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Mitigation of acrylamide-induced behavioral deficits, oxidative impairments and neurotoxicity by oral supplements of geraniol (a monoterpene) in a rat model

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ABSTRACT

In the recent past, several phytoconstituents are being explored for their potential neuromodulatory effects in neurological diseases. Repeated exposure of acrylamide (ACR) leads to varying degree of neuronal damage in experimental animals and humans. In view of this, the present study investigated the efficacy of geraniol (GE, a natural monoterpene) to mitigate acrylamide (ACR)-induced oxidative stress, mitochondrial dysfunction and neurotoxicity in a rat model and compared its efficacy to that of curcumin (CU, a spice active principle with multiple biological activities). ACR administration (50 mg/kg bw, *i.p.* 3 times/week) for 4 weeks to growing rats caused typical symptoms of neuropathy. ACR rats provided with daily oral supplements of phytoconstituents (GE: 100 mg/kg bw/d; CU: 50 mg/kg bw/d, 4 weeks) exhibited marked improvement in behavioral tests. Both phytoconstituents markedly attenuated ACR-induced oxidative stress as evidenced by the diminished levels of reactive oxygen species, malondialdehyde and nitric oxide and restored the reduced glutathione levels in sciatic nerve (SN) and brain regions (cortex – Ct, cerebellum – Cb). Further, both phytoconstituents effectively diminished ACR-induced elevation in cytosolic calcium levels in SN and Cb. Furthermore, diminution in the levels of oxidative markers in the mitochondria was associated with elevation in the activities of antioxidant enzymes. While ACR mediated elevation in the acetylcholinesterase activity was reduced by both actives, the depletion in dopamine levels was restored only by CU in brain regions. Taken together our findings for the first time demonstrate that the neuromodulatory propensity of GE is indeed comparable to that of CU and may be exploited as a therapeutic adjuvant in the management of varied human neuropathy conditions.

1. Introduction

Many herbal extracts/bioactives have been extensively used in various forms of traditional medicines such as Ayurveda. Phytoconstituents such as curcumin (CU), withanoloides, bacopasides have been demonstrated to possess preventive or curative effects in different neurodegenerative disorders and neuropathic conditions [1–3]. Geraniol (GE), an acyclic monoterpene present in essential oils of various spices and aromatic herbs is also commonly found in ginger, nutmeg, coriander, lemon, lemon-grass, lavender, etc. Besides its usage as a flavoring (owing to its aroma) agent in many foods/beverages, it is widely employed in a range of cleansing products, perfumes and cosmetics [4]. GE possesses both antioxidant and anti-inflammatory properties [5,6]. Recent studies in rats have shown that oral supplementation of GE effectively suppressed ferric nitrilotriacetate-induced renal oxidative stress, tumor incidence and hepatocarcinogenesis [5,7]. Further, GE also possesses anti-tumor activity against various cancer cells [8], marked anti-inflammatory, insecticidal and antimicrobial properties [4,9].

CU is an active principle of turmeric, a commonly used spice for culinary and medicinal purposes. Unlike GE, over-whelming research emphasizes a plethora of pharmacological properties of CU in different models of neurological disorders [10]. CU possesses antioxidant and anti-inflammatory properties [2,11]. It counteracts oxidative stress resulting due to different conditions such as ageing and diseases like pulmonary, metabolic, cardiovascular and autoimmune diseases [12]. Its therapeutic effects against neurodegenerative disorder and neuropathological conditions have been explicitly researched employing several models [13–15]. Hence, CU was chosen as a positive phytoconstituent while evaluating the efficacy of GE in this investigation.

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Acrylamide (ACR), a well-documented human neurotoxin is commonly used chemical for various industrial applications and gel electrophoresis in laboratories. ACR monomer is known to affect both central and peripheral nervous system and impacts both sensory and motor functions [16–18]. In the recent past, ACR has gained considerable attention due to its formation during heat processing of carbohydrate rich foods (eg., French fries, chips). Compelling evidence from different experimental models indicate its toxic effects leading to neuropathic signs [19–21]. The participation of oxidative stress and inflammatory responses in ACR neurotoxicity are widely accepted [18,22,23]. In several cell types, ACR induced cytotoxic effects *via* elevation in various types of oxidative markers such as reactive oxygen species (ROS), 3-nitrotyrosine and activation of Cox 2 and NOS [20–23]. ACR forms conjugates with reduced glutathione (GSH) and the resulting complex is metabolized by cytochrome P450 (subtype CYP 2E1) to form glycidamide. In rodent models, ACR caused lipid peroxidation, depletion in GSH levels and alterations in apoptotic markers [18,19,24].

