

Portulaca oleracea inhibit vincristine induced peripheral neuropathy: involvement of ATP-sensitive K⁺ channels

L. Mohana Rupa^{1*}, K. Mohan², N. Santhosh Kumar³

¹Department of Pharmacology,
Sri Sathya Sai Medical
College & Research Institute,
Tamil Nadu, India,

²Department of Anaesthesia,
Sri Sathya Sai Medical
College & Research Institute,
Tamil Nadu, India,

³Department of Biochemistry,
Shridevi Institute of Medical
Sciences & Research Hospital,
Tumkur, Karnataka, India

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***Correspondence to:**

Dr. L. Mohana Rupa,
Email: drrupa2014@gmail.
com

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ABSTRACT

Background: The *Portulaca oleracea* belonging to portulacaceae family. It is a herbaceous plant widely distributed throughout the world and used in traditional medicine for many ailments. The present study was to evaluate the antinociceptive action of petroleum ether extract of *P. oleracea* in vincristine induced peripheral neuropathic pain and the possible mechanisms involved.

Methods: Peripheral neuropathy was induced in rats by administration of vincristine sulfate (50 µg/kg i.p.) for 10 consecutive days. The cold tail hyperalgesia was assessed by cold water tail immersion test. To identify the possible mechanisms involved in the antinociceptive action of petroleum ether extract of *P. oleracea*, acetic acid writhing method was employed. Mice were pretreated with naloxone, glibenclamide before petroleum ether extract treatment to identify the involvement of opioid and potassium channels, respectively.

Results: The administration of petroleum ether extract of *P. oleracea* (100 and 200 mg/kg p.o.) for 10 days significantly attenuated vincristine-induced cold hyperalgesia. Pre-treatment with glibenclamide reversed the antinociceptive effect of *P. oleracea*, but the naloxone pre-treatment did not reverse the antinociceptive activity of *P. oleracea*.

Conclusion: The results of the present study reveal the antinociceptive effect of *P. oleracea* in vincristine induced peripheral neuropathy and involving ATP-sensitive potassium channels pathway.

Keywords: Antinociceptive effect, Neuronal mechanisms, Petroleum ether extract

INTRODUCTION

Neuropathic pain is one of the main side-effects of a wide number of antitumor agents.¹ Nowadays, there is no effective treatment to prevent or reverse this chemotherapy-induced neuropathy.² The mechanism(s) responsible for the pain syndrome are quite unknown.³

Many drugs such as cannabinoid,⁴ gabapentin,⁵ thalidomide and minocycline,⁶ neurotrophin⁷ and acetyl-L-carnitine⁸ have been successfully reduced hyperalgesia induced

by paclitaxel. However, their prolonged clinical use is hampered by their side-effects. It is apparent that due to the side-effects of the currently used drugs, there is a need for safe agents with minimal adverse effects. Recently, the search for the appropriate agents has been focused on plants.

Portulaca oleracea belonging to portulacaceae family. It contains many biologically active compounds such as free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinones, proteins,⁹

a-linolenic acid, b-carotene^{10,11} mono terpene glycoside¹² and N-trans-feruloyltyramine.¹³ It has also been found to contain vitamin C, oleoresins-I and II, saponins, tannins, saccharides, triterpenoids, 0a-tocopherol and glutathione.¹⁴

Number of studies had shown that *P. oleracea* exerts diverse biological actions such as antibacterial, antifungal,¹⁵ antifertility,¹⁶ muscle relaxant,¹⁷ wound healing properties,¹⁸ analgesic, and antiinflammatory activity.¹⁹

METHODS

The *P. oleracea* leaves were collected from local vegetable market at Guduvancherry, Tamil Nadu. The sample was authenticated by botanist at Government Degree College at Ammapettai, Sembakkam.

Animals

Either sex of rats weighing 150-200 g and mice 25-30 g were procured from the institutional animal house. The animals had free access to food and water ad libitum under strict hygienic conditions and maintained in room temperature of 25°C ± 1°C, relative humidity 45-55% and a 12:12 hr light/dark cycle. All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals and the study protocol was approved by the Institutional Animal Ethical Committee.

