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RAPID COMMUNICATION

Caffeic Acid Phenethyl Ester reduces Pro Inflammatory Cytokines in Moderate Swimming Test in Growing Rats Model

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Background: Caffeic acid phenethyl ester (CAPE) is a naturally occurring polyphenolic concentrated in propolis of honeybee hives. CAPE has been shown various physiological and pharmacologic properties. The aim of the present study was to investigate the effects of CAPE on proinflammatory markers in growing rats by performing the moderate swimming test.

Methods: A total number of 21 male Wistar albino rats were separated into three groups (n = 7): sedentary: negative control group; exercise: positive control group received vehicle orally and exercise + CAPE: CAPE treated group: treated with CAPE (20 mg/kg) orally 30 min before exercise, for 5 days. The animals were left free to swim in the tank, 20 minutes/day for 5 days. At 24 hours after finishing the experiment, rats were euthanised and blood was collected to analyze the level of serum interleukin IL-6 and tumor necrosis factor–α (TNF-α).

Results: Growing rats subjected to the moderate swimming test and in those treated with CAPE showed a lower level of TNF-α compared to the negative control. More interestingly, the one-way ANOVA data demonstrated a decreased level of proinflammatory IL-6 in the CAPE-treated group compared to the negative control.

Conclusion: Results of this study indicate that short-term administration of CAPE may modulate proinflammatory cytokine profiles during moderate exercise and may serve to boost the anti-inflammatory effects of exercise. Further studies are needed to evaluate the efficacy and safety of long-term administration of CAPE as an adjective anti-inflammatory agent.

Keywords: CAPE, swimming, rats, interleukins, proinflammatory

Introduction

Inflammation is an immune reaction to deleterious stimuli, such as damage and pathogens, which is initiated to protect the body and is accompanied by chemotaxis of leukocytes, adhesion and migration to the inflammatory site.[1] Inflammatory processes are strongly associated with several chronic diseases, such as diabetes,[2] osteoporosis,[3] inflammatory bowel disease,[4] cardiovascular diseases,[5] muscle wasting or physical frailty[6] and early mortality,[7] especially among elderly and sedentary persons.[8] Moreover, evidence shows that the risk of developing chronic inflammation can start early in childhood and its effects continue to affect the health of adulthood.[9]

Several mediators have been identified in the inflammatory mechanisms, including cytokines [interleukins (ILs) and tumor necrosis factor-α (TNF-α)].[10] Both are

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commonly used as proinflammatory cytokine biomarkers of inflammation. Earlier studies have shown that IL-6 and TNF-α induce neurotransmitter synthesis, which is associated with poor health in previously healthy subjects.[11]

Strenuous exercise acts as a stress factor during and after its performance and is capable of causing inflammation,[12–14] that would in turn help in limiting the tissue damage initially caused by the exercise.[15] Proinflammatory cytokine IL-6 and TNF-α have been shown to increase more with intense exercise compared with moderate exercise,[16] but still there are many discrepancies and differences regarding the impact of moderate exercise on proinflammation markers.[17]

Changes in inflammatory biomarkers due to exercise may be divided into acute "changes during and immediately following exercise training" and long-term effects "after resolving the effect of acute exercise activities phase".[18] On the other hand, performing regular moderate exercise bouts was identified as an anti-inflammatory after the washout of residual effects of acute exercise.[8]

Caffeic acid phenethyl ester (CAPE) is a naturally occurring polyphenolic concentrated in propolis of honeybee hives.[19] CAPE has been shown to have various physiological and pharmacologic properties, such as anti-inflammatory,[20] neuroprotective,[21] immunomodulatory,[22] antineoplastic[23] and antioxidant activities.[19] However, to the best of our knowledge, there is hardly any data in the literature regarding the anti-inflammatory/immunomodulatory effect of CAPE on exercise. Therefore, the effects of CAPE on proinflammatory markers in growing rats performing a moderate swimming test were observed in the present study.

Materials and Methods

Animal and Exercise Protocol

A total number of 21 male Wistar albino rats (weighing 200–300 grams) were obtained from the animal house at Imam Abdulrahman Bin Faisal University (IAU), Saudi Arabia, and were housed in cages with free access to food and water. They were kept in a separate air-conditioned (22 ± 2 °C) room, on a 12-hour light/12-hour dark cycle, with lights on at 7:00 a.m.

