Effect of coffee constituents, caffeine and caffeic acid on anxiety and lipopolysaccharide-induced sickness behavior in mice

Jayesh Mudgala,1, Sanchari Basu Mallik,a,b,1, Madhavan Nampoothiria, Manas Kinraa, Susan Hallb, Gary D. Grantb, Shailendra Anoopkumar-Dukieb, Andrew K. Davyb, C. Mallikarjuna Raoa, Devinder Arora,a,b,⁎,1

a Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal 576104, India
b School of Pharmacy and Pharmacology, MHIQ, QUM Network, Griffith University, Gold Coast, Australia

ARTICLE INFO

Keywords:
Caffeic acid
Caffeine
Sickness behavior
Neuroinflammation
Cytokines
Antioxidant

ABSTRACT

Caffeine (CAF) and polyphenolic compounds like caffeic acid (CA), chlorogenic acid and ferulic acid are commonly consumed through beverages and food. These constituents modulate immune system and impact the systemic inflammatory pathways. This study explored the modulatory effect of CA and CA+CAF on behavior and lipopolysaccharide (LPS) – induced neuroinflammation in mice. An array of behavioral tests suggest that CA (15mg/kg) and CA+CAF (10mg/kg + 5mg/kg) express anxiolytic-like properties. Systemic administration of LPS (1.5mg/kg) induced profound immobility, increased the systemic/brain cytokine (TNF-α and IL-6) levels and altered the host antioxidant defence in all the animals. Seven days pretreatment with CA alone and CA+CAF significantly reversed the LPS-induced behavioral changes and inflammatory markers. Our results suggest that the low doses of CA may offer a comparable anxiolytic and antidepressant effect when combined with CAF.

1. Introduction

Coffee is one of the most commonly consumed beverages over the world and contains many bioactive constituents such as caffeine (CAF), caffeic acid (CA), chlorogenic acid (CGA), ferulic acid (FA), pyrogallic acid and trigonelline (Hall et al., 2015; Hall, Yuen, & Grant, 2018; Karpinska, Świsłocka, & Lewandowski, 2017). CAF as the key constituent is possibly the most commonly consumed psychoactive constituent of coffee that has been correlated to many beneficial (mild to moderate use) as well as detrimental (overuse) effects of coffee consumption including the neurotoxicity and cardiotoxicity (Cano-Marquina, Tarin, & Cano, 2013). The pharmacologic effects of CAF on the nervous system are mediated through its effect on adenosine receptors as a non selective antagonist and stimulation of the cholineric system, that results in increased arousal and concentration as well as in decreased fatigue (Kim, Kwak, & Myung, 2015). Habitual CAF consumption through CAF containing beverages has been linked to decreased amyloid beta levels in brain (Arvandash et al., 2009), neuroprotective effects in animal models of Parkinson’s Disease, decreased oxidative stress and improved mitochondrial function (Xu, Liu, Pekar, & Lu, 2015). The phenolic constituents of coffee such as CA, CGA and FA have also been reported to possess antiinflammatory, antioxidant, antiapoptotic and neuroprotective properties (Szwajgier, Borowiec, & Pustelniak, 2017). These bioactive constituents of coffee have been reported to act individually as well as in combination to afford neuroprotection (Cao et al., 2011; Shukitt-Hale, Miller, Chu, Lyle, & Joseph, 2013). For instance, CAF has been reported to be synergistic with eicosanoyl-5-hydroxytryptamide (EHT), a fatty acid derivative of serotonin that is present in coffee, to prevent α-synuclein mediated toxicity in brain (Yan et al., 2018). At the same time, CAF in combination with CA has been reported to inhibit acetylcholinesterase (AChE) and monoamine oxidase (MAO) enzymes and stimulate Na⁺ - K⁺ -ATPase activity in the brain, demonstrating improved activity as compared to CA alone (Akomolafe et al., 2017).

