Neuropharmacological and Neuroprotective Effect of *Portulaca oleracea* Extract in Rats

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Abstract
Neurological disorders remain a significant global health challenge, necessitating the search for safer and more effective therapeutic agents. Portulaca oleracea *has attracted considerable attention because of its rich phytochemical composition and reported neuroprotective properties. This study investigated the neuropharmacological and neuroprotective effects of aqueous* Portulaca oleracea *extract using the rat phrenic nerve–diaphragm preparation. The effects of the extract on neuromuscular transmission were compared with those of pancuronium and D-tubocurarine, two established non-depolarizing neuromuscular blocking agents. Under control conditions, normal diaphragm contractions were observed following phrenic nerve stimulation. Administration of pancuronium and D-tubocurarine produced concentration-dependent inhibition of contractile responses, which were reversed after tissue washing. Similarly, the aqueous extract of* Portulaca oleracea *caused a marked reduction in contraction amplitude in a concentration-dependent manner, with substantial recovery following washing, indicating a reversible effect. Furthermore, physostigmine reversed both D-tubocurarine- and extract-induced blockades, suggesting the involvement of cholinergic mechanisms at the neuromuscular junction. The findings indicate that* Portulaca oleracea *possesses significant neuropharmacological activity and may interfere with cholinergic neurotransmission through mechanisms comparable to those of classical non-depolarizing neuromuscular blockers. These results provide additional evidence supporting the biological activity of* Portulaca oleracea *and highlight its potential as a source of bioactive compounds for further neuroprotective and neuromuscular research.*

Keywords: Portulaca oleracea*, neuropharmacology, neuroprotection, neuromuscular transmission, phrenic nerve–diaphragm preparation, physostigmine, D-tubocurarine, pancuronium.*

Introduction

Neurological disorders constitute a major global public health challenge because they are associated with increasing morbidity, disability, and mortality across different populations worldwide (Jalali & Rahbardar, 2023). The growing prevalence of neurodegenerative diseases, cognitive impairment, depression, and other nervous system disorders has intensified the search for effective and safer therapeutic agents capable of preventing neuronal damage and improving neurological outcomes (El Rabey et al., 2025). Conventional neuropharmacological therapies have demonstrated substantial benefits in the management of neurological diseases; however, their long-term use is often limited by adverse effects, high costs, and incomplete

therapeutic efficacy (Patwa, 2024). Consequently, considerable attention has been directed toward medicinal plants and naturally derived compounds as alternative or complementary interventions for neurological disorders (Kumar et al., 2022). Among these medicinal plants, *Portulaca oleracea* L., commonly known as purslane, has attracted scientific interest because of its rich phytochemical composition and broad pharmacological properties (Ghorani et al., 2023).

Portulaca oleracea is widely distributed across many regions of the world and has been traditionally utilized as both a food source and a medicinal plant for the treatment of various ailments (Kumar et al., 2022). The plant contains numerous bioactive compounds, including flavonoids, alkaloids, omega-3 fatty acids, polysaccharides, vitamins, and phenolic constituents that contribute to its therapeutic potential (Ghorani et al., 2023). These phytochemicals possess antioxidant, anti-inflammatory, immunoregulatory, and cytoprotective activities that may be beneficial in preventing cellular and tissue damage (Ghorani et al., 2023). Growing evidence suggests that oxidative stress and neuroinflammation are key mechanisms involved in the pathogenesis of many neurological disorders, making antioxidant-rich medicinal plants attractive candidates for neuroprotection (Jalali & Rahbardar, 2023). As a result, researchers have increasingly investigated the pharmacological effects of *Portulaca oleracea* in experimental models of disease and tissue injury (Daneva et al., 2026).

