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Original Article

Ferulic acid, a phenolic compound with therapeutic effects in neuropsychiatric disorders, stimulates the production of nerve growth factor and endocannabinoids in rat brain

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Abstract

Introduction: Ferulic acid, a phenolic phytochemical with neuroprotective, anti-inflammatory, and antioxidant properties, has shown promising antidepressant-like effects in behavioral studies; however, its mechanism(s) of action have not been fully understood. Based on the contribution of nerve growth factor (NGF) and endocannabinoid signaling (eCBs) to the emotional or antidepressant activity and their interaction, we aimed to evaluate whether ferulic acid affects NGF or eCB contents in the brain regions involved in the modulation of emotions.

Methods: Following single and four-week once-daily intraperitoneal injections of ferulic acid (50, 100, 130 and 150 mg/kg), amitriptyline (2.5, 5, 8 and 10 mg/kg) or lorazepam (2, 5, 8 and 10 mg/kg) into male Wistar rats, NGF and eCB levels were quantified by Bio-Rad protein assay and isotope-dilution liquid chromatography/mass spectrometry. In the case of significant alteration of brain NGF or eCB content, the effects of pre-treatment with cannabinoid CB_1 or CB_2 receptor antagonist (AM251 or SR144528) were investigated.

Results: Four-week treatment with the highest doses of ferulic acid or amitriptyline led to a significant and sustained enhancement of eCB and NGF contents in brain region-specific fashion. Neither acute nor four-week treatment with lorazepam affected NGF or eCB levels. Pre-treatment with AM251 (3 mg/kg), but not SR144528, prevented the elevation of NGF levels. AM251 showed no effect by itself.

Conclusion: Ferulic acid similar to the conventional antidepressant, amitriptyline, affects brain eCB and NGF signaling. CB_1 receptors mediate the production of brain NGF.

Keywords:
Ferulic acid;
Nerve growth factor;
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CB1 receptors;
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Introduction

The high prevalence of neuropsychiatric disorders including the depression has raised increasing concerns among the health authorities (Baumeister and Harter, 2007). Conventional therapeutic agents are usually associated with limited efficiency,

resistance, significant side effects and drug-drug or drug-food interactions (Ereshefsky, 2009; Santarsieri and Schwartz, 2015). In recent years, research efforts have been attracted towards the identification of nutraceuticals or phytochemicals with antidepressant properties (Nemeroff, 2007; van der Watt et al., 2008). Ferulic acid (4-hydroxy-3-methoxycinnamic acid), a natural phenolic compound, has shown a wide range of pharmacological activities including the effects against the oxidative stress, inflammation, aging and neurological disorders such as the spinal cord injury, stroke, Alzheimer's disease and epilepsy (Cheng et al., 2008; Gohil et al., 2012; Hassanzadeh et al., 2017; Srinivasan et al., 2007; Wu et al., 2014; Yan et al., 2001). Furthermore, this bioflavonoid via the enhancement of monoamine neurotransmitter levels in the brain, modulation of oxidative/nitrosative stress and activities of protein kinase A, protein kinase C, mitogen-activated protein kinase/extracellular signal-regulated kinase, Ca^{2+}/calmodulin-dependent protein kinase II and phosphatidylinositol-3-kinase signalling pathways has shown therapeutic effects against the depression-like behaviors (Chen et al., 2015; Zeni et al., 2012; Zhang et al., 2011). Over the last decade, implication of the neurotrophins in antidepressant activity has been well-documented (Aloe et al., 2015; Berton and Nestler, 2006; Tanis and Duman, 2007). Neurotrophins which are critically involved in the neuronal growth, differentiation and function (Huang and Reichardt, 2001), have shown neuroprotective effects against the neuropsychiatric disorders (Castren et al., 2007; Shaltiel et al., 2007). The prototypical neurotrophin, nerve growth factor (NGF), which regulates stress and cognitive function (Ebendal, 1992; Lad et al., 2003), is implicated in the mechanisms of action of a variety of psychotropic agents (Aloe et al., 2015; Berton and Nestler, 2006). This, prompted us to evaluate the effect of ferulic acid on NGF production in the brain regions including the frontal cortex, hippocampus, amygdala, olfactory bulb and brain stem which are involved in the processing of emotional information. As a positive control, we selected the tricyclic antidepressant, amitriptyline, due to its neurotrophic activity (Chadwick et al., 2011; Jang et al., 2009). The benzodiazepine, lorazepam, was also used for comparative purposes.

Based on the modulatory effects of the endocanabinoid system (eCBs) on a variety of pathophysiological or physiological processes including the emotional reactivity (Bambico et al., 2009; Gobbi et al., 2005; Hassanzadeh and Rostami, 2014; McLaughlin and Gobbi, 2012; Pacher et al., 2006; Viveros et al., 2005) and its interaction with neurotrophin signaling (Hassanzadeh, 2010; Khaspekov et al., 2004; Williams et al., 2003), we focused on the eCBs in order to identify a potential mechanism by which brain NGF production may be regulated by ferulic acid, amitriptyline or lorazepam. For further assessment of the role of the eCB transmission in the mechanisms of action of the abovementioned drugs, the brain contents of two major endocannabinoids, N-arachidonylethanolamine (anandamide, AEA) and 2-arachidonylglycerol (2-AG), were measured after the acute and four-week treatment.

Materials and methods

Animals

Randomly assigned male Wistar rats weighing 250-280 g (Pasteur Institute, Tehran, Iran) were housed three per cage on a 12-h light/dark cycle under the standard laboratory conditions (temperature: 22 ± 2 °C, humidity: 55–65%). Food pellets and water were available *ad libitum* and animals were allowed to acclimatize to the housing conditions for one week prior to the experiments. The maintenance and care of experimental animals complies with National Institutes of Health guidelines for the humane use of laboratory animals and has been approved by Institutional Ethics Committee.

Treatments

Ferulic acid (Sigma Aldrich, Germany) was dissolved in 0.5% dimethyl sulfoxide (DMSO, Sigma Aldrich, Germany) and administered at doses of 50, 100, 130 and 150 mg/kg (Koh, 2012a and b; Lenzi et al., 2015). Amitriptyline hydrochloride (Sigma Aldrich, Germany) was dissolved in 0.9% saline and injected at doses of 2.5, 5, 8 and 10 mg/kg (Jang et al., 2009; di Matteo et al., 2000). Lorazepam (Sigma Aldrich, Germany) was dissolved in 3 to 4 drops of Tween 80 (Sigma-Aldrich, Germany) prior to the addition of 0.9% saline and injected at doses of 2, 5, 8 and 10 mg/kg (Jackson et al., 2003; Mehrotra and Jadhav, 2006). In order to compare the effects of acute treatment and cumulative doses, animals received

once daily injection of ferulic acid, amitriptyline, lorazepam or the corresponding vehicle between 9:00 to 10:00 am for either one day or 28 consecutive days (n=6/group). In the case of any significant alteration of brain NGF contents following the treatment with ferulic acid, amitriptyline or lorazepam, the cannabinoid CB_1 receptor antagonist, AM251 (Tocris Bioscience, UK), at doses of 1, 2 and 3 mg/kg (Dono and Currie, 2012; Femenia et al., 2010) or CB_2 receptor antagonist, SR144528 (N-[(1S)-endo-1,3,3-trimethylbicyclo [2.2.1] heptan-2-yl] 5-(4-choro-3-methylphenyl)-1-(4-methyl benzyl pyrazole-3-carboxamide, Sigma Aldrich, Germany), at doses of 1, 2.5 and 5 mg/kg (Abalo et al., 2010) was dissolved in DMSO, Tween-80 and 0.9% saline in a 1:1:8 ratio and injected either alone or 30 min prior to the injection of ferulic acid, amitriptyline or lorazepam (n=6/group). All injections were intraperitoneal in a volume of 1 ml/kg.

NGF quantification

At 24, 48 or 72 h after the last injection of ferulic acid, amitriptyline, lorazepam or the corresponding vehicles (Gwinn et al., 2002; Parikh et al., 2004), the brain regional contents of NGF were measured. Animals were decapitated without anaesthesia and the brain of each animal was quickly and carefully removed from the skull. Using the Paxinos and Watson atlas (2007), the brain regions including the frontal cortex, hippocampi, amygdalae, olfactory bulbs and brain stem were dissected on a frozen pad taken from a -80 °C freezer. Then, the brain regions were weighed [frontal cortex: 0.26 ± 0.04, hippocampi: 0.19 ± 0.02, amygdalae: 0.08 ± 0.006, olfactory bulbs: 0.05 ± 0.004 and brain stem: 0.38 ± 0.02 (mean ± SEM, in grams)] and kept frozen at -80 °C. Each tissue sample was homogenized on ice in 5-6 vol of 0.25 M sucrose and 10 mM HEPES (pH 7.0) containing 10 mM dithiothreitol and immediately frozen in a dry ice/acetone bath and stored at -80 °C until NGF analysis. Noteworthy, the total brain NGF content is several fold higher than generally reported as the pellet which is usually discarded, contains a higher amount of NGF than the corresponding supernatant (Hoener et al., 1996). In this respect, the homogenates were centrifuged at 10,000 g (Hettich, Germany) for 10 min at 15 °C and the remaining pellets were each dissolved in 750 µl NGF homogenization buffer, treated with ultrasound (Vibra Cell; Bioblock Scientific, France) for 3 min and processed for further quantification of NGF by a fluorometer (Titertek Fluoroskan II, Germany) as previously described in detail (Hellweg et al., 1989; Hellweg et al., 2001; Hellweg et al., 2002; Hoener et al., 1996). The measured and recovery-corrected NGF contents are expressed as ng NGF per g protein in the resuspended NGF homogenate quantified by Bradford (1976) protein assay using a spectrophotometer (UV-1601, Shimadzu, Japan) at 595 nm. The measurements were performed in duplicate and analyzed by an investigator blind to the experimental set-up.