CA (3,4-dihydroxycinnamic acid, CA) is a highly bioactive polyphenol. It is a major natural phenol in dietary sources including, but not limited to, sage, thyme, Ceylon cinnamon, sunflower seeds, red grapes, barley and, rye, present as a simple ester with quinic acid or saccharides (Charrouf & Guillaume, 2007; Kinra et al., 2019; Mehrrota, Shanbhag, Chamallamudi, Singh, & Mudgal, 2011). Owing to its o-dihydroxyl

⁎ Corresponding author at: School of Pharmacy and Pharmacology, Gold Coast campus, Griffith University, Queensland 4222, Australia.
E-mail address: d.arora@griffith.edu.au (D. Arora).
1 JM, SBM, DA contributed equally to this work.

https://doi.org/10.1016/j.jff.2019.103638
Received 17 May 2019; Received in revised form 9 October 2019; Accepted 16 October 2019
1756-4646/ © 2019 Elsevier Ltd. All rights reserved.
functionality in the catechol ring system and its ability to transfer H-atom to the peroxyl radicals easily makes CA a very potent antioxidant molecule (Sopheap & Betty, 2002). Apart from that, CA has also proved its worth as a potent 5-lipoxygenase (LOX) inhibitor and has demonstrated its ability to downregulate NF-κB, IL-6 and, IL-1β in the inflammatory reaction (Kinra et al., 2019; Liang, Shi, Luo, & Yang, 2015).

The psychostimulant and immunomodulatory effects of caffeine have been extensively studied, however, there are very limited investigational studies exploring such roles of other coffee constituents like CA. Furthermore, in the presence of CA and different stressors, these constituents express differential biological activities (Akomolafe et al., 2017; Basu Mallik et al., 2016). In our earlier study we have shown that acute administration of CA was effective against lipopolysaccharide (LPS)-induced sickness behavior and neuroinflammation in mice (Basu Mallik et al., 2016). LPS within 60–90 min of administration produces a significant change in the behavior of animals that is characterised by significant decrease in locomotion, fatigue and cognitive impairment due to peripheral rise in cytokines which ultimately results in neuroinflammation by the augmentation of central inflammatory milieu (Bluthé, Michaud, Poli, & Dantzer, 2000; Dantzer, O’Connor, Freund, Johnson, & Kelly, 2008; Hart, 1988). With this background, the current study was designed to evaluate the modulatory role of CA alone and in combination with CAF on anxiety-like (anxiolytic or anxiogenic) behavior; and how these dietary constituents modulate the general behavior including the normal locomotion and depressive symptoms when challenged by stressors like LPS. Furthermore, in order to correlate these behavioral effects with the systemic biochemical changes, we measured neuronal and systemic proinflammatory cytokines and oxidative stress markers.

2. Material and methods

2.1. Animals

Male Swiss albino mice (20–30 g), obtained from the inbred strains of central animal research facility (CARF), Manipal Academy of Higher Education (MAHE) were employed in this study. All the experimental procedures were approved and performed in accordance with the guidelines set out by the Institutional Animal Ethics Committee (IAEC) of MAHE (Protocol No. IAEC/KMC/81/2016), in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). The animals were housed in groups of 6, under controlled laboratory conditions, maintained on 12 h day and night cycle with free access to food and tap water.

2.2. Chemicals and reagents

All the chemicals used in this study were of analytical grade. Caffeine, caffeic acid, lipopolysaccharides (Escherichia coli 0111:B4), 2-thiobarbituric acid, L-glutathione reduced, 5,5′-dithio-bis (2-nitrobenzoic acid) were purchased from Sigma-Aldrich Co. LLC (St Louis, MO, USA). Carboxymethylcellulose, sodium dihydrogen phosphate anhydrous, disodium hydrogen phosphate anhydrous and tri-chloroacetic acid were purchased from Merck Millipore Corporation (Merck KGaA, Darmstadt, Germany).

