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Ameliorative effect of 5–methoxyflavone on vincristine induced peripheral neuropathy in mice

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ABSTRACT

Flavonoids have been shown to possess antinociceptive effect mediated through the ionotropic $GABA_A$ receptors. In the present study,5-methoxyflavone was evaluatedfor ameliorative effect on vincristine induced peripheral neuropathy in mice. Peripheral neuropathy was induced by treating vincristine (0.1 mg/kg, i.p) once per day for 7 days. Administration of 5–methoxyflavone (50 mg/kg, i.p) significantly reduced the paw withdrawal response score in animals that developed cold allodynia due to vincristine treatment ($p < 0.01$). 5–methoxyflavone did not show significant withdrawal response with hair aesthesiometer in mechanical allodynia test. A significant and dose-dependent increase in the latency time to thermal response was observed after 5–methoxyflavone treatment in mice ($p < 0.01$). In conclusion, the present study has identified a novel and potential therapeutic utility of 5–methoxyflavone in the treatment of vincristine induced neuropathic pain.

KEYWORDS: 5-methoxyflavone;vincristine; nociception; peripheral neuropathy;

1. INTRODUCTION

Peripheral neuropathy is a debilitating condition frequently caused by anti-neoplastic drugs (vincristine, taxanes and platinum compounds), disease (diabetes) or trauma to peripheral nerves [1]. Depending on the treatment regimens, chemotherapy induced neuropathic pain can occur in 30-40% of patients and even as high as 75% under certain regimens [2]. Vincristine treatment has been reported to reduce the quality of life and the ability to perform daily activities [3]. In most cases, damage to the neural circuit can be reversed partially with cessation of treatment. Due to the debilitating manifestation of neuropathy like allodynia and hyperalgesia, there is an increased incidence in the discontinuation of chemotherapy treatment by cancer patients. Attenuation of the above neuropathy symptoms shall ensure greater patient compliance to chemotherapy. Currently available drugs such as anticonvulsants (gabapentin, carbamazepine), opioids (morphine, tramadol), tricyclic antidepressants (amitriptyline) and selective serotonin reuptake inhibitors have limited efficacy in amelioration of neuropathy manifestations because of the diverse aetiology and complex pathophysiology [4].

A few methoxy derivatives of flavones [5], [6]have been shown to possess significant anti-nociceptive effect in various animal models of pain. Among the compounds studied,5–methoxyflavone was reported to possessa very low ED_{50} for anti–nociceptiveaction [5].However, 5–methoxyflavone has not yet been investigated for its ameliorative effect in

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vincristine induced peripheral neuropathy. Hence it was considered interesting to explore the potential effect of 5–methoxyflavone in peripheral neuropathy induced by vincristine in mice.

2. METHODOLOGY

Animals

Adult Swiss albino mice of either sex weighing 20–25gwere procured from Govt. King Institute, Chennai. They were housed in groups of six in polypropylene cagesin a controlled environment (21–24°C), with free access to food and water and maintained on 12 h / 12 hday/night cycle. All the experiments were performed between 0900 and 1400 h to avoid circadian variations and to maintain uniformity. Each test group consisted of a minimum of six mice. The experiments were carried out after the approval of institutional animal ethics committee. Proper care of the animals during the experimental procedures have been taken in accordance with the directions of committee for the purpose of control and supervision of experiments on animals (CPCSEA), India.

Drugs & Chemicals

5–methoxyflavone (Fig.1) was purchased from Research organics, Chennai, India. 5–methoxyflavone was prepared as a fine suspension in 0.5% carboxymethylcellulose and injected i.p 30min before any procedure. All drugs were administered by i.p injection in a volume of 10ml/kg bodyweight.

Induction of peripheral neuropathy by vincristine[7]

After habituation to the test environment mice were treated with intraperitoneal injection of Vincristine (0.1mg/kg) for 7 daysto induce peripheral neuropathy.The treated mice were assessed for neuropathy on 8th day.The doses of 5–methoxyflavonewere selected based on the anti-nociceptive activity reported earlier[5].

