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Research Article

Role of Boerhaavia Diffusa L: A In Relief of Neuropathic Pain of Paclitaxel Induced Neuropathy

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Abstract
Background: *The present study was designed to investigate the neuropathic activity of Boerhaavia diffusa L. against Paclitaxel induced peripheral neuropathy in rats.*
Materials and Methods: *Peripheral neuropathy was induced by a single i.p injection of Paclitaxel 2mg/kg for four alternative days 0, 2,4,6. The ethanolic extract of Boerhaavia diffusa was administered 100, 200, 400 mg/kg, p.o. daily up to 28 days. Neuropathic pain was assessed via various behavioral parameters procedures viz., Eddy's hot plate test and cold water tail immersion test, pinprick, Acetone drop, photoactometer tests were performed to assess the degree of thermal, mechanical hyperalgesia, cold allodynia along with locomotor activity.*
Result: *In this study Paclitaxel induced rats showed decreased tail withdrawal latency, and increased paw withdrawal latency along with decreased locomotor activity was observed for various behavioural tests. After treating rats with Boerhaavia diffusa L. at various doses 100, 200, 400 mg/kg, p.o. daily up to 28 days, the animals showed improvement in tail and paw withdrawal latency and locomotor activity in dose dependent manner.*
Conclusion: *The present study reveals the supplementation of B. diffusa L., extract ameliorates the pacliatxel induced neuropathy via above mentioned actions.*
Keywords: *Neuropathic pain, Paclitaxel, Boerhaavia diffusa L.*

Introduction

Neuropathic pain (NP) is a manifestation of multiple and varied disorders that display both peripheral and central sensitization mechanisms.[1] It affects somatosensory components of the nervous system.[2] A prolonged sensory input from injured peripheral nerve generate secondary morphological changes in dorsal regulatory ganglion (DRG), and formation of new ion channels and receptors at the cellular level result in hyper excitability of neurons.[3] In fact, many pathological conditions like cancer, AIDS, long standing diabetes, lumbar disc syndrome, herpes infection, multiple sclerosis and stroke causative for peripheral nerve damage and pain.[4]

The prevalence of cancer is increasing globally; around 17 million new cases were estimated by the year 2020.[5,6] Approximately, 40% cancer patients are sufferers of peripheral neuropathy during chemotherapy treatment with vincristine, paclitaxel (Taxol), platinum compounds.[7] The neuropathy inducing potential of these drugs depends on their cumulative dose, time, and mechanism of binding.[8]

Paclitaxel induced peripheral neuropathy occur due to microtubule dysfunction in dorsal root ganglia, axons and schwann cells.[9] The neuropathy will begin as early as in 24 to 72 hours after administering single, high dose of Paclitaxel.[10] The generating symptoms starts from lower extremities like pins and needles, burning, decreased or increased sensitivity to pain endanger quality of life.[11] The type of pain and generating symptoms are poorly treated with available drugs.[12]

Preclinically, various studies have been reported on plant products like aconittuber, lindera angustifolia, teucrium polium, phyllanthus emblica, vochysia divergens, cannabis sativa, nigella sativa, ocimum sanctum and ginkgo biloba in management of neuropathy.[13] Therefore ample of scope for developing the novel medicinal agents from the plant origin for treating neuropathic pain.

The present chosen trailing herb bearing a **Latin name;** Boerhaavia diffusa Linn. (***Family:*** Nyctaginaceae) is a creeping herb grown in tropical regions of South America, India and Africa. Indian Ayurveda renowned it as 'Punarnava', rejuvenates itself from dried root in rainy seasons as well as rejuvenates body. The whole plant has medical benefits especially roots. There, the roots are employed for multiple purposes including liver, gallbladder, and kidney, renal and urinary disorders. It is a very good nerve rejuvenator and it is given in cases of sciatica or nervous weakness or even paralysis conditions to relive pain. Traditionally Punarnava root used as anticonvulsant, analgesic, expectorant, CNS depressant, laxative, diuretic, abortifaci and swelling.[14] The plant is rich in photochemicals like phytochemicals like Punarnavine (alkaloid), boeravinone (rotenoid), flavonoids, amino acids, liriodendrons (Lignans), B-sistosterol and stetracosanoic acid, ecosanoic, steroic and urosolic acid has proven to cure and control disease prognosis. [15]