2.3. Drug treatments

Animals were randomly assigned to five groups (n = 6), including, group I: saline (SAL); group II: LPS; group III: caffeic acid + LPS (CA + LPS); group IV: caffeic acid + caffeine + LPS (CA + CAF + LPS) and group V: imipramine + LPS (IMI + LPS). SAL and LPS groups were administered with vehicle, carboxymethylcellulose (CMC, 0.25% w/v) at a dose of 10 mL/kg. CA + LPS; CA + CAF + LPS and IMI + LPS groups, were treated with CA (15 mg/kg); CA + CAF (10 mg/kg + 5 mg/kg); and IMI (15 mg/kg) respectively. IMI, a standard tricyclic antidepressant drug served as a positive treatment control that acts by inhibiting the monoamine uptake mechanisms. All the treatments were administered by oral route (p.o.) and were given once daily for 7 days, whereas LPS was given by intraperitoneal (i.p.) injection on day 7. Anxiolytic effect of acute dosing of CA, CA + CAF and IMI was observed sixty minutes after dosing on day 1. Sixty minutes after the last dose of the treatments on day 7, sickness behavior was induced in all the animals except SAL group using LPS (1.5 mg/kg, i.p.). The treatment schedule and doses were selected based on our preliminary and published studies, along with an extensive literature search (Basu Mallik et al., 2016; Mudgal et al., 2019; Sahu et al., 2019). Behavioral assays were performed within 1–2 h of LPS administration and were video recorded. The recorded data was analysed by well-trained observers, including the blinded observers to reduce bias. The animals used for biochemical estimations were sacrificed via cervical dislocation 3 h post-LPS injection. Blood was collected via cardiac puncture and was allowed to clot undisrupted at room temperature for 60 min. Serum was isolated by centrifugation at 6000 rpm at 4°C. Serum and brain samples were rapidly collected and stored at −20 °C until further estimations. Brains were homogenised in ice cold phosphate buffer (0.1 M, pH 7.4) for estimation of anti-oxidant and cytokine levels.

2.4. Open field test

Open field test (OFT) was used to assess the effect of LPS and other treatment groups on exploratory behavior of the animals. Locomotor activity (LMA) was assessed in mice individually placed into a clean, novel glass arena (30 × 30 × 60 cm), that was divided into nine virtual quadrants (10 × 10 cm each). LMA was measured by counting the number of crossings, number of rearings, center square entries, time in center and number of groomings over a 5 min period. The apparatus was cleaned with 50% ethanol between the experiments (Biesmans et al., 2013).

2.5. Forced swim test

The forced swim test (FST) was employed to study the effect of treatment groups on LPS-induced behavioral despair. The mice were forced to swim individually in a transparent plexiglass cylindrical tank (30 × 20 cm) containing water maintained at 22 ± 2°C. The water level was adjusted to a height of 15 cm. After an initial period of vigorous activity the animals assume a type of immobile posture. A mouse is said to be immobile when it ceases struggling and makes minimal movement of limbs to keep the head above water. The total duration of test was 6 min with the immobility time recorded over the last 5 min of the study (Porsolt, Bertin, & Jalfre, 1978).

2.6. Tail suspension test

The tail suspension test (TST) was performed according to previously described methods (O’connor et al., 2009). Briefly, a small piece of medical adhesive tape was placed approximately 1–1.5 cm from the tip of the tail and the mice were hung individually for a period of 5 min at 15 cm away from the nearest surface. The tail climbing was prevented by placing plastic tubing around the tail prior to applying the tape. The duration of immobility was then measured during the final 5 min of the total 6 min of test.

2.7. Dark-light box test

The dark-light box apparatus (40 × 20 × 15 cm) consisted of two equal compartments, one dark and one lit, illuminated with 40 W fluorescent tube light. The two compartments communicate by means of a small door (7 × 7 cm). At the beginning of the test, each animal was placed in the lit box with its head facing the door of the dark box.
The amount of time spent in the light box, and the number of transitions between dark and lit box were recorded after the first entry in the dark box.

2.8. Estimation of serum and brain cytokine levels

Serum and brain tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were determined using murine TNF-α and IL-6 enzyme-linked immunosorbent assay (ELISA) kits (Novex®, Life Technologies, USA) respectively. The assays were performed as per manufacturer’s instructions. The results were interpolated from the standard curve derived from TNF-α and IL-6 standards provided by the manufacturer. Intra- and inter-assay percentage coefficient of variation (%CV) was 5.4 and 6.5 respectively for IL-6 ELISA kit, %CV was 6.5 (Intra-assay) and 5.7 (Inter-assay).

2.9. Reduced glutathione and malondialdehyde assay

Reduced glutathione (GSH) and malondialdehyde (MDA) in the brain homogenates were quantified as described earlier (Janero, 1990; Khan et al., 2013). GSH levels were estimated by measuring the absorbance of complex formed between GSH and dithionitrobenzene (DTNB; Ellman’s reagent) at 412 nm using a standard plot of GSH and expressed as µmoles/mg of protein. MDA estimation was done by measuring the molar extinction coefficient of chromophore (1.56 × 10⁻⁵ M⁻¹ cm⁻¹). The total protein estimation was carried out using Pierce™ BCA Protein Assay Kit, as per manufacturer’s instructions.

