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Article *in* Journal of Basic and Clinical Physiology and Pharmacology · April 2018

DOI: 10.1515/jbcpp-2016-0127

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https://doi.org/10.1515/jbcpp-2016-0127
Received August 18, 2016; accepted February 27, 2018

Abstract

Background: Peripheral neuropathy is the dose limiting side effect of many anticancer drugs. Flavonoids exhibit good antinociceptive effect in animal models. Their efficacy against different types of nociception has been documented. The present study investigated the effect of flavonol (3-hydroxy flavone), 3′,4′-dimethoxy flavonol, 6,3′-dimethoxy flavonol, 7,2′-dimethoxy flavonol and 7,3′-dimethoxy flavonol against paclitaxel-induced peripheral neuropathy in mice.

Methods: A single dose of paclitaxel (10 mg/kg, i.p.) was administered to induce peripheral neuropathy in mice and the manifestations of peripheral neuropathy such as tactile allodynia, cold allodynia and thermal hyperalgesia were assessed 24 h later by employing Von Frey hair aesthesiometer test, acetone bubble test and hot water tail immersion test, respectively. The test compounds were prepared as a suspension in 0.5% carboxymethyl cellulose and were administered s.c. in various doses (25, 50, 100 and 200 mg/kg). The above behavioral responses were assessed prior to and 30 min after drug treatment. In addition, the effect of test compounds on proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β) and free radicals was investigated by using suitable *in vitro* assays.

Results: A dose-dependent attenuation of tactile allodynia, cold allodynia and thermal hyperalgesia was evidenced in mice treated with flavonol derivatives. The test compounds inhibited TNF-α, IL-1β and free radicals in a concentration-dependent manner.

Conclusions: These results revealed that flavonol and its dimethoxy derivatives ameliorated the manifestations of paclitaxel-induced peripheral neuropathy in mice. The inhibition of proinflammatory cytokines and free radicals could contribute to this beneficial effect.

Keywords: cold allodynia; dimethoxy flavonols; flavonol; free radicals; paclitaxel; peripheral neuropathy; tactile allodynia; thermal hyperalgesia; TNF-α.

Introduction

Serious side effects are associated with the use of cancer chemotherapeutic agents including neurotoxicity manifesting as neuropathy [1] which affects patient compliance leading to discontinuation of therapy. Currently available drugs to mitigate neuropathy, such as antidepressants, anticonvulsants, baclofen and clonidine [2], are not very effective and also produce serious adverse effects. Hence, safe compounds that can ameliorate the manifestations of peripheral neuropathy are highly desirable. Flavonoids have been suggested to be a potential source for analgesic drugs and their efficacy against different types of nociceptive animal models has been documented [3, 4]. In addition, the antiallodynic activity of a few flavonoids in various animal models of peripheral neuropathy has been reported [5–7]. A recent study in our laboratory identified the potent antinociceptive activity of flavonol and its dimethoxy derivatives in various types of pain in mice (unpublished observation). Hence, it was considered interesting to study their efficacy against paclitaxel-induced peripheral neuropathy in mice. The involvement of proinflammatory cytokines (tumor necrosis factor-alpha [TNF-α], interleukin-1-beta [IL-1β]) and free radicals in nociception and pathogenesis of peripheral neuropathy has been well documented [8]. Hence, the present study also investigated the effect of flavonol and its dimethoxy derivatives on proinflammatory cytokines and free radicals by employing suitable *in vitro* assays.

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Materials and methods

Animals

Inbred male Swiss albino mice weighing 20–25 g were selected for the study. The animals were housed in a controlled environment, with free access to food and water and maintained on a 12 h/12 h natural day/night cycle. All the experiments were carried out between 09:00 and 13:00 h to avoid circadian variations and to maintain uniformity. The experimental protocol was approved by the institutional animal Ethics Committee (number-KN/COL/3406/2014). In all the experiments, each group consisted of six animals and each animal was used only once.

Drugs and chemicals used in this study

Flavonol, 3′,4′-dimethoxy flavonol, 6,3′-dimethoxy flavonol, 7,2′-dimethoxy flavonol and 7,3′-dimethoxy flavonol (Figure 1, Research Organics, Chennai, India) were prepared as a fine suspension in 0.5% carboxymethyl cellulose (HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India) and administered s.c. in mice. Paclitaxel (Sarabhai, Mumbai, India) diluted with physiological saline (Infutec Healthcare Limited, Indore, Khargone, Madhya pradesh, India) was used for inducing peripheral neuropathy. Acetone (Merck Life Science Pvt. Ltd., Pirojshanagar, Mumbai, Maharashtra, India) was used for the assessment of cold allodynia. Diagnostic kits (Cayman, USA) were used for *in vitro* assays of TNF-α and IL-1β.

Paclitaxel-induced neuropathy [9]

A single dose (10 mg/kg) of paclitaxel diluted in physiological saline (0.9% NaCl) was injected i.p. to mice and neuropathic manifestations were assessed 24 h later. A characteristic pattern of behavioral changes could be observed in mice treated with paclitaxel when exposed to tactile, cold and thermal stimuli. The above behavioral changes were measured prior to and 30 min after administration of