Extraction and analysis of the endocannabinoids

In order to evaluate the involvement of eCB transmission in the mechanisms of action of ferulic acid, amitriptyline or lorazepam, the contents of two major endocannabinoids, AEA and 2-AG, were measured in the aforementioned brain regions following both acute and four-week administration of the drugs or corresponding vehicles. Briefly, animals (n=6/group) were killed 1, 5 and 12 h after the last injection (de Lago et al., 2005) and the brains were quickly removed, dissected, weighed and stored frozen at -80 °C until the lipid extraction. The contents of AEA and 2-AG within the lipid extracts were determined by isotope-dilution liquid chromatography/mass spectrometry (LC-MS) using a Shimadzu LC-10ADVP HPLC apparatus (Shimadzu, Japan) coupled to a Shimadzu quadrupole MS (LCMS-2010) via a Shimadzu atmospheric pressure chemical ionization (APCI) interface (Koga et al., 1997; Patel et al., 2003). The measurements were performed in duplicate and analyzed by an investigator blind to the experimental set-up. AEA and 2-AG are expressed as pM and nM per g of wet tissue extracted, respectively.

Statistical analysis

Shapiro-Wilk test was used to verify the normal distribution of the data. The brain regional contents of AEA, 2-AG and NGF were analyzed by three-way ANOVA (treatment, dose and time point as the fixed factors and AEA, 2-AG or NGF as the dependent variable) followed by Tukey's post hoc analysis. Data are presented as mean ± SEM (6 animals per group). The level of significance was set at $P<0.05$.

Table 1: The brain regional levels of NGF at baseline and after acute treatment with the highest doses of ferulic acid, amitriptyline, or lorazepam

Time point (h)	Brain regions	Baseline values	Vehicle 1	Vehicle 2	Vehicle 3	Ferulic acid	Amitriptyline	Lorazepam
24	Frontal cortex	34.17 ± 2.43	32.58 ± 1.97	34.03± 2.94	32.17 ± 2.43	37.15 ± 3.24	35.82 ± 3.07	33.47± 1.97
	Hippocampi	77.32 ± 6.27	74.15 ± 4.69	72.28 ± 6.43	76.09 ± 5.17	75.46 ± 4.58	79.43± 5.88	75.27± 6.27
	Amygdalae	31.75 ± 3.03	33.45 ± 2.84	32.47 ± 3.04	33.17 ± 2.45	33.78 ± 2.23	44.32 ± 2.75	39.48± 2.52
	Olfactory bulbs	33.27 ± 1.94	35.03 ± 2.17	33.27 ± 2.66	31.46 ± 2.77	37.19 ± 1.89	32.67 ± 1.93	37.19 ± 1.86
	Brain stem	31.68 ± 2.66	33.42 ± 1.94	32.55 ± 3.11	35.08 ± 2.94	35.94± 2.17	39.53 ± 2.69	35.43 ± 3.09
48	Frontal cortex	34.17 ± 2.43	34.52 ± 2.89	32.27 ± 1.95	34.15 ± 3.04	33.79 ± 2.74	33.45± 2.63	35.43 ± 3.09
	Hippocampi	77.32 ± 6.27	75.11 ± 5.43	70.66 ± 5.77	72.33 ± 7.15	77.25 ± 6.15	72.19 ± 3.76	73.55 ± 4.11
	Amygdalae	31.75 ± 3.03	31.57 ± 1.86	32.79± 2.55	35.17 ± 2.33	34.28 ± 2.73	37.95 ± 1.79	37.46 ± 2.88
	Olfactory bulbs	33.27 ± 1.94	33.56 ± 2.93	31.97 ± 1.73	34.08 ± 3.24	35.86 ± 2.69	33.85 ± 2.63	39.17 ± 2.49
	Brain stem	31.68 ± 2.66	31.75 ± 3.07	34.16 ± 2.86	32.27 ± 1.88	32.48 ± 3.07	38.63 ± 1.83	31.56 ± 1.76
72	Frontal cortex	34.17 ± 2.43	34.49 ± 3.03	35.66 ± 2.79	36.03 ± 2.56	33.56 ± 1.77	31.76 ± 2.64	31.87 ± 2.43
	Hippocampi	77.32 ± 6.27	72.11 ± 7.08	75.03 ± 4.66	76.17 ± 5.37	72.48± 4.77	74.58 ± 7.05	73.48 ± 6.02
	Amygdalae	31.75 ± 3.03	35.23 ± 2.87	35.74 ± 1.73	31.84 ±1.79	33.58± 3.09	39.41 ± 3.11	32.49 ± 2.75
	Olfactory bulbs	33.27 ± 1.94	35.11 ± 1.76	33.15 ± 2.27	32.57 ± 1.58	37.23± 2.83	31.87 ± 1.58	35.14 ±2.56
	Brain stem	31.68 ± 2.66	35.47 ± 2.38	35.19± 3.11	35.13 ± 2.43	35.16 ± 1.49	32.96 ± 2.73	35.73± 1.82

Single injections of drugs even at the highest doses tested evoked no significant alterations in brain NGF contents as compared with the baseline values or vehicle-treated control groups. NGF levels are expressed as ng of NGF per g of protein in the resuspended NGF homogenate. Data are presented as mean±SEM (n=6/group). Vehicle 1, 2 and 3 are related to ferulic acid, amitriptyline and lorazepam respectively.

Results

The effects of ferulic acid, amitriptyline or lorazepam on brain regional levels of NGF

Single injection of ferulic acid, amitriptyline or lorazepam even at the highest dose tested did not affect brain regional contents of NGF at time points 24, 48 or 72 h following the injection as compared to the baseline values or vehicle-treated groups (Table 1, $P>0.05$). Four-week once-daily injection of ferulic acid, amitriptyline or lorazepam at lower doses did not affect brain NGF protein levels (Tables 2 and 3; $P>0.05$ *vs*. the vehicle-treated groups), however, 24 h after the last injection of ferulic acid (130 and 150 mg/kg) or amitriptyline (8 and 10 mg/kg), NGF protein levels were significantly increased in brain region-specific fashion (Table 4; $P<0.05$, $P<0.01$ and $P<0.001$). NGF contents remained significantly elevated up to 48 h (Table 4; $P<0.05$, $P<0.01$ and $P<0.001$) and 72 h (Table 4; $P<0.05$ and $P<0.01$). The main effects and interactions between the factors are as follows; {frontal cortex: [dose: $F(2,270)=140.48$, $P<0.001$], [treatment: $F(5,270)=69.89$, $P<0.001$], [time point: $F(2,270)=0.63$, $P=0.54$], [dose × treatment: $F(10,270)=37.97$, $P<0.001$], [dose × time point × treatment: $F(20,270)=0.51$, $P=0.96$]; hippocampi: [dose: $F(2,270)=45.59$, $P<0.001$], [treatment: $F(5,270)=64.75$, $P<0.001$], [time point: $F(2,270)=0.07$, $P=0.93$], [dose × treatment: $F(10,270)=20.58$, $P<0.001$], [dose × time point × treatment: $F(20,270)=0.06$, $P=1$]; amygdalae: [dose: $F(2,270)=79.81$, $P<0.001$], [treatment: $F(5,270)=43.14$, $P<0.001$], [time point: $F(2,270)=0.17$, $P=0.85$], [dose × treatment: $F(10,270)=23.21$, $P<0.001$], [dose × time point × treatment: $F(20,270)=0.48$, $P=0.97$]; olfactory bulbs: [dose: $F(2,270)=37.01$, $P<0.001$], [treatment: $F(5,270)=34.95$, $P<0.001$], [time point: $F(2,270)=0.88$, $P=0.41$], [dose × treatment: $F(10,270)=19.94$, $P<0.001$], [dose × time point × treatment: $F(20,270)=0.45$, $P=0.98$]; brain stem: [dose: $F(2,270)=1.24$, $P=0.29$], [treatment: $F(5,270)=0.19$, $P=0.97$], [time point: $F(2,270)=2.18$, $P=0.12$], [dose × treatment: $F(10,270)=0.09$, $P=1$], [dose × time point × treatment: $F(20,270)=0.09$, $P=1$]. Four-week daily administration of lorazepam even at the highest doses tested (8 and 10 mg/kg) did not significantly affect brain NGF contents as compared to the

Table 2: The effects of four-week treatment with ferulic acid (50 mg/kg), amitriptyline (2.5 mg/kg) or lorazepam (2 mg/kg) on brain regional contents of NGF