Cold allodynia (acetone bubble test)

Cold allodynia test was performed according to the method described [8][8]. The mice were housed and habituated for 10min in a transparent plastic box (7x7x13 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 pawand the paw withdrawal response was observed for a period of 20sec. The withdrawal responses were ranked as follows: 0 - noresponse, 1 - immediate withdrawal, 2- prolonged withdrawal and 3 - licking / biting of the hind paw. The response was measured three times in each paw alternatively at an interval of 1 min. The paw withdrawal response score was noted before drug treatment and 30 min after administration of 5–methoxyflavone in doses of 12.5, 25 or 50 mg/kg, i.p or morphine (10 mg/kg, s.c).

Tactileallodynia (Hair aesthesiometer test)

The hair aesthesiometer test has been used to explore the dynamic responses to a tactile stimulus [9]. 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

10min in a transparent plastic box (7x7x13 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 each hind paw at an interval of 30 sec. The paw withdrawal response was scored as: 0 - no response, 1 - move away from the filament, 2 - immediate flinching of the hind paw [9]. 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 drug treatment and 30 min after administration of 5–methoxyflavone in doses of 12.5, 25 or 50 mg/kg, i.p or morphine (10 mg/kg, s.c).

Thermal hyperalgesia (hot water tail immersion test)

Thermal hyperalgesia was assessed using hot water tail immersion method as previously described [10]. The mouse was restrained in a mouse holder and the tail (2-3 cm) was immersed in hot water bath maintained at 48 ± 0.5°C. The time taken to flick the tail from the hot water was recorded as the reaction time. A cut off time of 20 sec was maintained. The reaction time was noted before and 30 minutes after administration of the test compound. Any increase in the reaction time between these two readings is considered as anti-nociceptive response.

Statistical analysis

Data are expressed as mean ± S.E.M.Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's 't' test for multiple comparison for thermal hyperalgesia and paired 't' test for mechanical and cold allodynia tests. A p value < 0.05 was considered statistically significant (SPSS v.16).

3. RESULTS AND DISCUSSION

3.1. Effect of 5–methoxyflavone on vincristine induced peripheral neuropathy

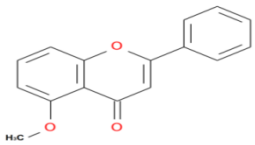

Fig 1. 5-methoxyflavone

Cold allodynia

An increase in the paw withdrawal response score observed with a non-noxious cold stimulus reflects the development of cold allodynia after vincristine administration. While vehicle treatment did not alter the response, a significant reduction was observed in paw withdrawal response score after morphine (10mg/kg, s.c) administration in mice. A significant reduction in paw withdrawal response score was observed with5–methoxyflavone(25 and 50 mg/kg, i.p) treatment when compared to their pretreatment values (Table – 1). Almost a similar reduction in the paw withdrawal response score was observed with both 25 and 50 mg/kg dose of 5–methoxyflavone (14.00 ± 1.07 and 14.00 ± 0.82).

Table 1. Effect of 5–methoxyflavone (5-MF) on vincristine induced cold allodynia in mice[£]

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Dose (mg/kg i.p)	Paw withdrawal response score	
	Before treatmen	After treatment
Vehicle	15.67 ± 0.88	13.67 ± 0.80
Morphine – 1(	16.50 ± 0.56	3.33 ± 1.09***
5 MF – 12.5	16.17 ± 0.98	15.33 ± 0.96
5 MF – 25	16.83 ± 0.48	14.00 ± 1.07*
5 MF – 50	17.67 ± 0.21	14.00 ± 0.82**

Each value represents the mean ± S.E.M. of six observations.* p< 0.05, ** p < 0.01 and *** p < 0.001 compared to respective score before treatment.£All treatment groups received vincristine (0.1 mg/kg, i.p) once daily for 7 days. Cold allodynia was determined before and 30 min after vehicle/morphine/5-methoxyflavone treatment on 8th day.

Tactileallodynia

Vincristine administration resulted in the development of tactile allodynia as evidenced by an increase in the paw withdrawal response score to an innocuous stimulus. In vehicle treated mice there was no significance in the paw withdrawal response score when compared to the pre treatment value (Table–2). However, morphine treatment demonstrated a significant reduction in the paw withdrawal response score when compared to its pre treatment value (Table–2). Treatment with 5–methoxyflavone in various doses did not show a significant reduction in the paw withdrawal response score when compared to their respective pre treatment values.