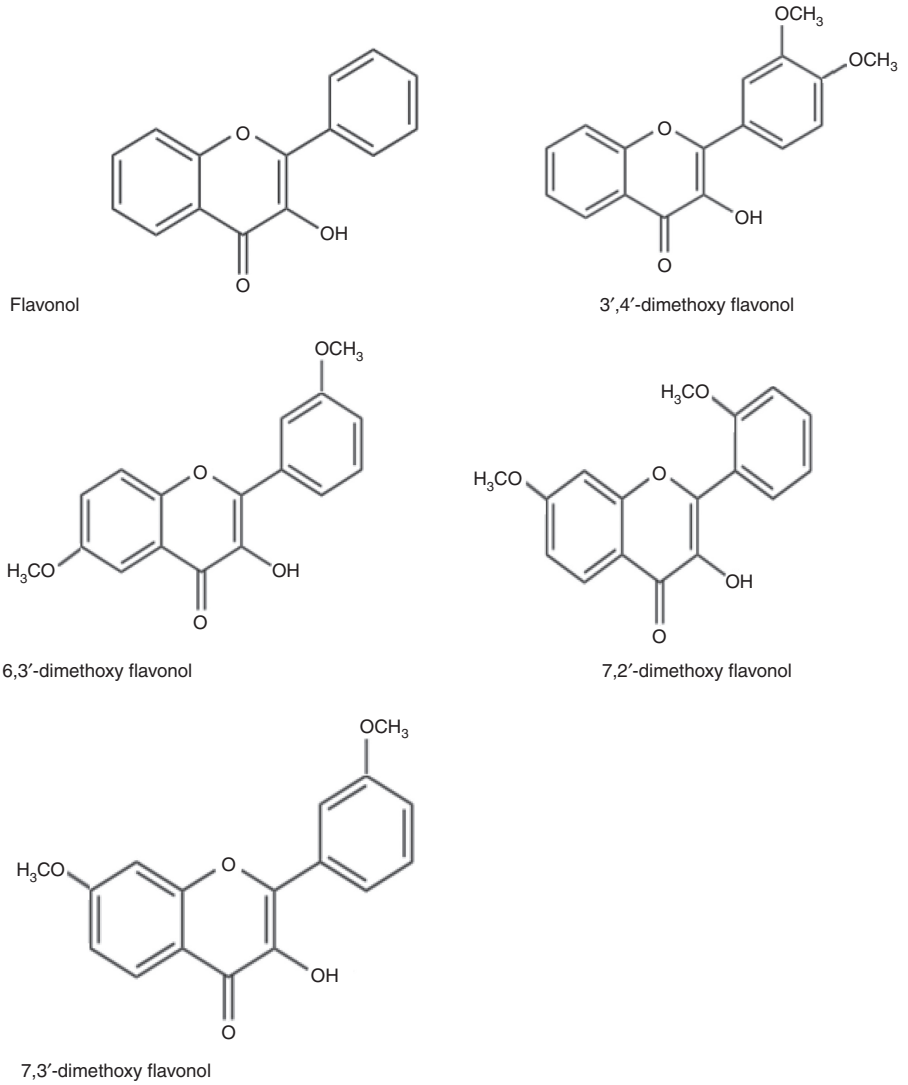

Figure 1: Structure of flavonol and dimethoxy flavonols used in this study.

various doses (25, 50, 100 and 200 mg/kg, s.c.) of test compounds (flavonol, 3′,4′-dimethoxy flavonol, 6,3′-dimethoxy flavonol, 7,2′-dimethoxy flavonol and 7,3′-dimethoxy flavonol) or morphine (10 mg/kg, s.c.) in different groups of paclitaxel treated mice.

Assessment of tactile allodynia [10]

Mice were placed individually in a plastic cage (13×7×7 cm) with a wire mesh bottom. After acclimatization for a period of 15 min, von Frey's hair aesthesiometer of 15 mm length was pressed perpendicularly against the plantar surface of the hind paws. The responses to the stimulus were ranked as follows:

0–No response
1–Move away from the filament
2–Immediate flinching or licking of the hind paw.

Stimulation with the von Frey hair aesthesiometer was applied five times to each hind paw at an interval of 30 s and the sum of 10 values served as paw withdrawal response score.

Assessment of cold allodynia [11]

Cold allodynia was assessed by touching each hind paw of the mouse with a bubble of acetone formed at the tip of a 1 mL syringe. The response of the mouse to acetone was noted for a period of 20 s and was graded to a four-point scale:

0–No response
1–Immediate withdrawal
2–Prolonged withdrawal
3–Licking/biting of the paw.

Acetone bubble was applied thrice to the hind paws with a gap of 1 min between each application and the scores recorded in both hind paws were added to obtain a paw withdrawal response score.

Thermal hyperalgesia [12]

Hot water tail immersion test was conducted for the assessment of thermal hyperalgesia. The mouse was restrained in a mouse holder and the tail was immersed in a hot water bath maintained at 48±0.5 °C. The time taken for the mouse to flick the tail from the hot water was considered as the reaction time. The reaction time was noted initially before any drug treatment and 30 min after drug treatment. A significant increase in mean reaction time between these two readings is an indication of attenuation of thermal hyperalgesia. A cut off time of 20 s was maintained to prevent any injury to the tail. The response was expressed as % maximum protective effect (MPE), which was calculated using a formula:

$$\%\ \text{MPE} = \frac{\text{Test latency} - \text{control latency}}{\text{Cut off time} - \text{control latency}} \times 100$$

Effect of flavonols on cytokines – IL-1β and TNF-α

Freshly heparinized human whole blood was used for this immunometric assay. This assay is based on a double antibody "sandwich" technique. A microwell plate supplied with the commercial kit (Cayman, USA) was coated with monoclonal antibodies specific for TNF-α or IL-1β. They will capture any TNF-α or IL-1β introduced in the well. Various concentrations of flavonols (dissolved and diluted in dimethyl sulfoxide) from 20 to 240 μM were added to the well. Some 50 μL of acetylcholinesterase (AchE), which binds selectively to a different epitope on the TNF-α or IL-1β molecule, was also added to the well. When TNF-α or IL-1β (standard or sample) was introduced to the above mix, the two antibodies form a sandwich by binding on opposite sides of the TNF-α or IL-1β molecule. The concentration of the analyte was then determined by measuring the enzymatic activity of the AchE by adding Ellman's reagent (which contains the substrate for AchE) to each well. The product of the AchE-catalyzed reaction has a distinct yellow color which shows strong absorbance at 412 nm. The intensity of this color determined spectrophotometrically is directly proportional to the amount of bound conjugate which in turn is proportional to the concentration of the TNF-α or IL-1β. Dexamethasone (20–240 μM) was used as a standard in TNF-α and IL-1β assay:

$$\%\ \text{of cytokine inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

Reactive oxygen species/free radical scavenging activity

The effect of various flavonols on free radical generation/scavenging was investigated against nitric oxide radicals and 2,2 diphenyl-1-picryl hydrazyl (DPPH) by *in vitro* methods.