2.10. Statistical analysis

All the data-sets were statistically analysed using GraphPad Prism 7.02 (Graph Pad Software Inc., San Diego, CA, USA). Results are expressed as means ± S.E.M. Comparison between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. A p value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Effect of CA, CA + CAF and IMI on anxiety-like behavior in mice

Acute administration of CA in the animals lead to a significantly increased LMA as observed by the number of line crossings and rearrings (62.4 ± 8.9 vs 33.0 ± 5.6, F[3, 22] = 10.8, p < 0.05 and 19.8 ± 4.7 vs 7.5 ± 1.8 of saline treated group, F[3, 22] = 5.9, p < 0.01; Fig. 1A and 1B). CA in combination with CAF (88.2 ± 8.5; 18.5 ± 2.0) and IMI (100.0 ± 18.8; 19.8 ± 5.9) produced a significant increase in horizontal locomotion (crossings) and vertical locomotion (rearrings) as compared to the saline treated group.

The center square entries were significantly increased by CA (6.4 ± 1.1), CA + CAF (7.6 ± 1.9), and IMI (11.6 ± 2.3) as compared to saline treated group (2.9 ± 0.6; F[3, 22] = 8.4, p < 0.05). However, time spent in the central arena was only improved by CA + CAF (17.5 ± 4.1), and IMI (24.7 ± 4.3; F[3, 23] = 1074, p < 0.05, Fig. 1C and D) without any significant effect of CA alone. Number of groomings were increased by CA (14.4 ± 1.9), and CA + CAF (18.4 ± 4.6; F[3, 22] = 5.9, p < 0.05, Fig. 1E).

In the dark-light box, both CA (13.0 ± 2.7), and CA + CAF (14.6 ± 3.3; F[3, 14] = 1.4, p < 0.05) significantly increased the number of transitions and the time spent in the light chamber (90.2 ± 10.5 s and 73.5 ± 11.1 s respectively, F[3, 14] = 4.1, p < 0.05), as compared to saline treated group (8.0 ± 1.5; 32.5 ± 11.4 respectively, Fig. 1F and 1G). IMI was only effective in increasing the time spent in light chamber (72.2 ± 13.2).

3.2. Effect of CA, CA + CAF and IMI on LPS-induced sickness behavior in mice

An acute administration of LPS in the animals significantly reduced the locomotor activity as assessed by the number of line crossings (5.9 ± 3.2 vs 54.2 ± 7.2 of saline treated group; Fig. 2A). Pretreatment of the animals with CA, and CA + CAF significantly improved the LMA (44.0 ± 10.4, and 26.0 ± 4.1 respectively, F[4, 35] = 10.8, p < 0.05). However, at the currently employed dose IMI did not show a significant improvement in this exploratory behavior (Fig. 2A).

In FST, LPS treatment produced a significantly increased immobility time (259.2 ± 11.4 s) as compared to saline treated group (108.0 ± 33.7 s) (Fig. 2B). Interestingly, all the treatments including; CA, CA + CAF, and IMI prevented this LPS-induced increase in the immobility time (118.0 ± 17.8 s, 152.2 ± 23.4 s, and 98.7 ± 15.9 s respectively, F[4, 23] = 8.1, p < 0.05). Similarly, the immobility time in the TST, was also significantly increased by LPS treatment (265.3 ± 9.7 s) as compared to saline treated group (118.2 ± 14.7 s) (Fig. 2C). Pretreatment of the animals with CA, CA + CAF and IMI significantly reduced this LPS-induced increase in the immobility time (170.5 ± 15.0 s, 168.0 ± 25.4 s, and 150.6 ± 32.3 s respectively, F[4, 22] = 7.9, p < 0.05).

