- ✓ Visualization of capillary-like structures using microscope.

Results

MTS assay

- Under treatment with CP and KP at the concentration of 50 µM, the ECs maintain their metabolic activity and do not sustain a toxic effect (Figure 2).
- Treatment with 75 and 100 µM CP and KP dramatically inhibited HMVECs growth ($p\leq0.05$).
- The results showed that KP presented more inhibitory effects on HMVECs growth than CP.

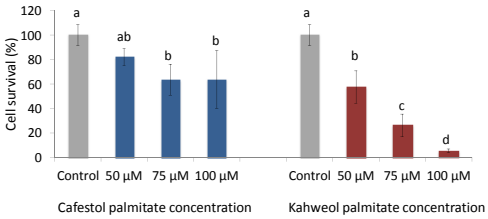

Figure 2. Effect of CP and KP on HMVECs viability evaluated by MTS assay.

TUNEL assay

- Both compounds induced apoptosis, though not reaching statistical significance ($p\geq0.05$) (Figure 3).
- The percentage of apoptotic cells was 6.6% vs. 93.4% of live cells for HMVECs treated with CP.
- Regarding KP, 14.4% apoptotic cells were observed vs. 85.6% of live cells.
- KP induced apoptosis more than CP (Figure 4).

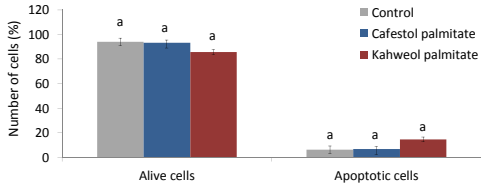

Figure 3. Effect of CP and KP (50 µM) on HMVECs apoptosis by TUNEL assay.

Migration assay

- CP and KP led to impairment of migratory capacity at concentration of 50 µM as compared to control (Figure 6).
- This inhibitory effect was not significant for CP ($p\geq0.05$) while it was more pronounced in the presence of KP ($p\leq0.05$).
- No significant differences of migration inhibition was observed between KP and CP (Figure 5)

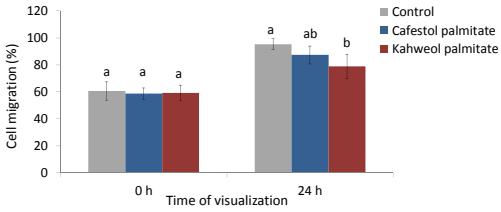

Figure 5. Effect of CP and KP (50 µM) on HMVECs migration.

BrdU assay

- Both compounds revealed significant anti-proliferative effects on HMVECs (Figure 3), without affecting cell viability (Figure 7).
- No significant difference was found between CP (29±4%; $p\leq0.05$ vs. control) and KP (51±5%; $p\leq0.05$ vs. control).

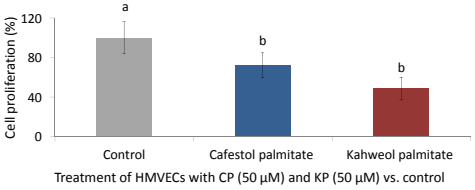

Figure 7. Evaluation of cell proliferation of HMVECs through BrdU assay.

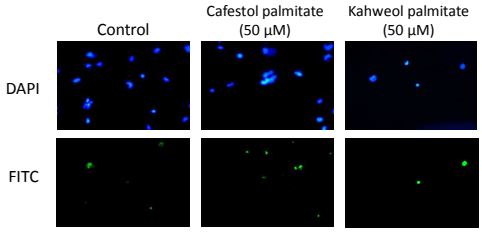

Figure 4. Fluorescent images of HMVECs treated with CP and KP vs. control. Green = apoptotic cells; Blue= Dapi-stained nuclei (Magnification 200X)

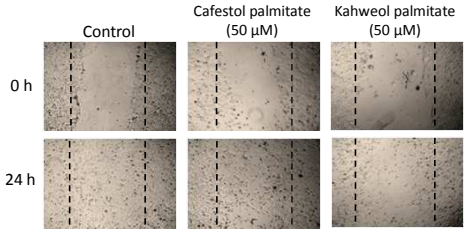

Figure 6. Images of HMVECs treated with CP and KP vs. control.

Matrigel assay

- Formation of capillary-like structure was inhibited by treating the HMVECs by CP and KP (Figure 8).
- KP represented more inhibitory effects than CP.

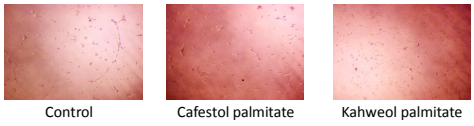

Figure 8. CP and KP inhibited tube formation of HMVECs on matrigel

Conclusion

- According to our results, CP and KP are able to prevent several steps involved in angiogenesis.
- This study revealed the potential application of CP and KP as promising strategy against angiogenesis-dependent disorders.
- Our findings further indicate that KP exerts more potent anti-angiogenic effects than CP, which may explain the more beneficial health effects reported for kahweol.

References

1. Cavin, C., Holzhaeuser, D., Scharf, G., Constable, A., Huber, W.W. & Schilter, B. (2002) Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity. *Food and Chemical Toxicology,* **40**(8), 1155-1163.
2. Cardenas, C., Quesada, A.R. & Medina, M.A. (2011) Anti-angiogenic and anti-inflammatory properties of kahweol, a coffee diterpene. *PLoS One,* **6**(8), e23407.
3. Wang, S., Yoon, Y.C., Sung, M.J., Hur, H.J. & Park, J.H. (2012) Antiangiogenic properties of cafestol, a coffee diterpene, in human umbilical vein endothelial cells. *Biochemical and Biophysical Research Communications,* **421**(3), 567-71.
4. Kim, H.G., Hwang, Y.P., Han, E.H., Choi, J.H., Kwon, K.-i., Chung, Y.C., Jeong, M.H., Jeong, T.C., Kang, W. & Jeong, H.G. (2012) The coffee diterpene kahweol inhibits metastasis by modulating expressions of MMPs and VEGF via STAT3 inactivation. *Food Chemistry,* **133**(4), 1521-1529.
5. Kölling-Speer, I., Strohschneider, S. & Speer, K. (1999) Determination of free diterpenes in green and roasted coffees. *Journal of High Resolution Chromatography,* **22**(1), 43-46.

Acknowledgment

This study was partially funded by FCT (UID/BIM/04293/2013) and FEDER funds through the Operational Programme for Competitiveness Factors e COMPETE, ON.2 e O Novo Norte E North Portugal Regional Operational Programme and National Funds through FCT – Foundation for Science and Technology, in the scope of the P2020 Partnership Agreement under the projects: UID/EQU/00511/2013-LEPABE and NORTE-07-0124-FEDER-000025 e RL2_ Environment & Health and SFRH/BD/79318/2011.