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# Caffeic acid phenethyl ester (CAPE) suppresses systemic inflammation in rat trauma model

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## ABSTRACT

**Aim:** This study was conducted to explain the anti-inflammatory activity of caffeic acid phenethyl ester (CAPE) in an experimental rat thoracic trauma model.
**Materials and Methods:** Forty adult (200–250 g) male Wistar albino rats were used. Rats were randomly divided into four groups: Control ($n$ = 10), trauma model ($n$ = 10), trauma model + CAPE ($n$ = 10), and CAPE ($n$ = 10) groups, respectively. CAPE treatment was administered intraperitoneally at a dose of 10 µmol/kg for 7 days. At the end of the seventh day, the rats were sacrificed under anesthesia. Serum interleukin 1-beta (IL-1β), IL-6, and IL-10 cytokine levels were determined as picogram per milliliter using the sandwich enzyme-linked immunosorbent assay method.
**Results:** In the trauma model, there were an increased IL-1β and IL-6 serum levels and decreased pro-inflammatory IL-10 serum levels compared to the control group ($p < 0.05$). CAPE treatment resulted in a decrease in IL-1β and IL-6 levels and an increase in IL-10 levels ($p < 0.05$).
**Conclusion:** CAPE administration suppresses systemic inflammation in the thoracic trauma model by reducing the expression of pro-inflammatory cytokines IL-1β and IL-6 and increasing the expression of anti-inflammatory IL-10.

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## KEYWORDS

Blunt chest trauma; IL-1β; IL-6; IL-10

## Introduction

Thoracic trauma can initiate a systemic inflammatory response after trauma and is considered the most sensitive trauma to multiple trauma [1,2]. It was reported that thoracic trauma causes systemic effects such as increased release of tumor necrosis factor – alpha (TNF-α) and interleukin (IL)-6 pro-inflammatory cytokines, other inflammation mediators, and activation of the complement system [3,4]. Moreover, it can cause secondary organ damage by increasing the number of polymorphonuclear leukocytes and other inflammatory cells in trauma patients [5,6]. Experimental studies have shown that complications such as lung injury [7,8], cardiac damage [9,10], hepatic injury [7,11], and immunodysfunction [4,12] develop after chest trauma in murine models. This may be a consequence of a systemic inflammatory response involving strong pro-inflammatory cytokine release (TNF-α, IL-6, and IL-1β) following trauma [13,14]. There are numerous studies in the literature, including the reduction of the pro-inflammatory response following trauma.

Caffeic acid phenethyl ester (CAPE) is an important biological component of propolis produced by worker honeybees [15]. *In vivo* and *in vitro* experimental studies have reported that CAPE exerts immunomodulatory effects by suppressing *T* cell proliferation and lymphokine production [16–18]. Also, CAPE has strong anti-inflammatory, anti-oxidant, anti-apoptotic, and anti-neoplastic effects [19]. The anti-inflammatory effect of CAPE was reported in a diabetic rat model [20], ischemia-reperfusion model [21], lipopolysaccharide-induced sepsis model [22], and hepatotoxic models [23–25]. Akgün et al. [26] have shown that CAPE administration reduces TNF and IL-6 levels as well as tissue damage in local spinal cord injury. However, no studies have examined the role of CAPE

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on inflammatory cytokines in thoracic trauma. In this study, the aim was to investigate the effect of CAPE on serum IL-1β, IL-6, and IL-10 inflammatory cytokine levels in experimental thoracic trauma induced in rats.