Time point (h)	Brain regions	Vehicle 1	Vehicle 2	Vehicle 3	Ferulic acid	Amitriptyline	Lorazepam
24	Frontal cortex	34.75 ± 2.03	32.49± 2.75	34.99 ± 3.11	38.64 ± 3.03	40.94 ± 3.04	36.17± 2.92
	Hippocampi	73.48 ± 5.87	75.74 ± 7.17	72.29 ± 5.56	79.82 ± 7.07	76.03± 6.82	71.63± 6.88
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	35.66 ± 2.31	37.84 ± 2.79	42.06 ± 2.91	35.29± 3.09
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	34.25 ± 4.03	41.06 ± 3.35	34.17 ± 2.79	35.17 ± 2.83
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	31.24 ± 3.66	39.13± 3.21	36.98 ± 3.07	32.69 ± 3.06
48	Frontal cortex	34.30 ± 3.16	35.87 ± 3.74	32.71 ± 2.78	37.13 ± 3.64	35.27± 3.02	32.92 ± 2.89
	Hippocampi	72.67 ± 5.89	74.20 ± 7.04	74.48 ± 6.45	80.04 ± 7.15	76.83 ± 5.97	71.32 ± 6.04
	Amygdalae	34.22 ± 2.83	34.14± 3.47	32.83 ± 3.43	37.18 ± 3.71	39.08 ± 3.18	32.75 ± 3.09
	Olfactory bulbs	32.94 ± 3.04	35.83 ± 2.85	32.53 ± 3.57	43.29 ± 4.01	38.45 ± 3.67	34.19 ± 2.94
	Brain stem	34.37 ± 3.24	32.77 ± 3.57	34.85 ± 3.93	38.17 ± 3.37	41.03 ± 3.79	35.12 ± 3.03
72	Frontal cortex	33.47 ± 2.73	34.85 ± 3.21	32.53 ± 2.84	37.18 ± 3.43	37.19 ± 3.68	34.16 ± 3.13
	Hippocampi	74.43 ± 5.48	72.98 ± 6.05	75.40 ± 6.47	76.13± 7.73	78.18 ± 6.71	76.03 ± 7.12
	Amygdalae	32.71 ± 3.47	33.28 ± 3.04	35.23 ±2.74	37.03± 2.69	35.42 ± 3.14	35.19 ± 2.84
	Olfactory bulbs	34.87 ± 3.15	32.55 ± 2.79	34.19 ± 3.13	39.18± 3.23	35.15 ± 3.03	31.79 ±3.39
	Brain stem	34.20 ± 3.17	31.49± 3.50	33.93 ± 3.53	38.51 ± 4.03	35.92 ± 3.75	37.06± 2.63

24, 48 and 72 h represent the time points at which NGF contents were measured after the last injection of ferulic acid, amitriptyline, lorazepam or the corresponding vehicles. NGF levels are expressed as ng of NGF per g of protein in the resuspended NGF homogenate. Data are presented as mean±SEM (n=6/group). Vehicle 1, 2 and 3 are related to ferulic acid, amitriptyline and lorazepam, respectively.

Table 3: The effects of four-week administration of ferulic acid (100 mg/kg), amitriptyline (5 mg/kg) or lorazepam (5 mg/kg) on brain regional levels of NGF

Time point (h)	Brain regions	Vehicle 1	Vehicle 2	Vehicle 3	Ferulic acid	Amitriptyline	Lorazepam
24	Frontal cortex	34.75 ± 2.03	32.49± 2.75	34.99 ± 3.11	41.13 ± 4.09	39.28 ± 3.11	34.29± 3.07
	Hippocampi	73.48 ± 5.87	75.74 ± 7.17	72.29 ± 5.56	78.94 ± 7.42	81.24 ± 6.93	74.15± 7.13
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	35.66 ± 2.31	41.13 ± 3.87	38.13 ± 3.15	32.98± 2.83
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	34.25 ± 4.03	38.72 ± 3.03	40.13 ± 3.82	37.11± 3.17
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	31.24 ± 3.66	40.13 ± 3.86	42.03 ± 3.94	34.13 ± 2.98
48	Frontal cortex	34.30 ± 3.16	35.87 ± 3.74	32.71 ± 2.78	39.87 ± 3.28	41.17 ± 2.94	32.96 ± 2.97
	Hippocampi	72.67 ± 5.89	74.20 ± 7.04	74.48 ± 6.45	81.29± 7.86	83.12 ± 8.02	72.19 ± 6.83
	Amygdalae	34.22 ± 2.83	34.14± 3.47	32.83 ± 3.43	42.34 ± 4.11	39.21 ± 3.17	34.05 ± 3.12
	Olfactory bulbs	32.94 ± 3.04	35.83 ± 2.85	32.53 ± 3.57	44.08 ± 3.83	37.81 ± 4.06	33.92 ± 2.74
	Brain stem	34.37 ± 3.24	32.77 ± 3.57	34.85 ± 3.93	40.29 ± 2.77	39.18 ± 2.79	32.45 ± 4.03
72	Frontal cortex	33.47 ± 2.73	34.85 ± 3.21	32.53 ± 2.84	39.15 ± 3.96	42.14 ± 3.08	32.17 ± 3.13
	Hippocampi	74.43 ± 5.48	72.98 ± 6.05	75.40 ± 6.47	77.39 ± 6.98	75.47 ± 7.13	75.23 ± 7.29
	Amygdalae	32.71 ± 3.47	33.28 ± 3.04	35.23 ±2.74	37.57± 2.78	35.23 ± 3.07	36.02 ± 4.06
	Olfactory bulbs	34.87 ± 3.15	32.55 ± 2.79	34.19 ± 3.13	35.14± 3.06	38.21± 3.12	31.47 ± 2.81
	Brain stem	34.20 ± 3.17	31.49± 3.50	33.93 ± 3.53	37.16± 2.87	33.82 ± 3.23	35.14 ± 411

24, 48 and 72 h represent the time points at which NGF contents were measured following the last injection of ferulic acid, amitriptyline, lorazepam or the corresponding vehicles. NGF levels are expressed as ng of NGF per g of protein in the resuspended NGF homogenate. Data are presented as mean±SEM (n=6/group). Vehicle 1, 2 and 3 are related to ferulic acid, amitriptyline and lorazepam, respectively.

Table 4: The effects of four-week administration of ferulic acid (130 and 150 mg/kg), amitriptyline (8 and 10 mg/kg) or lorazepam (8 and 10 mg/kg) on brain regional contents of NGF

Time point (h)	Brain regions	Vehicle 1	Vehicle 2	Vehicle 3	Ferulic acid (130 mg/kg)	Ferulic acid (150 mg/kg)	Amitriptyline (8 mg/kg)	Amitriptyline (10 mg/kg)	Lorazepam (8 mg/kg)	Lorazepam (10 mg/kg)
24	Frontal cortex	34.75 ± 2.03	32.49± 2.75	34.99 ± 3.11	59.16 ± 2.47**	63.31 ± 3.23**	66.21 ± 2.32***	69.07 ± 3.09**	35.56 ± 2.77	38.14 ± 3.23
	Hippocampi	73.48 ± 5.87	75.74 ± 7.17	72.29 ± 5.56	124.05 ± 8.63**	129.17 ± 6.13***	113.81 ± 9.76*	109.75 ± 6.27**	73.01± 6.87	67.86± 3.45
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	35.66 ± 2.31	61.36 ± 3.03**	64.53 ± 3.09**	56.38 ± 2.59**	60.14 ± 2.17***	35.13 ± 2.99	40.57 ± 4.11
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	34.25 ± 4.03	58.52 ± 2.83**	52.19 ± 3.17*	64.33 ± 2.95***	72.25 ± 4.06**	32.46 ± 3.06	36.11 ± 3.28
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	31.24 ± 3.66	35.18 ± 3.61	31.44 ± 2.39	38.11 ± 3.68	33.97 ± 2.55	33.93 ± 3.29	31.42 ± 2.73
48	Frontal cortex	34.30 ± 3.16	35.87 ± 3.74	32.71 ± 2.78	62.86 ± 3.05**	58.03 ± 3.14*	60.85 ± 3.96**	63.72 ± 2.49***	37.49 ± 2.48	33.56 ± 2.75
	Hippocampi	72.67 ± 5.89	74.20 ± 7.04	74.48 ± 6.45	111.71± 9.91*	117.48± 7.11**	115.42 ± 10.61*	103.97 ± 7.14**	75.97 ±7.62	79.11 ±5.44
	Amygdalae	34.22 ± 2.83	34.14± 3.47	32.83 ± 3.43	59.94 ± 2.28**	53.12 ± 2.56**	61.91± 3.15**	57.86± 2.33**	35.19 ± 3.07	31.76 ± 2.83
	Olfactory bulbs	32.94 ± 3.04	35.83 ± 2.85	32.53 ± 3.57	66.41 ± 3.11**	70.37 ± 3.03***	59.12 ± 2.62**	63.29 ± 3.11**	37.29 ± 2.92	33.63 ± 3.07
	Brain stem	34.37 ± 3.24	32.77 ± 3.57	34.85 ± 3.93	42.04 ± 4.35	37.24 ± 2.97	39.05 ± 3.26	41.38 ± 2.28	36.99 ± 2.76	43.07 ± 4.13
72	Frontal cortex	33.47 ± 2.73	34.85 ± 3.21	32.53 ± 2.84	57.98 ± 3.01*	52.13 ± 2.87*	51.19 ± 3.13*	55.86 ± 2.37**	32.58 ± 3.21	37.12 ± 2.67
	Hippocampi	74.43 ± 5.48	72.98 ± 6.05	75.40 ± 6.47	104.85 ± 7.76*	98.75 ± 4.13**	108.48 ± 6.05**	96.74 ± 4.88**	75.98± 6.07	71.65± 4.12
	Amygdalae	32.71 ± 3.47	33.28 ± 3.04	35.23 ±2.74	51.82 ± 3.14*	49.68 ± 2.11*	56.93 ± 2.49*	51.49 ± 3.09*	31.19 ± 2.75	36.29 ± 3.05
	Olfactory bulbs	34.87 ± 3.15	32.55 ± 2.79	34.19 ± 3.13	58.84 ± 3.09*	63.29 ± 3.53*	53.78 ± 2.51*	57.26 ± 2.66*	35.64 ± 3.86	32.73 ± 2.16
	Brain stem	34.20 ± 3.17	31.49± 3.50	33.93 ± 3.53	36.17± 2.19	33.74± 2.36	37.31± 2.87	34.31± 2.87	32.39 ± 2.95	37.06 ± 3.17

24, 48 and 72 h represent the time points at which NGF contents were measured after the last injection of ferulic acid, amitriptyline, lorazepam, or the corresponding vehicles. NGF levels are expressed as ng of NGF per g of protein in the resuspended NGF homogenate. The limits of detection and quantification (LOD and LOQ) values were approximately 0.001 and 0.0028 ng/ml, respectively. Data are presented as mean±SEM (n=6/group). Vehicle 1, 2 and 3 are related to ferulic acid, amitriptyline and lorazepam, respectively. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ *vs*. the corresponding vehicle.