Several studies have reported findings that support the neuroprotective potential of *Portulaca oleracea* in experimental animals. Martins et al. (2016) demonstrated that extracts of *Portulaca oleracea* protected dopaminergic neurons against 6-hydroxydopamine-induced lesions, suggesting a possible role in the prevention of neurodegenerative damage. Similarly, Patwa (2024) reported that *Portulaca oleracea* exhibited neuroprotective activity in rats exposed to acrylamide-induced neurotoxicity, indicating its capacity to mitigate neuronal injury. Evidence from an Alzheimer's disease model further showed that methanolic extract of *Portulaca oleracea* protected the brains of male rats and improved neuropathological outcomes associated with neurodegeneration (El Rabey et al., 2025). Additional support for its neurological benefits was provided by findings that *Portulaca oleracea* extract modulated neuroplasticity in a murine model characterized by non-alcoholic fatty liver disease and depression-like manifestations (Mitran et al., 2025). Collectively, these studies agree that *Portulaca oleracea* possesses biological properties capable of influencing neuronal survival, neuroplasticity, and brain function (Jalali & Rahbardar, 2023).

Beyond its direct effects on the nervous system, other investigations have highlighted the broader protective effects of *Portulaca oleracea* against systemic pathological conditions that may indirectly affect neurological health. Research involving diabetic animal models demonstrated that *Portulaca oleracea* extract improved physiological outcomes in spontaneously hypertensive rats with induced type 2 diabetes (Daneva et al., 2026). Protective effects against diabetes-associated inflammatory and reproductive dysfunctions have also been documented, suggesting potent anti-inflammatory and antioxidant actions (Rakhshandeh et al., 2022). Studies examining hypoxia-induced liver injury revealed that acetone extracts of *Portulaca oleracea* improved liver histopathology and reduced tissue damage (Maliza et al., 2026). Similar antioxidative properties were observed in experimental gastric lesions, where the plant extracts exerted gastroprotective effects through oxidative stress modulation (Ngouana et al., 2025). These findings support the argument that the therapeutic benefits of *Portulaca oleracea* may extend beyond individual organ systems and may be linked to common protective mechanisms such as reduction of oxidative stress and inflammation (Ghorani et al., 2023).

Despite the growing body of evidence supporting the medicinal value of *Portulaca oleracea*, important limitations remain in existing literature. Many studies have focused on specific

disease models such as Parkinsonian lesions, Alzheimer's disease, diabetes-related complications, or toxin-induced injury, thereby limiting the generalizability of their findings across diverse neuropharmacological contexts (Martins et al., 2016). Existing reviews have summarized the neurological benefits of the plant but have also emphasized the need for additional experimental validation regarding its mechanisms of action and behavioral effects (Jalali & Rahbardar, 2023). Furthermore, considerable variation exists in extraction methods, dosage regimens, treatment duration, and experimental designs across studies, making direct comparison of outcomes difficult (Kumar et al., 2022). Although antioxidant and anti-inflammatory activities are frequently proposed as major mechanisms underlying neuroprotection, the relationship between these mechanisms and observable neuropharmacological responses remains insufficiently characterized in many animal models (Ghorani et al., 2023; Agbo et al., 2016). Consequently, there is still a need for studies that comprehensively evaluate both neuropharmacological and neuroprotective effects of *Portulaca oleracea* extract using standardized experimental approaches in laboratory animals (Patwa, 2024).

The importance of addressing this knowledge gap extends to biomedical and medical sciences, particularly among students and researchers specializing in pharmacology, neuroscience, and experimental medicine who require evidence-based data on plant-derived neurotherapeutics (Jalali & Rahbardar, 2023). A clearer understanding of the neuropharmacological actions and neuroprotective mechanisms of *Portulaca oleracea* may contribute to the development of safer therapeutic strategies and provide a scientific basis for future translational research (El Rabey et al., 2025). Therefore, the present study investigates the neuropharmacological and neuroprotective effects of *Portulaca oleracea* extract in rats using an experimental animal model designed to evaluate behavioral, neurological, and biological responses following extract administration (Patwa, 2024). The study assesses the capacity of the extract to influence neurobehavioral functions while simultaneously examining its protective effects against neuronal damage through relevant biochemical and physiological indicators (Martins et al., 2016). By integrating neuropharmacological assessment with neuroprotective evaluation, this study seeks to provide comprehensive evidence that addresses existing limitations in literature and advances current understanding of the therapeutic potential of *Portulaca oleracea* in neurological disorders (Mitran et al., 2025).

