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Original article

Effect of embelin against 3-nitropropionic acid-induced Huntington's disease in rats

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ABSTRACT

3-Nitropropionic acid (3-NP) causes severe neurotoxicity in animals, which depicts Huntington's disease (HD) in humans. Embelin, the main active constituent of *Embelia ribes*, has been reported to possess various pharmacological actions, mainly anti-inflammatory, antioxidant, anticonvulsant and neuroprotective. The aim of the present study was to evaluate the neuroprotective effect of embelin against 3-NP induced experimental HD in rats. Adult Wistar rats were pretreated with vehicle/embelin (10 and 20 mg/kg p.o.) for 7 days. From 8th day onwards, embelin was co-treated with 3-NP (15 mg/kg, i.p.) for 7 days. At the end of the treatment schedule, animals were evaluated for behavioral alterations and brain homogenates were used for estimation of oxidative stress parameters (lipid peroxidation, reduced glutathione, catalase and glutathione-S-transferase). 2,3,5-Triphenyl tetrazolium chloride (TTC) stained brain slices were used for lesion size measurement. Administration of 3-NP significantly altered the behavioral and neuronal antioxidant status and caused significant neuronal damage in striatal region. Embelin, at both the tested doses, caused a significant reversal of behavioral and antioxidant status alterations and reversed the striatal neuronal damage induced by 3-NP. These findings suggest the neuroprotective effect of embelin against HD. The observed protective effect might be attributed to the antioxidant properties of embelin.

1. Introduction

Huntington's disease (HD) is a progressive and fatal neurological disorder, characterized by clinical triad movement disorder, dementia and psychiatric disturbance due to striatal-specific neuronal degeneration [1]. Although the exact cause of neuronal death in HD remains unknown, it has been postulated that the abnormal aggregation of the mutant huntingtin protein may cause toxic effects in neurons, leading to a cascade of pathogenic mechanisms associated with transcriptional dysfunction, oxidative stress, mitochondrial alterations, apoptosis, bioenergetic defects and subsequent excitotoxicity [2]. HD patients often exhibit deficits in executive tasks requiring planning, cognitive flexibility and problem solving. HD poses challenges for health and social-care professionals due to its complexity and unpredictability. With an incidence of 2–10 per 100,000, HD afflicts 30,000 people in USA and another 250,000 persons are genetically at risk [1].

Several animal models exists for HD such as stereotactic injection of kainic, quinolinic and ibotenic acids into specific region of the brain, but systemic administration of 3-nitropropionic acid (3-NP) is the recent and popularly used one [3,4]. 3-NP crosses the blood–brain barrier and at cellular level it is an irreversible inhibitor of the electron transport enzyme succinate dehydrogenase (SDH), a mitochondrial complex II enzyme, responsible for the oxidation of succinate to fumarate in Kreb's cycle. Subsequently it blocks the transport of electrons in oxidative phosphorylation, causing decreased ATP levels in brain. It affects normal brain electrical activity and oxidative stress has been suggested to play a role in 3-NP toxicity. Neurons are metabolically highly active hence, processes that affect the mitochondrial function invariably leads to neuronal death [5,6]. Thus, a major factor in 3-NP toxicity could be because of cellular and mitochondrial stress [7]. Accumulating data indicates that 3-NP produces free radicals and consequent disturbance of glutathione redox cycle [8] and the inflammation associated with 3-NP, also acts as a contributing

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factor for neuronal damage and further free radical generation [6]. In addition to striatum, the major site of toxicity, 3-NP also causes severe damage to the neurons present in other regions of brain like hippocampus, cortex and hypothalamus [9].

Natural products are important resources for the development of novel drug leads, which help to treat neurodegenerative diseases such as HD. Several natural products are reported to reduce toxicity in HD models including *Calendula officinalis* [10], *Withania somnifera* root extract [11], ginsenosides [12], Lig-8, a lignophenol derivative from *Bamboo lignin* [13], green tea (−)-epigallocatechin-gallate [14] and Sesamol from *Sesamum indicum* [15]. Many of the quininone and benzoquinone derivatives are also found to be useful in the pharmacotherapy for HD. Coenzyme Q_{10}, a naturally occurring benzoquinone derivative enhances mitochondrial complex I activity and its supplementation to HD patients may reduce impaired mitochondrial function in HD [16]. Geldanamycin, a benzoquinone ansamycin is known to activate a heat shock response and inhibit huntingtin aggregation in cell culture model of HD [17]. Idebenone, a quinone derivative was studied in one hundred patients with clinically diagnosed HD [18].

Many plant extracts, formulations and phytoconstituents with antioxidant and anti-inflammatory properties, and those possessing anticonvulsant activity have been reported to protect neuronal damage in different experimental models [10,19–22].