Nitrogen derived radical scavenging activity [13]

This assay is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. This can be estimated colorimetrically with Griess reagent. Some 3 mL of reaction mixture containing 2 mL of sodium nitroprusside in phosphate buffered saline and 1 mL of various concentrations (20, 30, 60, 120 and 240 μM) of the investigated flavonols dissolved in ethanol were incubated at 37 °C for 4 h. A control sample without the test compound was also incubated in an identical manner. After incubation, 0.5 mL of Griess reagent (100 mL of 1% sulfanilamide prepared in 3M HCl) was added to react with the reaction mixture. The absorbance of the chromophore formed was read at 546 nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of the control and test compound. A standard antioxidant vitamin C (20–240 μM) was used for comparison:

$$\%\ \text{of nitric oxide inhibition} = \frac{\text{Abs. control} - \text{Abs. test}}{\text{Abs. control}} \times 100$$

DPPH scavenging activity [14]

DPPH is a stable free radical, showing a deep violet color, characterized by an absorption band in ethanol solution at 517 nm.

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, the free radical DPPH is reduced to corresponding hydrazine. Stock solution of DPPH was prepared by dissolving 25 mg of DPPH in 100 mL of ethanol. Some 2 mL of reaction mixtures containing 1.9 mL of DPPH and 0.1 mL of the investigated flavonols in different concentrations (20, 30, 60, 120 and 240 μM) dissolved in ethanol were prepared. A control reaction mixture without the test compound was prepared in an identical manner. The reaction was allowed to complete in the dark for about 20 min. Then, the absorbance of the test mixture was read at 517 nm. The activity was compared with vitamin C, which was used as a standard antioxidant:

$$\% \text{ of DPPH inhibition} = \frac{\text{Abs. control} - \text{Abs. test}}{\text{Abs. control}} \times 100$$

Statistical analysis

The results were analyzed by one way analysis of variance followed by Dunnett's t-test or paired 't' test using SPSS 16 software. All p values less than 0.05 were considered statistically significant. All the IC_{50} values for *in vitro* studies were calculated by linear regression analysis.

Results

Effect of flavonol and dimethoxy flavonols on paclitaxel-induced tactile allodynia in mice

Stimulation of the mouse hind paw with the von Frey hair aesthesiometer produced aversive behavioral responses like flinching or licking of the paw, which were generally not observed in naïve mice. Treatment with morphine 10 mg/kg showed a significant reduction in the paw withdrawal response score when compared to the score obtained before drug treatment (Table 1). Treatment with various doses of flavonol and dimethoxy flavonols also produced a significant and dose-dependent reduction in the paw withdrawal response scores when compared to their respective pretreatment values (Table 1). However, the response observed with 7,2′-dimethoxy flavonol was significant only at the highest dose employed (200 mg/kg). Flavonol in the maximal employed dose (200 mg/kg) exhibited a greater reduction in the paw withdrawal response score when compared to dimethoxy flavonols.

Effect of flavonol and dimethoxy flavonols on paclitaxel-induced cold allodynia in mice

Application of a drop of acetone on the hind paw of a paclitaxel-treated mouse elicited aversive behavioral responses

Table 1: Effect of flavonol and dimethoxy flavonols (DMF) against paclitaxel-induced tactile allodynia in mice.[b]

Dose of the test compounds, mg/kg, s.c.	Paw withdrawal response score									
	Flavonol		3′,4′-DMF		6,3′-DMF		7,2′-DMF		7,3′-DMF	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
25	20.00 ± 0.00	19.83 ± 0.16	20.00 ± 0.00	19.16 ± 0.30	20.00 ± 0.00	18.50 ± 0.61	20.00 ± 0.00	19.33 ± 0.33	20.00 ± 0.00	16.83 ± 0.47[a]
50	20.00 ± 0.00	17.00 ± 1.52	19.83 ± 0.16	18.00 ± 0.85[a]	20.00 ± 0.00	18.50 ± 0.22[a]	20.00 ± 0.00	18.83 ± 0.54	20.00 ± 0.00	14.00 ± 0.36[a]
100	19.83 ± 0.16	7.66 ± 1.90[a]	19.83 ± 0.16	9.00 ± 0.68[a]	20.00 ± 0.00	12.66 ± 0.49[a]	20.00 ± 0.00	18.66 ± 0.80	20.00 ± 0.00	9.50 ± 0.42[a]
200	19.50 ± 0.22	1.33 ± 0.61[a]	19.83 ± 0.16	8.50 ± 1.38[a]	20.00 ± 0.00	8.66 ± 0.49[a]	20.00 ± 0.00	14.50 ± 1.56[a]	20.00 ± 0.00	5.16 ± 0.79[a]

Each value represents mean ± S.E.M of six observations. Paw withdrawal response score in vehicle-treated mice before treatment – 20.00 ± 0.00 and after treatment – 19.33 ± 0.33. Paw withdrawal response score in morphine treated mice before treatment – 20.00 ± 0.00 and after treatment – 3.50 ± 1.23[a]. [a]$p < 0.05$ compared to the value before drug treatment. [b]All treatment groups received paclitaxel (10 mg/kg, i.p.) 24 h prior to the test. Tactile allodynia was determined before and 30 min after vehicle/morphine/flavonol/dimethoxy flavonols treatment on the next day. 3′,4′-DMF, 3′,4′-dimethoxy flavonol; 6,3′-DMF, 6,3′-dimethoxy flavonol; 7,2′-DMF, 7,2′-dimethoxy flavonol; 7,3′-DMF, 7,3′-dimethoxy flavonol.

such as immediate withdrawal of the paw with licking or biting of the paw. Morphine treatment produced a significant reduction in the paw withdrawal response score when compared to its pretreatment value (Table 2). Treatment with different doses of flavonol and dimethoxy flavonols showed a significant and dose-dependent reduction in the paw withdrawal response scores when compared to the respective values obtained before drug administration (Table 2). Similar to the response observed in tactile allodynia, in this method also flavonol exhibited a maximal reduction in the paw withdrawal response score (200 mg/kg).