Materials and Methods

Study Design

This experimental study was conducted using the isolated rat phrenic nerve–diaphragm preparation to evaluate the neuropharmacological effects of aqueous *Portulaca oleracea* extract and to compare its activity with established neuromuscular blocking agents, pancuronium and D-tubocurarine. The ability of physostigmine to reverse the induced neuromuscular blockade was also assessed.

Materials

The apparatus used for the study included a dissecting set, kymograph, 100 mL organ bath, syringes (1, 2, 5, and 20 mL), weighing balance, Soxhlet extractor, beakers, heating mantle, Petri dishes, thread and needle, polygraph, recording paper, electrodes, and a Washington recorder. The drugs used were D-tubocurarine, pancuronium, and physostigmine. The whole plant of *Portulaca oleracea* was used for extract preparation.

Collection and Authentication of Plant Material

The whole plant of *Portulaca oleracea* was collected from the vicinity of the animal house, University of Jos, Nigeria. The plant material was authenticated by Mr. Okwonkwu at the Federal School of Forestry, Jos, Nigeria.

Preparation of Physiological Salt Solution

Tyrode solution was prepared by dissolving sodium chloride (40 g), potassium chloride (1.0 g), calcium chloride (1.0 g), sodium bicarbonate (5.0 g), sodium hydrogen phosphate (0.25 g), magnesium chloride (0.5 g), and glucose (5.0 g) in 5 L of distilled water. The solution was thoroughly mixed until complete dissolution of the salts was achieved.

Preparation of Aqueous Extract

Fresh stems and leaves of *Portulaca oleracea* were collected, air-dried, and pulverized into a fine powder. A 100 g sample of the powdered plant material was extracted with 250 mL of distilled water using a Soxhlet extractor at 100°C for 75 h. The resulting extract was concentrated to dryness using a rotary evaporator maintained at 40°C. The dried extract yielded approximately 5.28% of the original plant material and was stored at 4°C until use.

Preparation of Drug Solutions

The aqueous extract solution was prepared by dissolving 1 g of the dried extract in 10 mL of distilled water to obtain a stock concentration of 1×10^{-1} g/mL. D-Tubocurarine stock solution was prepared by diluting 1 mL of a 15 mg/1.5 mL ampoule with 9 mL distilled water to obtain 1×10^{-3} g/mL, followed by a further ten-fold dilution to obtain 1×10^{-4} g/mL. Pancuronium solution was similarly prepared from a 20 mg/2 mL ampoule to obtain a final concentration of 1×10^{-4} g/mL. Physostigmine solution was prepared by diluting 1 mL of a 1×10^{-2} g/mL stock solution with 9 mL distilled water to obtain a final concentration of 1×10^{-3} g/mL.

Preparation of the Phrenic Nerve–Diaphragm

Rats were humanely sacrificed, and the thoracic cavity was opened to expose the diaphragm and phrenic nerve. The phrenic nerve–diaphragm preparation was carefully isolated and mounted in a 100 mL organ bath containing Tyrode solution supplemented with double glucose. The phrenic nerve was positioned between platinum stimulating electrodes connected to an electrical stimulator, while separate electrodes were attached to the diaphragm muscle for direct stimulation. Muscle contractions were recorded through a transducer connected to a Washington recorder. The preparation was maintained at 37°C and continuously aerated. Nerve and muscle stimulation were delivered at a frequency of approximately 12 stimuli per minute using rectangular wave pulses of 0.5 ms duration at 5 V and 10 V, respectively. The tissue was allowed to equilibrate for 30 min before commencement of the experiments.

Effect of Pancuronium on the Phrenic Nerve–Diaphragm Preparation

Baseline responses to indirect (nerve) and direct (muscle) stimulation were recorded. Pancuronium (5×10^{-7} g/mL) was added to the organ bath and allowed to incubate for 10 min, after which responses were recorded. The tissue was subsequently washed with fresh Tyrode solution, and pancuronium at a concentration of 1×10^{-6} g/mL was incubated for another 10 min before recording the responses.