## Materials and Methods

All experimental procedures were conducted after receiving permission from the Local Ethics Committee (Permission number: 17/10/2017-7) at Namık Kemal University Application and Research Centre for Experimental Animals (NKU-DHUAM). Forty male Wistar albino rats were used in this study. Rats were housed under standard laboratory conditions (22°C ± 2°C; 60% humidity; and 12/12 dark light cycles) and were given a standard diet. Rats were divided into four groups ($n$ = 10): control, trauma, trauma + CAPE, and CAPE. Thoracic trauma model was applied as described by Raghavendran et al. [27]. A cylindrical weight was dropped from a certain height (0.5 m) onto the right hemithorax of rats under ketamine-xylazine anesthesia (50–15 mg/kg, intraperitoneally). Total energy transferred to the chest wall of the rat was 1.96 J ($E = m \times g \times h$, $m$: mass of the cylindrical weight (0.4 kg); $g$: gravity (9.8 m/s$^2$); and $h$: height from the platform (0.5 m). CAPE (Sigma Aldrich, C8221) treatment was applied for 7 days after thoracic trauma (10 μmol/kg, intraperitoneally, dissolved in dimethyl sulfoxide). At the end of the seventh day, rats were sacrificed under anesthesia (ketamine/xylazine; 90/10 mg/kg) and blood samples were taken from the heart. Blood samples were centrifuged at 2,500 rpm for 5 minutes (THERMO/HERAEUS Labofuge 400 R). Serum samples were stored at −80°C for enzyme-linked immunosorbent assay (ELISA) study.

Serum IL-1β (YL biont, YLA0030RA), IL-6 (YL biont YLA0031RA), and IL-10 (YL biont, YLA0440RA) levels were determined with the sandwich ELISA method according to the manufacturer's instructions as picogram per milliliter. Cytokine levels were calculated based on the absorbance of complex cytokines-antibodies (Multiskan Go microplate spectrophotometer, Thermo Scientific).

## Results

Serum IL-1β, IL-6, and IL-10 levels are shown in Table 1. In the trauma group, there were increased IL-1β and IL-6 pro-inflammatory cytokine levels compared to the control group ($p < 0.05$). Also, the trauma group had decreased anti-inflammatory IL-10 level compared to the control group. The trauma + CAPE group showed reduced pro-inflammatory IL-1β and IL-6 and increased anti-inflammatory IL-10 level ($p < 0.05$).

**Table 1.** Effect of CAPE on serum inflammatory cytokines IL-1β, IL-6, and IL-10 (pg/ml).

| | Control | Thoracic trauma | Thoracic trauma + CAPE | CAPE |
|---|---|---|---|---|
| IL-1β | 83.9 ± 10.7 | 150.0 ± 25.5[a] | 102.1 ± 11.6[b] | 81.8 ± 7.6 |
| IL-6 | 48.9 ± 6.0 | 94.1 ± 11.8[a] | 66.6 ± 16.2[b] | 51 ± 8.5 |
| IL-10 | 24.7 ± 5.1 | 10.9 ± 3.7[a] | 17 ± 3.4[b] | 23.1 ± 4.5 |

[a]$p < 0.05$ compared to control group.
[b]$p < 0.05$ compared to thoracic trauma group.

## Discussion

In the present study, a model of blunt chest trauma was induced and the effect of CAPE, an important component of propolis, on the release of serum cytokines IL-1β, IL-6, and IL-10 was investigated. Our study is the first in the literature to show that CAPE increases the level of anti-inflammatory IL-10 and inhibits the release of serum pro-inflammatory cytokines IL-1β and IL-6 in the thoracic trauma model.

Thoracic trauma, which is a source of high mortality and morbidity, may trigger a serum pro-inflammatory response and it leads to secondary damage of organs by activating the immune system and other inflammatory cells [8]. Cytokines are biological mediators that play an important role in the initiation and maintenance of the inflammatory response [28]. It was determined that the pro- and anti-inflammatory cytokines are in balance in the organism and that this balance is impaired in favor of pro-inflammatory cytokines in many diseases such as inflammatory bowel diseases [28,29], osteoarthritis [30], chronic obstructive pulmonary disease [31], rheumatoid arthritis [32], etc. Recent studies have shown that thoracic trauma is directly related to pro-inflammatory cytokine release [33,34].

