Neuroscience Letters 611 (2016) 106–111

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Lipopolysaccharide induced anxiety- and depressive-like behaviour in mice are prevented by chronic pre-treatment of esculetin

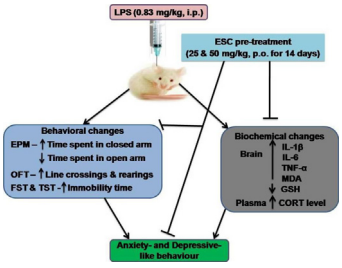
Kunjbihari Sulakhiya[a,*], Gohil Pratik Keshavlal[a], Babul B. Bezbaruah[c], Shubham Dwivedi[a], Satendra Singh Gurjar[b], Nitin Munde[a], Ashok Jangra[a], Mangala Lahkar[c], Ranadeep Gogoi[b]

[a] Laboratory of Neuroscience, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, Assam 781032, India
[b] Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, Assam 781032, India
[c] Department of Pharmacology, Gauhati Medical College, Guwahati, Assam 781032, India

HIGHLIGHTS

- ESC pre-treatment attenuated LPS-induced anxiety- & depressive-like behaviour in mice.
- LPS-induced elevated brain IL-1β, IL-6, and TNF-α level were reversed by ESC.
- Plasma CORT level was also decreased by ESC pre-treatment in LPS challenged mice. Chronic pre-treatment of ESC prevented LPS evoked oxidative stress in mouse brain.
- ESC could be effective in psychiatric disorders associated with neuroinflammation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 2 October 2015
Accepted 20 November 2015
Available online 24 November 2015

Keywords:
Anxiety
Depression
Esculetin
Lipopolysaccharide
Neuroinflammation
Oxidative stress

ABSTRACT

Inflammation and oxidative stress are involved in the pathophysiology of anxiety and depression. Esculetin (ESC), a coumarin derived potent antioxidant, also possessing anti-inflammatory and neuroprotective activity. This study investigated the effect of ESC in lipopolysaccharide (LPS)-induced anxiety- and depressive-like behaviour in mice. ESC (25 and 50 mg/kg, p.o.) was administered daily for 14 days, and challenged with saline or LPS (0.83 mg/kg; i.p.) on the 15th day. Behavioural paradigms such as elevated plus maze (EPM), open field test (OFT), forced swim test (FST) and tail suspension test (TST) were employed to assess anxiety- and depressive-like behaviour in mice post-LPS injection. Hippocampal cytokines, MDA and GSH level, and plasma corticosterone (CORT) were measured. ESC pre-treatment significantly ($P < 0.05$) attenuated LPS-induced anxiety-like behaviour by modulating EPM and OFT parameters. Moreover, LPS-induced increase in immobility time in FST and TST were also prevented significantly ($P < 0.05$) by ESC (50 mg/kg). ESC pre-treatment ameliorated LPS-induced neuroinflammation by attenuating brain IL-1β, IL-6, TNF-α level, and oxidative stress as well as plasma CORT level. In conclusion, the results suggest that ESC prevented LPS-induced anxiety- and depressive-like behaviour which may be governed by inhibition of cytokine production, oxidative stress and plasma CORT level. The results support the potential usefulness of ESC in the treatment of psychiatric disorders associated with inflammation and oxidative stress.

* Corresponding author. Fax: +91 3612337700.
E-mail addresses: niperkunj@gmail.com, kunj_pharma@yahoo.co.in (K. Sulakhiya).

http://dx.doi.org/10.1016/j.neulet.2015.11.031
0304-3940/

1. Introduction

According to WHO, the mental disorders are affecting approximately 450 million people out of which 10–20 million committing suicide every year globally [1]. In India, the lifetime prevalence of anxiety and depression is 18.7% and 24.4%, respectively, and co-morbidity of anxiety with depression is high about 87% [2]. Clinically, various drugs are used to treat anxiety and depression, but treatment outcome is not satisfactory due to low efficacy, delayed action, adverse effects of medicines as well as poor patient compliance [3,4]. These considerations implicate the search for novel anxiolytic and antidepressant agents having higher efficacy and lower toxicity with rapid onset of action.

Converging lines of evidence suggest that pro-inflammatory conditions including infection, chronic disease, immunotherapy and resulting cytokines are involved in the pathophysiology of affective disorders [5,6]. Increased level of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) in serum or plasma have been found in depressed individuals [7]. Furthermore, increased levels of circulating pro-inflammatory cytokines lead to hyperactivation of the HPA axis [8].

Numerous studies indicate that oxidative stress plays important role in the pathophysiology of anxiety and depression [4,9,10]. Increased lipid peroxidation, altered levels of antioxidant defences, such as glutathione (GSH), DNA damage and reduction in the level of antioxidative enzyme activities have been reported [7,9–11]. Thus, targeting oxidative stress with strong antioxidants could be a promising approach to offer protection against anxiety and depression.