Effect of D-Tubocurarine on the Phrenic Nerve–Diaphragm Preparation

Baseline responses were first obtained following nerve and muscle stimulation. D-Tubocurarine (1×10^{-6} g/mL) was then added to the organ bath and incubated for 10 min. The

responses were recorded, and the procedure was repeated to obtain additional recordings. Following the experiment, the tissue was allowed to rest for 15 min before subsequent testing.

Effect of Aqueous *Portulaca oleracea* Extract on the Phrenic Nerve–Diaphragm Preparation

Initial control responses to nerve and muscle stimulation were recorded. The aqueous extract (1×10^{-3} g/mL) was added to the organ bath and incubated for 10 min before recording the responses. The procedure was repeated to obtain replicate recordings. Subsequently, the concentration was increased to 1.5×10^{-3} g/mL and incubated for 10 min before further recordings were made.

Reversal of D-Tubocurarine- and *Portulaca oleracea*-Induced Blockade by Physostigmine

The phrenic nerve was stimulated for 2 min to establish a stable baseline response. D-Tubocurarine (1×10^{-6} g/mL) was then added to the organ bath. After 3 min, physostigmine (1×10^{-5} g/mL) was administered, and the responses were recorded. The same procedure was employed to assess the reversal of *Portulaca oleracea*-induced blockade.

Results

Effect of Pancuronium on Phrenic Nerve–Diaphragm Preparation

Figure 1 demonstrates the inhibitory effect of pancuronium on neuromuscular transmission in the phrenic nerve–diaphragm preparation. Under control conditions, the diaphragm exhibited normal contractions. Following administration of pancuronium, the contractile response was markedly reduced, indicating blockade of nicotinic acetylcholine receptors at the neuromuscular junction. The inhibition became more pronounced at higher concentrations of pancuronium. Following washing of the tissue, contractile activity was substantially restored, confirming that the blockade was reversible. These findings are consistent with the known pharmacological action of pancuronium as a non-depolarizing neuromuscular blocking agent.

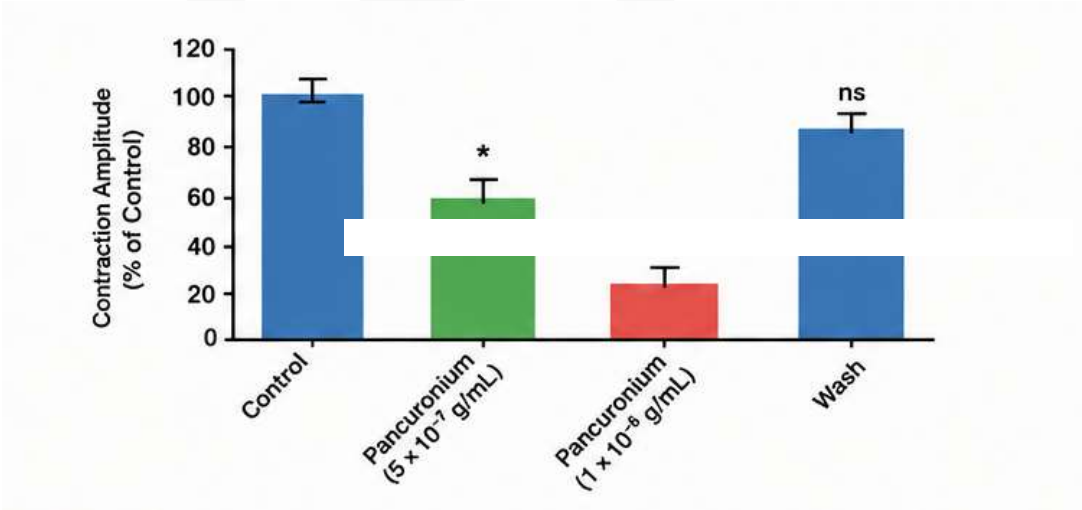


Figure 1: Effect of Pancuronium

Effect of D-Tubocurarine on Phrenic Nerve–Diaphragm Preparation

Figure 2 shows that D-tubocurarine produced a significant reduction in diaphragm contractions following stimulation of the phrenic nerve. The progressive decline in contractile response with increasing drug concentration indicates a dose-dependent neuromuscular blockade. The restoration of contractions after washing suggests that the effect was reversible and occurred through competitive antagonism of acetylcholine at nicotinic receptors. The observed pattern

closely resembles that produced by pancuronium, supporting the classification of D-tubocurarine as a classical non-depolarizing neuromuscular blocker.

