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Research Article

The Coffee-Specific Diterpenes Cafestol and Kahweol Induce Peripheral Antinociception in Rats by Activating the L-Arginine/Nitric Oxide/Cyclic Gmp Pathway

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Abstract

The L-arginine/nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway has been implicated as a molecular mechanism of antinociception produced by several antinociceptive agents, including μ-, κ-, and δ-opioid receptor agonists, nonsteroidal analgesics, cannabinoids, cholinergic agents, and α2C adrenoceptor agonists. In this study, we investigated whether cafestol and kahweol, both diterpenes present in coffee bean oil, could also activate the L-arginine/NO/cGMP pathway to elicit peripheral antinociception. The nociceptive threshold was measured by the rat paw pressure test, and the hyperalgesia was induced by intraplantar (i.pl.) injection of prostaglandin E2 (2 μg/paw). All drugs were locally administered (i.pl.) into the right hindpaw of male Wistar rats. The results showed that cafestol and kahweol (40 and 80 μg/paw) elicited a local antinociceptive effect, which was antagonized by the nonselective NO synthase (NOS) inhibitor L-NOArg (36 and 48 μg/paw) and by the selective neuronal NOS inhibitor L-NPA (36 and 48 μg/paw). However, selective endothelial and inducible NOS inhibitors L-NIO and L-NIL (96 μg/paw), respectively, did not alter the cafestol- and kahweol-induced peripheral antinociception. In addition, the soluble guanylyl cyclase inhibitor ODQ (50 and 100 μg/paw) blocked the action of cafestol, and the cGMP-phosphodiesterase inhibitor zaprinast (50 μg/paw) potentiated the antinociceptive effects of intermediate-dose (40 μg/paw) of these diterpenes. Furthermore, we found an increase in nitrite levels in rat paw homogenate, indicating that exogenous cafestol and kahweol induced NO release. Thus, our results suggest that both cafestol and kahweol stimulate the L-arginine/NO/cGMP pathway via neuronal NOS to induce peripheral antinociception.

Keywords: *Cafestol; Kahweol; Nitric Oxide; Cyclic GMP; Peripheral Antinociception*

Introduction

It is being increasingly acknowledged that foods and beverages contain non-nutritional constituents that may possess biological activities with beneficial health effects, such as antinociceptive and anti-carcinogenic properties [1,2]. The full assessment of such food components requires a thorough investigation of both efficacy and safety.

Cafestol and kahweol are examples of such biologically active food components. Kahweol and its dehydro derivative, cafestol (Figure 1), are naturally occurring diterpenes found only in the unsaponifiable lipid fraction of coffee. The levels of cafestol and kahweol in a coffee drink are significantly influenced by the brewing method. These diterpenes are extracted from ground coffee during brewing but are mostly removed by paper filters. Turkish, Scandinavian, and French press (cafetière) style coffees contain high levels of cafestol and kahweol, while filtered, percolated, and instant style coffees contain low levels of these diterpenes [3].

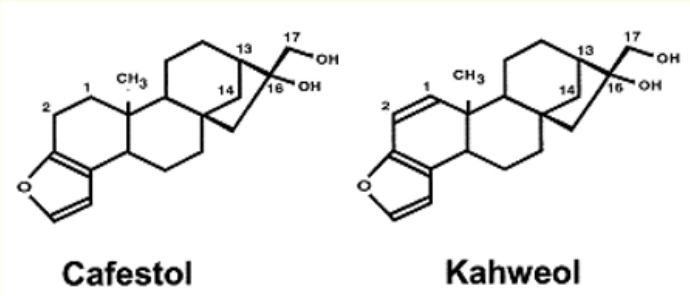

Figure 1: *Chemical structure of cafestol and kahweol.*

Cafestol and kahweol have been shown to exhibit both adverse and chemoprotective properties. It is well documented that a mixture of these diterpenes increases blood cholesterol in both human and animal models [4]. However, animal studies have shown that cafestol and kahweol also protect against well-known carcinogens [5]. The chemoprotective effects of cafestol and kahweol have thus far been linked to beneficial modifications in xenobiotic metabolism [6,7], antiangiogenic properties [8], and induction of apoptosis [9-11].

Prostaglandin E2 (PGE2) is the principal pro-inflammatory prostanoid, and it primarily contributes to one of the key features of inflammation: pain hypersensitivity [12]. Cafestol has inhibitory activity on PGE2 production *in vitro* [13-15], suggesting anti-inflammatory and analgesic activity for this diterpene. Studies have also shown that kahweol may suppress cell adhesion molecule expression, inhibit cyclooxygenase-2 and inducible nitric oxide synthase expression in macrophages via suppression of the pro-inflammatory transcription factor NF-kB *in vitro*, reducing PGE2 and NO levels [16,17]. In addition, Guzzo., *et al.* [18-20] demonstrated, for the first time, the antinociceptive effect of cafestol and kahweol and proposed that both diterpenes induce peripheral antinociception by releasing endogenous opioid peptides and noradrenaline.

It is well established that the L-arginine/nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway has an important role in the peripheral antinociception induced by μ- [21,22], κ- and δ-opioid receptor agonists [23,24]. Based on these observations, the present study investigated the involvement of the L-arginine/NO/cGMP pathway in mediating the peripheral antinociception caused by cafestol and kahweol.