Thus, the present study is aimed to investigate the neuropathic pain relieving activity, antioxidant activity of Boerhaavia diffusa L., against the Paclitaxel induced neuropathy in albino rats.

Materials and Methods

Chemicals and reagents

Paclitaxel was obtained from Hetero Health care LTD, Hyderabad, India and pregabalin was purchased from Swapnaroop drugs & pharmaceuticals, Aurangabad, Maharashtra, India. All other chemical were used are analytical grade.

Plant material collection and extraction: The plant Boerhaavia diffusa L., was gathered from local fields in the month of October-November, it was authenticated with a botanist. The roots were separated and thoroughly cleaned with distilled water, and shade dried until they were free from moisture. The dried roots were subjected to size reduction with a mechanical grinder until fine coarse powdered granules would obtain. The obtained granular powder was undergone cold maceration in 95% ethanol for a period of 72 hrs with frequent agitation in a closed neck glass bottle. The obtained visibly green coloured liquid containing soluble phytochemical constituent's undergone filtration through whatman # 1 filter paper and further concentrated at 45^{o} C on a water bath to obtain a sticky brownish semisolid paste.[16] The extracted semisolid paste was undergone drying in desiccators to remove moist and stored at below 10^{0} C. The obtained yield in % (2.54 gm w/w) was calculated with reference to the powder used for extraction.

According to OECD 420 guidelines study was conducted on healthy albino rats (150-200 g), sub maximal and supra maximal doses of 200 mg/kg were selected.[17]

Animal selection and induction of Neuropathy

After taking approval from Institutional Animal Ethical Committee (IAECH) healthy adult albino rats of either sex 150-200 gm were selected for the study. The animals were housed in clean, transparent polypropylene cages with six animals in each cage by maintaining standard laboratory conditions 12:12 hrs light and dark cycles, at 25±3^0 C and 35-60% humidity and had free accesses to water with standard chow diet. The study was approved by Institutional Animal Ethical Committee (IAEC).

Treatment Protocol

Six experimental groups were assigned as Control, Disease control, Standard, and three groups of test drugs at various doses, expressed as 0.9% N.S (p.o), Paclitaxel (i.p.) 2mg/kg, four alternative days 0,2,4,6 in early morning as described previously.[18] Pregabalin 10 mg/kg (p.o), B. Diffusa L. 100, 200, 400 mg/kg (p.o) by dissolving in distil water respectively 1 hour prior commencing the experiment. On 7th day the animals diagnosed neuropathic pain in 5 groups. From 7th day animals fed with Pregabalin and B.diffusa L., respectively one hour before doing the behavioural parameter tests. The pain threshold was assessed by cold water tail immersion test and paw heat hyperalgesia, acetone drop test, pinprick test, and actophotmeter test, were used to assess the Behavioural alterations according the standard methods[18]. The assessments were done on day 0 (before administering Paclitaxel) and after on days 7, 14, 21, 28 respectively. At the end of the study (i.e., 28th day) rats was sacrificed by administering thiopental-50 mg/kg, IP, and a 10%w/v nerve homogenate was prepared by mixing Triss-Hcl buffer (P^H 7.4). The nerve homogenate was further cooled in ice cold water at 4^0 C and centrifuged at 2500 rpm for 10 min. The obtained supernatant was subjected to estimate for total protein, total calcium**,** lipid peroxidation, superoxide dismutase levels [18] was estimated.

