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Effect of *Centella asiatica* leaf powder on oxidative markers in brain regions of prepubertal mice *in vivo* and its *in vitro* efficacy to ameliorate 3-NPA-induced oxidative stress in mitochondria ☆

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Abstract

Centella asiatica (CA) is a common medicinal plant used in the ayurvedic system of medicine to treat various ailments and as a memory enhancer. Despite its extensive usage in children, data on its ability to modulate neuronal oxidative stress in prepubertal rodents are limited. Hence in the present study we have addressed primarily two questions (i) whether dietary intake of CA leaf powder possess the propensity to modulate endogenous oxidative markers in mouse brain regions and (ii) the efficacy of CA aqueous extract to abrogate 3-nitropropionic acid (3-NPA)-induced oxidative stress in brain mitochondria *in vitro*. Prepubertal male mice were fed CA-incorporated diet (0.5 and 1%) for 4 weeks, and biochemical markers of oxidative stress in brain regions were determined. Mice fed CA showed significant diminution in the levels of malondialdehyde (30–50%), reactive oxygen species (32–42%) and hydroperoxide levels (30–35%), which was accompanied by enhanced activities of antioxidant enzymes in all brain regions. While the levels of reduced glutathione and total thiols were elevated, the protein carbonyl content was decreased in brain among CA-fed mice. Interestingly, the oxidative markers among brain mitochondria of CA-fed mice were also significantly diminished (malondialdehyde, 25%; ROS, 30%; hydroperoxides, 35% and protein carbonyls, 28%). Further, the aqueous extract of CA showed significant free radical scavenging activity determined in established chemical test systems (viz., DPPH, superoxide and hydroxyl radical scavenging activity). Furthermore, the aqueous extract of CA markedly ameliorated the 3-NPA induced oxidative stress response in brain mitochondria under *in vitro* exposure,. Taken together, these data suggest that CA has the propensity to modulate both endogenous and neurotoxicant induced oxidative impairments in the brain and may be effectively employed as a neuroprotective adjuvant to abrogate oxidative stress *in vivo*.

Keywords: *Centella asiatica*; Brain regions; Oxidative markers; Prepubertal mice mitochondria; 3-NPA; *In vitro*

Introduction

Centella asiatica (L) Urban (Apiaceae) is a slender creeping plant native to countries like India, Srilanka, Madagascar, South Africa and Malasia (Kartnig, 1988). It is used in the ayurvedic system of medicine to treat various ailments like headache, body ache, insanity, asthma, leprosy, ulcers, eczemas and wound healing (Chopra et al., 1956; Shukla et al., 1999; Suguna et al.,

Abbreviations: CA, *Centella asiatica*; CAAE, *Centella asiatica* leaf aqueous extract; LPO, lipid peroxidation; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; DCF, 2′,7′-dichloro-fluorescein; DCF-DA, 2′,7′-dichloro-fluorescein diacetate; 3-NPA, 3-nitropropiponic acid.

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1996). Earlier few clinical reports demonstrate the antidepressive–sedative effects (Chatterjee et al., 1992) of *Centella asiatica* (CA) powder and extracts as well as their ability to improve venous insufficiency (Kartnig, 1988). Further, studies have also shown the efficacy of CA extracts in improving memory and cognitive function in rats (Vaidyaratnam, 1994; Veerendra Kumar and Gupta, 2002, 2003).

Oxidative stress due to increase in free radical generation or impaired endogenous antioxidant mechanism is an important factor that has been implicated in various neurodegenerative diseases (Beal, 1995). The brain is highly susceptible to free radical damage because of its high utilization of oxygen and the presence of relatively low concentration of antioxidant enzymes and free radical scavengers. There have been efforts to find various therapeutic agents (both natural and synthetic) that could reduce oxidative stress and improve memory (Anekonda and Reddy, 2005). It has been postulated that the mechanistic basis of the neuroprotective activity of antioxidants rely not only on the general free radical trapping or antioxidant activity *per se* in neurons, but also on the suppression of genes induced by pro-inflammatory cytokines and other mediators released by glial cells (Wang et al., 2006). Recently, herbal treatments have been used in animal and cellular models of Alzheimer's disease (AD) and in clinical trials with AD subjects (Anekonda and Reddy, 2005). These extracts have multifunctional properties (such as procholinergic, antioxidant, antiamyloid, anti-inflammatory) and their increased human usage globally necessitates a better understanding of their potential for alleviating or reducing various neurological pathologies. Recent clinical data suggest the efficacy of a combination of antioxidants in preventing AD (Behl, 2005).