Materials and Methods

Animals

All experiments were performed on 180 - 220g male Wistar rats (from CEBIO-UFMG). The rats were housed in a temperature-controlled room (23 ± 1°C) on an automatic 12h light/dark cycle (06:00 - 18:00h). All tests were conducted during the light phase (08:00 - 15:00h). Food and water were freely available until the onset of the experiments. All animal procedures and protocols were approved by the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais (UFMG), protocol 50/2013.

Measurement of hyperalgesia

Hyperalgesia was induced by subcutaneous injection of PGE2 (2 µg) into the plantar surface of the hind paw. Hyperalgesia was measured according to the paw pressure test [25,26]. An analgesimeter (Ugo-Basile, Italy) was used, fitted with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the hind paw. The weight in grams (g) required to elicit the nociceptive response of paw flexion was determined as the nociceptive threshold. A cut-off value of 300g was used to reduce the possibility of paw damage. The nociceptive threshold was measured in the right paw and defined as the average of the three consecutive trials recorded before and 3h after the PGE2 injection. The threshold was calculated as the difference between these two averages (Δ of nociceptive threshold) and is expressed in grams.

Drugs and substances

The following drugs were used in the present study: cafestol acetate (Caf; Sigma, St. Louis, MO), kahweol (Axxora, San Diego, CA, USA), *N*G-Nitro-L-arginine (L-NOArg; RBI, USA), *N*W-Propyl-L-arginine (L-NPA; Sigma), *N*5-(1-Iminoethyl)-L-ornithine dihydrochloride (L-NIO; Sigma), and *N*6-(1-Iminoethyl)-L-lysine hydrochloride (L-NIL; Sigma) were dissolved in isotonic saline. ODQ (1H-[1,2,4]Oxadiazolo[4,3a] quinoxalin-1-one; RBI) and Zaprinast (1,4-Dihydro-5-[2-propoxyphenyl]-7H-1,2,3-triazolo [4,5d] pyrimidine-7-one; Sigma) were dissolved in 10% dimethylsulfoxide (DMSO), and PGE2 (Sigma) was dissolved in 2% ethanol in saline. All drugs were administered subcutaneously using an injected volume of 50 ml/paw, with the exception of PGE2, where an injected volume of 100 ml/paw was used.

Experimental protocol

Both cafestol and kahweol were administered subcutaneously in the right hind paw 2:55h after local injection of PGE2. In the protocol used to determine whether cafestol and kahweol were acting outside the injection paw, PGE2 was injected into both hindpaws, while cafestol and kahweol were singly administered 2:55h later into the left paw, after which the nociceptive threshold was measured in the right paw. L-NOArg, L-NPA, L-NIO, and L-NIL were administered 30 minutes before cafestol or kahweol; ODQ and zaprinast were administered 10 and 60 minutes, respectively, before cafestol or kahweol.

The protocols for the dose and timing of administration of each drug used in this study were derived from the literature and pilot experiments. Overall, two experimenters were necessary to conduct these experiments, with measurements made by one unaware of the treatments.

Nitrite determination

Nitrite (NO_2^-) levels were measured using the Griess reaction [27]. Cafestol (80 µg/paw) and kahweol (80 µg/paw) were administered 2:55h after local administration of PGE2 (2 µg). After 5 minutes, the animals were killed by cervical dislocation, and the plantar surface of the rat paw was collected. This tissue from each animal was homogenized in 900 µl of homogenization buffer containing the following: 30 mM Tris-HCl, pH 6.8, 5 mM EDTA, 250 mM sucrose, 30 mM KCl, 2% â-mercaptoethanol, PMSF (100 ìg/ml), benzamidine (5 ìg/ml), aprotinin (2 ìg/ml), and leupeptin (2 ìg/ml). Samples were then centrifuged (12,000g, 4°C, 15 minutes). Briefly, 100 µl of the homogenate was applied to a microliter plate well, followed by 100 µl of Griess reagent [0.2% (w/v) naphthyleneethylenediamine and 2% (w/v) sulfanilamide in 5% (v/v) phosphoric acid]. After 10 minutes of color development at room temperature, the absorbance was measured with a microplate reader (Titertek Multiskan MCC/340, Flow Lab, McLean, VA) at 545 nm. Each sample was assayed in duplicate wells. The NO_2^- standard reference curves were made with sodium NO_2^- in distilled water at concentrations of 100, 50, 25,12.5, 6.25, 3.13, and 1.56 M. The assay's detection limit is ~1.5 mol/L in distilled water.

Statistical analysis

All results are presented as means ± SEM. The data obtained from the nitrite determination were evaluated using Student's t-test, and the other data were analyzed using one-way analysis of variance (ANOVA) and the post hoc Bonferroni test for multiple comparisons.