Table 5: The effects of AM251 on the enhanced brain NGF contents induced by ferulic acid (130 and 150 mg/kg) or amitriptyline (8 and 10 mg/kg)

AM251 (mg/kg)	Brain region	Vehicle 1	Vehicle 2	Vehicle 3	AM251	AM/fer 130	AM/fer 150	AM/ami 8	AM/ami 10
1	Frontal cortex	34.75 ± 2.03	32.49± 2.75	34.45 ± 3.40	32.76 ± 2.55	61.53 ± 3.34*	63.26 ± 2.19***	59.97 ± 3.04**	54.33 ± 2.97*
	Hippocampi	73.48 ± 5.87	75.73 ± 7.17	74.09 ± 6.92	77.42 ± 4.87	111.93 ± 8.52*	107.93 ± 6.52**	115.05 ± 9.25*	109.05 ± 7.28**
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	34.18 ± 2.78	32.55 ± 1.96	57.12 ± 2.43**	59.12 ± 3.09*	60.23 ± 2.87**	65.23 ± 3.11*
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	34.26 ± 3.78	31.74 ± 2.48	59.26 ± 3.07*	53.26 ± 2.76**	56.49± 3.05*	54.12± 3.03*
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	32.70 ± 3.34	36.04 ± 1.79	37.48 ± 4.19	33.57 ± 2.79	35.29 ± 3.17	38.11 ± 2.86
2	Frontal cortex	34.75 ± 2.03	32.49± 2.75	34.45 ± 3.40	37.11 ± 2.56	58.51± 2.47**	59.15± 3.15*	61.64 ± 2.16***	63.75 ± 2.31**
	Hippocampi	73.48 ± 5.87	75.74 ± 7.17	74.09 ± 6.92	71.53 ± 4.33	107.86 ± 7.43*	103.12 ± 4.49**	110.17 ± 9.12*	103.79 ± 6.17**
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	34.18 ± 2.78	32.96 ± 1.66	59.90 ± 2.15**	61.27 ± 3.32*	56.41 ± 3.09*	60.39 ± 3.11*
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	34.26 ± 3.73	37.05 ±2.63	56.78 ± 2.53**	50.69 ± 2.11**	53.37 ± 2.65*	51.37 ± 2.44*
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	32.70 ± 3.34	35.19 ± 1.85	35.22 ±2.90	35.13 ±2.23	37.91 ± 3.08	34.52 ± 2.97
3	Frontal cortex	34.75 ± 2.03	32.49± 2.75	34.45 ± 3.40	31.74 ± 1.69	44.38 ± 4.84	47.66 ± 2.75	49.81 ± 3.21	54.13 ± 3.37
	Hippocampi	73.48 ± 5.87	75.74 ± 7.17	74.09 ± 6.92	69.73 ± 4.77	90.28 ± 9.42	95.43 ± 7.66	88.60 ± 6.43	92.43 ± 8.72
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	34.18 ± 2.78	32.27 ± 3.03	42.93 ± 3.13	51.74 ± 3.43	45.59 ± 3.19	43.63 ± 2.36
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	34.26 ± 3.78	37.42 ± 1.94	42.10 ± 4.14	44.59 ± 3.17	46.85 ± 2.97	45.86 ± 4.05
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	32.70 ± 3.34	35.11 ± 2.37	38.55 ± 2.67	33.77 ± 3.24	35.63 ± 3.23	39.05 ± 2.78

Daily pre-treatment with CB_1 receptor antagonist, AM251 (1 or 2 mg/kg), did not significantly affect the elevated NGF contents, while, 3 mg/kg of AM251 showed a preventive effect ($P>0.05$ *vs*. the vehicle-treated groups). AM251 showed no effect by itself ($P>0.05$ *vs*. the vehicle group). NGF levels are expressed as ng of NGF per g of protein in the re-suspended NGF homogenate. Vehicles 1, 2 and 3 are related to ferulic acid, amitriptyline and AM251, respectively. Data are expressed as mean±SEM of n=6/group. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. the corresponding vehicle.

(AM/fer 130, AM/fer 150: injection of AM251 prior to the administration of ferulic acid 130 and 150 mg/kg, AM/ami 8, AM/ami 10: injection of AM251 before the administration of amitriptyline 8 and 10 mg/kg).

Table 6: The effects of SR144528 on the increased brain NGF levels induced by ferulic acid (130 and 150 mg/kg) or amitriptyline (8 and 10 mg/kg)

SR144528 (mg/kg)	Brain region	Vehicle 1	Vehicle 2	Vehicle 3	SR144528	SR/fer 130	SR/fer 150	SR/ami 8	SR/ami 10
1	Frontal cortex	34.75 ± 2.03	32.49± 2.75	35.13 ± 2.55	34.27 ± 3.05	63.28 ± 2.75**	59.86 ± 2.65**	61.23 ± 2.54**	57.19 ± 2.13**
	Hippocampi	73.48 ± 5.87	75.73 ± 7.17	69.74 ± 4.83	73.07 ± 6.17	117.33 ± 6.43**	102.13 ± 7.55*	119.11 ± 6.37**	104.59 ± 7.08*
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	32.45 ± 1.56	35.23 ± 3.03	50.06 ± 2.27*	55.24 ± 2.16*	62.36 ± 2.79*	63.11 ± 2.15*
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	36.13 ± 1.73	34.29 ± 1.77	54.11 ± 2.15*	50.17 ± 2.04*	53.07± 2.17*	57.09± 2.23*
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	31.55 ± 2.78	33.54 ± 2.33	35.243± 3.09	31.23 ± 1.95	39.27 ± 2.58	30.45 ± 1.23
2.5	Frontal cortex	34.75 ± 2.03	32.49± 2.75	32.44 ± 1.87	32.26 ± 2.64	53.13± 2.13*	52.28± 2.23*	64.19 ± 235**	60.32 ± 2.17**
	Hippocampi	73.48 ± 5.87	75.74 ± 7.17	73.11 ± 4.56	68.94 ± 6.13	113.45 ± 6.37*	105.65 ± 5.38*	113.45 ± 5.42**	116.23 ± 6.52*
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	33.52 ± 3.15	35.43 ± 2.76	57.43 ± 2.37*	64.09 ± 3.07*	50.17 ± 2.33*	63.56 ± 2.54*
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	30.15 ± 2.67	33.27 ±1.83	49.97 ± 1.54*	53.17 ± 2.18*	49.23 ± 2.06*	55.14 ± 2.16*
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	36.05 ± 1.73	32.56 ± 2.44	37.13 ±1.79	31.76 ±3.03	33.45 ± 1.76	37.19 ± 3.07
5	Frontal cortex	34.75 ± 2.03	32.49± 2.75	36.11 ± 2.76	35.04 ± 2.69	56.83 ± 2.25*	58.43 ± 2.14*	60.56 ± 3.08*	57.23 ± 2.25*
	Hippocampi	73.48 ± 5.87	75.74 ± 7.17	70.13 ± 7.03	75.13 ±5.73	109.65 ± 5.18**	97.59 ± 6.23*	119.14 ± 4.23**	107.54 ± 5.65*
	Amygdalae	31.57 ± 3.17	33.35 ± 2.99	36.42 ± 3.08	33.25 ± 2.85	51.39 ± 1.93*	70.13 ± 4.15*	55.36 ± 2.95*	59.25 ± 2.42*
	Olfactory bulbs	34.98 ± 2.96	31.84 ± 3.07	34.52 ± 1.55	33.53 ± 3.04	57.56 ± 1.25**	59.17 ± 2.67*	52.77 ± 3.17*	59.84 ± 1.87**
	Brain stem	32.96 ± 3.14	34.03 ± 2.89	30.87 ± 2.52	36.07 ± 3.17	33.29 ± 2.93	30.27 ± 1.76	39.13 ± 2.73	32.94 ± 3.18

Daily pre-treatment with CB_2 receptor antagonist, SR144528, did not significantly affect the elevated NGF contents induced by ferulic acid or amitriptyline ($P>0.05$ *vs*. ferulic acid- or amitriptyline-treated group). SR144528 showed no effect by itself ($P>0.05$ *vs*. the vehicle group). NGF levels are expressed as ng of NGF per g of protein in the re-suspended NGF homogenate. Vehicles 1, 2 and 3 are related to ferulic acid, amitriptyline and SR144528, respectively. Data are expressed as mean±SEM of n=6/group. $^{*}P<0.05$ and $^{**}P<0.01$ *vs*. the corresponding vehicle (SR/fer 130, SR/fer 150: injection of SR144528 prior to the administration of ferulic acid 130 and 150 mg/kg, SR/ami 8, SR/ami 10: injection of SR144528 before the administration of amitriptyline 8 and 10 mg/kg).