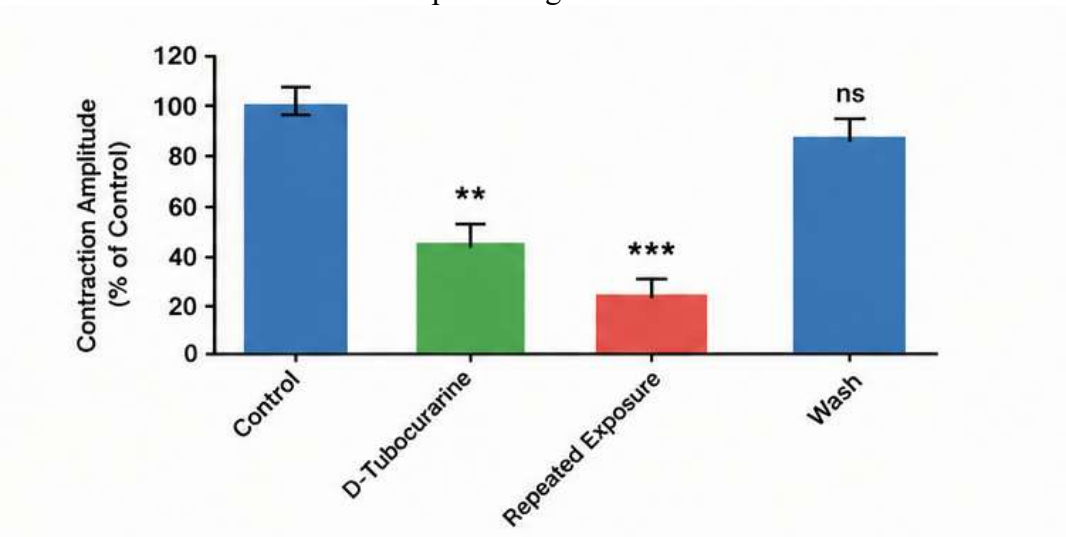


Figure 2: Effect of D-Tubocurarine

Effect of Aqueous Extract of *Portulaca oleracea* on Phrenic Nerve–Diaphragm Preparation

Figure 3 illustrates the effect of the aqueous extract of *Portulaca oleracea* on neuromuscular transmission. Administration of the extract reduced the amplitude of diaphragm contractions compared with the control response. The reduction was greater at higher concentrations, suggesting a concentration-dependent inhibitory action. The recovery of contractions after washing indicates that the effect of the extract was reversible. These observations suggest that the extract possesses neuromuscular depressant properties and may interfere with cholinergic transmission at the neuromuscular iunction.