Probabilities of less than 5% ($P < 0.05$) were considered to be statistically significant. Statistical analyses were performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Peripheral antinociceptive effect of cafestol and kahweol

The administration of cafestol and kahweol (40 and 80 µg) into the right hind paw produced an antinociceptive response ($P < 0.05$) against PGE2-induced hyperalgesia (2 µg/paw) in a dose-dependent manner (Figure 2A and 2B). Cafestol and kahweol at the 20 µg or vehicles did not alter ($P > 0.05$) the PGE2-induced hyperalgesia. Cafestol and kahweol, even at the highest dose (80 µg/paw), did not alter ($P > 0.05$) the animals' baseline threshold in the absence of hyperalgesia (left paw). Although the 80 µg/paw dose of cafestol and kahweol reversed the hyperalgesia induced by PGE2, this dose alone did not alter the nociceptive threshold (Figure 2A and 2B). When administered into the left paw, cafestol and kahweol at a dose of 80 µg did not produce an antinociceptive effect in the right paw, indicating that the effect is limited to a peripheral site of action (Figure 2A and 2B, inset).

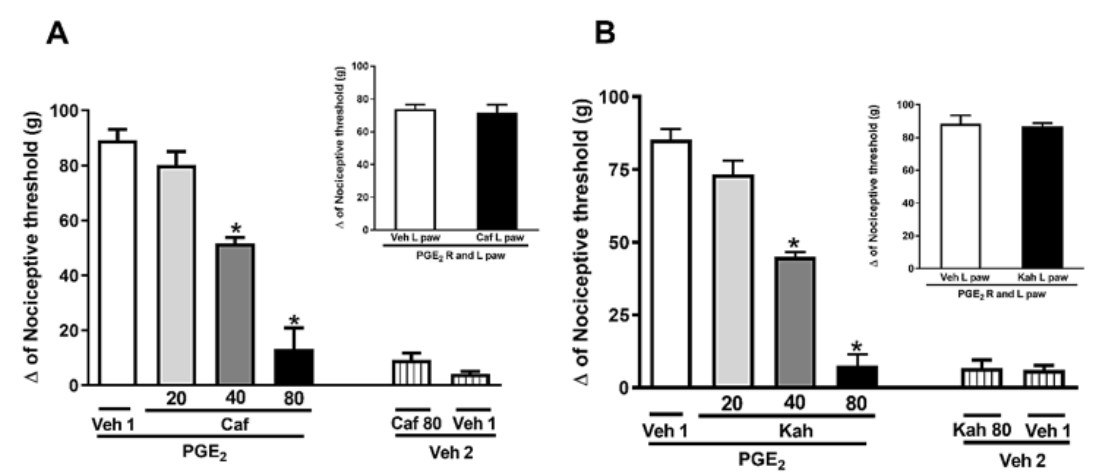

Figure 2: *Effect of cafestol and kahweol on PGE_2-induced hyperalgesia in rats. Cafestol (A, Caf; 20, 40, and 80 µg/paw) and kahweol (B, Kah; 20, 40, and 80 µg/paw) were administered 2:55h after local administration of PGE_2 (2 µg). Antinociceptive response was measured by the paw pressure test, as described in the Methods section. Each column represents the mean ± S.E.M. (n = 5). *indicates a significant difference from the (PGE_2 + Veh 1)-injected group ($P < 0.05$, ANOVA + the Bonferroni test). Veh 1 = DMSO 10% in saline, Veh 2 = Ethanol 2% in saline. Inset: Exclusion of outside paw antinociceptive effect of both cafestol and kahweol. PGE_2 (2 µg) was administered in both hindpaws, right (R) and left (L). Cafestol and kahweol (80 µg/paw) were administered 2:55 h after PGE_2 in the left hind paw (Caf or Kah L paw). Antinociceptive responses were measured in the right hindpaw, as described in section experimental protocol. Each column represents the mean ± S.E.M (n = 5).*

Involvement of the L-arginine/NO/cGMP pathway in cafestol- and kahweol-induced peripheral antinociception

The antinociceptive effect of both cafestol and kahweol (80 µg/paw) was antagonized ($P < 0.05$) by the nonselective NOS inhibitor L-NOArg (36 and 48 µg/paw) in a dose-dependent manner. L-NOArg did not induce hyperalgesia or antinociception when used alone (Figure 3A and 3B). Similarly, the selective neuronal NOS (nNOS) inhibitor L-NPA (36 and 48 µg/paw) blocked the action of cafestol and

kahweol. L-NPA did not induce hyperalgesia or antinociception when given alone (Figure 4A and 4B). The selective endothelial (eNOS) and inducible (iNOS) inhibitors L-NIO and L-NIL (96 µg/paw) were not able to block the antinociceptive effect induced by cafestol and kahweol (Figure 5A and 5B). The soluble guanylyl cyclase inhibitor ODQ (50 and 100 µg/paw) abolished the antinociceptive effect of cafestol and kahweol in a dose-dependent manner and had no effect when injected alone (Figure 6A and 6B). The intraplantar administration of the cGMP-phosphodiesterase inhibitor zaprinast (50 µg/paw) potentiated the peripheral antinociceptive effect of cafestol and kahweol at the 40 µg/paw dose. When administered alone, zaprinast did not alter the control groups (Figure 7A and 7B).