Ethics Approval

The study protocol was reviewed and approved by the ethical committee (IRB-UGS-2019-01-311) in compliance with the institutional animal care and use committee at IAU and conducted in accordance with international guidance on animal welfare.

Study Design

Rats were separated into three groups (n = 7): sedentary: negative control group; exercise: positive control group received vehicle orally, and exercise + CAPE: CAPE treated group: treated with CAPE (20 mg/Kg, at a Sigma-Aldrich company) orally 30 min before exercise, for 5 days. The dose of CAPE was chosen because it has previously been shown to cause the maximal effect against inflammation and lipid peroxidation in rodents.[24]

Exercise Training Protocol

Swimming exercise was performed in a similar manner as described elsewhere.[25] Briefly, animals were placed in a water tank at a temperature of 32°C ± 1°C between 9 and 11 AM. The animals were left free to swim in the tank for 20 minutes/day for 5 days. At the end of each training session, rats were towel-dried and returned to their respective cages. The individual body weight of rats was recorded before the start and completion of the experiment, respectively. At 24 hours after finishing the experiment, rats were anesthetized with 5% Sevoflurane with 95% of oxygen flow meter for blood collection from inferior vena cava. Near 10 mL of blood was collected in a plain vacutainer. Serum was separated by centrifugation at 2400 x g. Serum samples were stored at −20°C before Cytokines analysis. Rats were killed by decapitation and discarded following the Bio-Medical Waste Rule.

Cytokines Analysis

The level of serum interleukin IL-6 and Tumor Necrosis Factor– ά (TNF- ά) were estimated by using commercial ELISA kits (ELABSCIENCE, USA). The results were expressed in pg/mL for IL-6 and TNF- ά. The sensitivity of the assays for detection of cytokine level was as stated in the kit literature of the manufacturer.

Statistical Analysis

The statistical analysis of data was performed using IBM SPSS Statistics for Windows, version 24.0. The data are reported as a mean± standard error. The statistical evaluation was conducted using one-way ANOVA, followed by

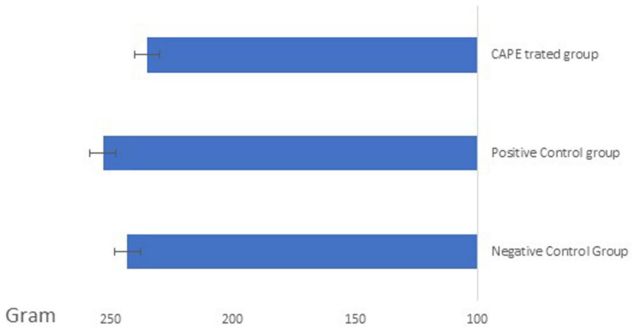

Figure 1 Baseline values of body weight (gram) for the study groups.

the Tukey HSD multiple comparison test. The value of p <0.05 was considered to be statistically significant.

Results

There were no significant differences in the baseline body weight between the study groups, as shown in Figure 1. The experiment's protocol did not show any significant changes in the body weight measurements among the study groups when compared with baseline values, as appeared in Figure 2.

Growing rats subjected to the moderate swimming test showed a lower level of TNF-α compared to the negative control. Administration of CAPE caused an additional reduction in the level of TNF-α of these animals. The one-way ANOVA of CAPE X moderate swimming test showed a significant reduction in TNF-α ($F(2,18) = 26.4$, $p<0.001$)), as presented in Table 1.

More interestingly, the one-way ANOVA of data demonstrated a decreased level of proinflammatory IL-6 ($F(2,18) = 6.7$, $p=0.006$)) in the CAPE treated group compared to the negative control (Table 1).

Discussion

This study provides the first demonstration that the administration of CAPE for 5 days in moderate swimming test decreased the levels of proinflammatory cytokines (IL-6, TNF-α) in growing rats. However, in contrast, moderate exercise without supplementation decreased TNF-α compared to the negative group.