3.3. Effect of CA, CA + CAF and IMI on LPS-induced serum and brain TNF-α and IL-6.

Both brain and serum cytokines, TNF-α, and IL-6 were quantified. Acute administration of LPS produced a significant increase in serum and brain TNF-α levels (985.8 ± 73.1 vs 229.8 ± 35.6 pg/ml of saline serum, and 440.1 ± 29.3 vs 262.7 ± 2.4 pg/mg of protein of saline brain; Fig. 3A and 3B). Seven day pretreatment of the animals with CA, CA + CAF and IMI significantly reduced this increase in TNF-α levels in serum (511.1 ± 79.2 vs 797.3 ± 8.9; 523.2 ± 95.3 respectively, F[4, 9] = 14.2, p < 0.05) and brain (112.1 ± 48.1; 46.1 ± 27.3; 152.9 ± 36.0 respectively, F[4, 14] = 12.0, p < 0.05). Similarly, acute administration of LPS produced a significant increase in serum and brain IL-6 levels (590.2 ± 88.5 vs 262.3 ± 124.3 pg/ml of saline serum, and 1353.1 ± 119.1 vs 397.2 ± 83.3 pg/mg of protein of saline brain; Fig. 3C and 3D). Pretreatment of the animals with CA, CA + CAF and IMI significantly reduced the LPS-induced increase in IL-6 in serum (178.156 ± 87.348, 237.652 ± 103.088, 227.088 ± 65.659 respectively, F[4, 19] = 7.3), and brain (397.5 ± 119.1, 848.2 ± 61.3, 818.9 ± 130.8 respectively, F[4, 12] = 9.1, p < 0.05).

3.4. Effect of CA, CA + CAF and IMI on LPS-induced serum and brain MDA and GSH.

In the brain homogenates, LPS administration resulted in significant lipid peroxidation, as assessed by increase in MDA levels (nM/mg of protein) (1128 ± 168.3 vs 400.5 ± 41.3 of saline treated group; Fig. 3E) and decreased total GSH levels (µmoles/mg of protein) (2.2 ± 0.2 vs 4.2 ± 0.4 of saline treated group; Fig. 3F). Pretreatment of the animals with CA, CA + CAF and IMI significantly improved the MDA levels (749.2 ± 30.7; 589.2 ± 40.5; 519.3 ± 93.4 respectively, F[4, 17] = 8.54, p < 0.05) as well as GSH levels (3.6 ± 0.2; 4.2 ± 0.5; 4.2 ± 0.5 respectively, F[4, 18] = 3.4, p < 0.05).

4. Discussion

This study is an extension of our previous work, where we established the effect of acute administration of CA in LPS-induced sickness behavior and effect of coffee in LPS-induced depressive-like behavior (Basu Mallik et al., 2016; Hall, Arora, Anoopkumar-Dukie, & Grant, 2016). In the present study, we have determined both, acute effects of CA and CA + CAF on behavioral parameters, as well as their chronic effects on LPS-induced neuroinflammation.

As observed in OFT, 7 day pretreatment protocol of CA significantly
Fig. 1. Effect of caffeic acid (CA), Caffeic acid + caffeine (CA + CAF), and imipramine (IMI) on number of crossings (A); number of rearings (B); center square entries (C); time in center (D); number of groomings (E); number of transitions in dark-light box (F) and time spent in light box (G). *p < 0.05 as compared to saline (SAL) group.
increased the horizontal and vertical activities as observed by number of crossings and rearings. Furthermore, in the DLB, the number of transitions and the total time spent in the light chamber was significantly increased. All these observations are suggestive of anxiolytic properties of CA. It is noteworthy that we employed only half of the dose of CA (15 mg/kg) in 7 day protocol as compared to 30 mg/kg used in our earlier acute CA administration study.

Our results are in alignment with the reported studies by Takeda, Tsuji, Inazu, Egashira, and Matsumiya (2002) and Pereira et al. (2006), where both the studies propose the anxiolytic and antidepressant properties of CA within the low dose. Interestingly, the results of Takeda et al., are suggestive of a possible mechanism other than the monoamine reuptake and monoamine oxidase inhibition (Takeda et al., 2002). Since the focus of our current study was to evaluate the effect of CA + CAF on these behavioral and biochemical parameters, the doses of both CA and CAF were further reduced in the combination group (10 mg/kg and 5 mg/kg respectively). CA + CAF significantly increased the exploratory behavior in both the OFT and DLB, suggestive of a synergistic effect of this combination in producing anxiolytic-like effect.