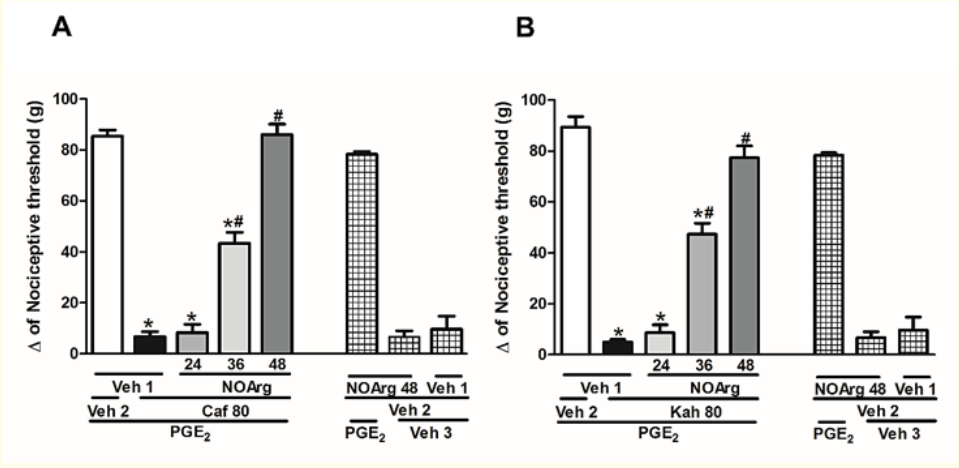

Figure 3: *Antagonism by L-NOArg of the peripheral antinociception induced by cafestol and kahweol in hyperalgesic paws (PGE_2, 2 µg). L-NOArg (NOArg; 24, 36, and 48 µg/paw) was administered 30 minutes before cafestol (A, Caf; 80 µg/paw) and kahweol (B, Kah; 80 µg/paw). Each column represents the mean ± S.E.M. (n=5). * and # indicate significant differences compared to (PGE_2 + Veh 1 + Veh 2) and (PGE_2 + Veh 1 + Caf 80 or Kah 80)-injected groups, respectively (P < 0.05, ANOVA + the Bonferroni test). Veh 1 = Saline, Veh 2 = DMSO 10% in saline, Veh 3 = Ethanol 2% in saline.*

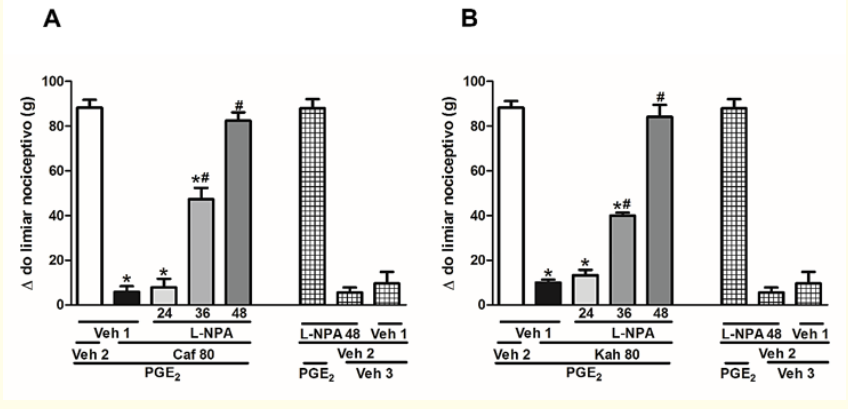

Figure 4: *Antagonism by L-NPA of the peripheral antinociception induced by cafestol and kahweol in hyperalgesic paws (PGE_2, 2 µg). L-NPA (24, 36 and 48 µg/paw) was administered 30 minutes before cafestol (A, Caf; 80 µg/paw) and kahweol (B, Kah; 80 µg/paw). Each column represents the mean ± S.E.M. (n = 5). * and # indicate significant differences compared to (PGE_2 + Veh 1 + Veh 2) and (PGE_2 + Veh 1 + Caf 80 or Kah 80)-injected groups, respectively (P < 0.05, ANOVA + the Bonferroni test). Veh 1 = Saline, Veh 2 = DMSO 10% in saline, Veh 3 = Ethanol 2% in saline.*