Statistical Analysis

The results were expressed as mean ± SD. The data was entered in Microsoft excel and analysis was done by using multiple comparison tests by using ANOVA followed by Bonferroni t-test. The statistical analysis was done by using the Sigma Stat 3.5.values of P < 0.05 were considered statistically significant.

Results

Behavioural Parameters

Cold water tail immersion test

This group also showed similar results with B. diffusa L. 100 mg/kg (7.833±0.752, 8.83±0.983, 9.16±0.983, 9.83±0.983), 200 mg/kg (8.50±1.048, 9.66±632, 10.50±1.516, 10.83±1.471), 400 mg/kg p.o (10.16±1.471, 10.16±1.505, 12.00±1.264, 12.50±1.048) were shown significant (p<0.001) value as above group in comparison with disease control group from day 7 to 28 days. Whereas tail withdrawal latency of Paclitaxel induced rats was decreased in comparison with normal rats from day 7 to 28 day (10.16±0.752, 10.66±0.816, 10.83±0.752, 11.00±1.264). [Table-I]

Eddy's hot plate test: Paclitaxel treated rats showed decreased paw withdrawal latency in comparison with normal rats from day 7 to 28 days (8.33±0.816, 7.16±0.752, 6.00±0.894, 5.00±0.632). The higher dose of B. diffusa L., 400 mg/kg only given early significant (P < 0.01) response from day 7 (11.16±1.169, 12.50±1.048, 12.16±0.752, 14.00±0.632) in comparison with and remaining two doses 100, 200 mg/kg were unsuccessful. [Table-II]

Pinprick Test: Rats treated with Paclitaxel causative for development of mechanical hyperalgesia observed as increased paw withdrawal latency from a nicking object in comparison with normal rats from day 7 to 28 (10.33±0.816, 12.50±0.547, 14.16±0.752, 15.33±0.816). The dose 400 mg/kg of B. diffusa were shown a significant (P<0.001) early response in decreased in paw withdrawal latency from day 7 (7.33±1.36, 8.66±1.211, 10.16±1.16, 11.5±1.048) and dose 200 mg/kg were given late

significant response (P<0.05) from day 14 (10.33±1.211, 11.50±1.048, 12.66±1.032) in comparison with Paclitaxel. [Table-III]

Acetone drop test: The acetone increases paw withdrawal latency in Paclitaxel treated rats (14.06±1.063, 23.72±2.560, 32.89±1.834, 43.06±2.323) in comparison with normal rats from day 7 to 28. All three doses of B. diffusa were successful in decreasing paw withdrawal latency. The test dose 400 (9.00±0.843, 13.94±1.597, 16.78±2.810, 17.94±2.776) and 200 mg/kg (10.44±1.004, 17.22±1.109, 21.28±2.081, 27.33±2.271) of B. diffusa were shown early significant results on day 7 (P< 0.001) and dose 100 mg/kg (19.67±2.044, 26.83±2.834, 35.72±3.172) was on day 14 (P< 0.01). [Table-IV]

Locomotor Activity Test: Paclitaxel treated rats showed decreased locomotor activity in comparison with normal rats from day 7 to 28 (42.67±7.033, 38.83±6.494, 35.67±6.154, 39.33±7.005). All the tree doses of B. diffusa were successful in improving locomotor activity in comparison with diseased control rats. The early significant effect was observed with B. diffusa dose 100 mg/kg dose on 21st day (52.33±5.086, 55.83±4.535); 200 mg/kg (56.17±6.616, 64.67±6.772, 69.67±6.218) on day 14, and with 400 mg/kg day 7 (54.33±5.538, 63.83±6.464, 71.50±7.007, 78.33±7.554) significantly (P<0.001). [Table-V]

In all behavioural parameters pregabalin showed early significant results (P<0.001) from day 7 to 28 days.