Effect of flavonol and dimethoxy flavonols on paclitaxel-induced thermal hyperalgesia in mice

Treatment with morphine showed a significant increase in the mean reaction time (15.14 ± 0.48 s) offering 75.68% protection against paclitaxel – induced thermal hyperalgesia (Table 3). Similarly, treatment with various doses of flavonol and its dimethoxy derivatives elicited a dose-dependent and significant increase in the mean reaction time. A maximal protective effect of 28.9% was observed with flavonol (200 mg) and other dimethoxy flavonols in a similar dose, which offered protection ranging from 13.35% (7,3′-dimethoxy flavonol) to 27.11% (3′,4′-dimethoxy flavonol) (Table 3).

Effect of flavonol and dimethoxy flavonols on TNF-α

Flavonol and dimethoxy flavonols inhibited TNF-α in a concentration-dependent manner. At a concentration of 20 μM, the inhibitory activity on TNF-α ranged between 20.46% and 26.72% for these compounds (Figure 2). At a concentration of 240 μM, the inhibition ranged from 73.84% to 86.14% with 7,2′-dimethoxy flavonol exerting maximum inhibition. The IC_{50} value of dexamethasone was 31.32 μM and that of test compounds ranged from 59.56 μM (3′,4′-dimethoxy flavonol) to 98.48 μM (6,3′-dimethoxy flavonol).

Effect of flavonol and dimethoxy flavonols against IL-1β

A concentration-dependent inhibition of IL-1β was evident with flavonol and different dimethoxy flavonols. At a concentration of 20 μM, the inhibitory activity on IL-1β ranged between 26.68% and 35.6% for these compounds

Table 2: Effect of flavonol and dimethoxy flavonols (DMF) against paclitaxel-induced cold allodynia in mice.[b]

Dose of the test compounds, mg/kg, s.c.	Paw withdrawal response score									
	Flavonol		3′,4′-DMF		6,3′-DMF		7,2′-DMF		7,3′-DMF	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
25	17.50 ± 0.22	17.16 ± 0.30	18.00 ± 0.00	16.66 ± 0.61	18.00 ± 0.00	17.66 ± 0.21	18.00 ± 0.00	17.33 ± 0.33	18.00 ± 0.00	16.33 ± 0.42[a]
50	17.50 ± 0.22	12.33 ± 1.30[a]	18.00 ± 0.00	14.16 ± 0.79[a]	17.83 ± 0.16	15.66 ± 0.55[a]	18.00 ± 0.00	16.66 ± 0.61	18.00 ± 0.00	11.83 ± 0. 40[a]
100	17.50 ± 0.34	11.33 ± 2.27[a]	18.00 ± 0.00	10.00 ± 1.46[a]	18.00 ± 0.00	16.00 ± 0.44[a]	17.83 ± 0.16	15.66 ± 0. 61[a]	18.00 ± 0.00	10.16 ± 0. 47[a]
200	18.00 ± 0.00	4.50 ± 0.76[a]	18.00 ± 0.00	8.16 ± 1.49[a]	18.00 ± 0.00	6.00 ± 0.36[a]	18.00 ± 0.00	11.83 ± 1.40[a]	18.00 ± 0.00	7.00 ± 0.57[a]

Each value represents mean ± S.E.M of six observations. Paw withdrawal response score in vehicle treated mice before treatment – 18.00 ± 0.00 and after treatment – 17.66 ± 0.21. Paw withdrawal response score in morphine treated mice before treatment – 18.00 ± 0.00 and after treatment – 2.66 ± 1.25[a]. [a]$p < 0.05$ compared to the value before drug treatment. [b]All treatment groups received paclitaxel (10 mg/kg, i.p.) 24 h prior to the test. Cold allodynia was determined before and 30 min after vehicle/morphine/flavonol/dimethoxy flavonols treatment on the next day. 3′,4′-DMF, 3′,4′-dimethoxy flavonol; 6,3′-DMF, 6,3′-dimethoxy flavonol; 7,2′-DMF, 7,2′-dimethoxy flavonol; 7,3′-DMF, 7,3′-dimethoxy flavonol.

Table 3: Effect of flavonol and dimethoxy flavonols (DMF) against paclitaxel-induced thermal hyperalgesia in mice.[b]

Dose of the test compounds, mg/kg, s.c.	Mean increase in reaction time (s)				
	Flavonol	3′,4′-DMF	6,3′-DMF	7,2′-DMF	7,3′-DMF
25	0.88 ± 0.48 (3.35)	0.04 ± 0.01 (0.15)	0.13 ± 0.05 (0.60)	0.64 ± 0.11[a] (3.15)	0.50 ± 0.08[a] (2.45)
50	2.17 ± 0.72 (9.8)	1.92 ± 0.28[a] (9.55)	0.86 ± 0.10[a] (4.25)	1.54 ± 0.10[a] (7.65)	0.70 ± 0.09[a] (3.45)
100	5.08 ± 0.95[a] (24.35)	4.18 ± 0.43[a] (20.86)	1.28 ± 0.09[a] (6.35)	2.80 ± 0.40[a] (11.95)	1.72 ± 0.09[a] (8.55)
200	5.99 ± 0.82[a] (28.9)	5.43 ± 0.45[a] (27.11)	4.39 ± 0.42[a] (21.91)	4.54 ± 0.59[a] (22.66)	2.68 ± 0.19[a] (13.35)

Each value represents mean ± S.E.M of six observations. Mean increase in reaction time after vehicle treatment – 0.01 ± 0.01. Mean increase in reaction time after morphine treatment – 15.14 ± 0.48[a] (75.68). [a]$p < 0.05$ compared to the value observed with vehicle treatment. [b]All treatment groups received paclitaxel (10 mg/kg, i.p.) 24 h prior to the test. Thermal hyperalgesia was determined before and 30 min after vehicle/morphine/flavonol/dimethoxy flavonols treatment on the next day and the mean increase in reaction time calculated. Values in parenthesis indicate maximal protective effect. 3′,4′-DMF, 3′,4′-dimethoxy flavonol; 6,3′-DMF, 6,3′-dimethoxy flavonol; 7,2′-DMF, 7,2′-dimethoxy flavonol; 7,3′-DMF, 7,3′-dimethoxy flavonol.