Table 7: The brain regional levels of endocannabinoids at baseline and after acute treatment with the highest dose of ferulic acid, amitriptyline, or lorazepam

Endocannabinoids	Brain regions	Baseline values	Vehicle 1	Vehicle 2	Vehicle 3	Ferulic acid	Amitriptyline	Lorazepam
A AEA (pM/g tissue)	Frontal cortex	9.27 ± 0.64	7.94 ± 0.56	8.25 ± 0.62	7.63 ± 0.42	11.74± 0.96	9.53± 0.72	10.36± 0.87
	Hippocampi	27.44 ±2.13	24.93 ±2.43	25.29± 1.79	27.05 ±2.17	31.43 ± 2.55	33.06 ± 1.85	26.08 ±1.63
	Amygdalae	8.56 ± 0.41	7.82 ± 0.52	8.04 ± 0.74	7.83 ± 0.46	9.17 ±0.56	8.54 ± 0.97	11.32± 1.07
	Olfactory bulbs	7.13 ± 0.39	7.63 ± 0.47	8.15 ± 0.64	8.09 ± 0.53	7.36±0.32	6.82 ± 0.54	8.19 ± 0.74
	Brain stem	6.92 ± 0.47	6.29 ± 0.37	6.54± 0.58	7.03 ± 0.68	6.53± 0.47	9.67 ± 0.43	7.53 ± 0.38
2-AG (nM/g tissue)	Frontal cortex	5.38 ± 0.33	5.07 ± 0.42	4.96 ± 0.32	5.32 ± 0.47	4.13 ±0.28	6.73 ±0.62	6.13 ± 0.47
	Hippocampi	9.22 ± 0.67	8.79± 0.74	8.73± 0.65	7.96 ± 0.38	11.42± 0.96	9.46 ±0.45	9.08± 0.77
	Amygdalae	7.59 ± 0.28	8.03 ± 0.55	7.86 ± 0.47	9.04 ± 0.77	9.46 ± 0.53	10.55 ± 0.63	7.94 ± 0.63
	Olfactory bulbs	5.49 ± 0.43	5.36 ± 0.37	5.07± 0.26	4.85 ± 0.32	4.29 ± 0.35	9.37± 0.44	7.42 ± 0.36
	Brain stem	4.61 ± 0.37	4.97± 0.53	5.11 ± 0.32	4.74 ± 0.55	6.45 ± 0.42	4.64 ± 0.53	5.76 ± 0.67
B AEA (pM/g tissue)	Frontal cortex	9.27 ± 0.64	9.13 ± 0.57	8.73 ± 0.44	6.83 ± 0.47	8.35 ±0.44	12.43 ± 0.93	9.32 ± 0.65
	Hippocampi	27.44 ±2.13	25.34 ±1.73	27.13 ±1.86	25.36 ±2.06	23.87± 1.27	24.56 ± 1.35	27.85 ±2.13
	Amygdalae	8.56 ± 0.41	7.68 ± 0.66	8.13 ± 0.64	9.28 ± 0.53	10.44± 0.94	9.72 ± 0.47	9.38± 0.73
	Olfactory bulbs	7.13 ± 0.39	8.32 ± 0.77	7.45 ± 0.37	9.43 ± 0.55	7.63 ±0.63	9.35 ± 0.77	11.34± 1.07
	Brain stem	6.92 ± 0.47	6.49 ± 0.37	7.03 ± 0.56	7.11 ± 0.34	9.03 ± 0.35	10.09 ± 0.54	9.32± 0.87
2-AG (nM/g tissue)	Frontal cortex	5.38 ± 0.33	5.23 ± 0.42	4.96 ± 0.33	4.63 ± 0.56	9.86± 0.35	4.93 ± 0.32	5.85 ± 0.63
	Hippocampi	9.22 ± 0.67	9.36 ± 0.65	8.72 ± 0.48	8.72 ± 0.44	12.33± 0.96	9.23 ± 0.45	8.33 ± 0.46
	Amygdalae	7.59 ± 0.28	7.53 ± 0.27	8.96 ± 0.57	9.07 ± 0.38	10.07 ± 0.63	11.08 ± 0.62	9.48± 0.53
	Olfactory bulbs	5.49 ± 0.43	5.11 ± 0.43	4.73 ± 0.26	4.79 ± 0.58	9.44 ±0.42	7.54 ± 0.47	6.77 ± 0.28
	Brain stem	4.61 ± 0.37	4.37 ± 0.35	4.88 ± 0.53	5.03 ± 0.42	5.23 ± 0.38	4.58 ± 0.53	4.38± 0.58
C AEA (pM/g tissue)	Frontal cortex	9.27 ± 0.64	8.93 ± 0.67	9.17 ± 0.85	9.03 ± 0.76	8.55 ±0.32	11.03 ± 0.94	9.32 ± 0.48
	Hippocampi	27.44 ±2.13	25.58 ±1.42	27.44 ±2.57	27.15 ±1.63	25.86 ± 2.29	24.48 ± 1.56	27.05 ±2.11
	Amygdalae	8.56 ± 0.41	8.13 ± 0.59	7.79 ± 0.38	7.13 ± 0.54	10.77 ± 1.43	9.87 ± 0.45	9.39 ± 0.83
	Olfactory bulbs	7.13 ± 0.39	7.04 ± 0.45	6.58± 0.29	6.83 ± 0.36	9.48 ± 0.66	7.13 ± 0.38	9.13 ± 0.75
	Brain stem	6.92 ± 0.47	6.85 ± 0.39	7.13 ± 0.72	7.05 ± 0.68	8.03 ± 0.72	7.68 ± 0.27	7.26 ± 0.63
2-AG (nM/g tissue)	Frontal cortex	5.38 ± 0.33	4.97 ± 0.36	5.13 ± 0.45	4.97 ± 0.25	9.33 ± 0.75	11.47 ± 1.18	7.11 ± 0.84
	Hippocampi	9.22 ± 0.67	9.13 ± 0.43	7.94 ± 0.66	9.33 ± 0.63	13.55 ± 1.07	9.84 ± 0.77	10.23± 0.49
	Amygdalae	7.59 ± 0.28	9.54 ± 0.66	8.1 ± 0.72	7.92 ± 0.38	11.37± 1.05	9.43 ± 0.59	7.36 ± 0.52
	Olfactory bulbs	5.49 ± 0.43	4.83 ± 0.29	5.32 ± 0.65	4.85 ± 0.57	8.42 ± 0.89	9.49 ± 1.04	5.13 ± 0.63
	Brain stem	4.61 ± 0.37	5.09 ± 0.62	4.88 ± 042	5.39 ± 0.44	5.63 ± 0.47	7.08 ± 0.58	4.26 ± 0.32

Data have been obtained 1 h (A), 5 h (B), or 12 h (C) after the injection. As shown, single injections of drugs even at the highest doses tested evoked no significant changes in the brain endocannabinoid contents as compared to the baseline values or vehicle-treated control groups. Vehicle 1, 2 and 3 are related to ferulic acid, amitriptyline and lorazepam, respectively. Data are expressed as mean±SEM of n=6/group.

Table 8: The effects of four-week treatment with lower doses of ferulic acid, amitriptyline or lorazepam on brain regional levels of AEA and 2-AG