There is a gap in knowledge about the effect of short-term moderate intensity exercise on proinflammatory cytokines; indeed, several published reports are contradictory.[17] These discrepancies could be attributed to the intensity of the effort related to the type of the exercise and types of muscle contraction.[17] Meanwhile, our findings are consistent with previous data showing that short-term moderate exercise training reduces the levels of proinflammatory biomarkers.[26]

A limited number of studies have studied the anti-inflammatory effect of CAPE in exercise models. Previous studies have reported the protective anti-inflammatory effect of CAPE against eccentric exercise-induced skeletal muscle damage in animals[27] and acute swimming exercise.[28] In this context, supplementation of CAPE during moderate intensity exercise could exert beneficial effects and bring about a reduction in systemic inflammation via a decrease in proinflammatory cytokine production during exercise bout[29] as well as during the growing life span.[9]

Evidence has found that local application of CAPE increased the apoptosis of leukocytes and significantly

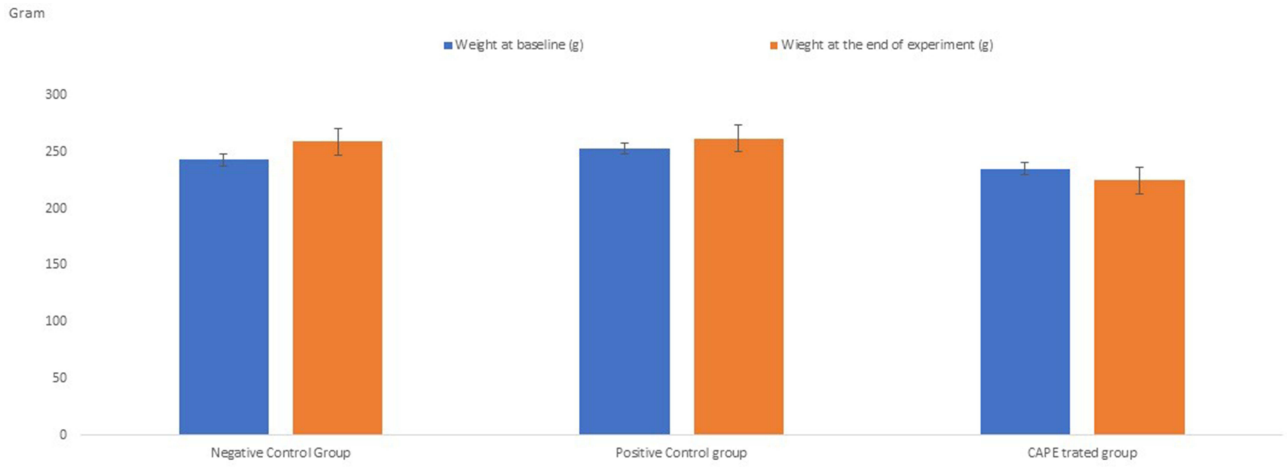

Figure 2 Baseline body weight (gram) vs weight at the end of experiment of the study groups.

Table 1 The Effect of Caffeic Acid Phenethyl Ester on Pro Inflammatory Cytokines.

	Negative Control Group	Positive Control Group	CAPE Treated Group
	Mean±SEM (*p* value)	**Mean±SEM (*p* value)**	**Mean±SEM (*p* value)**
IL-6	220.3±2.0	197.3±2.0	140.5±2.0# (0.006)
TNF-α	39.1±0.9	32.5±0.9# (<0.001)	31.2±0.6# (<0.001)

Note: #Significant compared to negative control Group.

decreased levels of monocytes and neutrophils in the inflammatory site exudate.[30] In the same line, CAPE administration in situ inhibited NF-κB activation via the phosphorylation of p65 and suppression of IκB-α degradation. Moreover, data showed that mRNA expression levels of inflammatory biomarkers induced by NF-κB transcriptional activation, including inducible nitric oxide synthase, IL-2, interferon-γ, IL-6 and nuclear factor-α, have all been reduced significantly by CAPE treatment.[31]

