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Standardized fruit extract of *Momordica charantia L* protect against vincristine induced neuropathic pain in rats by modulating GABAergic action, antimitotoxic, NOS inhibition, anti-inflammatory and antioxidative activity

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ABSTRACT

In the present experimental work, we investigate the protective potential of standardized *Momordica charantia L* fruit extract [gallic acid (GA) 6%; >8% bitters {momordicosides K (3%) and L (2%); and momordicines I (2%) and II (3%)}] (MC) comparable to its marker compound gallic acid in chemo-toxic neuropathy induced by vincristine [75 μg/kg intra-peritoneal (i.p.) for 10 consecutive days] in rats. An array of behavioral examinations was carried out on days 1st, 12th and 21st, followed by various biochemical and histopathological studies at the end. Vincristine significantly induced cold and dynamic mechanical allodynia, mechanical and heat hyperalgesia; functional deficit in walking and a rise in the levels of TNF-α, IL 6, mitochondrial complexes, myeloperoxidase (MPO), thiobarbituric acid reactive substances (TBARS), and nitrite along with decrease in glutathione (GSH). Administration of MC [400 and 800 mg/kg, *per oral* (p.o.)], GA (30 mg/kg, p.o.) and gabapentin (100 mg/kg, p.o.) attenuated the vincristine induced behavioral and biochemical changes. MC demonstrated superior antinociceptive activity in comparison to GA. Histopathological evaluation also divulged defending the effects of MC. Pretreatment of saclofen (1 mg/kg, i.p.), picrotoxin (1 mg/kg, i.p.) upturned the antinociceptive action of MC, but ingestion of GABA (100 mg/kg, i.p.) potentiated the action of MC. Additionally, pretreatment with L-arginine [nitric oxide (NO) donor; 100 mg/kg, i.p.] inverted the antinociceptive action of MC; whereas, aminoguanidine (iNOS inhibitor) and 7-nitroindazole (nNOS inhibitor) potentiated it. Besides this a PPARγ antagonist BADGE did not amend the effect of MC. Corroboratively, the attenuating effect of MC in vincristine induced neuropathy is attributed to its modulating action on GABAergic system along with antimitotoxic, NOS inhibition, anti-inflammatory and anti oxidative activities.

1. Introduction

Chemoneuropathy ruins a throbbing, onerous difficulty of cancer treatment for patients receiving plant alkaloids; viz vincristine and paclitaxel, until now the cause and persistence of this conditions are not completely documented (Jaggi and Singh, 2012a; LaPointe et al., 2013).

Research studies from last decade revealed that increased release of cytokines such as TNF-alpha and IL-1, IL-6 along with NO from glial cells, macrophages and LC cells (Siau et al., 2006; Mangiacavalli et al., 2010) are associated with vincristine induced peripheral neuropathy. In addition, mitochondrial dysfunction (Broyl et al., 2010) and oxidative stress (generation of reactive oxygen species in DRG neurons and free radicals) produce neuronal cytotoxicity in cancer chemotherapy-induced peripheral neuropathy [CIPN] (Kim et al., 2010; Muthuraman et al., 2011). Albeit, in depth the mechanisms of vincristine led neuropathy are not yet completely revealed.

Momordica charantia L. (Cucurbitaceae) MC commonly known as bitter gourd, Karela (In India) and balsam pear is an important medicinal vegetable crop. Earlier, oil extracted from the seed of MC, when applied topically to the patients of spondylitis, rheumatoid arthritis and diabetic neuropathy demonstrated relief from pain (Pushpa Khanna, 2005). Moreover, study done by Nerurkar et al. (2011) showed that the methanolic extract of bitter melon fruit [catechin (3% w/w), quercetin (0.62% w/w), trans-chalone (0.3% w/w), caffeine (0.25% w/w); while, GA, caffeic acid, and flavones (less than 0.01% w/w) in BM methanolic extracts] lowers the expression of neuro-inflammatory markers such as NF-κB1, IL-16, IL-22 as well as IL-17R in the brains of mice fed with high-fat diet. Further MC fruit extract showed relief from acetic acid induced writhing pain in rats (Ullah et al., 2012). Recently our

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study demonstrated that administration of standardized fruit powder of MC produced antinociceptive action against injury induced (tibial and sural nerve transection) neuropathic pain in rats via PPARγ agonistic activity, TNFα inhibition and antioxidant potential (Jain et al., 2014). Therefore, we decided to explore the potential of MC in chemo-toxic (vincristine induced) painful peripheral neuropathy in rats with more mechanistic approaches on NOS system i.e. iNOS and nNOS; antimitotoxic potential; action on GABA and PPARγ receptor along with histopathological evaluation of sciatic nerve and spleen.

2. Materials and methods

2.1. Experimental animals

Wistar rats of both sexes, weighing 200–250 g (procured from NIPER, Mohali) were employed in the present study. They were housed in animal cages with free access to water and standard laboratory pellet chow diet (Hindustan Liver Limited, India). The animals in cages were kept in the departmental animal house (temperature maintained at 18 to 23 °C; relative humidity maintained at 30 to 35%) and were exposed to the normal cycle of light and dark. The experimental protocol (Table 1) was approved by the institutional animal ethics committee (IAEC) and the care of the animals was carried out as per the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) (Registration no. 574/02/ab/CPCSEA), Ministry of environment and forest, Government of India. Experiments were performed after acclimatization of rats for one week.