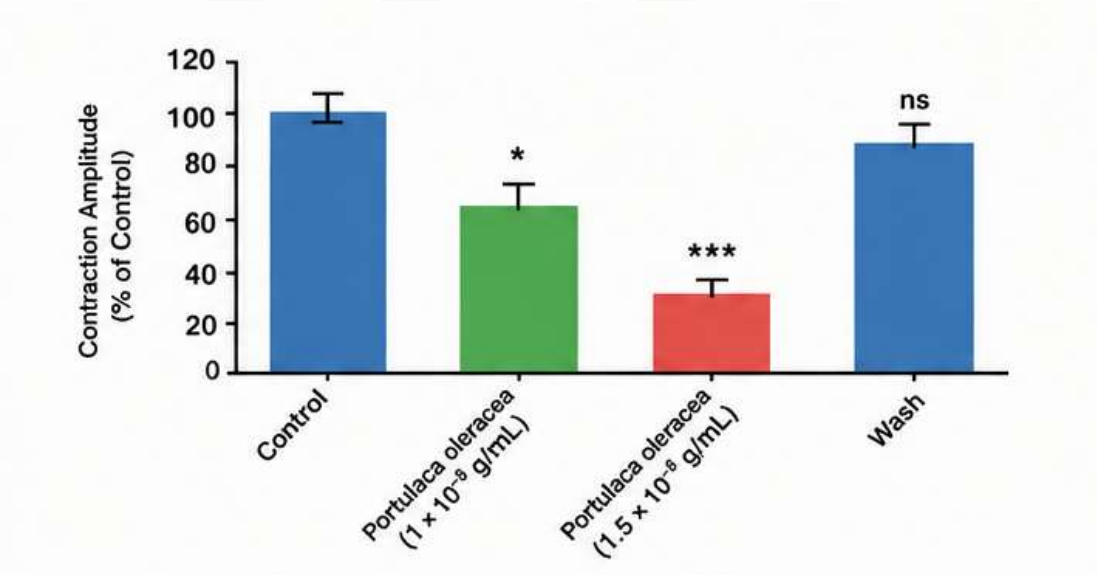


Figure 3: Portulaca oleracea Extract

Reversal Effect of Physostigmine on D-Tubocurarine and *Portulaca oleracea* Extract-Induced Blockade

Figure 6 demonstrates the ability of physostigmine to reverse the neuromuscular blockade produced by D-tubocurarine and the aqueous extract of *Portulaca oleracea*. Administration of D-tubocurarine markedly reduced diaphragm contractions, confirming its inhibitory effect on neuromuscular transmission through competitive blockade of nicotinic acetylcholine receptors at the neuromuscular junction. Similarly, treatment with *Portulaca oleracea* extract resulted in a reduction in contractile response, indicating neuromuscular depressant activity. Following the addition of physostigmine, a significant restoration of diaphragm contractions was observed in both groups. The recovery of contractile activity suggests that physostigmine increased the availability of acetylcholine at the neuromuscular junction by inhibiting acetylcholinesterase, thereby overcoming the blockade. The reversal of the extract-induced inhibition by physostigmine further indicates that the neuromuscular depressant effect of *Portulaca oleracea* may involve interference with cholinergic neurotransmission. These findings suggest that the mechanism of action of the extract may be similar, at least in part, to that of competitive neuromuscular blocking agents and supports the involvement of cholinergic pathways in its neuropharmacological activity.