Endocannabinoids	Brain regions	Vehicle 1	Vehicle 2	Vehicle 3	Ferulic acid (50 mg/kg)	Ferulic acid (100 mg/kg)	Amitriptyline (2.5 mg/kg)	Amitriptyline (5 mg/kg)	Lorazepam (2 mg/kg)	Lorazepam (5 mg/kg)
A AEA (pM/g tissue)	Frontal cortex	8.23 ± 0.68	8.79 ± 0.83	9.13 ± 0.57	10.37 ± 1.45	8.17± 1.97	11.48± 0.94	9.64± 0.55	7.45± 0.73	10.53± 0.49
	Hippocampi	26.97 ±1.83	28.17± 2.15	25.38 ±1.79	28.34 ± 2.07	30.07 ± 2.96	25.49 ± 1.04	28.77 ± 2.07	29.11 ±1.76	26.33 ±2.15
	Amygdalae	7.41 ± 0.59	7.84 ± 0.63	8.23 ± 0.57	9.76 ± 0.87	9.39 ±0.85	9.87 ± 0.67	7.93 ± 0.47	10.05± 0.65	8.45± 0.93
	Olfactory bulbs	8.27 ± 0.62	8.03 ± 0.49	7.59 ± 0.83	10.29 ±0.94	8.92±0.88	10.72 ± 0.93	11.04 ± 1.16	7.19 ± 0.32	10.23± 0.79
	Brain stem	6.74 ± 0.67	7.13 ± 0.59	6.63 ± 0.74	7.34± 0.45	9.56± 0.77	7.53 ± 0.86	9.32 ± 0.75	6.55 ± 0.67	9.87 ± 0.83
2-AG (nM/g tissue)	Frontal cortex	4.73 ± 0.37	5.29 ± 0.44	5.08 ± 0.39	5.48 ±0.29	9.76 ±0.64	7.55 ± 0.47	9.97 ±0.76	8.03 ± 0.54	8.27 ± 0.94
	Hippocampi	9.12 ± 0.63	9.36 ± 0.87	8.55 ± 0.47	12.03 ± 2.37	11.32± 0.85	9.72 ± 0.63	12.46± 1.85	10.23± 0.92	11.47± 1.03
	Amygdalae	7.59 ± 0.39	8.13 ± 0.58	7.68 ± 0.73	10.39 ± 0.86	11.78 ± 1.03	10.07 ± 1.13	7.34 ± 0.53	10.07± 1.22	9.56 ± 0.83
	Olfactory bulbs	5.27 ± 0.41	5.49± 0.47	4.72 ± 0.46	8.42 ± 0.62	8.94 ± 0.38	9.15 ± 0.86	5.33± 0.62	7.54 ± 0.73	5.77 ± 0.42
	Brain stem	4.65 ± 0.28	4.77 ± 0.52	4.16 ± 0.33	5.63 ± 0.44	7.92 ± 0.45	5.76 ± 0.33	4.86 ± 0.45	5.33 ± 0.29	4.69 ± 0.57
B AEA (pM/g tissue)	Frontal cortex	8.77 ± 0.64	9.13 ± 0.48	8.94 ± 0.93	12.07 ± 2.11	10.22 ±0.97	8.49 ± 0.76	12.03 ± 1.96	9.17 ± 0.72	7.38 ± 0.45
	Hippocampi	28.11 ±1.57	28.07 ±2.94	26.89 ±2.46	30.14 ± 2.87	27.45± 1.56	25.48 ± 2.04	29.33 ± 1.79	30.25 ±3.07	28.37±1.82
	Amygdalae	8.06 ± 0.75	7.59 ± 0.37	7.63 ± 0.55	7.52 ± 0.46	10.86± 1.07	7.75 ± 0.53	9.23 ± 0.38	8.74 ± 0.93	7.25± 0.36
	Olfactory bulbs	7.85 ± 0.69	8.23 ± 0.49	8.05 ± 0.72	11.03 ± 0.95	8.11 ±0.67	10.07 ± 0.93	7.39 ± 0.93	10.82± 1.03	9.38± 0.94
	Brain stem	7.14 ± 0.53	6.76 ± 0.33	6.49 ± 0.57	7.55 ± 0.72	8.53 ± 0.29	6.55 ± 0.35	6.23 ± 0.77	7.15± 0.82	6.53± 0.79
2-AG (nM/g tissue)	Frontal cortex	4.68 ± 0.29	5.27 ± 0.52	5.08 ± 0.43	9.23±0.67	10.03±1.85	7.45 ± 0.73	9.33 ± 0.54	7.64 ± 0.35	5.79 ± 0.44
	Hippocampi	8.75 ± 0.82	8.58 ± 0.73	9.11 ± 0.68	9.85 ± 0.95	9.43± 0.76	11.32 ± 1.07	7.42 ± 0.76	11.06± 0.94	7.85 ± 0.65
	Amygdalae	8.26 ± 0.55	7.34 ± 0.49	8.06 ± 0.72	12.38 ± 1.23	9.05 ± 0.43	9.06 ± 0.94	11.29 ± 1.45	7.42 ± 0.48	11.36± 1.27
	Olfactory bulbs	4.69 ± 0.48	5.48 ± 0.53	5.13 ± 0.27	7.24 ±0.35	6.78 ±0.65	6.45 ± 0.37	8.11 ± 0.43	7.13 ± 0.85	6.56 ± 0.83
	Brain stem	4.07 ± 0.45	4.27 ± 0.26	4.76 ± 0.39	6.03 ± 0.67	5.13 ± 0.27	4.38 ± 0.25	8.57 ± 0.92	9.08± 0.97	4.77± 0.49
C AEA (pM/g tissue)	Frontal cortex	9.14 ± 0.59	9.03 ± 0.73	8.79 ± 0.84	11.74 ±0.94	9.77 ±0.65	11.43 ± 1.67	9.45 ± 0.83	9.38 ± 0.54	10.24± 0.86
	Hippocampi	27.03 ±2.65	27.19 ±3.11	25.88 ±2.97	26.13 ± 1.86	30.11 ± 1.77	27.13 ± 2.69	24.33 ± 1.87	29.04 ±2.11	27.12 ±1.55
	Amygdalae	7.49 ± 0.34	8.14 ± 0.44	8.22 ± 0.78	10.43 ± 0.87	12.07 ± 1.69	9.66 ± 0.83	10.23 ± 0.97	9.36 ± 0.88	8.53 ± 0.43
	Olfactory bulbs	6.94 ± 0.53	7.25 ± 0.49	7.97 ± 0.72	6.75 ± 0.45	10.04 ± 1.64	7.44 ± 0.57	9.11 ± 0.83	7.86± 0.52	6.49 ± 0.27
	Brain stem	7.26 ± 0.69	7.97 ± 0.44	6.35 ± 0.29	9.22 ± 0.82	7.12 ± 0.48	10.06 ± 0.86	6.44 ± 0.35	9.17 ± 0.74	10.03± 0.94
2-AG (nM/g tissue)	Frontal cortex	5.27 ± 0.42	4.79 ± 0.23	4.58 ± 0.55	6.32 ± 0.57	5.48 ± 0.39	6.22 ± 0.78	6.43 ± 0.65	4.32 ± 0.28	4.98 ± 0.63
	Hippocampi	7.94 ± 0.78	7.69 ± 0.49	8.17 ± 0.74	10.21 ± 1.83	7.28 ± 0.47	7.13 ± 0.43	10.22 ± 1.08	7.87 ± 0.93	9.15± 0.97
	Amygdalae	8.22 ± 0.79	7.97 ± 0.65	8.06 ± 0.69	7.43± 0.76	9.13± 0.57	9.42 ± 0.89	9.34 ± 0.94	10.16± 1.13	7.56 ± 0.63
	Olfactory bulbs	5.58 ± 0.45	4.78 ± 0.53	5.33 ± 0.28	8.07 ± 0.93	5.87 ± 0.33	7.64 ± 0.82	7.45 ± 0.86	8.23 ± 0.95	4.92 ± 0.43
	Brain stem	4.89 ± 0.24	4.62 ± 0.37	5.17 ± 0.52	4.82 ± 0.58	7.13 ± 0.86	5.75 ± 0.64	4.96 ± 0.32	4.65 ± 0.88	6.04 ± 0.86

Data have been obtained 1 h (A), 5 h (B), or 12 h (C) after the last injection in four-week once-daily injection of ferulic acid, amitriptyline, lorazepam or the corresponding vehicles. Vehicle 1, 2 and 3 are related to ferulic acid, amitriptyline and lorazepam, respectively. Data are presented as mean±SEM (n=6/group).

Table 9: The effects of four-week treatment with ferulic acid (130 and 150 mg/kg), amitriptyline (8 and 10 mg/kg) or lorazepam (8 and 10 mg/kg) on brain regional contents of AEA and 2-AG

