




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 frailty⁶ 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 antineoplastic²³ 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 $32^{\circ}\text{C} \pm 1^{\circ}\text{C}$ 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 $2400 \times g$. 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- α (TNF- α) were estimated by using commercial ELISA kits (ELABSCIENCE, USA). The results were expressed in pg/mL for IL-6 and TNF- α . 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 \pm standard error. The statistical evaluation was conducted using one-way ANOVA, followed by

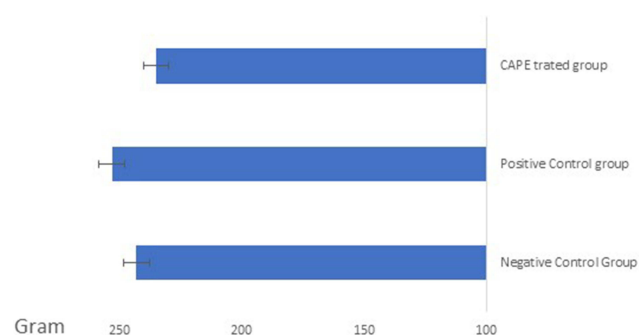


Figure 1 Baseline values of body weight (gram) for the study groups.

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 animals²⁷ 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 bout²⁹ as well as during the growing life span.⁹

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

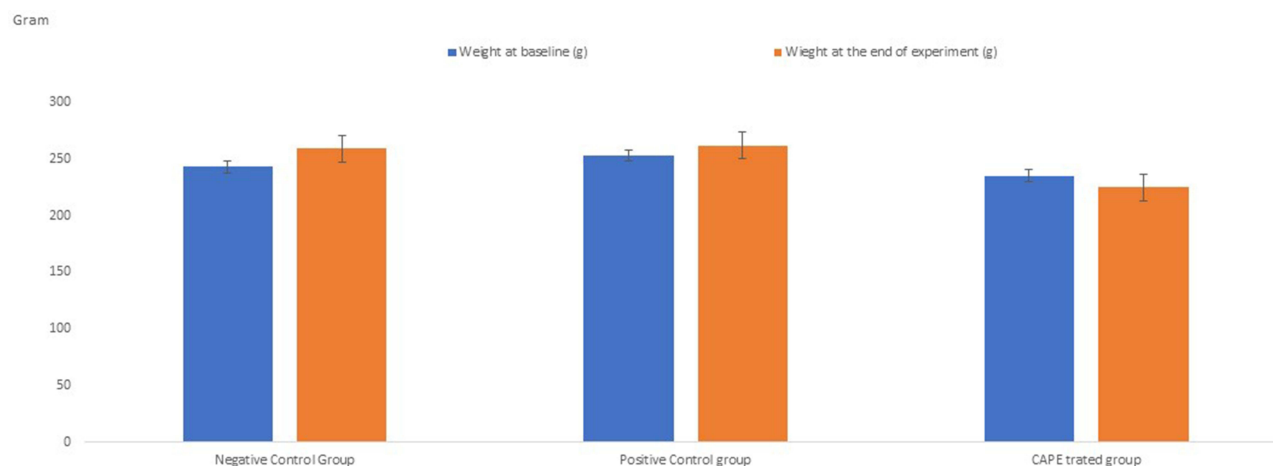


Figure 2 Baseline body weight (gram) vs weight at the end of experiment of the study groups.

Table I 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.

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