Table 2. Effect of 5–methoxyflavone (5-MF) on vincristine induced tactile allodynia in mice £

Dose (mg/kg, i.	Paw withdrawal response score	
	Before treatment	After treatment
Vehicle	19.33 ± 0.67	18.83 ± 0.83
Morphine – 1(	18.67 ± 0.67	7.33 ± 0.84*
5 MF – 12.5	20.00 ± 0.00	19.33 ± 0.67
5 MF – 25	19.67 ± 0.33	19.67 ± 0.33
5 MF – 50	19.60 ± 0.40	17.60 ± 1.60

Each value represents the mean ± S.E.M. of six observations.*p < 0.001 compared to respective score before treatment.£All treatment groups received vincristine (0.1 mg/kg, i.p)

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once daily for 7 days. Tactile allodynia was determined before and 30 min after vehicle/morphine/5-methoxyflavone treatment on 8th day.

Thermal hyperalgesia

The mean increase in the reaction time observed in vehicle treated animals was 0.19 ± 0.12 sec. A significant increase in the mean reaction time was observed with morphine (10 mg/kg) treatment. A dose-dependent increase in the thermal latency was observed with different doses of 5–methoxyflavonetreatment (Table–3). In a dose of 50 mg/kg, 5–methoxyflavone showed a maximum increase in the mean reaction time (2.73 ± 0.33 sec).

Table 3. Effect of 5–methoxyflavone (5-MF) on vincristine induced thermal hyperalgesia in mice [£]

Dose (mg/kg, i.p)	Mean increase in reaction time (sec)
Vehicle	0.19 ± 0.12
Morphine – 10	15.94 ± 1.18 **
5 MF – 12.5	1.07 ± 0.14 *
5 MF – 25	1.38 ± 0.19 *
5 MF – 50	2.73 ± 0.33 *

Each value represents the mean ± S.E.M. of six observations. * $p < 0.01$ and ** $p < 0.001$ compared to vehicle treatment. [£]All treatment groups received vincristine (0.1 mg/kg, i.p) once daily for 7 days. Thermal hyperalgesia was determined before and 30 min after vehicle/morphine/5-methoxyflavone treatment on 8th day. The mean increase in reaction time represents the difference in the latency time recorded before and after treatment with the vehicle / test compound

Studies have reported that flavonoids such as myricitrin[11], quercetin[12]and rutin[13] inhibit various types of neuropathy in animal models. A recent report indicated that, 6–methoxyflavanone significantly attenuated diabetes induced mechanical allodynia as well as vulvodynia, probably through interactions with GABAergic and opioidergic receptors [14]. In the present study, vincristine administration resulted in characteristic behavioural changes such as mechanical allodynia, cold allodynia and thermal hyperalgesia pertaining to neuropathy in mice. Administration of 5–methoxyflavone significantly reduced the paw withdrawal response score in animals that developed cold allodynia due to vincristine treatment. However, only a similar degree of attenuation of cold allodynia was evident with 25 and 50 mg/kg treatment of 5–methoxyflavone (Table–1). A dose-dependent and significant prolongation of latency to the thermal response was observed after 5–methoxyflavone administration in animals exhibiting

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thermal hyperalgesia due to vincristine treatment. However, the response was of much lesser magnitude when compared to morphine treatment (Table–3). The neuropathic changes manifesting as cold allodynia and thermal hyperalgesia resulting from vincristine administration could be effectively attenuated by 5–methoxyflavone administration. However, the same type of attenuation was not evident in vincristine induced tactile allodynia by 5–methoxyflavone administration (Table–2). Methoxylated flavones have been shown to effectively suppress the manifestations of peripheral neuropathy due to paclitaxel administration in mice [15, 16].

4. CONCLUSION

The present study has identified the efficacy of a monomethoxy substitutedflavone (5–methoxyflavone) in attenuating the neuropathic changes due to vincristine, widely used anticancer drugs. More studies on the effect of 5–methoxyflavone on neuropathy induced by other cancer chemotherapeutic drugs (oxaliplatin and paclitaxel) will bring out the possible therapeutic utility of 5–methoxyflavone in this debilitating condition.

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