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Piperine and curcumin exhibit synergism in attenuating D-galactose induced senescence in rats

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ABSTRACT

Aging is associated with progressive decline in mental abilities and functional capacities. Postmitotic tissues are most vulnerable to alteration due to oxidative damage leading to behavioral and biochemical changes. We hypothesized that the anatomical and functional facets of the brain could be protected with powerful antioxidants such as piperine and curcumin by examining their effects individually and in combination in delaying senescence induced by D-galactose. Young adult male Wistar rats were treated with piperine (12 mg/kg) alone, and curcumin (40 mg/kg) alone; and in combination for a period of 49 days by the oral route with treatment being initiated a week prior to D-galactose (60 mg/kg, i.p.). A control group, D-galactose alone and naturally aged control were also evaluated. Behavioral tests, hippocampal volume, CA1 neuron number, oxidative parameters, formation of lipofuscin like autofluorescent substances, neurochemical estimation, and histopathological changes in CA1 region of hippocampus were established. Our results suggest that the combination exerted a superior response compared to monotherapy as evidenced by improved spatial memory, reduced oxidative burden, reduced accumulation of lipofuscin; improvement in signaling, increase in hippocampal volume and protection of hippocampal neurons. We speculate that the powerful antioxidant nature of both, augmented response of curcumin in the presence of piperine and enhanced serotoninergic signaling was responsible for improved cognition and prevention in senescence.

1. Introduction

Senescence is an inevitable biological phenomenon characterized by a decline in physiological function and metabolic processes leading to an alteration in the functional capacity of organs and tissues. Aging is characterized by loss of neuronal dendrites (Dlugos, 2008), reduction in neuronal density (Hwang et al., 2006), altered synthesis of neurotransmitters (Allard et al., 2011), Purkinje cell atropy (Hadj-Sahraoui et al., 2001), sensory changes (Seminowicz et al., 2009) and metabolic alterations (McKinney et al., 2008).

Chemicals can accelerate the process of aging by inducing changes in various organ systems. D-Galactose (D-Gal) induces memory impairment and alters motor skills in experimental animals (Wei et al., 2005). D-Gal facilitates deterioration of cholinergic neurons in the basal forebrain (Ypsilanti et al., 2008) and affects neurogenesis in the hippocampus (Liu et al., 2010). In addition, it alters calcium homeostasis (Moyer Jr. et al., 2009), neurotransmitter synthesis and mitochondrial function (Kong et al., 2006). D-Gal promotes cumulation of free radicals thereby deteriorating neuronal cells (Hua et al., 2007). Owing to the above reasons, D-Gal has been widely employed to experimentally induce aging in rodents. Furthermore, postmitotic cells amass lipofuscin inclusions during aging due to cellular oxidative damage (Brunk et al., 1992).

Changes in environment and lifestyles can trigger premature senescence. Perturbation in the prooxidant and antioxidant balance is common with advancing age. Further, the pathological changes associated with aging can add to the social and economic burden. This urged us to focus our attention on naturally available antioxidants such as piperine and curcumin, as oxidative stress plays a putative role in neurodegeneration. Piperine is the main alkaloid in the fruits of black pepper, long pepper, and other piper species. Piperine prevents oxidative stress (Selvendiran and Sakthisekaran, 2004), depression (Li et al., 2007), and inflammation. It possesses immunomodulatory and cytoprotective action (Pathak and Khandelwal, 2007). Piperine has gained recognition in the management of central nervous system disorders such as Alzheimer's disease (Chonpathompikunlert et al., 2010). On the other hand, curcumin, a yellow pigment present in the Indian spice turmeric (*Curcuma longa*), is accepted as an antioxidant (Vajragupta et al., 2003; Motterlini et al., 2000) and

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anti-inflammatory (Naik et al., 2011). It prevents neurological diseases (Bhutani et al., 2009), reduces focal brain ischemia (Jiang et al., 2007), is a neuroprotective (Tang et al., 2009); minimizes methotrexate induced oxidative stress, and hepatotoxicity (Banji et al., 2011).

Although piperine and curcumin exhibited beneficial response in central nervous system disorders, their effect in delaying senescence alone and in combination has not been elucidated. As piperine and curcumin are potential antioxidants, we hypothesized that they could prevent the progression of aging effectively and extensively. The present study attempted to investigate and compare the effects of piperine and curcumin alone, and in combination in reversing D-Gal induced changes in memory, neurochemistry, and morphology of the hippocampus. In addition, we investigated the effect of piperine and curcumin in attenuating D-gal induced oxidative damage, and accumulation of lipofuscin like autofluorescent substances in the brain in rats.