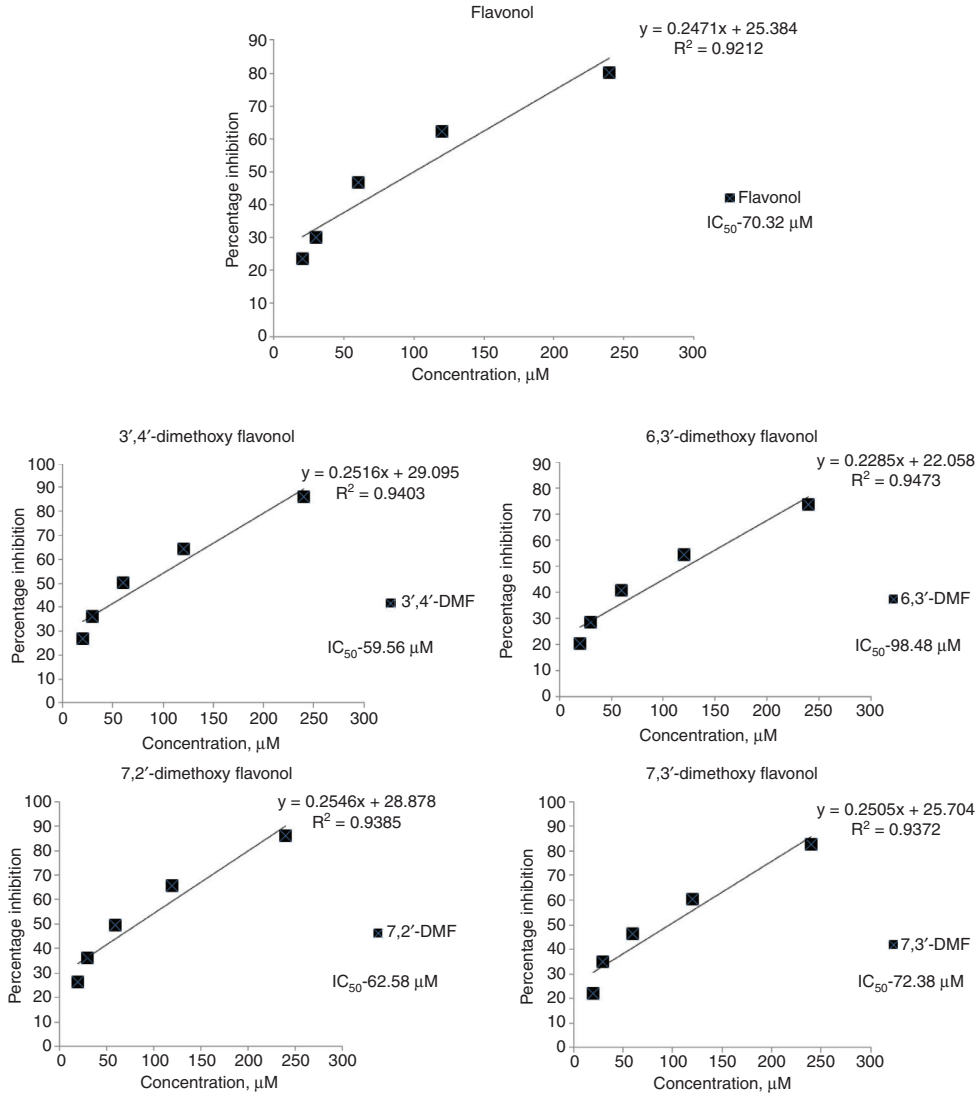

Figure 2: Tumor necrosis factor-alpha (TNF-α) inhibitory activity of flavonol and dimethoxy flavonols.

(Figure 3). At a concentration of 240 µM, the inhibition ranged from 82.84% to 92.38% with 7,3′-dimethoxy flavonol exerting maximum inhibition. The IC_{50} value of dexamethasone was 35.21 µM and that of test compounds ranged from 37.58 µM (3′,4′-dimethoxy flavonol) to 67.54 µM (7,2′-dimethoxy flavonol).

Effect of flavonol and dimethoxy flavonols on free radicals

Nitric oxide scavenging activity

Flavonol and various dimethoxy flavonols exhibited a concentration-dependent inhibition of nitric oxide radical generation. In the lowest concentration employed (20 µM), the percentage inhibition of nitric oxide radical generation for all test compounds ranged between 30.18% and 39.42% (Figure 4). In the highest concentration employed (240 µM), the inhibition of nitric oxide radical generation ranged between 68.82% and 79.74% (Figure 4). The IC_{50} values of 3′,4′-dimethoxy flavonol (40.58 µM) and 7,2′-dimethoxy flavonol (39.46 µM) were almost similar to the IC_{50} value of the standard drug ascorbic acid (41.22 µM). The IC_{50} values of flavonol, 6,3′-dimethoxy flavonol and 7,3′-dimethoxy flavonol ranged between 48.28 and 90.78 µM, which were higher than that of ascorbic acid.

DPPH scavenging activity

Flavonol and various dimethoxy flavonols exhibited a concentration-dependent inhibition of DPPH radical