The observed effects of CAPE along with moderate intensity swimming test in reducing levels of pro-inflammatory cytokines were independent of the changes in rats' body weight, which confirm the direct effect of CAPE on the inflammatory cascades, since losing body fat has been involved in the reduction of serum IL-6 and TNF-α.[32]

Conclusion

Results of this study indicate that short-term administration of CAPE may modulate proinflammatory cytokine profiles during moderate exercise and may serve to boost the anti-inflammatory effects of exercise. Further studies are needed to evaluate the efficacy and safety of long-term administration of CAPE as an adjective anti-inflammatory agent.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*. 2007;7:678–689. doi:10.1038/nri2156
2. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol*. 2019;11(3):45–63.
3. Abrahamsen B, Bonnevie-Nielsen V, Ebbesen EN, Gram J, Beck-Nielsen H. Cytokines and bone loss in a 5-year longitudinal study hormone replacement therapy suppresses serum soluble interleukin-6 receptor and increases interleukin-1-receptor antagonist: the Danish Osteoporosis Prevention Study. *J Bone Miner Res*. 2000;5(8):1545–1554. doi:10.1359/jbmr.2000.15.8.1545
4. Axelrad JE, Lichtiger S, Yajnik V. Inflammatory bowel disease and cancer: the role of inflammation, immunosuppression, and cancer treatment. *World J Gastroenterol*. 2016;22(20):4794–4801. doi:10.3748/wjg.v22.i20.4794
5. Schumacher A, Seljeflot I, Sommervoll L, Christensen B, Otterstad JE, Arnesen H. Increased levels of markers of vascular inflammation in patients with coronary heart disease. *Scand J Clin Lab Invest*. 2002;62(1):59–68. doi:10.1080/003655102753517217
6. Pedersen M, Bruunsgaard H, Weis N, et al. Circulating levels of TNF-alpha and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. *Mech Ageing Dev*. 2003;24(4):495–502. doi:10.1016/S0047-6374(03)00027-7
7. Bruunsgaard H. The clinical impact of systemic low-level inflammation in elderly populations. *Dan Med Bull*. 2006;53(3):285–309.
8. Allen J, Sun Y, Woods JA. Exercise and the regulation of inflammatory responses. *Prog Mol Biol Transl Sci*. 2015. doi:10.1016/bs.pmbts.2015.07.003
9. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med*. 2019;25(12):1822–1832. doi:10.1038/s41591-019-0675-0
10. Stow JL, Murray RZ. Intracellular trafficking and secretion of inflammatory cytokines. *Cytokine Growth Factor Rev*. 2013;24(3):227–239. doi:10.1016/j.cytogfr.2013.04.001