(Cold water tail immersion: 12.16±0.983, 12.16±0.983, 12.83±1.471, 13.16±1.329; Eddy's hot plate: 11.66±0.816, 13.00±0.632, 14.16± 0.408, 15.16±0.752; Pin prick test: 6.00±1.095, 7.33±1.211, 8.66±1.032, 9.50±1.048; Acetone drop test: 6.56±0.544, 8.11±1.109, 8.61±1.163, 7.89±1.004; Locomotor activity test: 60.67±4.227, 74.17±5.345, 83.50±5.753, 91.17±5.947).

Biochemical Parameters

Effect of B. diffusa on Lipid peroxidation levels (TBARS), total protein, total calcium, superoxide desmutase levels (nM/gm of wet tissue) in sciatic nerve

In 28days of study Paclitaxel treated rats were shown increased levels of lipid per oxidation (TBARS) levels (54.55±10.296), calcium levels (26.7±2.654) and decreased levels of total protein (34.16±4.633) and superoxide dismutase levels (13.15±6.696) was noted significantly (P<0.05) in comparison with normal control rats**.** The test dose 400 mg/kg of B. diffusa reduces the raised lipid peroxidation (TBARS) levels (36.50±6.992) and improves total protein levels (53.91±3.679) significantly (P<0.001); while the superoxide dismutase levels were more successfully raised by 400 mg/kg (28.05±6.105), 200 mg/kg (23.33±4.190) test doses significantly (P<0.001) in Paclitaxel induced rats. The raised calcium levels due to Paclitaxel therapy were successfully blocked by 200 (21.20±1.930), 400 mg/kg (18.18±1.772) doses of B. diffusa significantly (P<0.001). [Table-VI]

Table-I: Effects of B. diffusa L., on cold water tail immersion test in paclitaxel induced rats

Treatment groups	Reaction time (Sec) (n=6)				
	Day-0	Day-7	Day-14	Day-21	Day-28
Normal Control	11.5±1.04	10.16±0.752	10.66±0.816	10.83±0.752	11.00±1.264
Paclitaxel (Disease control)	11.33±1.03	5.166±0.752	4.50±1.048	3.833±0.752	3.50±0.547
Paclitaxel + Pregabalin (10 mg/kg)	11.41±1.42	12.16±0.983	12.16±0.983	12.83±1.471	13.16±1.329
Paclitaxal + B. diffusa (100 mg/kg)	11±1.41	7.833±0.752 **	8.83±0.983 ***	9.16±0.983 ***	9.83±0.983 ***
Paclitaxel + B. diffusa (200 mg/kg)	11.33±0.81	8.50±1.048 ***	9.66±1.632 ***	10.50±1.516 ***	10.83±1.471 ***
Paclitaxel + B. diffusa (400 mg/kg)	11.83±1.47	10.16±1.471 ***	11.33±1.505 ***	12.00±1.264 ***	12.50±1.048 ***

*P<0.05, **P<0.01, ***P<0.001 compared with disease group.

Table-II: Effects of B. diffusa L., on thermal hyperalgesia by Eddy's hot plate test in paclitaxel induced rats

Treatment groups	Reaction time (Sec) (n=6)				
	Day-0	Day-7	Day-14	Day-21	Day-28
Normal control	11.83±1.16	11.66±1.032	12.16±0.752	12.66±1.032	12.33±1.032
Paclitaxel (Disease control)	12±1.26	8.33±0.816	7.16±0.752	6.00±0.894	5.00±0.632
Paclitaxel + Pregabalin (10 mg/kg)	12±1.26	11.66±0.816	13.00±0.632	14.16±0.408	15.16±0.752
Paclitaxel + B. diffusa (100 mg/kg)	12.16±1.47	9.66±1.211	10.833±0.752	11.66±0.516	12.16±0.752
Paclitaxel + B. diffusa (200 mg/kg)	12.33±0.81	10.50±1.378	11.83±1.169	12.83±0.983	13.33±0.816
Paclitaxel + B. diffusa (400 mg/kg)	11.83±1.47	11.16±1.169 **	12.50±1.048 ***	13.16±0.752 ***	14.00±0.632 ***

*P<0.05, **P<0.01, ***P<0.001 compared with disease group.