Similarly, 7 days of chronic treatment with IMI had a comparable effect in the employed tests for the assessment of anxiety-like behavior. In the second part of our study, animals were challenged with LPS after 7 days of pretreatment with CA, CA + CAF, and IMI. Peripheral injection of LPS is known to mediate a number of acute effects including changes in behavior like social withdrawal, decreased food intake and fever (Painsipp, Köfer, Sinner, & Holzer, 2011; Yirimiya, Tio, & Taylor, 1996). Since the blood brain barrier (BBB) loses its integrity during systemic inflammatory conditions, as imposed by LPS administration and becomes leaky (Varatharaj & Galea, 2017) leading to development of neuroinflammation. This sickness behavior has been associated with the release of proinflammatory cytokines including TNF-α and IL-1β, mediated by their actions on vagal cytokine receptors (Szentirmai & Krueger, 2014). In the present study, LPS administration significantly reduced the LMA as demonstrated by a significant decrease in the number of crossings in OFT, and all the animals expressed a typical corner occupancy behavior. Similarly, the immobility time was significantly increased by LPS administration, as observed in the FST and TST. Pretreatment of the animals with CA, CA + CAF and IMI significantly improved the locomotor activity and reduced the immobility time in both FST as well as TST. The lack of any effect of IMI on LPS-induced locomotor activity has been reported in our previous studies (Basu Mallik et al., 2016; Hall et al., 2016). Furthermore, LPS produced a significant increase in both serum and brain inflammatory cytokines, TNF-α and IL-6. LPS binds to toll-like receptors (TLRs) 2 and 4 which are present in both neurons and microglia (Park et al., 2012; Yang et al., 1998). This binding of LPS to microglial membrane bound TLR4 leads to upregulation and translocation of NF-kB transcription factor and thus stimulates cytokines and chemokines productions through MyD-88 as well as TRIF-dependent pathways essential for immune and inflammatory responses (Burton, Sparkman, & Johnson, 2011; Kawasaki & Akira, 2010; Kawasaki, Takemura, Standley, Akira, & Kawai, 2013; Li & Verma, 2002). Increased levels of proinflammatory cytokines IL-6 and TNF-α, have been reported to be pivotal in mediating sickness behavior (Burton et al., 2011; Burton, Ryttych, Freund, & Johnson, 2013), therefore these two proinflammatory markers were chosen to be determined in this study. Further, cytokines like TNF-α and IL-6 are known to be the central mediators of depression, in both humans and rodents. Scientific evidence states that LPS challenge to microglial cells induces the release of TNF-α and IL-6. IL-6 further regulates the release of interleukin 1 (α and β), the other major cytokines involved in depression (Minogue, Barrett, & Lynch, 2012). Human trials in patients suffering from depression also co-relate plasma levels of TNF-alpha and IL-6 with neuro-inflammation (O’Brien, Scott, & Dinan, 2004).
Pretreatment of the animals with CA significantly reduced the LPS-induced upregulation of TNF-α and IL-6 in both serum and brain homogenates. A similar trend was observed with low dose combination of CA+CAF and IMI pretreated animals. It is noteworthy that the results from chronic administration of these treatments produced a relatively better protection against the systemic and neuronal inflammatory cytokines (Basu Mallik et al., 2016). CA is well known phenolic compound present in coffee and has been reported to decrease the production of inflammatory mediators such as nitric oxide and inducible nitric-oxide synthase, prostaglandin E2 and cyclooxygenase-2, and TNF-α (Yang, Hong, Lee, Kim, & Lee, 2013). Even though CAF is one of the major component of caffeinated coffee, however, there are evidences that suggest that coffee is superior to CAF alone in providing beneficial effects (Cao et al., 2011; Shukitt-Hale et al., 2013). Furthermore, it is CAF or caffeinated coffee (but not decaffeinated coffee) that protects against Alzheimer’s pathology (Arnedash & Cao, 2010). Results from our own lab have shown that caffeinated coffee was found to be more neuroprotective as compared

Fig. 3. Effect of caffeic acid (CA), caffeic acid + caffeine (CA + CAF), and imipramine (IMI) on LPS-induced changes in TNF-α (pg/mL in serum) (A) and (pg/mg of protein in brain homogenates) (B); IL-6 (pg/mL in serum) (C) and (pg/mg of protein in brain homogenates) (D); MDA levels (nmoles/mg of protein) (E) and total GSH levels (μmoles/mg of protein in brain homogenates) (F). *p < 0.05 as compared to saline (SAL) group; #p < 0.05 compared with LPS group.
to CAF alone, whereas decaffeinated coffee did not show any neuroprotective effects in depressive like behavior in mice (Hall et al., 2016).