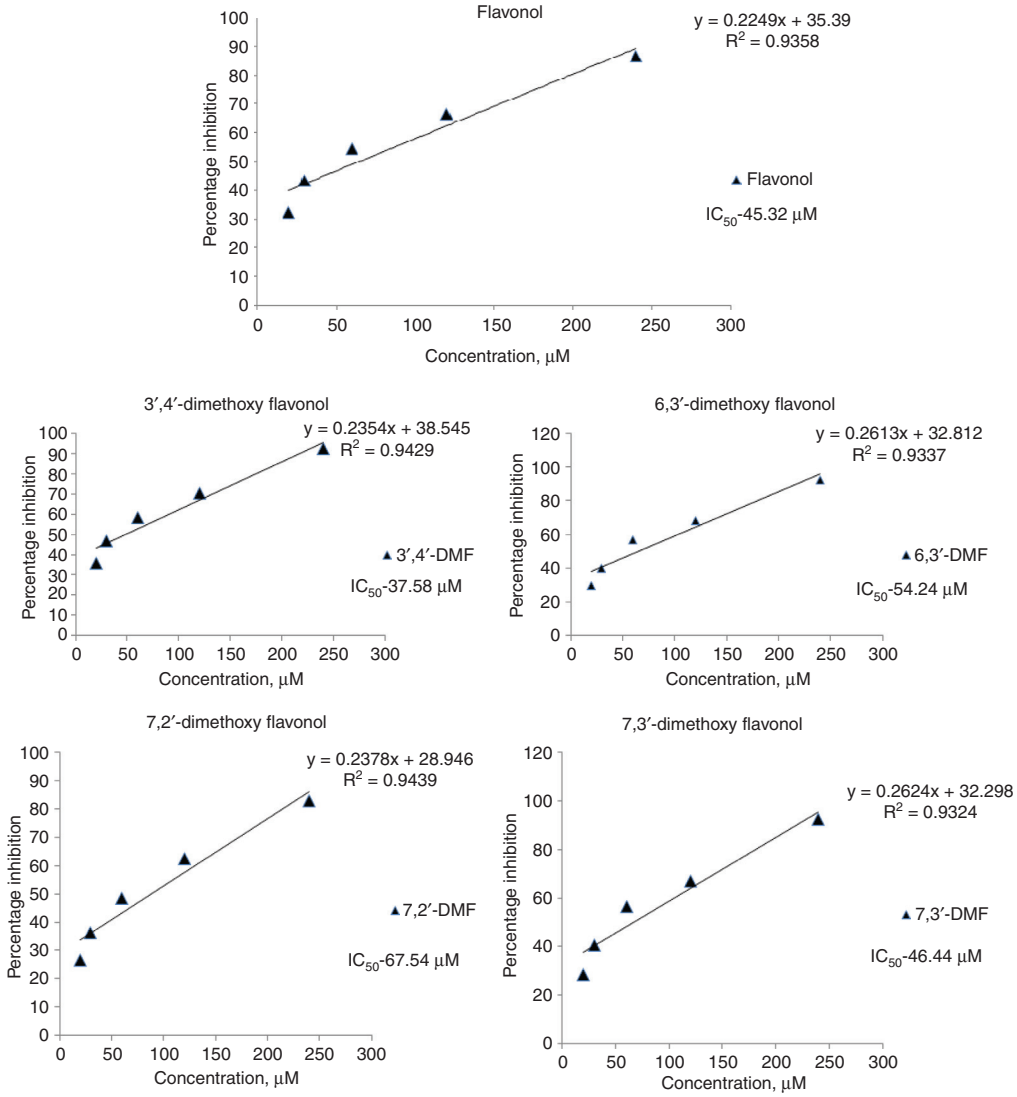

Figure 3: Interleukin-1-beta (IL-1β) inhibitory activity of flavonol and dimethoxy flavonols.

generation. In the lowest concentration employed (20 μM), the percentage inhibition of DPPH radical generation for all test compounds ranged between 27.64% and 34.02% (Figure 5). In the highest concentration employed (240 μM), the inhibition of DPPH radical generation ranged between 81.24% and 86.14% (Figure 5). The IC_{50} value of 3′,4′-dimethoxy flavonol (43.62 μM) was less than the IC_{50} value of the standard drug ascorbic acid (48.12 μM). The IC_{50} values of flavonol, 6,3′-dimethoxy flavonol, 7,2′-dimethoxy flavonol and 7,3′-dimethoxy flavonol ranged between 50.56 and 59.46 μM, which were higher than that of ascorbic acid.

Discussion

A variety of drugs are available for the management of neuropathic pain of different etiology [15]. These drugs include opioid analgesics, anticonvulsants, antidepressants, topical treatments (lidocaine patch, capsaicin), NMDA receptor antagonists, baclofen and clonidine [2]. However, the efficacy of these drugs in the treatment of neuropathic pain is not totally satisfactory and many inherent adverse effects associated with these drugs are also a cause for concern. Hence, investigation on safe compounds effective in ameliorating neuropathic pain

