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Phytochemical Screening and Evaluation of Protective Effect of Methanolic Extract of *Cleodendrum viscosum* Leaves in Rat Model of Vincristine Induced Peripheral Neuropathy

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Abstract

Chemotherapy induced peripheral neuropathy is a disabling pain condition resulting from cancer therapy. However, no scientific data available for many herbal drugs which are locally used and evaluating these drugs would be worth to have scientific approach of using them. In this study an indigenous plant called *Clerodendrum viscosum* leaves have been used to evaluate the effect of plant in vincristine induced peripheral neuropathy in rat model. Vincristine sulfate was administered to Male sprague dawley rats to induce neuropathy. Pain behavior was assessed by Hot plate, Cold plate, sciatic function index and formalin test were also estimated. Animals were sacrificed and the sciatic nerve excised for histopathological studies. The whole preclinical studies revealed that the aforementioned plant extract exhibited less neuronal damage. The study concluded that methanolic extract of *Cleodendrum Viscosum* leaves can used against chemotherapy induced peripheral neuropathy.

Keywords*:* Cancer, Chemotherapy, Hotplate, Neuropathy, Histopathology, Vincristine.

INTRODUCTION

Cancer is currently a leading cause of mortality worldwide [1]. However, thanks to advances in medicine and modern technology, the availability of sensitive tests and diagnostic methods to detect cancer at an early stage and the use of increasingly effective treatments, including chemotherapeutic agents, the number of cancer survivors is rising: It is expected to increase by 35%, from 13.7 million in 2012 to 18 million, by 2022 [2]. Although these survivors may have beaten cancer, many of them have poor outcomes due to a number of syndromes that reduce the quality of life as a consequence of cancer treatment, including pain, which they often experience for a long time after completing their cancer treatment [3].

Drugs used in cancer chemotherapy constitute an extremely effective tool in arresting the progression of cancer since they have numerous targets and mechanisms of action aimed at eliminating rapidly dividing cancer cells. Unfortunately, these drugs also affect normal cells and structures of the body, causing various deleterious and sometimes even devastating side effects, Chemotherapy induced peripheral neuropathy is considered as a prevalent adverse effect, Millions of cancer patients are still facing this consequence [4].

Chemotherapy induced peripheral neuropathy is mainly involved in a sensory peripheral neuropathy though some patients experience motor symptoms such as weakness and autonomic neuropathy, and it is often distributed in a “stocking and glove” manner, causing symptoms such as pain, allodynia, loss of sensation, paresthesia, numbness, tingling, and gait disturbance. This can cause significant loss of functional abilities and negatively affect quality of life, which can lead to dose reductions, discontinuation of treatment, and ultimately affect overall survival [5]. The prime objective of the present study is to develop the use of herbal medicines against this adverse effect, because

Phytomedicines has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [6].

Clerodendrum viscosum is widely distributed in tropical and subtropical regions of the world particularly in India. Extracts from different parts of the plant have been used elaborately in traditional system of medicine, it is well known as a medicinal plant because of wide therapeutic uses. *Clerodendrum viscosum* is a prominent plant in ayurveda and used as a remedy against tumors, skin diseases, snake bite, scorpion sting, intestinal infections, and kidney dysfunctions since ages [7]. The purpose of this study is to evaluate the effectiveness of the leaves of *Cleodendrum Viscosum* in rat model of peripheral neuropathy by creating Analgesic and Anti-inflammatory Screening Models.

MATERIALS AND METHODS

Collection and processing of plant sample

The *Clerodendrum Viscosum* leaves were collected from nearby local areas during the month of December 2021 and January 2022. The leaves were washed with water thoroughly to remove all the solid particles and shade dried in room temperature for about 7-10 days. Then the dried leaves were grinded to coarse powder using an electrical grinder and this grinding process makes the plant parts exposed to the solvents for easy penetration to extract the phytoconstituents and then the powdered sample were stored in a sterile airtight container**.**

Extraction of plant material

The dried powdered sample of the leaves were weighed 10g and soaked in 150ml of methanol in a Soxhlet apparatus by continuous heat exposure for 48hours till the solution becomes clear. The extract obtained was collected and concentrated. The concentrated extract was then weighed and was subjected to preliminary phytochemical screening and biological activity studies [8].

Preliminary Phytochemical Screening

Preliminary phytochemical screening is conducted for methanolic extract of *Clerodendrum viscosum* to identify various phytoconstituents such as Carbohydrates, Proteins, Amino acids, Glycosides, Flavanoids and Alkaloids [9].

Pharmacological Studies

Experimental Animals

Thirty adult male Sprague dawley rats with a body weight of 200 - 220g were used for this study. Rats were grouped and housed (n=6 per cage) in a room with controlled temperature (21 ± 2 0ºC) and 12 hour light-dark cycle was maintained. All the rats had free access to food and water ad libitum. All the experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), with the approval certificate number IAEC/NCP/2022/1. The experiments were performed in accordance to the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines for ethical use of animals

Induction of Peripheral neuropathy

Induction of peripheral neuropathy by Vincristine was induced in rats by intraperitoneal administration by Vincristine sulphate (3µg/kg) once per day for 14 consecutive days.