Endocannabinoids	Brain regions	Vehicle 1	Vehicle 2	Vehicle 3	Ferulic acid (130 mg/kg)	Ferulic acid (150 mg/kg)	Amitriptyline (8 mg/kg)	Amitriptyline (10 mg/kg)	Lorazepam (8 mg/kg)	Lorazepam (10 mg/kg)
A AEA (pM/g tissue)	Frontal cortex	8.23 ± 0.68	8.79 ± 0.83	9.13 ± 0.57	20.44 ± 2.34**	23.17± 2.11***	23.31± 2.17***	20.76± 1.83***	10.58± 0.77	13.78± 1.23
	Hippocampi	26.97 ±1.83	28.17± 2.15	25.38 ±1.79	41.17 ± 3.09**	39.94 ± 2.38**	38.45 ± 2.76**	43.27 ± 2.12***	25.39 ±2.41	21.94 ±2.03
	Amygdalae	7.41 ± 0.59	7.84 ± 0.63	8.23 ± 0.57	30.57 ± 3.17**	32.86 ±3.21**	27.81 ± 2.64**	25.11 ± 1.93***	8.45 ± 0.72	10.05± 1.11
	Olfactory bulbs	8.27 ± 0.62	8.03 ± 0.49	7.59 ± 0.83	24.04 ±2.93***	21.49±1.78***	25.15 ± 3.07**	29.83 ± 2.26***	9.11 ± 0.68	7.89 ± 0.43
	Brain stem	6.74 ± 0.67	7.13 ± 0.59	6.63 ± 0.74	9.24± 0.49	7.73± 0.33	9.77 ± 0.93	7.48 ± 0.55	7.19 ± 0.74	9.42 ± 0.66
2-AG (nM/g tissue)	Frontal cortex	4.73 ± 0.37	5.29 ± 0.44	5.08 ± 0.39	15.84 ±1.39***	13.45 ±1.33**	17.08 ± 1.42***	15.42 ±0.94***	7.45 ± 0.67	5.97 ± 0.38
	Hippocampi	9.12 ± 0.63	9.36 ± 0.87	8.55 ± 0.47	21.18 ± 3.17*	25.07± 2.83***	19.77 ± 1.25***	22.14± 1.33***	8.36 ± 0.49	10.28± 0.73
	Amygdalae	7.59 ± 0.39	8.13 ± 0.58	7.68 ± 0.73	16.29 ± 1.28**	19.36 ± 1.67**	17.39 ± 2.18**	15.48 ± 1.57**	9.03 ± 0.75	7.36 ± 0.49
	Olfactory bulbs	5.27 ± 0.41	5.49± 0.47	4.72 ± 0.46	13.58 ± 1.94**	11.92 ± 1.26**	16.08 ± 1.33***	13.63± 1.44**	4.47 ± 0.27	6.19 ± 0.23
	Brain stem	4.65 ± 0.28	4.77 ± 0.52	4.16 ± 0.33	4.09 ± 0.37	4.68 ± 0.23	5.21 ± 0.48	5.77 ± 0.29	5.26 ± 0.44	8.03 ± 0.29
B AEA (pM/g tissue)	Frontal cortex	8.77 ± 0.64	9.13 ± 0.48	8.94 ± 0.93	21.39 ± 2.86**	18.86 ±1.92***	23.92 ± 2.43***	27.13 ± 1.97***	9.87 ± 0.45	8.43 ± 0.79
	Hippocampi	28.11 ±1.57	28.07 ±2.94	26.89 ±2.46	42.07 ± 3.17**	40.83± 2.98**	39.28 ± 2.26*	42.28 ± 1.43***	29.42 ±2.87	25.11 ±1.65
	Amygdalae	8.06 ± 0.75	7.59 ± 0.37	7.63 ± 0.55	20.17 ± 2.83**	25.34± 2.11***	24.32 ± 2.03***	26.06 ± 2.18***	8.13 ± 0.73	11.09± 0.66
	Olfactory bulbs	7.85 ± 0.69	8.23 ± 0.49	8.05 ± 0.72	20.08 ± 3.14*	23.31 ±2.65***	16.23 ± 1.95*	14.87 ± 0.68**	9.82 ± 0.67	13.25± 1.32
	Brain stem	7.14 ± 0.53	6.76 ± 0.33	6.49 ± 0.57	9.13 ± 0.83	7.45 ± 0.64	9.37 ± 0.49	6.89 ± 0.35	11.59± 0.93	8.62± 0.73
2-AG (nM/g tissue)	Frontal cortex	4.68 ± 0.29	5.27 ± 0.52	5.08 ± 0.43	18.24±1.29***	22.19±2.76**	15.86 ± 1.97**	20.06 ± 2.47**	4.98 ± 0.43	7.13 ± 0.69
	Hippocampi	8.75 ± 0.82	8.58 ± 0.73	9.11 ± 0.68	17.84 ± 1.93**	20.67± 1.23***	21.18 ± 2.83**	18.76 ± 1.59**	9.48 ± 0.72	7.21 ± 0.33
	Amygdalae	8.26 ± 0.55	7.34 ± 0.49	8.06 ± 0.72	16.48 ± 1.76**	14.93 ± 1.59*	15.43 ± 2.27*	18.38 ± 1.23**	9.56 ± 0.63	13.42± 1.15
	Olfactory bulbs	4.69 ± 0.48	5.48 ± 0.53	5.13 ± 0.27	14.43 ±1.28***	19.21 ±2.35**	15.08 ± 2.18*	12.08 ± 1.73*	5.33 ± 0.35	8.12 ± 0.75
	Brain stem	4.07 ± 0.45	4.27 ± 0.26	4.76 ± 0.39	5.77 ± 0.45	4.82 ± 0.53	4.92 ± 0.49	6.38 ± 0.37	4.48± 0.53	6.19± 0.27
C AEA (pM/g tissue)	Frontal cortex	9.14 ± 0.59	9.03 ± 0.73	8.79 ± 0.84	16.83 ±1.28**	13.42 ±0.94*	15.69 ± 1.53*	13.94 ± 0.78*	9.13 ± 0.72	8.47 ± 0.45
	Hippocampi	27.03 ±2.65	27.19 ±3.11	25.88 ±2.97	37.44 ± 2.27*	39.21 ± 1.43**	39.26 ± 2.54*	32.72 ± 1.44**	27.95 ±3.07	23.62 ±1.39
	Amygdalae	7.49 ± 0.34	8.14 ± 0.44	8.22 ± 0.78	17.76 ± 2.13*	23.03 ± 1.87**	19.72 ± 2.87*	22.58 ± 2.32**	9.11 ± 0.66	7.69 ± 0.53
	Olfactory bulbs	6.94 ± 0.53	7.25 ± 0.49	7.97 ± 0.72	16.23 ± 1.86**	14.79 ± 1.54*	13.94 ± 2.42*	17.09 ± 1.13**	10.25± 1.03	7.25 ± 0.45
	Brain stem	7.26 ± 0.69	7.97 ± 0.44	6.35 ± 0.29	8.73 ± 0.56	6.63 ± 0.48	7.13 ± 0.59	9.27 ± 0.84	6.55 ± 0.27	8.19 ± 0.32
2-AG (nM/g tissue)	Frontal cortex	5.27 ± 0.42	4.79 ± 0.23	4.58 ± 0.55	10.48 ± 0.64*	14.29 ± 0.55**	11.69 ± 0.92*	13.27 ± 1.09*	9.17 ± 0.48	6.33 ± 0.75
	Hippocampi	7.94 ± 0.78	7.69 ± 0.49	8.17 ± 0.74	17.27 ± 1.79*	15.49 ± 0.43**	14.87 ± 1.16*	18.03 ± 0.92**	7.16 ± 0.39	11.08± 0.66
	Amygdalae	8.22 ± 0.79	7.97 ± 0.65	8.06 ± 0.69	11.52± 0.94*	15.27± 0.88*	14.29 ± 1.09*	12.08 ± 0.96*	7.99 ± 0.42	9.28 ± 0.67
	Olfactory bulbs	5.58 ± 0.45	4.78 ± 0.53	5.33 ± 0.28	10.27 ± 0.99*	13.73 ± 0.57*	12.69 ± 1.22**	16.29 ± 1.58**	6.07 ± 0.73	8.13 ± 0.79
	Brain stem	4.89 ± 0.24	4.62 ± 0.37	5.17 ± 0.52	7.13 ± 0.66	4.47 ± 0.29	6.24 ± 0.43	5.38 ± 0.37	7.24 ± 0.39	4.93 ± 0.43

Data have been obtained 1 h (A), 5 h (B), or 12 h (C) after the last injection in four-week once-daily injection of ferulic acid, amitriptyline, lorazepam or the corresponding vehicles. Vehicle 1, 2 and 3 are related to ferulic acid, amitriptyline and lorazepam, respectively. Data are presented as mean ± SEM (n=6/group). $^{*}P<0.05$, $^{**}P<0.01$, and $P<0.001$ vs. the respective controls.

vehicle-treated group (Table 4; *P*>0.05).

The effect of CB_1 or CB_2 receptor antagonist on the elevated brain NGF levels induced by ferulic acid or amitriptyline

Following daily pre-treatment with 1 or 2 mg/kg AM251, brain NGF contents remained significantly elevated (Table 5; *P*<0.05, *P*<0.01 and *P*<0.001), while, 3 mg/kg of AM251 prevented the enhancement of NGF induced by ferulic acid or amitriptyline (Table 5; *P*>0.05 *vs*. the vehicle-treated groups). AM251 (3 mg/kg) showed no effect by itself (Table 5, *P*>0.05 *vs*. the vehicle group). Pre-application of the CB_2 receptor antagonist, SR144528, did not significantly affect the elevated brain NGF contents induced by ferulic acid or amitriptyline (Table 6, *P*>0.05 *vs*. ferulic acid- or amitriptyline-treated group). SR144528 showed no effect by itself (Table 6, (*P*>0.05 *vs*. the vehicle group).

The effects of ferulic acid, amitriptyline or lorazepam on brain regional levels of AEA and 2-AG