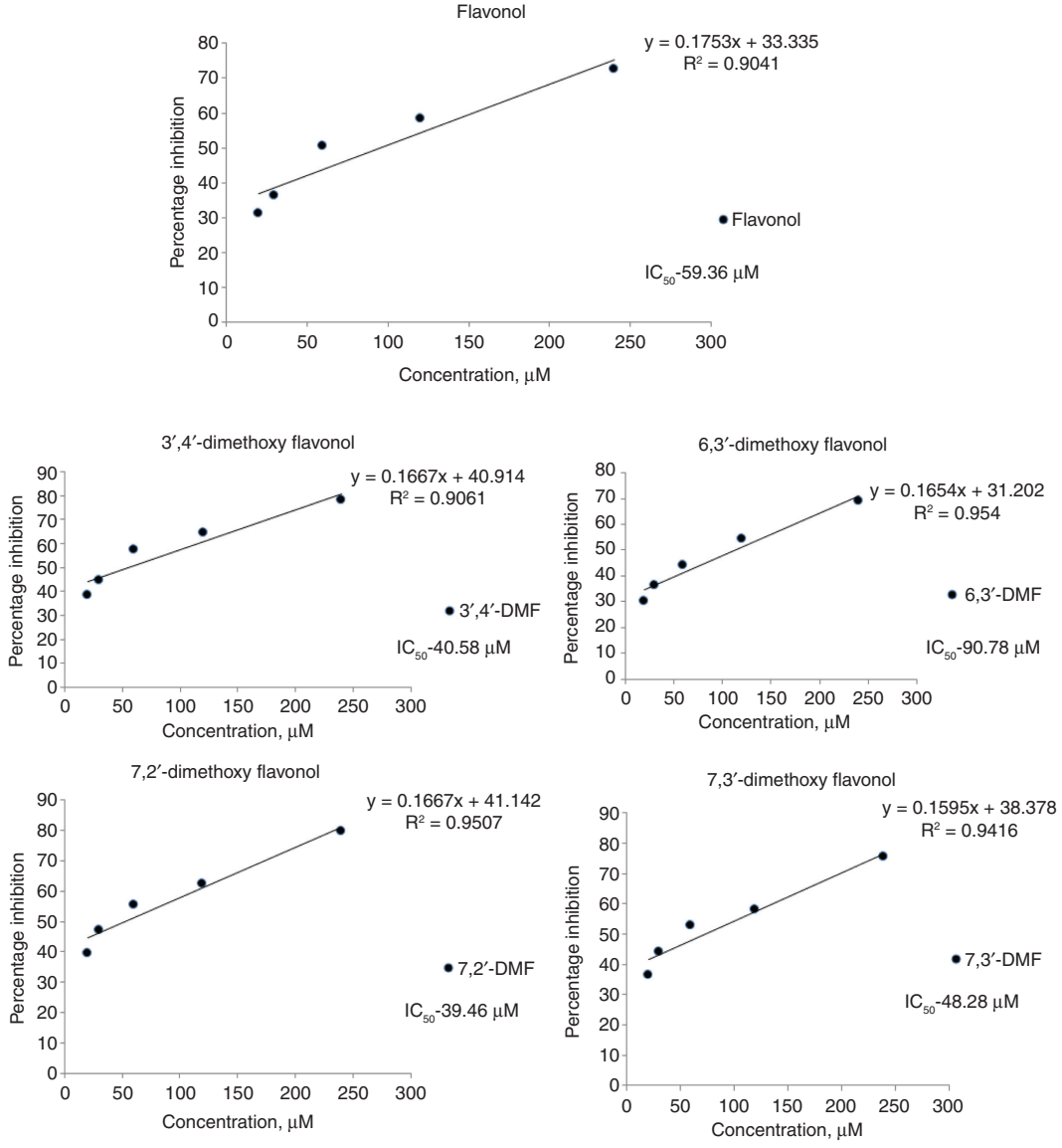

Figure 4: Nitric oxide scavenging activity of flavonol and dimethoxy flavonols.

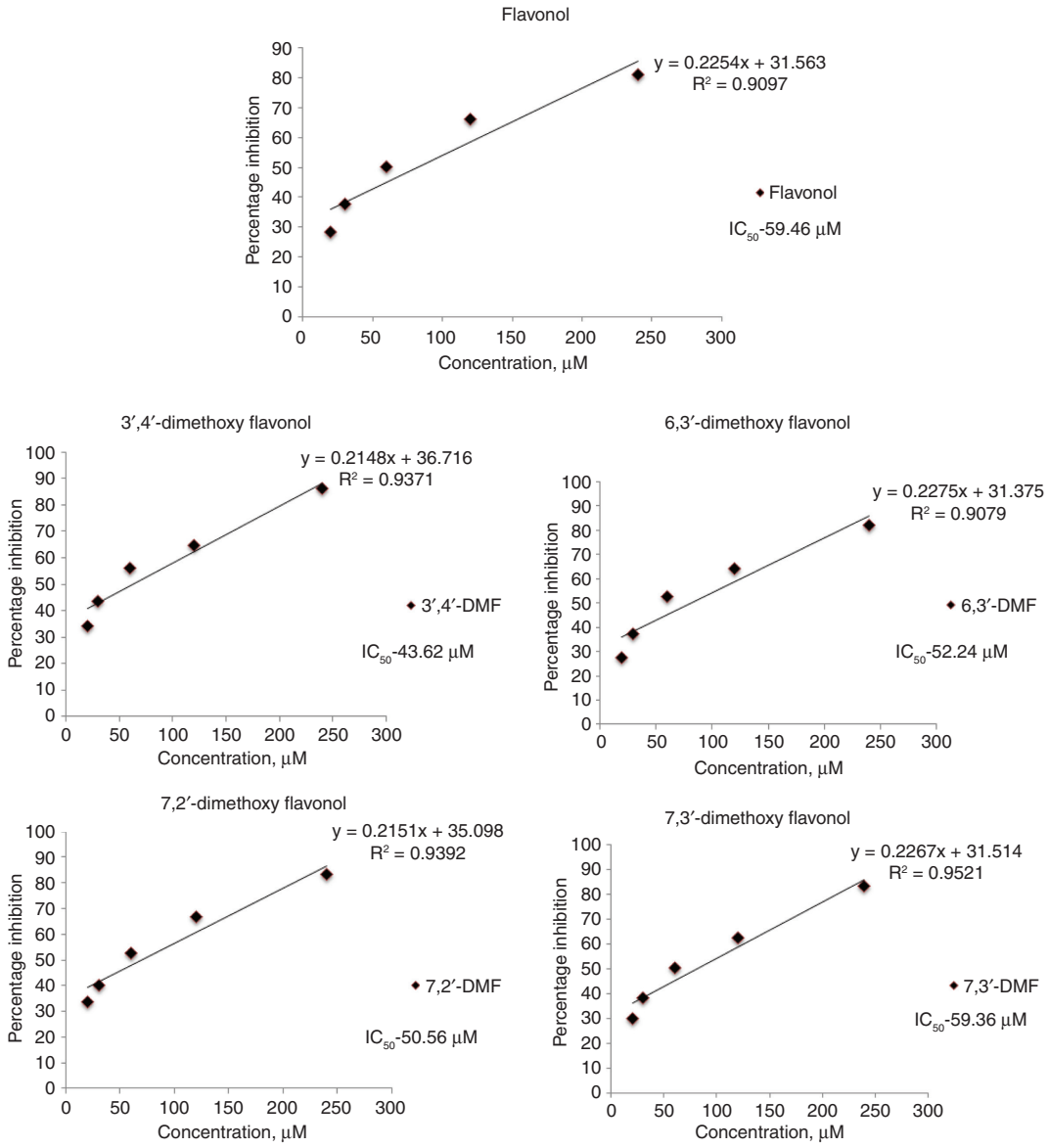

Figure 5: 2,2 Diphenyl-1-picryl hydrazyl (DPPH) scavenging activity of flavonol and dimethoxy flavonols.

is imminent. Earlier literature reported the antiallodynic activity of a few flavonoids in various animal models of neuropathy. Meotti et al. [5] reported the antiallodynic activity of myricitrin in neuropathic pain induced by partial ligation of the sciatic nerve. Another study reported the preventive effects of flavonoids rutin and quercetin against anticancer drug oxaliplatin-induced peripheral neuropathy in mice [6]. Epigallocatechin-3-gallate and its derivative, significantly attenuated thermal hyperalgesia induced by chronic constriction nerve injury in mice [7].