Preparation of extract

Portulaca leaves were shade dried, and 1 kg of coarse powder was soaked in 4 L of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was 10% w/w.²⁰

Drugs and chemicals

Vincristine sulfate (Chandra Bhagat Pharma Pvt., Ltd., Mumbai, India), was dissolved in normal saline. Ethosuximide (Apollo Life Sciences Private Limited, India) was used as a standard drug for comparison. Naloxone hydrochloride (Endo labs, USA); glibenclamide (Dr. Reddy's laboratories, India); All the reagents used in the present study were of analytical grade.

Induction of neuropathic pain by vincristine

The induction of peripheral neuropathy was done in rats by administration of vincristine sulfate (50 µg/kg i.p.) for 10 consecutive days.²¹

Assessment of neuropathic pain

Cold-hyperalgesia test (Cold water tail immersion test)

In this method, the rat was restrained in a rat holder, and the tail was immersed in a cold water bath maintained at 0-4°C. The reaction time to flick the tail from the cold water was recorded for each rat. The reaction time was noted initially before *P. oleracea* extract treatment and 30 min after the *P. oleracea* extract treatment. A significant increase in mean reaction time (s) between these two readings is an indication of antinociceptive response. A cut-off time of 20 s was maintained.²²

Investigation on the mechanisms of action

Further experiments were carried out to elucidate the possible mechanisms by which the *P. oleracea* exert their antinociceptive action. The 200 mg/kg dose of *P. oleracea* was selected for this.

To assess the possible mechanisms involved in the antinociceptive action of *P. oleracea*, mice were pre-treated with the following interacting agents (i.p.) 15 mins prior to the administration of petroleum ether extract of *P. oleracea*. The antinociceptive response was recorded 30 mins after *P. oleracea* extract treatment by acetic acid writhing method.

- Opioid antagonist naloxone 5 mg/kg;²³
- ATP-sensitive potassium channel (K⁺_{ATP}) blocker glibenclamide 10 mg/kg;²⁴

Experimental protocol

Group I: vehicle control group

Normal saline was administered to rats for 10 consecutive days. The behavioral test was employed on 11th day.

Group II: ethosuximide group

Ethosuximide (i.p. 450 mg/kg) was used as a standard drug and for 10 consecutive days, starting from the day 1, 30 mins prior to vincristine administration. The behavioral test was employed on 11th day.

Group III: petroleum ether extract of *P. oleracea* group

The petroleum ether extract of *P. oleracea* (100, 200 mg/kg p.o.) was administered for 10 consecutive days, starting from the day 1, 1 h prior to vincristine administration. The behavioral test was employed on 11th day.

Statistical analysis

All the results were expressed as mean ± standard error mean. Statistical analysis was done using paired "t" test and

a SPSS (Software Package Used for Statistical Analysis) software version 16.0. The $p < 0.05$ was considered to be statistically significant.

RESULTS

The present study the mean increase in the reaction time of vehicle treated control animals was not significant. However, ethosuximide treatment resulted in a significant increase in reaction time. 100 and 200 mg/kg of *P. oleracea* offered maximum antinociceptive active against vincristine induced cold tail hyperalgesia (Table 1 and Figure 1).

Pre-treatment with glibenclamide significantly attenuated the antinociceptive effect of *P. oleracea*. However, the naloxone pre-treatment did not produce any change in the antinociceptive activity of *P. oleracea*. (Table 2 and Figure 2).

DISCUSSION

In the present study, 10 days administration of vincristine (50 $\mu\text{g}/\text{kg}$, i.p.) led to significant development of tail cold hyperalgesia, which was significantly attenuated by petroleum ether extract of *P. oleracea*. Vincristine has been used as a chemotherapeutic agent for the treatment

of several malignancies including breast cancer, leukemia, lymphomas, and primary brain tumors.²⁵ However, clinical use of vincristine has been associated with the development of neurotoxicity of peripheral nerve fibers with resultant sensory-motor neuropathy. Among all the chemotherapeutic agents, vincristine produces predictable and uniform neurotoxicity in all the patients even at the therapeutic doses.²⁶ Painful paresthesias are usually the first symptoms in the majority of patients with this dose dependent neuropathy. The pinprick and temperature senses are more affected than vibration sense. In the later stages of disease, loss of axons and motor functions become more prominent.²⁷ Vincristine does not readily cross the blood-brain barrier,²⁸ but has dramatic effects on the peripheral nervous system causing microtubule disorganization and endoneurial swelling in myelinated as well as in unmyelinated sensory axons.^{29,30}

Antinociceptive activity of *P. oleracea* may be due to the presence of active phytoconstituents in the petroleum ether extract, such as flavonoids, phenols and alkaloids.¹⁹ This interesting observation indicates that the extracts can be a potential source for the treatment of chronic pain. However, detailed study like isolation of active molecule and characterization is required to confirm the phytochemical response for the activity.