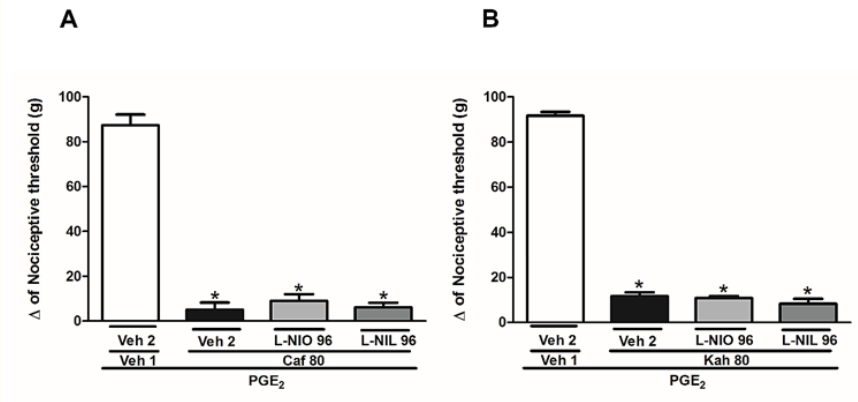

Figure 5: *Effect of intraplantar administration of L-NIO and L-NIL on peripheral antinociception induced by cafestol and kahweol in hyperalgesic paws (PGE_2, 2 µg). L-NIO and L-NIL (96 µg/paw) were administered 30 min before cafestol (A, Caf; 80 µg/paw) and kahweol (B, Kah; 80 µg /paw). Each column represents the mean ± S.E.M. (n = 5). *Indicates significant differences compared to (PGE_2 + Veh 1 + Veh 2)-injected group (P < 0.05, ANOVA + the Bonferroni test). Veh 1 = Saline, Veh 2 = DMSO 10% in saline, Veh 3 = Ethanol 2% in saline.*

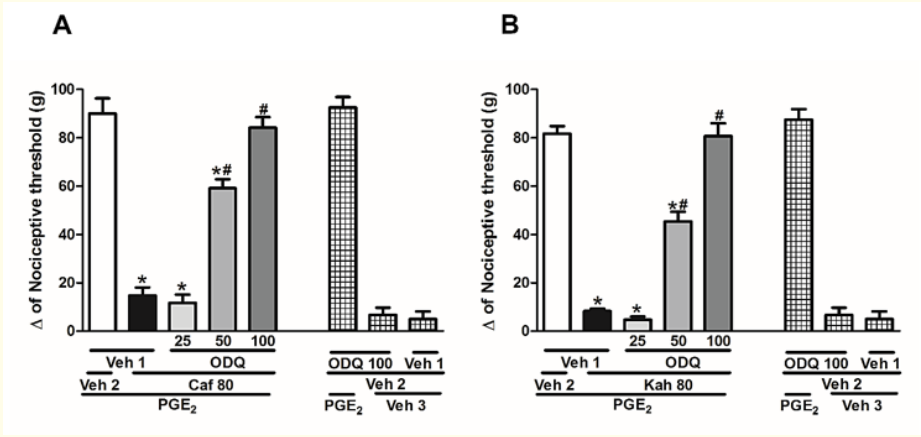

Figure 6: *Antagonism by ODQ of the peripheral antinociception induced by cafestol and kahweol in hyperalgesic paws PGE2 (2 µg). ODQ (µg/paw) was administered 10 minutes before cafestol (A, Caf; 80 µg/paw) and kahweol (B, Kah; 80 µg /paw). Each column represents the mean ± S.E.M. (n = 5). * and # indicate significant differences compared to (PGE_2 + Veh 1 + Veh 2) and (PGE_2 + Veh 1+ Caf 80 or Kah 80)-injected groups, respectively (P < 0.05, ANOVA + the Bonferroni test). Veh 1 = DMSO 10% in saline, Veh 2 = DMSO 10% in saline, Veh 3=Ethanol 2% in saline.*

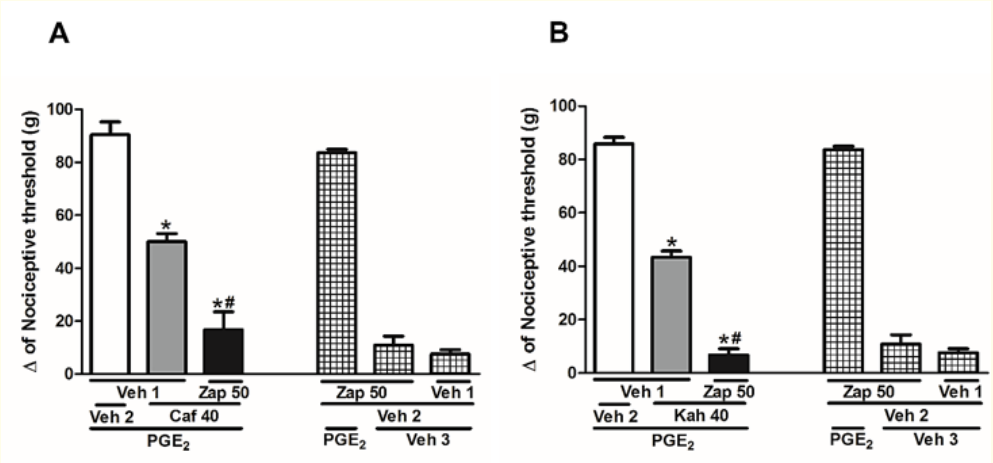

Figure 7: *Increase in the peripheral antinociceptive effect of cafestol and kahweol by intraplantar administration of the cGMP-phosphodiesterase inhibitor zaprinast (Zap, µg/paw). Zap was administered 60 minutes before cafestol (A, Caf; 80 µg/paw) and kahweol (B, Kah; 80 µg/paw). * and # indicate significant differences compared to (PGE2 + Veh 1 + Veh 2) and (PGE2 + Veh 1 + Caf 80 or Kah 80)-injected groups, respectively (P < 0.05, ANOVA + the Bonferroni test). Veh 1 = DMSO 10% in saline, Veh 2 = DMSO 10% in saline, Veh 3 = Ethanol 2% in saline.*

Effect of cafestol and kahweol on the NO_2^- levels in the homogenized plantar surface of the rat paw

Figure 8 shows that injection of cafestol and kahweol into the paw significantly increased NO_2^- levels compared with the control group.