Flavonol was reported to possess antinociceptive effect in mice [16]. Methoxylated flavone compounds were suggested to have better bioavailability [17]. Hence, in the present study, a few dimethoxy flavonol derivatives were chosen for investigation. These compounds exhibited good antinociceptive effect in mice when tested by acetic acid writhing, formalin nociception and hot water tail immersion methods (unpublished observation). Based on these evidences, the present study investigated the effect of flavonol and its dimethoxy derivatives against paclitaxel-induced peripheral neuropathy in Swiss albino mice. Hidaka et al. [9] introduced a single dose of paclitaxel (10 mg/kg) -induced painful neuropathy method in mice which is similar to severe muscle pain in humans followed by chemotherapeutic

treatment. In this model, the development of painful neuropathy was observed after 24 h and lasted for 72 h, suggesting the recovery in functions of impaired sensory neurons.

Significant attenuation of the manifestations of paclitaxel-induced peripheral neuropathy was evident in mice treated with different flavonols. Maximum attenuation of neuropathic responses was recorded with all the flavonols in a dose of 200 mg/kg against tactile allodynia and cold allodynia (Tables 1 and 2). However, the response observed with 7,2′-dimethoxy flavonol was less when compared to that of other flavonols. The MPE offered by 7,3′-dimethoxy flavonol against thermal hyperalgesia was 13.35%, whereas the response with other compounds ranged between 21.91% and 28.9% (Table 3). Notwithstanding the magnitude of response observed in different tests, the above results clearly reveal the protective effect of flavonol and its dimethoxy derivatives against neuropathic manifestations induced by a widely used anticancer drug paclitaxel.

Flavonoids rutin and quercetin have been reported to offer protection against oxaliplatin-induced peripheral neuropathy in mice [6], and myricitrin [5] was effective in attenuation of allodynia due to partial ligation of the sciatic nerve. Taken together, these results suggest that flavonoids could be a potential source for identification of an effective drug in the management of neuropathy.

In the present study, flavonol was found to exert maximum efficacy in the amelioration of neuropathic manifestations after paclitaxel administration. Introduction of two methoxy groups in various positions of the flavonol has retained the efficacy but not augmented the effect. Further investigations on their effect against neuropathy induced by other chemotherapeutic drugs like vincristine and oxaliplatin can be expected to provide more information in this regard.

Effect of flavonol and dimethoxy flavonols against TNF-α and IL-1β

Nobiletin, a hexamethoxy flavone, has been reported as an anti-inflammatory drug based on its inhibitory effect on pro-inflammatory cytokines such as IL-1α, IL-1β, TNF-α and IL-6 in mouse macrophages [18]. Recent studies reported the inhibition of TNF-α and IL-1β activity by dihydroxy flavones [19] and dimethoxy flavones [20]. Hence, it was considered interesting to screen flavonol and its dimethoxy derivatives for their effect on TNF-α and IL-1β.

Treatment with flavonol and dimethoxy flavonols resulted in a significant and concentration-dependent inhibitory activity on TNF-α (Figure 2). 7,2′-Dimethoxy flavonol and 3′,4′-dimethoxy flavonol showed a higher degree of inhibition of TNF-α when compared to other flavonols. Similarly, a significant and concentration-dependent inhibitory activity on IL-1β was recorded for all the tested compounds (Figure 3). However, 7,3′-dimethoxy flavonol, 3′,4′-dimethoxy flavonol and 6,3′-dimethoxy flavonol exhibited a higher degree of inhibition of IL-1β compared to 7,2′-dimethoxy flavonol and flavonol. The results of the present study are in agreement with the previous reports on flavonoids against proinflammatory cytokines TNF-α and IL-1β. Inflammatory cytokines (TNF-α and IL-1β) are involved in allodynia and hyperalgesia following nerve injury after treatment with anticancer drugs like paclitaxel and vincristine by sensitizing A δ and C fibers [21]. The inhibition of TNF-α and IL-1β by flavonol derivatives observed in the present study may contribute to the protection against paclitaxel-induced neuropathic manifestations.

Free radical scavenging activity of flavonol and dimethoxy flavonols

The involvement of reactive oxygen species in different types of pain and in particular chemotherapy-induced pain [22] has been well documented. The involvement of free radicals in paclitaxel-induced peripheral neuropathy has been proposed [8]. A free radical scavenger phenyl N-tert-butyl nitrone [23] ameliorated mechanical allodynia due to paclitaxel-induced peripheral neuropathy in rats. It is a well-known fact that many beneficial effects of flavonoids are mainly attributed to their potent antioxidant activity [24, 25]. Recent reports indicate the antioxidant property of dihydroxy flavones [19] and dimethoxy flavones [20] by inhibiting reactive oxygen species.

In the present study, a dose-dependent inhibition of nitric oxide generation was recorded for flavonol and its dimethoxy derivatives. In a maximal concentration of 240 μM, the % inhibition of nitric oxide generation by test compounds ranged between 68.82% and 79.94% (Figure 4). A dose-dependent inhibition of DPPH free radical generation was also observed for all the investigational compounds with almost a similar maximum effect ranging between 81.24% and 86.14% (Figure 5). The antioxidant activity of the flavonols may also be responsible for protection against neuropathic manifestations.

Conclusions

Flavonol and its dimethoxy derivatives significantly ameliorated the manifestations of paclitaxel-induced peripheral neuropathy in mice. Inhibition of inflammatory cytokines and free radicals recorded in the present study may contribute to the beneficial effects of flavonols.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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