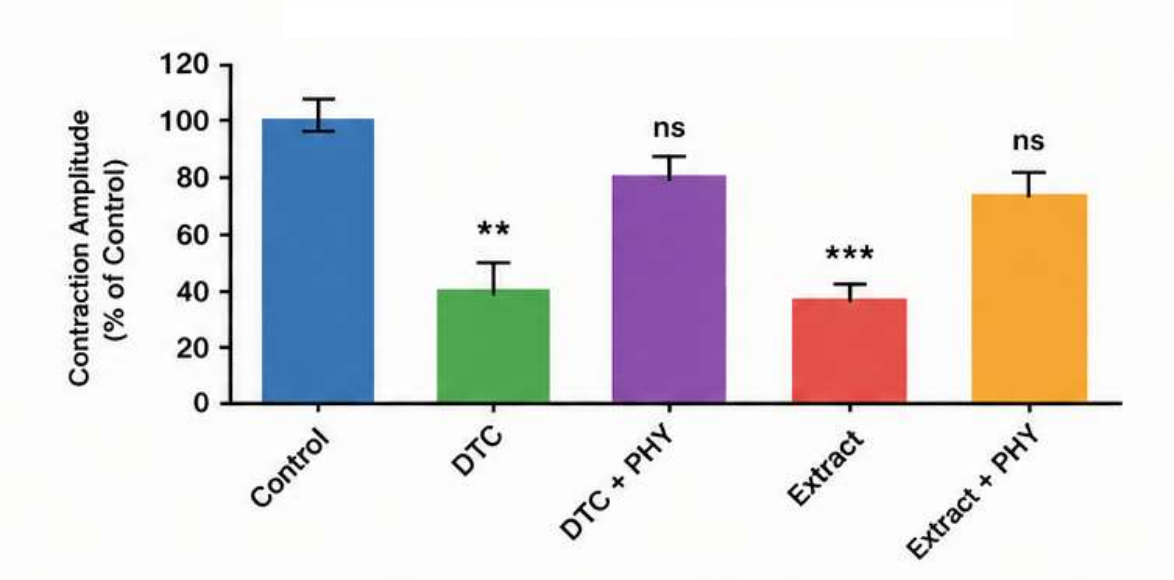


Figure 4: Reversal by Physostigmine

Discussion

The present study investigated the neuropharmacological effects of pancuronium, D-tubocurarine, and aqueous extract of *Portulaca oleracea* on the phrenic nerve–diaphragm preparation, with particular emphasis on their influence on neuromuscular transmission and the ability of physostigmine to reverse induced blockade. The findings demonstrated that pancuronium and D-tubocurarine produced marked inhibition of diaphragm contractions following phrenic nerve stimulation, while the aqueous extract of *Portulaca oleracea* also reduced contractile responses in a concentration-dependent and reversible manner. Furthermore, physostigmine antagonized the blockade induced by D-tubocurarine and the plant extract, suggesting involvement of cholinergic mechanisms at the neuromuscular junction.

The inhibitory effect of pancuronium observed in this study is consistent with its established pharmacological profile as a non-depolarizing neuromuscular blocking agent. Pancuronium acts by competitively antagonizing acetylcholine at nicotinic receptors located on the motor

end plate, thereby preventing depolarization and subsequent muscle contraction. The progressive reduction in diaphragm contractions observed following pancuronium administration supports this mechanism of action. The restoration of contractile activity after washing further confirms the reversible nature of the blockade and indicates that the observed effect was pharmacological rather than toxic. Although the cited studies primarily focused on the neuroprotective and pharmacological activities of *Portulaca oleracea*, the reversible blockade produced by pancuronium in the present study provides a well-established experimental standard against which the effects of the plant extract can be compared (Jalali & Rahbardar, 2023).

Similarly, D-tubocurarine produced a significant and concentration-dependent reduction in diaphragm contractions. The findings align with the classical description of D-tubocurarine as a competitive antagonist of nicotinic acetylcholine receptors at the neuromuscular junction. The recovery of muscle contractions following washing suggests that receptor occupancy by the drug was reversible and that normal neuromuscular transmission resumed once the antagonist was removed. The similarity between the effects of pancuronium and D-tubocurarine observed in this study confirms the sensitivity of the phrenic nerve–diaphragm preparation as a model for evaluating agents that influence neuromuscular transmission. The ability of physostigmine to reverse D-tubocurarine-induced blockade further supports the involvement of competitive antagonism because acetylcholinesterase inhibition increases the concentration of acetylcholine available to compete for receptor binding sites.

The aqueous extract of *Portulaca oleracea* produced a notable reduction in diaphragm contractions, indicating that the plant possesses neuromuscular depressant activity. The concentration-dependent nature of the inhibition suggests a pharmacologically mediated effect rather than nonspecific tissue impairment. Moreover, the substantial recovery of contractile responses following washing indicates that the action of the extract was reversible. These observations suggest that one or more phytochemical constituents of *Portulaca oleracea* may interfere with neuromuscular transmission, either through modulation of acetylcholine release, interaction with nicotinic receptors, or alteration of postsynaptic membrane responsiveness. The findings are significant because they demonstrate a direct pharmacological effect of the plant extract on peripheral neuromuscular function.

The observed neuromuscular inhibitory activity of *Portulaca oleracea* is partially supported by previous reports describing the effects of the plant on the nervous system. Jalali and Rahbardar (2023) reviewed evidence indicating that *Portulaca oleracea* and its bioactive constituents possess diverse neuropharmacological properties, including modulation of neurotransmitter systems and regulation of neuronal excitability. Similarly, Kumar et al. (2022) reported that the plant contains numerous bioactive compounds capable of exerting significant pharmacological effects on different physiological systems. The rich composition of flavonoids, alkaloids, omega-3 fatty acids, and phenolic compounds identified in *Portulaca oleracea* may contribute to the neuromuscular effects observed in the present study through interactions with cholinergic pathways or ion channel function. The findings therefore extend existing knowledge by demonstrating that the pharmacological actions of *Portulaca oleracea* may involve peripheral neuromuscular mechanisms in addition to previously reported central nervous system effects.