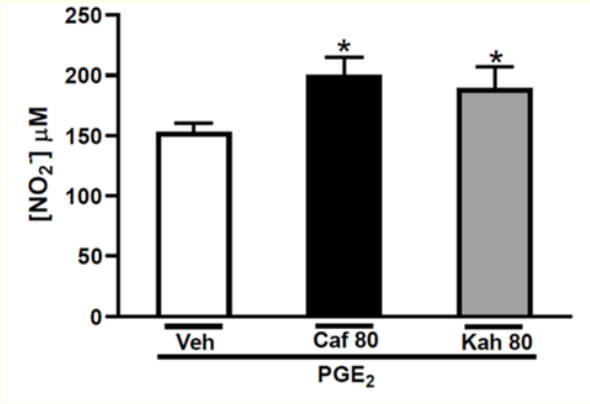

Figure 8: *Effect of cafestol and kahweol injection on nitrite concentration [NO_2^-] in the homogenized paw tissue. Cafestol (A, Caf; 80 µg/paw) and kahweol (B, Kah; 80 µg/paw were administered 2:55h after local administration of PGE_2 (2 µg). The tissue of the plantar surface of the rat hind paw was collected 3 hours after local administration of PGE2. Each column represents the mean ± S.E.M. (n = 5). *Indicates a significant difference in comparison with (PGE2 + Veh 1)-injected group (P < 0.05, Student's t test.). Veh = DMSO 10% in saline.*

Discussion and Conclusion

Nitric oxide is synthesized from the oxidation of the terminal guanidine nitrogen of L-arginine, which is then converted into L-citrulline. This reaction is catalyzed by a family of enzymes known as NOS, which exists in three isoforms. There are two constitutive forms of the enzyme: neuronal NOS (nNOS) and endothelial NOS (eNOS), and an inducible form (iNOS) [28]. Once generated, NO activates the guanylate cyclase enzyme, which directly increases intracellular cGMP levels [29]. The L-arginine/NO/cGMP pathway has been proposed as a peripheral antinociception mechanism of many drugs, such as the nonsteroidal analgesic drugs dipyrone, ketorolac and diclofenac [30-33]; the cholinergic agonist acetylcholine [34]; the angiotensin peptide Ang-(1-7) [35]; the anesthesic ketamine [36]; the α2 adrenoceptor agonist xylazine [37]; the cannabinoid agonist anandamide [38] and opioid agonists [21-24,39].

In the present study, the participation of the L-arginine/NO/cGMP pathway was evaluated in the peripheral antinociception induced by the injection of cafestol and kahweol into the rat paw PGE2-induced hyperalgesia test. Ferreira., *et al*. [40] showed that a single injection of PGE2 can sensitize nociceptors to chemical and mechanical stimuli. This method has an advantage over other hyperalgesic tests because it eliminates the possibility that the peripheral analgesic effect results from blocking the release or the action of mediators produced during the inflammatory process.

As previously demonstrated, both cafestol and kahweol exhibit potent peripheral antinociceptive effects [18-20]. To exclude the possibility that cafestol (80 µg/paw) and kahweol (80 µg/paw) produced antinociception by acting at sites outside the paw, we used the strategy of evaluating the efficacy of ipsilateral versus contralateral paw administration. PGE2 was administered to both hindpaws, thereby creating identical tissue conditions and an equal likelihood that the agents would reach sites beyond the injected paw. In this experiment, cafestol and kahweol proved ineffective at producing antinociception in the contralateral paw. This indicates that, at this dose (80 µg/paw), cafestol and kahweol only acted locally. For this reason, the dose of 80 µg/paw was used in subsequent experiments to evaluate the peripheral antinociceptive mechanism of cafestol and kahweol.

To investigate whether the antinociceptive effect of cafestol and kahweol is dependent on the L-arginine/NO/cGMP pathway, experiments based on NO biosynthesis were performed.

In the present results, pretreatment with the nonspecific NOS inhibitor L-NOArg and the soluble guanylyl cyclase inhibitor ODQ antagonized the peripheral antinociceptive effect induced by cafestol and kahweol in a dose-dependent manner, suggesting that this effect was due to the induced formation of the second messenger NO and the consequent activation of soluble guanylyl cyclase.

After confirming that NOS participates in the antinociception induced by both cafestol and kahweol, the possible involvement of specific NOS isoforms in this effect was then investigated. The results suggest that cafestol and kahweol specifically activate nNOS to produce NO. We excluded the possibility that eNOS and iNOS are involved in this process. Previous studies have shown that nNOS is the isoform involved in the peripheral antinociception induced by several analgesics, including opioid agonists [41], xylazine, acetylcholine, anandamide, and the nonsteroidal analgesics dipyrone and diclofenac [42]. The neuronal isoform of NOS is required for the central antinociceptive effect induced by nitrous oxide (N_2O) in mice [43,44] and in the peripheral antinociceptive effects of the *Crotalus durissus terrificus* venom in a rat paw hyperalgesic model [45].