2. Methods

2.1. Drugs and chemicals

Piperine and serotonin were purchased from Sigma Chemical Company, USA. D-Galactose and thiobarbituric acid were purchased from S.D. Fine Chemicals, Hyderabad, India. Bovine serum albumin, o-pthalaldehyde, 5, 5′-di thio bis (2-nitro benzoic acid) and curcumin were procured from Sisco Research Laboratories, Mumbai, India. All the other reagents and chemicals used were of analytical grade.

2.2. Subjects

Wistar rats were obtained from the National Institute of Nutrition, Hyderabad and were allowed to acclimatize for a period of 1-week. They were housed in a temperature controlled room (22 ± 2 °C) on alternative 12 h light–dark cycles. All animals were given access to food and water ad libitum. The experiments were performed in accordance with the guidelines provided by the Council for the Purpose of Control and Safety of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India and the experimental protocol has been approved by the Institutional Animal Ethical Committee (IAEC) bearing number NCOP/IAEC/32/2011. 36 healthy young adult male Wistar rats aged between 3 and 4 months (180–200 g) and six old rats (350–370 g; >20 months) were used for the induction of ageing. D-Gal was administered in a dose of 60 mg/kg to young rats ($n=6$/group) to examine changes in behavior. The duration of treatment selected was 42 days as previous reports suggest that duration of 6–8 weeks can produce behavioral impairments. Piperine was reported to exert an effect on neuronal tissues in a dose ranging from 3 to 89 mg/kg (Li et al., 2007); therefore, 6 and 12 mg/kg was used for the study. Curcumin was utilized in a dose of 20 and 40 mg/kg as it is safe up to a dose of 3.6 g/kg (Anand et al., 2007).

2.3. Experimental protocol

Rats were randomly allocated into seven groups of six animals each. Group 1 were young animals which received methyl cellulose solution (2%, in distilled water) orally for 49 days and served as the control; Group 2 were naturally aged animals which received methyl cellulose (2%, in distilled water) orally for 49 days; Group 3 were young animals which received the vehicle for 7 days prior to the daily administration of D-Galactose alone (D-Gal, 60 mg/kg, i.p.) for 42 days; Group 4 were young animals which received curcumin (20 mg/kg suspended in 2% aqueous methyl cellulose) orally for 49 days; Group 5 were young animals treated with piperine (6 mg/kg, suspended in 2% aqueous methyl cellulose) orally for 49 days; Group 6 received piperine (6 mg/kg) and curcumin (20 mg/kg) suspended in 2% aqueous methyl cellulose and administered orally to young animals for 49 days; Group 7 animals received piperine (12 mg/kg) and curcumin (40 mg/kg) suspended in 2% aqueous methyl cellulose administered orally to young animals for 49 days; Groups IV–VII received D-Gal (60 mg/kg; i.p.) for a total of 42 days. Monotherapy and combination therapy with piperine and curcumin were initiated 7 days prior to D-Gal treatment (Table 1).

2.4. Morris water maze

The water maze consisted of a large circular pool (180 cm in diameter and 38 cm in height) with dark walls and filled with tap water. An escape platform (9 cm in diameter) was submerged 0.5 cm below the surface of water. Each rat was subjected to training for 6 days, three trials each day with 5–7 min inter-trial-interval. During the navigation test, the time required to locate the escape platform (escape latency) was determined, and after locating this platform the animal was allowed to remain on it for 2 s. On day-7, the platform was removed and the number of crossings through the site where the original platform was located was recorded (Morris et al., 1982).

2.5. Determination of hippocampal volume

Animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p. injection) and killed. The hippocampus was separated, placed in fixative solution, and sectioned (40 μm) in the coronal plane and stained. Volumes of the pyramidal neurons within CA1–CA3 subfields, granular cell layer of the dentate gyrus and subiculum (without presubiculum and parasubiculum) were estimated on the basis of the Cavalieri principle (Gundersen et al., 1988). The sampling procedures for selecting the sections were based on the proposals suggested by Gundersen and Jensen

Table 1
Time line and treatment schedule of piperine, curcumin and combination therapy.

S. no.	Group/treatments	Total duration of treatment
1	Control (Methyl cellulose solution, orally)	49 days
2	Aged (Methyl cellulose solution, orally)	49 days
3	Vehicle from 1st–7th day + D-Gal (60 mg/kg, i.p.) from 7th–49th day	49 days
4	Curcumin alone (20 mg/kg, orally) from 1st–49th day + D-Gal from 7th–49th day	49 days
5	Piperine alone(6 mg/kg, orally) from 1st–49th day + D-Gal from 7th–49th day	49 days
6	Combination therapy; Piperine (6 mg/kg, orally) + Curcumin (20 mg/kg, orally) from 1st–49th day + D-Gal (60 mg/kg, i.p.) from 7th-49th day	49 days
7.	Combination therapy; Piperine (12 mg/kg, orally) + Curcumin (40 mg/kg, orally) from 1st–49th day + D-Gal (60 mg/kg, i.p.) from 7th–49th day	49 days