2.2. Drugs and chemicals

Vincristine sulfate (Cipla Pvt. Ltd., Mumbai; Batch No. J20394), GA, saclofen, picrotoxin, γ-aminobutyric acid (GABA), 2,6-dichlorophenolindophenol sodium, rotenone, adenosine 5′-triphosphate disodium salt hydrate, β-Nicotinamide adenine dinucleotide, antimycin A, HEPES, EGTA, 1,1,3,3 tetra methoxy propane, 7-nitroindazole, aminoguanidine (all from Sigma Aldrich, USA), DTNB [5,5′-dithio, bis (2-nitro benzoic acid)], BSA (bovine serum albumin) (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), thiobarbituric acid (HIMEDIA, Mumbai, India), Folin–Ciocalteu's phenol reagent (Merck Ltd., Mumbai, India), Gabapentin (≥98% purity) and BADGE (Cayman chemicals, New Delhi) were procured for the present study. All the reagents used in the present study were of analytical grade.

2.3. Procurement of MC

M. charantia L dry fruit powder (Batch No: MC/11009) was a generous gift from Natural Remedies, Bangalore. The standardized *M. charantia L* powder contains >8% bitters [Mainly momordicosides K (3%) and L (2%), and momordicines I (2%) and II (3%)], GA (6%) heavy metals (not more than 10 ppm) and was free from any microbes. It was stored in air-tight container at low temperature (below 25 °C) and suspended in 0.5% w/v carboxy methyl cellulose (CMC) solution before oral administration. The voucher specimen of *M. charantia L* dry fruit powder was deposited in pharmacognosy division, department of pharmacy, Banasthali university (Voucher specimen no. DPCOG/VS/2013/166).

2.4. Induction of neuropathic pain by vincristine

Peripheral painful neuropathy was induced in rats by administration of vincristine sulfate (75 μg/kg, i.p. for 10 consecutive days) as per the method described by Authier et al., 1999.

2.5. Behavioral examination

2.5.1. Cold allodynia (acetone drop test)

The cold allodynia was assessed by spraying a 100 μL of acetone onto the surface of the paw, without touching the skin. The response of the rat to acetone was noted for 20 s and was graded to a 4-point scale as defined by Flatters and Bennett, 2004; viz 0, no response; 1, quick withdrawal, flick or stamp of the paw; 2, prolonged withdrawal or repeated flicking; 3, repeated flicking of the paw with licking of the paw. Acetone was applied thrice to the hind paw, with a gap of 5 min between the acetone applications and the individual scores noted in 20 second interval were added to obtain a single score over a cumulative period of 60 s. The minimum score was 0, while the maximum possible score was 9.

2.5.2. Mechanical hyperalgesia (pin-prick test)

According to the method of Erichsen and Blackburn-Munro (2002) the mechanical hyperalgesia was assessed by the pin-prick test. The surface of the injured hind paw was touched with the point of the bent 18 gauge needle (at 90° to the syringe) at intensity sufficient to produce a reflex withdrawal response. The duration of the paw withdrawal was recorded in seconds.

2.5.3. Heat hyperalgesia (hot plate test)

The thermal nociceptive threshold, as an index of thermal hyperalgesia, was assessed using a hot plate, maintained at a temperature of 52.5 ± 1.0 °C. The rat was placed on the hot plate and nociceptive

Table 1
Protocol of experimental design.

For analysis of protective effect of standardized MC			
Groups	n	Substance	Description
I	6	Normal control	Only fodder and water.
II	6	Vincristine control	Vincristine (VIN, 75 μg/kg; i.p.) was administered daily for 10 days
III	6	Vehicle in VIN	Carboxy methylcellulose (0.5% w/v; p.o. for day 11 to 21st) was administered in vincristine treated rats
IV & V	6	MC & gabapentin per se	MC & gabapentin (800 mg/kg; & 100 mg/kg p.o.) daily, respectively for days 11 to 21st in normal rats.
VI & VII	6	MC in VIN	MC (400 and 800 mg/kg; p.o. daily for days 11 to 21st) was administered to VIN treated rats
VIII	6	Gabapentin in VIN	Gabapentin (100 mg/kg; p.o. daily for days 11 to 21st) was administered to VIN treated rats
IX	6	Gallic acid in VIN	Gallic acid (GA 30 mg/kg; p.o. daily for days 11 to 21st) was administered to VIN treated rats
For analysis of the possible mechanism of action of standardized MC			
X & XI	6	MC & GA in VIN	MC (100 mg/kg; i.p.); GA (10 mg/kg i.p.) daily for days 11 to 16th was administered to VIN treated rats
XII	6	Saclofen in VIN	Saclofen ($GABA_B$ receptor antagonist, 1 mg/kg, i.p.); 30 min prior to administration of MC in VIN treated rats on 16th day.
XIII	6	Picrotoxin in VIN	Picrotoxin (a non-competitive $GABA_A$ receptor antagonist, 1 mg/kg, i.p.);30 min prior to administration of MC in VIN treated rats on 16th day.
XIV	6	GABA in VIN	GABA (100 mg/kg i.p.); 30 min prior to administration of MC in VIN treated rats on the 16th day.
XV	6	BADGE in VIN	BADGE (30 mg/kg i.p.); 30 min before administration of MC (100 mg/kg; i.p.) on the 16th day.
XVI	6	L-arginine in VIN	L-arginine (NO donor; 100 mg/kg i.p.); 30 min before administration of MC (100 mg/kg; i.p.) on the 16th day
XVII	6	7-nitroindazole in VIN	7-nitroindazole (nNOS inhibitor; 20 mg/kg i.p.), 30 min before administration of MC (100 mg/kg; i.p.) on the 16th day
XVIII	6	Aminoguanidine in VIN	Aminoguanidine (iNOS inhibitor; 50 mg/kg i.p.); 30 min before administration of MC (100 mg/kg; i.p.) on the 16th day