According to Koesling and Friebe [46], activation of guanylate cyclase converts guanosine 5'-triphosphate (GTP) to cGMP, thereby increasing intracellular cGMP levels and eliciting antinociception [30,47]. The cGMP action is limited by degradation via a specific phosphodiesterase [48]. Thus, the inhibition of cGMP hydrolysis could increase the action of substances that signal via cGMP [47]. In the present study, the cGMP phosphodiesterase inhibitor zaprinast was shown to enhance the antinociceptive effect of an intermediate dose of cafestol and kahweol, thereby validating our hypothesis.

Previous studies have shown that after NOS-induced NO release, NO is rapidly oxidized to two stable breakdown products, NO_2^- and NO_3^- [49]. NO_2^- levels are a good indirect measure of the NO levels [50]. In the present study, we verified that injection of cafestol and kahweol into the rat paw increases NO_2^- levels, thereby strengthening our hypothesis.

Considering that cafestol and kahweol induce peripheral antinociception by releasing endogenous opioid peptides [20,21], and that opioid agonists can induce peripheral antinociception by activating the L-arginine/NO/cGMP pathway, we believe that both cafestol and kahweol lead to the release of endogenous opioids, subsequently causing the local generation of NO and cGMP.

The present study concludes that the L-arginine/NO/cGMP pathway and nNOS activation are involved in the peripheral antinociceptive effects induced by cafestol and kahweol.

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Conflicts of Interest

We declare no conflict of interest.

Bibliography

1. C Reilly. "Functional foods - a challenge for consumers". *Trends in Food Science and Technology* 5.4 (1994): 121-123.
2. F Bellisle., *et al*. "Functional food science and behaviour and psychological functions". *British Journal of Nutrition* 80.S1 (1998): S173-S193.
3. G Gross., *et al*. "Analysis of the content of the diterpenes cafestol and kahweol in coffee brews". *Food and Chemical Toxicology* 35.6 (1997): 547-554.
4. R Urgert and MB Katan. "The cholesterol-raising factor from coffee beans". *Annual Review of Nutrition* 17 (1997): 305-324.
5. C Cavin., *et al*. "Cafestol and kahweol, two coffee-specific diterpenes with anticarcinogenic activity". *Food and Chemical Toxicology* 40.8 (2002): 1155-1163.
6. WW Huber., *et al*. "Effects of coffee and its chemopreventive components kahweol and cafestol on cytochrome P450 and sulfotransferase in rat liver". *Food and Chemical Toxicology* 46.4 (2008): 1230-1238.
7. WW Huber., *et al*. "Enhancement of the chemoprotective enzymes glucuronosyl transferase and glutathione transferase in specific organs of the rat by the coffee components kahweol and cafestol". *Archives of Toxicology* 76.4 (2002): 209-217.
8. SH Ferreira and M Nakamura. "II - Prostaglandin hyperalgesia, the peripheral analgesic activity of morphine, enkephalin and opioid antagonists". *Prostaglandins* 18.2 (1979): 191-200.
9. JY Kim., *et al*. "Suppressive effects of the kahweol and cafestol on cyclooxygenase-2 expression in macrophages". *FEBS Letters* 569.1-3 (2004): 321-326.
10. HG Kim., *et al*. "The coffee diterpene kahweol inhibits tumor necrosis factor-alpha-induced expression of cell adhesion molecules in human endothelial cells". *Toxicology and Applied Pharmacology* 217.3 (2006): 332-341.
11. JY Kim., *et al*. "Suppressive effects of the kahweol and cafestol on cyclooxygenase-2 expression in macrophages". *FEBS Letters* 569.1-3 (2004): 321-326.

12. JY Kim., *et al*. "The coffee diterpene kahweol suppress the inducible nitric oxide synthase expression in macrophages". *Cancer Letters* 213.2 (2004): 147-154.

13. LS Guzzo., *et al*. "Cafestol, a coffee-specific diterpene, induces peripheral antinociception mediated by endogenous opioid peptides". *Clinical and Experimental Pharmacology and Physiology* 39.5 (2012): 412-416.

14. LS Guzzo., *et al*. "Involvement of endogenous opioid peptides in the peripheral antinociceptive effect induced by the coffee specific diterpene kahweol". *Pharmacological Reports* 67.5 (2015): 1010-1015.

15. SH Ferreira., *et al*. "The molecular mechanism of action of peripheral morphine analgesia: stimulation of the cGMP system via nitric oxide release". *European Journal of Pharmacology* 201.1 (1991): 121-122.

16. FA Maegawa and CR Tonussi. "The L-arginine/nitric oxide/cyclic-GMP pathway apparently mediates the peripheral antihyperalgesic action of fentanyl in rats". *Brazilian Journal of Medical and Biological Research* 36.12 (2003): 1701-1707.

17. Amarante LH and Duarte IDG. "The κ-opioid (±)-bremazocine elicits peripheral antinociception by activation of the L-arginine/nitric oxide/cyclic GMP pathway". *European Journal of Pharmacology* 454.1 (2002): 19-23.