Raghavendran et al. [35] showed increased IL-1β levels in bronchoalveolar lavage in a rat blunt chest trauma model. Similarly, Ates et al. [36] showed elevated serum IL-1β and TNF-α concentrations in blunt chest trauma model in rats. In addition to an increase in IL-1β, an increase in NO release and nuclear factor kappa-beta (NF-κβ) expression was also observed. In our study, the serum level of IL-1β

decreased significantly in the CAPE-treated group compared to the thoracic trauma group ($p < 0.05$). Ak et al. [37] reported that in a rat acute spinal injury model, CAPE treatment facilitates local tissue healing by lowering serum levels of TNF-α and IL-1β. In a methotrexate-induced hepatorenal injury model, CAPE administration was reported to reduce serum TNF-α and IL-1 levels as well as lower malondialdehyde, glutathione peroxidase, and myeloperoxidase levels compared to a model group.

IL-6 is a proinflammatory cytokine with paracrine and endocrine effects, which plays an important role in response to environmental factors such as infection and injury [38,39]. IL-6 is transported to the liver via the bloodstream, locally synthesized at the onset of inflammation and provides induction of acute phase proteins such as C-reactive protein (CRP), serum amyloid albumin, and haptoglobin [40]. Iraz et al. [41] showed increased serum IL-6 and CRP levels in lipopolysaccharide-induced lung injury model. In a rat burn model, increased TNF-α, interferon–gamma, and IL-6 levels were reported [42]. In our study, consistent with the literature, induction of thoracic trauma resulted in a significant increase in serum IL-6 levels compared to the control group ($p < 0.05$). CAPE treatment provided a significant reduction in serum IL-6 levels. In a murine *Helicobacter pylori*-induced gastric injury model, CAPE administration was reported to reduce levels of IL-6 and NF-κβ, similar to our study [43].

IL-10 is an immunosuppressive cytokine secreted mainly by macrophages and other immunological system cells [44]. It also has a therapeutic effect in many inflammatory diseases by inhibiting and suppressing immune system cell activation and functions [45]. Taniguchi et al. reported that the level of IL-6 and IL-10 increased significantly in a study of 22 patients with abdominal and thoracic trauma. Also, they reported that the ratio of IL-6/IL-10 decreased from days 0 to 4 after trauma [46]. In a peripheral nerve injury model, Mietto et al. [47] showed that IL-10 plays an important role in modulating inflammation and promoting regeneration in peripheral nerves in rats. It was reported that different agents applied in many experimental models inhibit tissue damage by increasing IL-10 synthesis [48,49]. In our study, IL-10 serum levels were determined on the seventh day following chest trauma and a significant increase was observed in the CAPE-treated group compared to the thoracic trauma group. Linard et al. [50] reported that CAPE administration suppressed the expression of TNF and IL-6 and found a significant increase in IL-10 levels in the intestines in a radiation-induced acute rat model. Similar to this result, the CAPE administration in our study also increased the level of IL-10 significantly.

TNF-α is a "master regulator" cytokine that mediates inflammatory response [16]. TNF-α stimulation is associated with the activation of NF-κβ, mitogen-activated protein kinases, and caspase cascade [51]. In this study, CAPE may have inhibited systemic inflammation by blocking TNF-α and NF-κβ activation.

In conclusion, in the current study, clearly, the CAPE administration inhibited pro-inflammatory IL-1β and provided an increase in pro-inflammatory IL-10 level in the thoracic trauma model in rats. However, there is a need for more extensive studies to explain the association of CAPE therapy with TNF-α, NF-κβ, and inflammatory cytokines. These results suggest that CAPE may have potential as an alternative agent in traumatic and inflammatory disorders.

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## References

[1] Giannoudis PV, Harwood PJ, Loughenbury P, Van Griensven M, Krettek C, Pape H-C. Correlation between IL-6 levels and the systemic inflammatory response score: can an IL-6 cutoff predict a SIRS state? J Trauma Acute Care Surg 2008; 65(3):646–52.