One of the long term objectives of our laboratory is to obtain experimental evidence on the neuroprotective propensity of different phytoconstituents against ACR-induced oxidative stress and neurotoxicity in the *Drosophila* system and validate the same in a rodent model [18,25]. In this regard, previously we demonstrated the neuroprotective efficacy of GE and CU in *Drosophila* system [26]. Accordingly, we hypothesized that GE may attenuate ACR-induced manifestations of behavioral aberrations, oxidative stress, mitochondrial dysfunctions and neurotoxicity in a rat model. We compared the propensity of GE with that of CU, a well-known spice active principle under similar conditions.

2. Materials and methods

2.1. Chemicals

Acrylamide (⩾99%), curcumin, geraniol (98%), thiobarbituric acid (TBA), 1,1,3,3-tetra methoxypropane, 2′,7′-dichloro-fluorescein (DCF), 2′,7′-dichloro-fluorescein diacetate (DCFH-DA) and other fine chemicals were procured from M/s Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Animals and care

Adult (8–9 weeks old) male albino rats (CFT-Wistar strain), were drawn from the stock colony of Institute Animal Facility. Animals housed (2 per cage) in rectangular polypropylene cages in a controlled atmosphere (12 h light/dark cycle) were provided with commercial chow diet and water *ad libitum*. Experiments were conducted strictly in accordance with approved guidelines by the Institute Animal Ethical Committee regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India. Handling and care of animals was strictly in compliance with the guidelines by the 'Institutional Ethics Committee' (Registration number: 49/1999/CPCSEA).

2.3. ACR dose selection

Based on our previous study, the dosage of 50 mg/kg bw, thrice a week for a period of 4 weeks was selected [18]. Earlier, other researchers have also employed a similar dosing regimen in order to induce neuropathy in rat models.

2.4. Dosage determination and the effects of different doses of geraniol and curcumin

In a preliminary study, the effect of oral supplementation (for 4 weeks) of both GE and CU on the endogenous levels of oxidative markers in brain regions was ascertained. The dosages (mg/kg bw/d; *po*) of phytoconstituents employed were: CU (25-low dose; 50-mid dose; and 100-high dose; and GE (50-low dose; 100-mid dose; and 200-high dose). Terminally, rats from both control and treatment groups were sacrificed; brain was excised and dissected to isolate cortex (Ct) and cerebellum (Cb). Biochemical markers of oxidative stress and antioxidant related enzymes were analyzed in brain regions. For the modulatory study, GE and CU were used at dosages of 100 and 50 mg/kg bw, respectively.

2.5. Experimental design: modulatory effects of CU and GE against ACR induced neuropathy

Adult rats (8–9 weeks old) were randomly assigned to four groups. Group I rats served as control and received the vehicle-edible oil only. Group II, III and IV rats were administered with ACR (50 mg/kg bw, *i.p.*, thrice a week) for 4 weeks. In addition, Groups III and IV rats received the phytoconstituents, CU (50 mg/kg bw/d,) and GU (100 mg/kg bw/d,), respectively in vegetable oil. Rats of all the groups were monitored for the development of signs of neuropathy. Daily feed intake and weekly body weights were recorded throughout the experimental period of 4 weeks. Rats of all the groups were subjected to behavioral tests each week as described below. Terminally rats were subjected to necropsy; the brain and SN were excised and processed for biochemical analysis. Brain regions-cortex (Ct) and cerebellum (Cb) were sub-dissected on ice. Markers of oxidative stress *viz*., reactive oxygen species (ROS), hydroperoxides (HP), lipid peroxidation (LPO), protein carbonyls (PC) and reduced glutathione (GSH) were determined in SN and brain regions.

2.5.1. Behavioral tests

Rats from both control and treatment groups were subjected to a battery of behavioral tests for the assessment of neuropathic signs (sensory and motor deficits).

The 'Behavioral index' (gait score) was assessed as described earlier [18]. A trained, observer not involved with the experimental protocol was employed for the purpose. Rats placed individually in a transparent box of suitable dimension were observed for 2 min and assigned the subjective gait scores (1–4). A score of 1 – an *unaffected or normal* rat; 2 – a rat *marginally affected* characterized by weakness, slight ataxia, active, foot splay; 3 – *moderately affected* rat characterized by foot splay with limb spread during ambulation and 4 – a rat *severely affected* exemplified with above symptoms together with the inability to support body weight, dragging hind-limbs and inability to rear. Average of all the scores per group were calculated and presented as *mean* ± SE for each group.

Hot hyperalgesia (thermal sensitivity) was assessed among all rats of different groups by tail immersion test. Tail of each rat was immersed in water maintained at a temperature of 52 ± 0.5 °C. The tail withdrawal latency (flicking response) or signs of struggle were observed and recorded. A cut-off time of 12 s was maintained in all cases [27]. Average of three trails/rat was carried out and the mean ± SE was calculated for each group.

Cold allodynia (sensitivity to cold stimuli) was assessed among all rats of various groups by tail immersion test. Tail of each rat was immersed in water maintained at a temperature of 10 ± 0.5 °C. The tail withdrawal latency (flicking response) or signs of struggle were observed and recorded. A cut-off time of 30 s was maintained in all