Table-III: Effects of B. diffusa L., on prick induced hyperalgesia by pin prick test in paclitaxel induced rats:

Treatment groups	Reaction time (Sec) (n=6)				
	Day-0	Day-7	Day-14	Day-21	Day-28
Normal control	3.16±0.75	2.66±0.816	2.83±0.752	3.33±0.516	3.50±0.547
Paclitaxel (Disease control)	3.5±0.83	10.33±0.816	12.50±0.547	14.16±0.752	15.33±0.816
Paclitaxel + Pregabalin (10 mg/kg)	2.83±0.75	6.00±1.095	7.33±1.211	8.66±1.032	9.50±1.048
Paclitaxel + B. diffusa (100 mg/kg)	3.16±0.40	9.66±1.505	11.16±1.329	12.66±1.032	13.83±0.752
Paclitaxel + B. diffusa (200 mg/kg)	2.83±0.98	9.00±0.894	10.33±1.211 *	11.50±1.048 ***	12.66±1.032 ***
Paclitaxel + B. diffusa (400 mg/kg)	2.83±1.16	7.33±1.366 ***	8.66±1.211 ***	10.16±1.16 ***	11.5±1.048 ***

*P<0.05, **P<0.01, ***P<0.001 compared with disease group.

Table-IV: Effects of B.diffusa L., on acetone drop test in paclitaxel induced rats

Treatment Groups	Reaction time (Sec) (n=6)				
	Day-0	Day-7	Day-14	Day-21	Day-28
Normal control	6±0.596	5.78±0.344	5.94±0.491	5.89±0.455	5.67±0.298
Paclitaxel (Disease control)	6±0.471	14.06±1.063	23.72±2.560	32.89±1.834	43.06±2.323
Paclitaxel + Pregabalin (10 mg/kg)	6±0.516	6.56±0.544	8.11±1.109	8.61±1.163	7.89±1.004
Paclitaxal + B. diffusa (100 mg/kg)	6.05±0.53	12.72±1.063	19.67±2.044 **	26.83±2.834 ***	35.72±3.172 ***
Paclitaxel + B. diffusa (200 mg/kg)	6.05±0.45	10.44±1.004 ***	17.22±1.109 ***	21.28±2.081 ***	27.33±2.271 ***
Paclitaxel + B. diffusa (400 mg/kg)	5.88±0.45	9.00±0.843 ***	13.94±1.597 ***	16.78±2.810 ***	17.94±2.776 ***

*P<0.05, **P<0.01, ***P<0.001 compared with disease group.

Table-V: Effects of B. diffusa L., on locomotor activity in paclitaxel induced rats

Treatment groups	Reaction time (Sec) (n=6)				
	Day-0	Day-7	Day-14	Day-21	Day-28
Normal control	113.5±6.53	114.83±6.940	116.00±6.293	116.33±4.926	117.50±5.822
Paclitaxel (Disease control)	112.83±6.5	42.67±7.033	38.83±6.494	35.67±6.154	39.33±7.005
Paclitaxel + Pregabalin (10 mg/kg)	113.5±6.44	60.67±4.227	74.17±5.345	83.50±5.753	91.17±5.947
Paclitaxel + B. diffusa (100 mg/kg)	114.83±6.49	45.33±4.320	48.50±4.506	52.33±5.086 ***	55.83±4.53 ***
Paclitaxel + B. diffusa (200 mg/kg)	112.66±5.78	50.17±5.636	56.17±6.616 ***	64.67±6.772 ***	69.67±6.21 ***
Paclitaxel + B. diffusa (400 mg/kg)	115±5.40	54.33±5.538 *	63.83±6.463 ***	71.50±7.007 ***	78.33±7.55 ***

*P<0.05, **P<0.01, ***P<0.001 compared with disease group.