The results of this study also show agreement with evidence suggesting that *Portulaca oleracea* possesses biologically active compounds capable of modifying neural function. Martins et al. (2016) demonstrated that extracts of *Portulaca oleracea* exerted protective effects on dopaminergic neurons exposed to neurotoxic injury. Likewise, El Rabey et al. (2025) reported neuroprotective effects of methanolic *Portulaca oleracea* extract in an experimental model of Alzheimer's disease. Mitran et al. (2025) further demonstrated that the plant extract influenced

neuroplasticity and neurological outcomes in a murine model of depression associated with metabolic dysfunction. Although these studies focused primarily on neuroprotection rather than neuromuscular transmission, they collectively support the concept that *Portulaca oleracea* contains pharmacologically active constituents capable of modifying nervous system activity. The present findings contribute additional evidence by demonstrating direct effects on neuromuscular communication.

However, some aspects of the current findings appear to differ from the predominant emphasis of previous studies. Most published investigations have highlighted the antioxidant, anti-inflammatory, cytoprotective, and neurorestorative properties of *Portulaca oleracea* rather than neuromuscular inhibitory actions. For example, Ghorani et al. (2023) described the plant as a potent source of antioxidant and immunoregulatory compounds that generally protect cellular function against oxidative damage. Similarly, Rakhshandeh et al. (2022), Daneva et al. (2026), Maliza et al. (2026), and Ngouana et al. (2025) reported protective effects in models of diabetes, hypoxia-induced injury, and gastric lesions. These studies did not report neuromuscular depression, which may reflect differences in experimental models, tissue preparations, extract types, dosage levels, and study objectives. Therefore, the neuromuscular inhibitory effect observed in the present study should not necessarily be interpreted as contradictory to the reported protective properties of the plant but rather as evidence that the extract may exert multiple pharmacological actions depending on the biological system under investigation.

The reversal of both D-tubocurarine- and *Portulaca oleracea*-induced blockade by physostigmine provides important insight into the possible mechanism underlying the extract's action. Physostigmine inhibits acetylcholinesterase and thereby increases the concentration of acetylcholine within the synaptic cleft. The restoration of contractile responses following physostigmine administration suggests that the blockade produced by the plant extract may involve interference with cholinergic neurotransmission at the neuromuscular junction. This observation raises the possibility that certain phytochemical constituents of *Portulaca oleracea* may act through competitive or partially competitive interactions with nicotinic receptors. Nevertheless, the exact mechanism cannot be conclusively established from the present study and requires further investigation involving receptor-binding studies, electrophysiological analyses, and isolation of specific active compounds.

A limitation of the present study is that it did not identify the individual phytochemicals responsible for the observed neuromuscular effects. Previous studies have shown that *Portulaca oleracea* contains numerous biologically active compounds with diverse pharmacological activities, making it difficult to determine which constituents contributed to the observed responses (Kumar et al., 2022). In addition, only the overall extract was evaluated, and therefore the specific pathways involved in neuromuscular inhibition remain uncertain. Future studies should focus on fractionation of the extract, characterization of active constituents, and detailed mechanistic investigations to clarify the molecular basis of the observed effects.

Conclusion

The present study demonstrated that aqueous extract of *Portulaca oleracea* produces a reversible, concentration-dependent inhibition of neuromuscular transmission in the rat phrenic nerve–diaphragm preparation. The observed effect was comparable to those of pancuronium and D-tubocurarine, indicating potential interference with cholinergic signaling at the neuromuscular junction. Reversal of the blockade by physostigmine suggests involvement of nicotinic acetylcholine receptor-mediated mechanisms. These findings provide evidence of the

neuropharmacological activity of *Portulaca oleracea* and highlight its potential as a source of bioactive compounds for further investigation in neuromuscular and neuroprotective research.

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