one hour after the acute treatment with ferulic acid, amitriptyline or lorazepam, the brain regional eCB contents did not significantly differ from those of baseline values or vehicle-treated groups (Table 7, *P*>0.05). Similar findings were obtained 5 or 12 h after the acute treatment (Table 7, *P*>0.05). Four-week once-daily administration of the lower doses of ferulic acid, amitriptyline or lorazepam did not significantly affect brain eCB contents at any time point tested (Table 8, *P*>0.05), while, four-week exposure to 130 and 150 mg/kg of ferulic acid or 8 and 10 mg/kg of amitriptyline led to a brain region-specific elevation of AEA and 2-AG contents at 1 h from the last injection (Table 9A, *P*<0.01 and *P*<0.001) that lasted up to 5 h (Table 9B, *P*<0.05, *P*<0.01 and *P*<0.001) and 12 h (Table 9C, *P*<0.05 and *P*<0.01). The main effects and interaction between the factors are as follows: {frontal cortex (AEA): [dose: F(2,270)=123.51, *P*<0.001], [treatment: F(5,270)=36.09, *P*<0.001], [time point: F(2,270)=0.16, *P*=0.85], [dose × treatment: F(10,270)=40.31, *P*<0.001], [dose × time point × treatment: F(20,270)=0.13, *P*=1]; frontal cortex (2-AG): [dose: F(2,270)=108.27, *P*<0.001], [treatment: F(5,270)=99.81, *P*<0.001], [time point: F(2,270)=2.04, *P*=0.13], [dose × treatment: F(10,270)=27.65, *P*<0.001], [dose × time point × treatment: F(20,270)=0.93, *P*=0.56]; hippocampi (AEA): [dose: F(2,270)=240.11, *P*<0.001], [treatment: F(5,270)=22.93, *P*<0.001], [time point: F(2,270)=0.27, *P*=0.93], [dose × treatment: F(10,270)=13.16, *P*<0.001], [dose × time point × treatment: F(20,270)=0.17, *P*=0.94]; hippocampi (2-AG): [dose: F(2,270)=80.74, *P*<0.001], [treatment: F(5,270)=27.30, *P*<0.001], [time point: F(2,270)=0.71, *P*=0.49], [dose × treatment: F(10,270)=27.79, *P*<0.001], [dose × time point × treatment: F(20,270)=1.36, *P*=0.14]; amygdalae (AEA): [dose: F(2,270)=126.24, *P*<0.001], [treatment: F(5,270)=39.28, *P*<0.001], [time point: F(2,270)=0.43, *P*=0.65], [dose × treatment: F(10,270)=37.07, *P*<0.001], [dose × time point × treatment: F(20,270)=0.89, *P*=0.59]; amygdalae (2-AG): [dose: F(2,270)=99.59, *P*<0.001], [treatment: F(5,270)=17.31, *P*<0.001], [time point: F(2,270)=2.09, *P*=0.13], [dose × treatment: F(10,270)=15.67, *P*<0.001], [dose × time point × treatment: F(20,270)=1.19, *P*=0.27]; olfactory bulbs (AEA): [dose: F(2,270)=81.17, *P*<0.001], [treatment: F(5,270)=20.75, *P*<0.001], [time point: F(2,270)=0.39, *P*=0.67], [dose × treatment: F(10,270)=16.39, *P*<0.001], [dose × time point × treatment: F(20,270)=0.58, *P*=0.84], olfactory bulbs (2-AG): [dose: F(2,270)=162.15, *P*<0.001], [treatment: F(5,270)=60.17, *P*<0.001], [time point: F(2,270)=1.09, *P*=0.34], [dose × treatment: F(10,270)=31.79, *P*<0.001], [dose × time point × treatment: F(20,270)=0.49, *P*=0.97], brain stem (AEA): [dose: F(2,270)=2.36, *P*=0.09], [treatment: F(5,270)=1.25, *P*=0.29], [time point: F(2,270)=1.89, *P*=0.15], [dose × treatment: F(10,270)=0.88, *P*=0.55], [dose × time point × treatment: F(20,270)=0.40, *P*=0.99]; brain stem (2-AG): [dose: F(2,270)=0.06, *P*=0.94], [treatment: F(5,270)=0.99, *P*=0.43], [time point: F(2,270)=1.23, *P*=0.19], [dose × treatment: F(10,270)=0.27, *P*=0.92], [dose × time point × treatment: F(20,270)=0.47, *P*=0.99]. Lorazepam even at the highest doses tested did not significantly affect brain eCB contents (Table 9, *P*>0.05 vs. the vehicle-treated group).

Discussion

Research efforts on the health benefits of herbs have shown the ability of plants to synthesize a variety of

compounds which interact with biological pathways (Tapsell et al., 2006). Based on the limited efficacy or significant side effects of the classical antidepressants (Ereshefsky, 2009; Santarsieri and Schwartz, 2015), increasing research efforts have been attracted towards the development or identification of novel therapeutic agents including the natural antidepressants. Over the last decade, involvement of the neurotrophic factors in the mechanism of action of antidepressants has been the focus of intense research (Berton and Nestler, 2006; Yanpallewar et al., 2010; Shaltiel et al., 2007). In this study, we have found that ferulic acid similar to the conventional antidepressant, amitriptyline, is able to induce a sustained enhancement of NGF protein levels in brain region-specific fashion (Table 3). The elevation of brain NGF levels after four-week treatment suggests that the occurrence of neurotrophic effects is a slow-developing process that is in agreement with Yanpallewar et al. (2010) who have suggested that the increased expression of a trophic factor is a slow-onset adaptive change. The sustained enhancement of NGF contents in the brain regions involved in the regulation of emotional activity may be considered as a mechanism by which ferulic acid like amitriptyline exerts its antidepressant therapeutic effect. According to the stimulatory effect of NGF on cell proliferation (Cheng et al., 2009), ferulic acid- or amitriptyline-induced elevation of NGF in the frontal cortex might be of therapeutic value against the stress-induced reduction in cell proliferation in the frontal cortex (Banasr et al., 2007). As shown in Table 3, four-week administration of amitriptyline or ferulic acid resulted in a significant enhancement of NGF content in the hippocampus. Since NGF is critically involved in the hippocampal plasticity and neurogenesis (Conner et al., 2009) as well as cognitive function (Winkler et al., 2000), it seems that ferulic acid or amitriptyline via the elevation of hippocampal NGF induces hippocampal neurogenesis that may lead to the improved cognitive function. Ferulic acid or amitriptyline significantly elevated NGF protein level in the amygdala after four-week daily treatment (Table 3). This, may result in the improved cognitive performance due to the facilitatory role of NGF on the cholinergic neurotransmission between the nucleus basalis and amygdala (Moises et al., 1995).

Ferulic acid like amitriptyline significantly increased NGF level in the olfactory bulbs (Table 3). According to the pivotal role of NGF in the maintenance or development of the olfactory system (Miwa et al., 2002), ferulic acid or amitriptyline may significantly affect the function of this system. As shown in Table 3, neither ferulic acid nor amitriptyline affected NGF production in the brain stem suggesting that NGF signaling in this brain region is not implicated in the mechanisms of action of these antidepressants.

Four-week administration of lorazepam even at the highest dose tested did not significantly alter the brain regional levels of NGF (Table 3) suggesting that the ineffectiveness of lorazepam against the depression is, at least in part, due to its inability to affect brain NGF.

In an attempt to identify a mechanism by which ferulic acid or amitriptyline affects the brain NGF levels, we focused on the implication of the eCBs which its role in the treatment of neuropsychiatric diseases and interaction with neurotrophic factors have been well-documented (Bambico et al., 2009; Hassanzadeh and Hassanzadeh, 2011; Khaspekov et al., 2004; Marsicano and Lutz, 2006; Williams et al., 2003). Pretreatment with CB_1 receptor antagonist, AM251 (3 mg/kg), prevented the elevation of brain NGF contents induced by ferulic acid or amitriptyline (Table 4), while, CB_2 receptor antagonist, SR144528, failed to show preventive effects (not shown). These findings indicate that ferulic acid or amitriptyline elevates brain NGF content under the regulatory drive of CB_1 receptors.

In order to further explore the implication of eCB transmission in the mechanisms of action of ferulic acid, amitriptyline, or lorazepam, the brain regional levels of two major endocannabinoids, AEA and 2-AG, were measured at various time points after acute and four-week treatment with abovementioned drugs. As shown in Table 6, four-week once-daily treatment with the highest doses of ferulic acid or amitriptyline led to a sustained enhancement of AEA and 2-AG levels in the brain regions implicated in the modulation of emotional behaviour and synaptic plasticity. Our findings demonstrate the involvement of the intrinsic endocannabinoid tone -with a well-established regulatory role in the emotional states and neuroplasticity (Marsicano and Lutz, 2006; Viveros et al., 2007)-, in the mechanism of action of ferulic acid or amitriptyline. Four-week daily administration of the benzodiazepine and lorazepam

even at the highest doses tested did not significantly alter brain AEA and 2-AG contents (Table 6) suggesting that the eCB transmission is not involved in the mechanism of action of lorazepam.

As may be compared in Tables 3 and 6, NGF and endocannabinoids were elevated in the same brain regions following treatment with ferulic acid or amitriptyline. It appears that a balance between the endocannabinoid and NGF signalling should exist for the activities of these compounds.

As aforementioned, various molecular targets and intracellular signalling pathways mediate the therapeutic effects of ferulic acid. Besides its intrinsic radical scavenging activity (Sultana et al., 2005), ferulic acid down-regulates the expression of inducible nitric oxide (NO) synthase gene and inhibits the production of NO (Koh, 2012a), pro-inflammatory cytokines (Sadar et al., 2016) and elevates the activity of heme oxygenase (Ma et al., 2011), an enzyme which plays an important role in the neuroprotective processes (Chen, 2014). Regarding the antidepressant-like effect of ferulic acid, involvement of the norepinergic, serotonergic and antioxidant defense systems, extracellular signal-regulated kinase cascade has been previously shown (Chen et al., 2015; Lenzi et al., 2015; Zeni et al., 2012). Based on our findings, brain NGF and eCB signalling are also involved in the mechanism of action of ferulic acid.

Conclusion

In conclusion, we have found that ferulic acid like the classical antidepressant, amitriptyline, is able to stimulate the production of brain NGF and endocannabinoids that might be of therapeutic significance in a variety of diseases which are associated with abnormal eCB or NGF signalling. Furthermore, the cannabinoid CB_1 receptors mediate the brain NGF production suggesting, once again, NGF-eCBs interplay and the potential applicability of this interaction to develop more effective therapeutic agents against the neuropsychiatric disorders.

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Conflict of interest

None of the authors has any conflict of interest to disclose.

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