Several types of ion channels play vital roles in the initiation of the pain signal and conduction of

Table 1: Effect of *Portulaca oleracea* on vincristine induced cold water hyperalgesia each column represents the mean \pm SEM of six rats.

Groups	Before drug treatment	After drug treatment
Vehicle	1.79 \pm 0.40	2.13 \pm 0.39
Ethosuximide	1.66 \pm 0.47	7.68 \pm 0.34**
<i>Portulaca oleracea</i> 100 mg/kg p.o	1.73 \pm 0.29	3.67 \pm 0.29*
<i>Portulaca oleracea</i> 200 mg/kg p.o	1.82 \pm 0.28	5.13 \pm 0.19**

** $p < 0.001$, * $p < 0.05$ when compared to vehicle - treated group. SEM: Standard error mean, *P. oleracea*: *Portulaca oleracea*

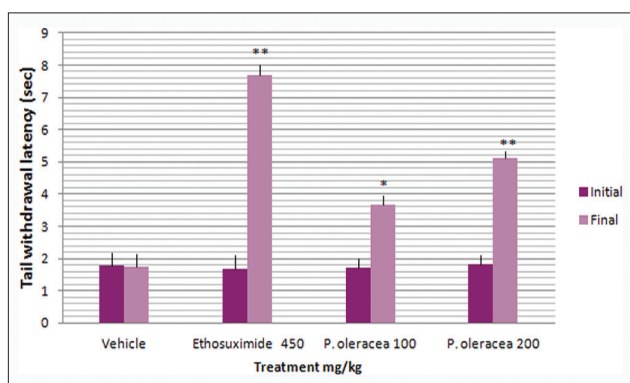


Figure 1: Effect of *Portulaca oleracea* on vincristine induced cold water hyperalgesia.

Table 2: The effect of pre-treatment with Naloxone, Glibenclamide in *P. oleracea* – induced antinociception against acetic acid-induced abdominal writhing. Each column represents the mean \pm SEM of six mice.

Groups	Before antagonist treatment	After antagonist treatment
Naloxone+ <i>P. oleracea</i>	14.91 \pm 1.46	15.23 \pm 2.09
Glibenclamide+ <i>P. oleracea</i>	14.91 \pm 1.46	29.59 \pm 0.21

SEM: Standard error mean, *P. oleracea*: *Portulaca oleracea*

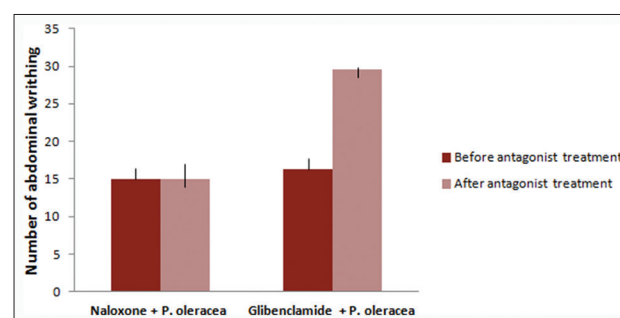


Figure 2: Mechanisms involved in *Portulaca oleracea* – antinociception.

nociception. The behavioral and electrophysiological results obtained by Du et al.,³¹ confirmed the presence of K^+_{ATP} channel in nociceptors. A variety of antinociceptive agents such as diclofenac,³² clonidine,³³ and resveratrol³⁴ have been shown to recruit K^+_{ATP} channel in eliciting their antinociceptive effect. The attenuation of the antinociceptive response elicited by *P. oleracea* by glibenclamide pre-treatment confirms the participation of K^+_{ATP} channels.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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