Embelia ribes Burm. (Family: Myrsinaceae) is a medicinal plant used traditionally as anti-inflammatory agent to relieve rheumatism and fever [23]. Its fruits are used as brain tonic, in the treatment of mental disorders, dyspnoea, diseases of the heart, etc. [24]. *E. ribes* is an important ingredient of a number of ayurvedic formulations in India [25]. Bhandari et al., have reported the dyslipidemic and antioxidant activity of ethanolic extract of *E. ribes* in streptozotocin-induced diabetes in rats [26]. Its aqueous [27] and ethanolic extracts [28] were reported for their potent neuroprotective effects.

Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone, Fig. 1) is a naturally occurring alkyl substituted hydroxy benzoquinone and a major constituent from all the parts of *E. ribes*. Embelin is reported to possess anti-inflammatory, analgesic [29], antioxidant [30], antidiabetic activities [31], anticonvulsant [32]. Neuroprotective effect of embelin against transient global ischemia in rats were reported by us [22]. Based on the above bases, the present study was designed to investigate the neuroprotective potentials of embelin against sub chronic administration of 3-NP induced Huntington's disease in rats.

2. Experimental

2.1. Chemicals

1-Chloro-2,4-dinitrobenzene (CDNB) and 3-NP are obtained from Sigma–Aldrich, St. Louis, USA. Reduced glutathione (GSH), 5,5′-dithiobis-2-nitrobenzoic acid, thiobarbituric acid, trichloroacetic acid and 2,3,5-triphenyl tetrazolium chloride (TTC) were purchased from Hi-Media Laboratories Pvt., Ltd., Mumbai, India. All the other chemicals used were of analytical grade.

2.2. Isolation of embelin

The berries of *E. ribes* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India. The plant specimen was authenticated by Prof. K. Siddappa, Department of Botany, Sree Siddaganga College of Arts, Science and Commerce, Tumkur, Karnataka, and kept in the college herbarium (SSCP11PC0013). Embelin was isolated according to our previously published protocol [32] and purity was matched with authentic sample.

2.3. Animals

Adult Wistar rats (190–220 g) bred in animal house of Sree Siddaganga College of Pharmacy, Tumkur, Karnataka, India were used. The animals were housed under standard laboratory conditions, maintained on a 12 h light: 12 h dark cycle and free access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (SSCPT/IAEC/73/2009–2010) and conducted according to CPCSEA guidelines, Govt. of India.

2.4. Experimental design

Embelin was suspended in distilled water using 1% v/v Tween-80 and administered orally to experimental animals at a constant volume of 3 ml/kg for 14 days. 3-NP was diluted with saline (pH 7.4) and administered by intra-peritoneal route at a dose of 15 mg/kg, for seven days to induce the toxicity [6,33]. Two doses of embelin (10 and 20 mg/kg, p.o.) were selected based on our earlier studies [22,32]. Embelin alone at the dose of 50 mg/kg was found to be safe in our earlier study [22] and hence, the effect of embelin on normal rats was not studied.

Rats were randomly divided into four groups of 8 animals in each and treated as follows: Groups I and II, normal and 3-NP alone treated groups, respectively, were treated with 1% Tween-80 (3 ml/kg/day). Groups III and IV were treated with a suspension of embelin in 1% Tween-80 at the doses of 10 and 20 mg/kg/day, respectively. All these treatments were given for 14 days orally. From days 8 to 14, after 1 h of the above treatments normal saline 3 ml/kg/day, i.p. was given to Group I, and 3-NP (15 mg/kg/day, i.p.) was administered to groups II–IV. Body weights were measured on regular basis and the percent changes in body weights from days 1 to 15 were calculated [6]. On day 14 of the experiment, after 3 h of 3-NP administration, rats were evaluated for behavioral parameters. On day 15 after body weight measurements, rats were euthanized and brains were isolated for biochemical estimations, lesion area measurements and histopathological studies.

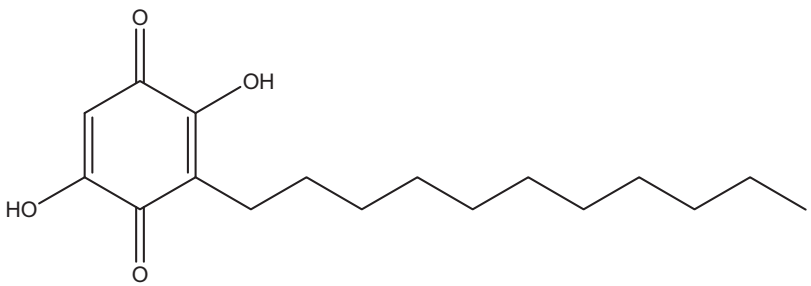

Fig. 1. Structure of embelin.