Despite the extensive human usage of CA, knowledge with regard to its ability to modulate endogenous oxidative markers in brain regions in experimental animals is lacking. Earlier studies showed that oral administration of an aqueous extract of CA reduces brain malondialdehyde levels and increases the glutathione levels in whole brain (Veerendra Kumar and Gupta, 2002) of adult rats. Recently, CA was demonstrated to accelerate nerve regeneration *in vivo* and increased neurite elongation *in vitro* (Soumyanath et al., 2005). Notable bioactive compounds of CA are the triterpene saponins, madecassocide and asiaticoside with their respective ursane type sapogenins viz., madecassic and asiatic acid (Mangas et al., 2006; Wijeweera et al., 2006). Further, CA is reported to contain numerous caffeic acid derivatives and flavonols and in particular quercetin, kaempferol, catechin, rutin and naringin (Zainol et al., 2003), some of which have been shown to be potent antioxidants (Hussin et al., 2007). However, studies describing the potential of dietary CA to mitigate oxidative markers in various brain regions *in vivo* have not been attempted either in adult or prepubertal rodents.

3-Nitropropionic acid (3-NPA), a mitochondrial toxin, causes preferential neuronal degeneration in the striatum and produces anatomical changes similar to Huntington's disease in experimental animals (Beal et al., 1993). Enhanced ROS generation and MDA levels have been demonstrated in brain regions of rats challenged with 3-NPA, indicating the vital role of oxidative stress in the manifestation of neurotoxicity (Fu et al., 1995). Earlier, 3-NPA-induced neurotoxicity has been shown to be attenuated by taurine, and *S*-allylcysteine (Tadros et al., 2005; Herrera-Mundo et al., 2006). However, attempts to modulate 3-NPA-induced oxidative stress either *in vivo* or *in vitro* by specific phytochemicals have not been attempted. In view of this, we are investigating the propensity of CA extracts to modulate 3-NPA-induced oxidative response both *in vitro* and *in vivo* in various brain regions of prepubertal mice.

In the present study, we have addressed two issues: (a) whether dietary *Centella asiatica* leaf powder could significantly reduce the levels of endogenous oxidative markers in different brain regions of prepubertal mice and (2) the *in vitro* efficacy of an aqueous extract of *Centella asiatica* against 3-nitropropionic acid (3-NPA)-induced oxidative stress response in brain mitochondria.

Materials and methods

Chemicals

Thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane, 2′, 7′-dichloro-fluorescein (DCF), 2′, 7′-dichloro-fluorescein diacetate (DCF-DA) and other fine chemicals were procured from M/s Sigma Chemical Co., St Louis, USA. BHT (butylated hydroxyl toluene), DPPH (1,1-diphenyl-2-picrylhydrazyl), PMS (phenazine methosulphate), NADH (nicotinamide adenine dinucleotide reduced), NBT (nitroblue tetrazolium), trichloro acetic acid (TCA) were procured from M/s Sisco research Laboratories, Mumbai, India. All other chemicals used were of analytical grade

Animals and care

Prepubertal male mice (CFT-Swiss, 4-week old) were drawn from the stock colony of the 'institute animal house facility'. They were housed in rectangular polypropylene cages (three per cage) kept on racks built of slotted angles, and the cages were provided with dust-free paddy husk as a bedding material. The animals were housed in a controlled atmosphere with a 12 h light/dark