Table-VI: Effect of B. diffusa on Lipid peroxidation levels (TBARS), total protein, total calcium, superoxide dismutase levels (nM/gm of wet tissue) in sciatic nerve:

Treatment groups	TBARS	Total Protein	Total Calcium	Super Oxide Dismutase (SOD)
Normal control	17.61±4.906	74.08±8.181	8.54±1.324	45.09±7.080
Paclitaxel (Disease control)	54.55±10.296	34.16±4.633	26.7±2.654	13.15±6.696
Paclitaxal+Pregabalin (10 mg/kg)	28.72±6.024	64.16±3.204	14.83±2.676	35.50±4.399
Paclitaxel+ B. diffusa (100 Mg/Kg)	47.05±6.965	39.83±4.854	22.93±2.068	22.37±3.328
Paclitaxel+ B. diffusa 200 Mg/Kg	42.05±7.864	44.91±5.453	21.20±1.930 ***	23.33±4.190 *
Paclitaxel+ B. diffusa 400 Mg/Kg	36.50±6.992 ***	53.91±3.679 ***	18.18±1.772 ***	28.05±6.105 ***

*P<0.05, **P<0.01, ***P<0.001 compared with disease group.

Discussion

The purpose of present study is to examine the effectiveness of B. diffusa L., against Paclitaxel induced neuropathic pain assessed in terms of behaviour parameters, oxidative stress markers in sciatic nerve during 28 day study period. The results indicate the B. diffusa L., effectively inhibits Paclitaxel induced neuropathic pain from the day-7.

In the present study, by administering Paclitaxel (2 mg/kg, i.p) for 4 days leads to significant development of allodynia, hyperalgesia was noted in rodents from day-7. Similar findings are reported from previous studies Mangaiarkkarasi [19] who used Paclitaxel as neuropathic pain inducing agent. Paclitaxel always affects somatosensory system rather than motor system,[20] where as vincristine disturbs both motor and sensory functions. [21]

Paclitaxel influence calcium dysregulation from swollen mitochondria[22] involved in activation of calpin and caspases results in neuronal excitation.[23] B. diffusa has well documented for limiting calcium conduction in nerve tissue, this could be due to the presence of liriodendrin has proven for its calcium limiting property and anticonvulsant property proven by. [24, 25]

In addition Paclitaxel induced neuropathy decreases locomotor activity, similar findings were reported by Ray and Trammel[26] during their four week study with Paclitaxel for assessing locomotor activity, sleep time, fatigue nature in rodents. The relation between sleep and neuropathic pain is bidirectional and may develop stress and sleep disorders.[27] Stress intern raises corticosterone levels.[28] However, rats treated with higher doses of B. diffusa L., were shown improvement in locomotor activity from day-14. Similar findings are reported with Meera sumanth and Mustafa [29] in their antistress study by taking B. diffusa.

Further, Paclitaxel has been reported to cause neuropathic pain by producing reactive oxygen species. [30] These reactive oxygen species involve in raising of lipid peroxidation levels in sciatic nerve. [31] Paclitaxel potentially inhibit anti oxidant such as superoxide dismutase, will produce increased levels of superoxide anions and other reactive intermediates involve in generation of neuropathic pain.[32] B. diffusa effectively improves the super oxide dismutase levels, and reduces lipid peroxidation levels due to its anti oxidant property.[33,34]

On the basis of data in our hand and with support from the literature, it may be proposed that B. diffusa significantly attenuates the neuropathic pain induced by Paclitaxel. This could be due to its multiple effects; anticonvulsant, antioxidant, antistress, anti-inflammatory properties.

Conclusion

The present research open gates for the management of neuropathic pain with B. diffusa root extract due to its vast phytochemical properties.

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