Vaccines or bacterial endotoxins (lipopolysaccharide or LPS) are most commonly employed for activation of immune-inflammatory pathway to recognize neurobiological mechanisms linking inflammation to anxiety and depression, in both healthy human volunteers and in rodents [12]. Systemic administration of LPS activates the innate immune system triggered by the release of pro-inflammatory cytokines (e.g. IL-1β, IL-6, and TNF-α) both in the periphery and brain [4,13] and exhibits anxiety- and depressive-like behaviour in animals [11,12,14].

Esculetin (6,7-dihydroxy-2H-1-benzopyran-2-one; ESC), a coumarin derivative, is isolated from various plant species such as *Artemisia scoparia, Artemisia capillaries*, *Ceratostiggma willmottianum*, and *Citrus limonia* that are used as folk medicines [15,16]. ESC possessing diverse biological activities, such as antioxidant [15,17], anti-inflammatory [18], anti-proliferative [19], antidepressant and cognitive enhancer [22]. The potent antioxidant effect of ESC is attributed to its free radical scavenging, reduction of superoxide anion generation and lipid peroxidation activity, as well as its antioxidant enzyme restoring, GSH/GSSG ratio augmenting and cytochrome c oxidase (COX) increasing activity which collectively prevents oxidative damage [17,20–22]. It showed neuroprotective effects against NMDA-induced neurotoxicity in cultured primary cortical neurons, middle cerebral artery occlusion induced cerebral ischemia/reperfusion (I/R) injury and MPTP induced Parkinson's disease in mice [23,24]. ESC, is nontoxic at low doses, can cross the blood-brain barrier easily that makes it an ideal candidate for the brain disorders [24]. Thus, based on the anti-inflammatory, antioxidant and neuroprotective activity of ESC, the present study was initiated to evaluate the effect of chronic pre-treatment of ESC against LPS-induced anxiety- and depressive-like behaviour in mice.

2. Materials and methods

2.1. Materials

Lipopolysaccharide from *Escherichia coli* (L-3129, serotype 0127:B8) and esculetin were purchased from Sigma–Aldrich, St. Louis, MO, USA. IL-1β, IL-6, and TNF-α immunoassay kits were purchased from Invitrogen Co., Carlsbad, CA, USA. Corticosterone ELISA kit was purchased from Abnova Corp., Taiwan. All other chemicals were of analytical grade unless mentioned otherwise.

2.2. Animals

Adult male Swiss albino mice (Weighing, 22–28 g) were obtained from Pasteur institute, Shillong, Meghalaya, India. The animals were housed and acclimatised for two weeks under controlled environment (Temperature—22 ± 1°C and 12 h light/dark cycles) prior to experimentation, and the same conditions were maintained throughout the experiment. Standard laboratory animal feed (Pranav Agro Industries Ltd., Pune, India) and water were provided ad libitum. All the experiments were conducted between 10.00 and 17.00 h, in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Gauhati Medical College and Hospital, Guwahati, Assam, India.

2.3. Drug treatment

On the day of administration fresh solutions of LPS and ESC were prepared from 1 mg/ml stock solutions. The doses of ESC (25 and 50 mg/kg, BW) were selected based on previous studies conducted regarding its protective role in disease conditions [15,16,22]. ESC, dissolved in 0.5% Carboxy Methyl Cellulose (CMC), was orally administered once daily for 14 days prior to, and on the same day of LPS injection. LPS was dissolved in sterile, endotoxin-free normal saline (0.9% w/v NaCl) and injected intraperitoneally at the dose of 0.83 mg/kg of body weight [4]. Both LPS and ESC were administered at the dose level of 10 ml/kg.

2.4. Experimental design

Animals were randomly divided into six experimental groups ($n = 8$) for behavioural and biochemical assessment. The group I and II were treated with vehicle (0.5% CMC, p.o.) for 14 days and then challenged with Saline and LPS (0.83 mg/kg, i.p.) respectively on the 15th day. Group I served as a vehicle control group while Group II served as a LPS control group. Group III and IV were treated orally with ESC at the doses of 25 and 50 mg/kg respectively, for 14 days and then challenged with LPS on the 15th day. Group V and VI were treated orally with ESC at the doses of 25 and 50 mg/kg respectively, for 14 days and then challenged with Saline on the 15th day.

Anxiety-like behaviour was assessed by EPM and OFT 3 h and 4 h, respectively post-LPS or saline challenge. Depressive-like behaviour was assessed by FST and TST after 24 and 28 h, respectively post-LPS or Saline challenge. Behavioural and biochemical analysis were performed on different group of animals to avoid the possible consequences of behavioural testing on biochemical parameters. The animals used for biochemical estimation were sacrificed by decapitation after 24 h of saline or LPS challenge, the hippocampus was quickly removed on an ice-cold metal plate, and