[2] Recknagel S, Bindl R, Kurz J, Wehner T, Ehrnthaller C, Knöferl MW, et al. Experimental blunt chest trauma impairs fracture healing in rats. J Orthop Res 2011; 29(5):734–9.

[3] Knöferl MW, Liener UC, Seitz DH, Perl M, Brückner UB, Kinzl L, et al. Cardiopulmonary, histological, and inflammatory alterations after lung contusion in a novel mouse model of blunt chest trauma. Shock 2003; 19(6):519–25.

[4] Seitz DH, Perl M, Liener UC, Tauchmann B, Braumüller ST, Brückner UB, et al. Inflammatory alterations in a novel combination model of blunt chest trauma and hemorrhagic shock. J Trauma Acute Care Surg 2011; 70(1):189–96.

[5] Hietbrink F, Koenderman L, Rijkers GT, Leenen LP. Trauma: the role of the innate immune system. World J Emerg Surg 2006; 1(1):15.

[6] Recknagel S, Bindl R, Brochhausen C, Göckelmann M, Wehner T, Schoengraf P, et al. Systemic inflammation induced by a thoracic trauma alters the

cellular composition of the early fracture callus. J Trauma Acute Care Surg 2013; 74(2):531–7.
[7] Neunaber C, Oestern S, Andruszkow H, Zeckey C, Mommsen P, Kutter D, et al. Cytokine productive capacity of alveolar macrophages and Kupffer cells after femoral fracture and blunt chest trauma in a murine trauma model. Immunol Lett 2013; 152(2):159–66.
[8] Wu X, Song X, Li N, Zhan L, Meng Q, Xia Z. Protective effects of dexmedetomidine on blunt chest trauma–induced pulmonary contusion in rats. J Trauma Acute Care Surg 2013; 74(2):524–30.
[9] Duran L, Guzel A, Akdemir H, Demir F, Karadeniz C, Alacam H, et al. Cardioprotective effect of aminoguanidine in combination with steroid therapy after blunt chest trauma. Bratisl Lek Listy 2014; 115(9):538–43.
[10] Kara H, Avcı A, Akinci M, Degirmenci S, Bayir A, Ak A. Blunt chest trauma as a cause of acute myocardial infarction. Acta Clin Belg 2014; 69(5):367–70.
[11] Wagner N, Dieteren S, Franz N, Köhler K, Mörs K, Nicin L, et al. Ethyl pyruvate ameliorates hepatic injury following blunt chest trauma and hemorrhagic shock by reducing local inflammation, NF-kappaB activation and HMGB1 release. PLoS One 2018; 13(2):e0192171.
[12] Huber-Lang M, Lambris JD, Ward PA. Innate immune responses to trauma. Nat Immunol 2018; 19:327–341.
[13] Moore FA, Moore EE. Evolving concepts in the pathogenesis of postinjury multiple organ failure. Surg Clin North Am 1995; 75(2):257–77.
[14] Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma. Injury 2007; 38(12):1336–45.
[15] Chu J, Zhang X, Jin L, Chen J, Du B, Pang Q. Protective effects of caffeic acid phenethyl ester against acute radiation-induced hepatic injury in rats. Environ Toxicol Pharmacol 2015; 39(2):683–9.
[16] Armutcu F, Akyol S, Ustunsoy S, Turan FF. Therapeutic potential of caffeic acid phenethyl ester and its anti-inflammatory and immunomodulatory effects (Review). Exp Ther Med 2015; 9(5):1582–1588.
[17] Firat U, Senol S, Gelincik I, Kapan M, Tokgoz O, Tekin R, et al. The effects of caffeic acid phenethyl ester (CAPE) on bacterial translocation and inflammatory response in an experimental intestinal obstruction model in rats. Eur Rev Med Pharmacol Sci 2015; 19(10):1907–14.