18. DF Pacheco., *et al*. "δ-Opioid receptor agonist SNC80 elicits peripheral antinociception via δ1 and δ2 receptors and activation of the L-arginine/nitric oxide/cyclic GMP pathway". *Life Sciences* 78.1 (2005): 54-60.

19. Green AF and Young PA. "A comparison of heat and pressure analgesiometric methods in rats". *British Journal of Pharmacology* 6.4 (1951): 572-585.

20. Randall LO and Sellito JJ. "A method for measurement of analgesic activity on inflamed tissues". *Archives Internationales de Pharmacodynamie et de Therapi* 111.4 (1957): 409-419.

21. LC Green., *et al*. "Analysis of nitrate, NO_2^- and ^{15}N.nitrate in biological fluids". *Analytical Biochemistry* 126.1 (1982): 131-138.

22. A Mulsh. "NO synthases: mechanism of activation, identity of NOx and expression in human cells". *Research in Immunology* 142.7 (1991): 561-565.

23. S Moncada., *et al*. "Nitric oxide: physiology, pathophysiology and pharmacology". *Pharmacological Reviews* 43.2 (1991): 109-142.

24. IDG Duarte., *et al*. "Analgesia by direct antagonism of nociceptor sensitization involves the L-arginine-nitric oxide-cGMP pathway". *European Journal of Pharmacology* 217.2-3 (1992): 225-227.

25. GG Lázaro-Ibáñez., *et al*. "Participation of the nitric oxide-cyclic GMP-ATP-sensitive K^+ channel pathway in the antinociceptive action of ketorolac". *European Journal of Pharmacology* 426.1-2 (2001): 39-44.

26. CR Tonussi and SH Ferreira. "Mechanism of diclofenac analgesia: direct blockade of inflammatory sensitization". *European Journal of Pharmacology* 251.2-3 (1994): 173-179.

27. MI Ortiz., *et al*. "The NO-cGMP-K^+ channel pathway participates in the antinociceptive effect of diclofenac, but not of indomethacin". *Pharmacology Biochemistry and Behavior* 76.1 (2003): 187-195.

28. IDG Duarte., *et al*. "Acetylcholine induces peripheral analgesia by the release of nitric oxide". *European Journal of Pharmacology* 186.2-3 (1990): 289-293.

29. TRL Romero and IDG Duarte. "α2-Adrenoceptor agonist Xylazine induces peripheral antinociceptive effect by activation of the L-arginine/nitric oxide/cyclic GMP pathway in rat". *European Journal of Pharmacology* 613.1-3 (2009): 64-67.

30. GML Reis. "Opioid receptor and NO/cGMP pathway as a mechanism of peripheral antinociceptive action of the cannabinoid receptor agonist anandamide". *Life Sciences* 85.9-10 (2009): 351-356.

31. MI Ortiz., *et al*. "Probable activation of the opioid receptor-nitric oxide-cyclic GMP-K^+ channels pathway by codeine". *Pharmacology Biochemistry and Behavior* 82.4 (2005): 695-703.

32. SH Ferreira. "Prostaglandins, aspirin like drugs and analgesia". *Nature* 240.102 (1972): 200-203.

33. TM Cunha., *et al*. "Morphine peripheral analgesia depends on activation of the PI3Kγ/AKT/nNOS/NO/KATP signaling pathway". *Proceedings of the National Academy of Sciences of the United States of America* 107.9 (2010): 4442-4447.

34. TRL Romero., *et al*. "The neuronal NO synthase participation in the peripheral antinociception mechanism induced by several analgesic drugs". *Nitric Oxide* 25.4 (2011): 431-435.

35. S Li., *et al*. "Antagonism of nitrous oxide antinociception in mice by antisense oligodeoxynucleotide directed against neuronal nitric oxide synthase enzyme". *Behavioural Brain Research* 152.2 (2004): 361-363.

36. JL Cope., *et al*. "Antagonism of the antinociceptive effect of nitrous oxide by inhibition of enzyme activity or expression of neuronal nitric oxide synthase in the mouse brain and spinal cord". *European Journal of Pharmacology* 626.2-3 (2010): 234-238.

37. G Picolo and Y Cury. "Peripheral neuronal nitric oxide synthase activity mediates the antinociceptive effect of *Crotalus durissus terrificus* snake venom, a delta and kappa-opioid receptor agonist". *Life Sciences* 75.5 (2004): 559-573.

38. D Koesling and A Friebe. "Soluble guanylyl cyclase: structure and regulation". *Reviews of Physiology, Biochemistry and Pharmacology* 135 (1999): 41-65.

39. IDG Duarte and SH Ferreira. "The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine-nitric oxide-cGMP pathway". *European Journal of Pharmacology* 221.1 (1992): 171-174.

40. SD Rybalkin., *et al*. "Regulation of cGMP specific phosphodiesterase (PDE5): phosphorylation in smooth muscle cells". *Journal of Biological Chemistry* 277.5 (2002): 3310-3317.

41. M Kelm. "Nitric oxide metabolism and breakdown". *Biochimica et Biophysica Acta* 1411.2-3 (1999): 273-289.

42. NS Bryan and MB Grisham. "Methods to detect nitric oxide and its metabolites in biological samples". *Free Radical Biology and Medicine* 43.5 (2007): 645-657.

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