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Effect of certain trimethoxy flavones on paclitaxel - induced peripheral neuropathy in mice

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ABSTRACT

Background: The anti - nociceptive effect of 7, 2′, 3′ – trimethoxy flavone, 7, 2′, 4′ – trimethoxy flavone, 7, 3′, 4′ – trimethoxy flavone and 7, 5, 4′ – trimethoxy flavone against inflammatory, neurogenic and thermal pain in mice was reported earlier. The present study was designed to investigate the effect of the above trimethoxy flavones in amelioration of peripheral neuropathy induced by paclitaxel.

Methods: Peripheral neuropathy was induced in mice by administration of a single i.p. dose (10 mg/kg) of paclitaxel. The manifestations of peripheral neuropathy such as tactile allodynia, cold allodynia and thermal hyperalgesia were assessed 24 h later by employing hair aesthesiometer test, acetone bubble test and hot water tail immersion test respectively. Further, the role of inflammatory cytokines like TNF – α, IL - 1β and free radicals in the action of trimethoxy flavones was investigated using *in vitro* assays.

Results: The test compounds dose dependently attenuated paclitaxel - induced tactile allodynia, cold allodynia and thermal hyperalgesia in mice. The test compounds inhibited TNF – α, IL - 1β and free radicals in a concentration dependent manner.

Conclusions: The investigated trimethoxy flavones attenuated paclitaxel – induced peripheral neuropathy in mice. The inhibition of cytokines and free radicals in addition to many neuronal mechanisms reported earlier may contribute to this beneficial effect.

1. Introduction

Peripheral neuropathy is a dose - limiting side effect of several classes of anticancer drugs including paclitaxel, oxaliplatin, cisplatin, vincristine and bortezomib[1]. These drugs produce typical manifestations of neuropathy within a week, which includes spontaneous pain, allodynia and hyperalgesia, that results in discontinuation of chemotherapy schedule by cancer patients. Attenuation of peripheral neuropathy shall ensure greater patient compliance to chemotherapy. Currently

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available drugs such as anticonvulsants (e.g. gabapentin, carbamazepine), local anesthetics (lidocaine), opioids (e.g. tramadol, morphine), tricyclic antidepressants (amitryptyline) and selective serotonin reuptake inhibitors have limited efficacy in amelioration of peripheral neuropathy because of the diverse aetiology and complex pathophysiology[2]. In addition, these drugs *per se* produce numerous side effects[3]. Thus, there is an unmet clinical need and a challenge to develop more effective therapies for the management of peripheral neuropathy.

A few reports indicate that flavonoids such as myricitrin[4], quercetin and rutin[5,6] were inhibit various types of neuropathy in animal models. In a recent study, four trimethoxy flavone derivatives (7, 2′, 3′ – trimethoxy flavone, 7, 2′, 4′ – trimethoxy flavone, 7, 3′, 4′ – trimethoxy flavone and 7, 5, 4′ – trimethoxy flavone) have been shown to possess antinociceptive property in mice[7]. Based on the aforementioned information, it was considered interesting to explore the potential effect of these trimethoxy flavones in peripheral neuropathy induced by a chemotherapeutic drug, paclitaxel in mice. Proinflammatory cytokines (TNF – α, IL - 1β) and free radicals are implicated in the pathogenesis of peripheral neuropathy.[8] Hence, the effect of trimethoxy flavones on the above provocative factors was also considered for investigation

2. Materials and methods

2.1. *Animals*

Adult Swiss albino mice of either sex weighing 25 – 30 g were used in the present study. These were obtained from the animal house of the Meenakshi Medical College and Research Institute. The animals had free access to food and water and maintained at 24 ± 1° C temperature in a 12 h day/ 12 h night cycle. All the experiments were carried out between 09.00 and 13.00 hours to avoid circadian variation. The experiments were performed after approval of the protocol by the institutional animal ethics committee.