[18] Wang LC, Lin YL, Liang YC, Yang YH, Lee JH, Yu HH, et al. The effect of caffeic acid phenethyl ester on the functions of human monocyte-derived dendritic cells. BMC Immunol 2009; 10:39; doi:10.1186/1471-2172-10-39
[19] Iraz M, Ozerol E, Gulec M, Tasdemir S, Idiz N, Fadillioglu E, et al. Protective effect of caffeic acid phenethyl ester (CAPE) administration on cisplatin-induced oxidative damage to liver in rat. Cell Biochem Funct 2006; 24(4):357–61.
[20] Celik S, Erdogan S. Caffeic acid phenethyl ester (CAPE) protects brain against oxidative stress and inflammation induced by diabetes in rats. Mol Cell Biochem 2008; 312(1–2):39–46.
[21] Aydogan H, Gurlek A, Parlakpinar H, Askar I, Bay-Karabulut A, Aydogan N et al. Beneficial effects of caffeic acid phenethyl ester (CAPE) on the ischaemia-reperfusion injury in rat skin flaps. J Plast Reconstr Aesthet Surg 2007; 60(5):563–8.
[22] Koksel O, Ozdulger A, Tamer L, Cinel L, Ercil M, Degirmenci U, et al. Effects of caffeic acid phenethyl ester on lipopolysaccharide-induced lung injury in rats. Pulm Pharmacol Ther 2006; 19(2):90–5.
[23] Kart A, Cigremis Y, Karaman M, Ozen H. Caffeic acid phenethyl ester (CAPE) ameliorates cisplatin-induced hepatotoxicity in rabbit. Exp Toxicol Pathol 2010; 62(1):45–52.
[24] Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M. Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. J Ethnopharmacol 2005; 97(2):273–80.
[25] Alp H, Pinar N, Dokuyucu R, Sahan M, Oruc C, Kaplan I, et al. Protective effects of intralipid and caffeic acid phenethyl ester (CAPE) on hepatotoxicity and pancreatic injury caused by dichlorvos in rats. Biochem Genet 2016; 54(6):803–815.
[26] Akgun B, Ozturk S, Artas G, Erol FS. Effects of intrathecal caffeic acid phenethyl ester (CAPE) on IL-6 and TNF-α levels and local inflammatory responses in spinal cord injuries. Turk Neurosurg 2018; 28(4):625–629.
[27] Raghavendran K, Davidson BA, Helinski JD, Marschke CJ, Manderscheid P, Woytash JA, et al. A rat model for isolated bilateral lung contusion from blunt chest trauma. Anesth Analg 2005; 101(5):1482–9.
[28] Cavaillon J-M. Pro-versus anti-inflammatory cytokines: myth or reality. Cell Mol Biol (Noisy-le-grand) 2001; 47(4):695–702.
[29] Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014; 14(5):329.
[30] Vernal R, Velasquez E, Gamonal J, Garcia-Sanz JA, Silva A, Sanz M. Expression of proinflammatory cytokines in osteoarthritis of the temporomandibular joint. Arch Oral Biol 2008; 53(10):910–5.
[31] Tang Y, Guan Y, Liu Y, Sun J, Xu L, Jiang Y. The role of the serum IL-33/sST2 axis and inflammatory cytokines in chronic obstructive pulmonary disease. J Interferon Cytokine Res 2014; 34(3):162–8.
[32] Pope RM, Shahrara S. Possible roles of IL-12-family cytokines in rheumatoid arthritis. Nat Rev Rheumatol 2013; 9(4):252.
[33] Wu XJ, Liu HM, Song XM, Zhao B, Leng Y, Wang E, et al. Penehyclidine hydrochloride inhibits TLR4 signaling and inflammation, and attenuates blunt chest trauma and hemorrhagic shock-induced acute lung injury in rats. Mol Med Rep 2018; 17(5):6327–36.