Chronic coffee and CAF intake also modulate the antioxidant system by reducing the lipid peroxidation of membranes, increasing glutathione and increasing the activity of antioxidant enzymes (Abreu, Silva-Oliveira, Moraes, Pereira, & Moraes-Santos, 2011). We have earlier shown that acute administration of CA alone (30 mg/kg) significantly reduces the lipid peroxidation and protects the glutathione depletion caused by LPS (Basu Mallik et al., 2016). In our current study both CA and CA + CAF significantly protected against LPS-induced oxidative stress and reduced the lipid peroxidation and prevented the depletion of glutathione antioxidant stores. Protection against oxidative stress may be one of the mechanisms by which CA and CA + CAF could have shown a prominent effect in both behavioral and biochemical functions.

In conclusion the results of our study suggest that administration of CA alone and CA + CAF showed a very competitive and comparable protection in behavioral and inflammatory markers against LPS-induced changes. However, the MDA and GSH data suggests of a better protection with the combination. Furthermore, under un-challenged conditions the CA + CAF group showed synergistic anxiolytic properties. This would indicate that various pharmacodynamic and pharmacokinetic mechanisms might be involved in translating these effects of dietary polyphenols and hydroxycinnamates. With the data in hand it is plausible that CA, CA + CAF could be acting via a combination of mechanisms including; modulation of LPS-TLR signalling, reducing oxidative stress and direct inhibition of inflammatory cascades.

Further studies investigating the exact mechanism and possible therapeutic potential of dietary cinnamates in modulating the neuroinflammatory pathways will substantiate these preliminary findings.

5. Animal ethics statement

All the experimental procedures were approved and performed in accordance with the guidelines set out by the Institutional Animal Ethics Committee (IAEC) of MAHE (Protocol No. IAEC/KMC/81/2016), in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

6. Statement

Caffeic acid and caffeine are commonly consumed in food and beverages. Caffeine interferes with the bioavailability of other coffee constituents and modulates their biological activity. This study was designed to evaluate the effect of caffeic acid and its combination with caffeine on behavioral and neuroinflammatory changes produced by systemic inflammation.

7. Essence of approach

We employed an array of behavioral models to investigate the effect of caffeic acid and caffeine on the normal exploratory behavior of the animals, that tells us about the anxiolytic or anxiogenic properties of these ingredients. Furthermore, to establish an inflammation-induced depression model, systemic administration of lipopolysaccharide (LPS) was used. Inflammatory markers from serum and brain were investigated by employing established biochemical methods.

8. A list of the specific major novel contributions

We found that caffeic acid alone and in combination with low doses of caffeine possess anxiolytic effects. Moreover, they also offered a significant protection against LPS-induced neuroinflammation and systemic changes.

9. Backroad of current hypothesis

Our current study was based on the following published data from our lab:

2. This study explored the effects of acute administration of caffeic acid alone in LPS-induced sickness behavior.

This study explored an impact of coffee on LPS-induced IDO enzyme induction that plays a pivotal role in kynurenic acid metabolic pathways and produces neuroinflammation. Furthermore, this study was based on depressive-like behavior that appears 24 h after the LPS administration.

With these two papers, we were very much interested in investigating the combined and chronic effects of these two dietary constituents (caffeic acid and caffeine) on normal behaviour, as well as the possible additive/synergistic effect in systemic and neuroinflammatory conditions. These polyphenols express differential biological activities based on the dose, route and in combination with other similar compounds. Furthermore, we employed a broad range of behavioral and biochemical assays in current study to substantiate our hypothesis.

Acknowledgements

The authors thank the Dr. K.S.R. Pai, HOD, Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education (MAHE), Manipal for providing facilities to carry out this project, and Mr. Sridhara Prabhu, CARF, MAHE, Manipal for facilitating the animal experiments.

Funding sources

This study was financially supported by QUM Network Research Grant Scheme 2017 to DA.

Declaration of Competing Interest

The authors declare no conflict of interest associated with this publication.

References


Burton, M. D., Sparkman, N. L., & Johnson, R. W. (2011). Inhibition of interleukin-6 trans-signaling in the brain facilitates recovery from lipopolysaccharide-induced...


Kawasaki, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immu-


Kim, Y. S., Kwak, S. M., & Myung, S. K. (2015). Caffeine intake from coffee or tea and messenger phosphatidylinositol-5-phosphate facilitates antiviral innate immune sig-