2.2. *Drugs and Chemicals*

The trimethoxy flavones (Research Organics, Chennai, India) used in the study; 7, 2′, 3′ - trimethoxy flavone, 7, 2′, 4′ - trimethoxy flavone, 7, 3′, 4′ - trimethoxy flavone and 7, 5, 4′ - trimethoxy flavone (Fig. 1) were prepared as a fine suspension in 0.5% carboxy methyl cellulose (CMC) and injected s.c to mice. Morphine sulphate (Pharma Chemico Laboratories, Solan, Himachal Pradesh, India), Paclitaxel (Adley Formulations, Haryana, India) and Acetone (Merck Specialities Private Limited, Mumbai, India) were used in the study. Diagnostic kits (Cayman, USA) were used for *in vitro* assay of IL - 1β and TNF – α.

2.3. *Induction of peripheral neuropathy by paclitaxel*

Peripheral neuropathy was induced[9] in mice according to the method described by Hidaka *et al.*, (2009). Mice were injected i.p. with a single dose of 10 mg/kg paclitaxel diluted in normal saline (0.9% NaCl) just before use. The manifestations of peripheral neuropathy (tactile allodynia, cold allodynia and thermal hyperalgesia) were assessed 24 h after paclitaxel administration.

2.4. *Tactile allodynia (Hair aesthesiometer test)*

The hair aesthesiometer test has been used to explore the dynamic responses to a tactile stimulus. The response to hair aesthesiometer has been described as allodynia because normal mice never withdraw from this stimulus. The mice were housed and habituated for 10 min in a transparent plastic box (7 X 7 X 13 cm) secured on a raised steel frame with the floor made of wire mesh. After the adaptation period, a 15 mm length of hair aesthesiometer was applied five times perpendicularly against the plantar skin of the each hind paw at an interval of 30 seconds. The paw withdrawal response was ranked as follows: 0 - no response, 1 - move away from the stimulus, 2 - immediate flinching of the hind paw [10]. The sum of the ten values noted from both hind paws served as the paw withdrawal response score. The paw withdrawal response score was noted prior to the drug treatment and 30 min after administration of various trimethoxy flavones in doses of 25, 50, 100 or 200 mg/kg, s.c. or morphine (10 mg/kg, s.c.).

2.5. *Cold allodynia (acetone bubble test)*

Cold allodynia test[11] was performed according to the method described by Flatters and Bennett, (2004). The mice were housed and habituated for 10 min in a transparent plastic box (7 X 7 X 13 cm) secured on a raised steel frame with the floor made of wire mesh. After the adaptation period, acetone bubble formed at the tip of a one ml syringe was applied to the plantar skin of hind paw and the paw withdrawal response was observed for a period of 20 sec. The withdrawal responses were ranked as follows: 0 - no response, 1 - immediate withdrawal, 2 - prolonged withdrawal, 3 – licking/biting of the hind paw. The response was measured three times in each paw alternatively at an interval of 1 min. The sum of six values served as the paw withdrawal response score. The paw withdrawal response score was noted before drug treatment and 30 min after administration of various trimethoxy flavones in doses of 25, 50, 100 or 200 mg/kg, s.c. or morphine (10 mg/kg, s.c.).

2.6. *Thermal hyperalgesia (hot water tail immersion test)*

Thermal hyperalgesia[12] was assessed using hot water tail immersion method as previously described by Lauren *et al.*, (2009). The mouse was restrained in a mouse holder and the tail (2 - 3 cm) of the mouse was immersed in hot water maintained at 48 ± 0.5 °C temperature. The time taken to flick the tail from the hot water was taken as the reaction time. A cut off time of 20 seconds was maintained. The reaction time was noted prior to the drug treatment and 30 min after drug treatment. Any increase in reaction time between these two readings is considered as anti - nociceptive response. Different trimethoxy flavones in doses of 25, 50, 100 or 200 mg/kg or morphine 10 mg/kg were administered s.c. to various groups

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