[34] Kalbitz M, Weber B, Braun C, Lackner I, Haffner-Luntzer M, Huber-Lang M, et al. Cardiac damage after experimental blunt chest trauma and multiple trauma with hemorrhagic shock. Am Assoc Immnol 2018; 166:54.
[35] Raghavendran K, Davidson BA, Woytash JA, Helinski JD, Marschke CJ, Manderscheid PA, et al. The evolution of isolated bilateral lung contusion from blunt chest trauma in rats: cellular and cytokine responses. Shock 2005; 24(2):132.
[36] Ateş G, Yaman F, Bakar B, Kısa U, Atasoy P, Büyükkoçak Ü. Evaluation of the systemic antiinflammatory effects of levosimendan in an experimental blunt thoracic trauma model. Ulus Travma Acil Cerrahi Derg 2017; 23(5):368–76.
[37] Ak H, Gulsen I, Karaaslan T, Alaca İ, Candan A, Koçak H, et al. The effects of caffeic acid phenethyl ester on inflammatory cytokines after acute spinal cord injury. Ulus Travma Acil Cerrahi Derg 2015; 21:96–101.
[38] Mesquida M, Leszczynska A, Llorenç V, Adán A. Interleukin-6 blockade in ocular inflammatory diseases. Clin Exp Immunol 2014; 176(3):301–9.
[39] Rohleder N, Aringer M, Boentert M. Role of interleukin-6 in stress, sleep, and fatigue. Ann N Y Acad Sci 2012; 1261(1):88–96.
[40] Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 2014; 6(10):a016295.
[41] Iraz M, Iraz M, Esrefoglu M, Aydin MS. Protective effect of\beta-glucan on acute lung injury induced by lipopolysaccharide in rats. Turk J Med Sci 2015; 45(2):261–7.
[42] Abali AES, Karakayali H, Ozdemir BH, Bayraktar N, Abbas OL, Haberal M. Destructive pulmonary effects of smoke inhalation and simultaneous alterations in circulating IL-6, TNF-α, and IFN-γ levels at different burn depths: an experimental study on rats. J Burn Care Res 2013; 34(3):334–41.
[43] Toyoda T, Tsukamoto T, Takasu S, Shi L, Hirano N, Ban H, 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):1786–95.
[44] Trifunović J, Miller L, Debeljak Ž, Horvat V. Pathologic patterns of interleukin 10 expression—a review. Biochem Med (Zagreb) 2015; 25(1):36–48.
[45] Tian G, Li J-L, Wang D-G, Zhou D. Targeting IL-10 in auto-immune diseases. Cell Biochem Biophys 2014; 70(1):37–49.
[46] Taniguchi T, Koido Y, Aiboshi J, Yamashita T, Suzaki S, Kurokawa A. The ratio of interleukin-6 to interleukin-10 correlates with severity in patients with chest and abdominal trauma. Am J Emerg Med 1999; 17(6):548–51.
[47] Mietto BS, Kroner A, Girolami EI, Santos-Nogueira E, Zhang J, David S. Role of IL-10 in resolution of inflammation and functional recovery after peripheral nerve injury. J Neurosci 2015; 35(50):16431–42.
[48] Zhou X-L, Yang Q-S, Ni S-Z, Tu X-P, Zhao Y, Xu B, et al. Protective effects of lipoxin A4 in testis injury following testicular torsion and detorsion in rats. Mediators Inflamm 2014; 2014:898056.
[49] Cao Y-Z, Tu Y-Y, Chen X, Wang B-L, Zhong Y-X, Liu M-H. Protective effect of Ulinastatin against murine models of sepsis: inhibition of TNF-α and IL-6 and augmentation of IL-10 and IL-13. Exp Toxicol Pathol 2012; 64(6):543–7.
[50] Linard C, Marquette C, Mathieu J, Pennequin A, Clarençon D, Mathé D. Acute induction of inflammatory cytokine expression after γ-irradiation in the rat: effect of an NF-κB inhibitor. Int J Radiat Oncol Biol Phys 2004; 58(2):427–34.
[51] Sabio G, Davis RJ. TNF and MAP kinase signalling pathways. Semin Immunol 2014; 26(3):237–45; doi: 10.1016/j.smim.2014.02.009