11. Starkie RL, Hargreaves M, Rolland J, Febbraio MA. Heat stress, cytokines, and the immune response to exercise. *Brain Behav Immun*. 2005;19(5):404–412. doi:10.1016/j.bbi.2005.03.005
12. Silveira LS, de Moura Mello Antunes B, Luis Araujo Minari A, Vagner Thomatieli Dos Santos R, Rosa Neto JC, Santos Lira F. Macrophage polarization: implications on metabolic diseases and the role of exercise. *Crit Rev Eukaryot Gene Expr*. 2016;19 (5):404–412. doi:10.1615/CritRevEukaryotGeneExpr.2016015920
13. Pendersen BK, Toft AD. Effects of exercise on lymphocytes and cytokines. *Br J Sports Med*. 2000. doi:10.1136/bjsm.34.4.246
14. Bernecker C, Scherr J, Schinner S, Braun S, Scherbaum WA, Halle M. Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners. *Scand J Med Sci Sport*. 2013;23 (2):207–214. doi:10.1111/j.1600-0838.2011.01372.x
15. Oishi Y, Manabe I. Macrophages in inflammation, repair and regeneration. *Int Immunol*. 2018. doi:10.1093/intimm/dxy054
16. Brenner IKM, Natale VM, Vasiliou P, Moldoveanu AI, Shek PN, Shephard RJ. Impact of three different types of exercise on components of the inflammatory response. *Eur J Appl Physiol Occup Physiol*. 1999;80(5):452–460. doi:10.1007/s004210050617
17. Cerqueira É, Marinho DA, Neiva HP, Lourenço O. Inflammatory effects of high and moderate intensity exercise—a systematic review. *Front Physiol*. 2020;10. doi:10.3389/fphys.2019.01550
18. Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. *Eur J Clin Invest*. 2017;47(8):600–611. doi:10.1111/eci.12781
19. Göçer H, Gülçin I. Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. *Int J Food Sci Nutr*. 2011;62 (8):821–825. doi:10.3109/09637486.2011.585963
20. Cho MS, Park WS, Jung WK, et al. Caffeic acid phenethyl ester promotes anti-inflammatory effects by inhibiting MAPK and NF-κB signaling in activated HMC-1 human mast cells. *Pharm Biol*. 2014;52(7):926–932. doi:10.3109/13880209.2013.865243
21. Tolba MF, Azab SS, Khalifa AE, Abdel-Rahman SZ, Abdel-Naim AB. Caffeic acid phenethyl ester, a promising component of propolis with a plethora of biological activities: a review on its anti-inflammatory, neuroprotective, hepatoprotective, and cardioprotective effects. *IUBMB Life*. 2013;65(8):699–709. doi:10.1002/iub.1189
22. Park JH, Lee JK, Kim HS, et al. Immunomodulatory effect of caffeic acid phenethyl ester in Balb/c mice. *Int Immunopharmacol*. 2004;4 (3):429–436. doi:10.1016/j.intimp.2004.01.013
23. Lee YJ, Liao PH, Chen WK, Yang CC. Preferential cytotoxicity of caffeic acid phenethyl ester analogues on oral cancer cells. *Cancer Lett*. 2000;153(1–2):51–56. doi:10.1016/S0304-3835(00)00389-X
24. Macías-Pérez JR, Beltrán-Ramírez O, Vásquez-Garzón VR, et al. The effect of caffeic acid phenethyl ester analogues in a modified resistant hepatocyte model. *Anticancer Drugs*. 2013;24(4):394–405. doi:10.1097/CAD.0b013e32835e9743
25. Cechella JL, Leite MR, Dobrachinski F, et al. Moderate swimming exercise and caffeine supplementation reduce the levels of inflammatory cytokines without causing oxidative stress in tissues of middle-aged rats. *Amino Acids*. 2014;46(5):1187–1195. doi:10.1007/s00726-014-1679-1
26. Middelbeek RJW, Motiani P, Brandt N, et al. Exercise intensity regulates cytokine and klotho responses in men. *Nutr Diabetes*. 2021;11(1):5. doi:10.1038/s41387-020-00144-x
27. Shen YC, Yen JC, Liou KT. Ameliorative effects of caffeic acid phenethyl ester on an eccentric exercise-induced skeletal muscle injury by down-regulating NF-κB mediated inflammation. *Pharmacology*. 2013;91(3–4):219–228. doi:10.1159/000348412
28. Akil M. Effects of caffeic acid phenethyl ester and oxidative stress caused by the exhaustion exercise on endotelial damage. *Arch Ital Biol*. 2020;158(2):37–44. doi:10.12871/00039829202021
29. Yudkin JS. Inflammation, obesity, and the metabolic syndrome. *Horm Metab Res*. 2007;39(10):707–709. doi:10.1055/s-2007-985898
30. Orban Z, Mitsiades N, Burke TR, Tsokos M, Chrousos GP. Caffeic acid phenethyl ester induces leukocyte apoptosis, modulates nuclear factor-kappa B and suppresses acute inflammation. *Neuroimmunomodulation*. 2000;7(2):99–105. doi:10.1159/000026427
31. Toyoda T, Tsukamoto T, Takasu S, et al. Anti-inflammatory effects of caffeic acid phenethyl ester (CAPE), a nuclear factor-κB inhibitor, on Helicobacter pylori-induced gastritis in Mongolian gerbils. *Int J Cancer*. 2009;125(8):1786–1795. doi:10.1002/ijc.24586
32. Mathieu P, Poirier P, Pibarot P, Lemieux I, Després JP. Visceral obesity the link among inflammation, hypertension, and cardiovascular disease. *Hypertension*. 2009;53(4):577–584. doi:10.1161/HYPERTENSIONAHA.108.110320