Experimental design

Group I served as normal control in which rats received normal saline per oral (p.o.). Group II served as Vincristine control in which rats were administered normal saline (p.o.), 1 hour before Vincristine injection (3µg/kg) for 14 consecutive days. Group III, IV and V rats received Pregabalin (10mg/kg /day) (p.o.); Methanolic extract of *Cleodendrum Viscosum* (400 mg/kg/day) (p.o.) and Methanolic extract of *Cleodendrum Viscosum* (200 mg/kg/day) (p.o.), respectively 1 hour before Vincristine injection (3µg/kg) for 14 Consecutive days. The behavioral tests like thermal hyperalgesia (hot plate), thermal allodynia (Cold plate), and sciatic functional index were performed on different days such as 0, 7, 10 and 14. On the day 14th the rats were subjected to formalin test. Thereafter all the rats were sacrificed under deep ether anesthesia and subjected to histopathological analysis was carried out in sciatic nerves.

Hot plate test

In this test rats were individually placed on a hot plate Eddy's hot plate with the temperature adjusted to 55± 1º C. The latency to the first sign of the paw licking or jump response to avoid the heat was taken as the index of the pain threshold: The cut off time was 10seconds in order to avoid the damage to the paw [10].

Cold plate test

Rats were placed on ice platform submerged approximately 1 cm below the surface of the cold water (4ºC) such the hairy and glabrous skin on the rat fleet were in contact with the cold water. The latency prior to the first reaction was recorded with a cut off time of 30 seconds [11].

Determination of Sciatic Functional Index (SFI)

The rats were subjected to toes print analysis to measure the SFI. The tests was carried out in an 8.2× 42 cm corridor with darkness in one end and covered with sheet of paper. The hind paw of rats was dipped in black Indian ink and the animal allowed to walking freely in the corridor. The analysis of the foot prints was done by considering the toes lengths from each other. Thus the distance from the heel to the third toe - print length (PL), Distance from the first toe to the fifth toe spread (TS), and distance from the second toe to the fourth-intermediary toe spread (ITS) were measured [12, 13].

Formalin test

This test was performed at the end of other behavioral assessment. The formalin test was done in each rat after acclimatization within a period of 15minute in an observation box. Then the animals were administered with the control and test drug prior to formalin injection. The 2.5% formalin of 0.1ml was injected to the sub plantar region. The nociception evaluation was done by quantification of the paw licking and paw elevation time parameters. The earlier (acute) phase was recorded at between 0-10 minutes, the delayed phase was recorded at between 20-40 minutes and with a resting period being noted between 10-20 minutes in between the acute phase and the delayed phase [14].

Histopathological Examination

Sciatic nerves were immersed in 10% buffered formalin for 24 hours followed by decalcification in 5% formic acid, processed for paraffin embedding sections at 5µ thickness. The sections were stained with hematoxylin-eosin and evaluated under light microscope with 10 X magnifications.

Statistical Analysis

All the results were expressed as Mean ± standard deviation. The data were statistically analyzed by one way analysis of variance (ANOVA) followed by post hoc tukey's multiple comparison test for formalin test. Two way ANOVA (treatment Vs duration) followed by Bonferroni's test was used for hot plate test cold plate test and sciatic functional index, Probability values of less than 0.05 was considered as significant. The analysis was carried out using Graph pad prisom software.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening of the extract

The result of preliminary phytochemical screening of methanolic extract of *Clerodendrum viscosum* leaf shows the presence of carbohydrates, alkaloids, glycosides, flavonoids, tannins & phenolic compounds.

Effect of Methanolic extract of *Clerodendrum viscosum* on Hot plate test

The animals treated with vincristine showed a significant increases ($p<0.001$) in hyperalgesia induced by hot plate was observed by the behavior of animals like hind paw licking, lifting or jumping from the surface of the hot plate. The effect was compared with 0^{th}day. The data exhibited a time dependent improvement in analgesia. The nociceptive response was significantly ($p<0.01$) improved in methanolic extract 200mg/kg treated animals when compared with vincristine administered group on day 10 and 14. Animals treated with extract 400 mg/kg showed significant improvement from day 7 onwards. However the results revealed that the test drugs of both dose level at 200mg/kg and 4000mg/kg can reinstate the pathophysiology of thermal analgesia.

Table 1: Effect of Methanolic extract of *Clerodendrum viscosum* on Hot plate test

	Group I	Group II	Group III	Group IV	Group V
Days					
0 day	7.94±0.52	8.14±0.77	7.84±.57	8.46±0.67	8.07±0.58
7^{th} day	7.5±0.37	7.30±0.63*	6.22±0.56	6.74±.77	6.94±0.35
10^{th} day	7.73±0.38	3.94±0.53***	4.42±0.27$^{\#}$	4.98±0.11$^{\#}$	7.37±0.37$^{\#\#\#}$
14^{th} day	7.49±0.45	4.25±0.60***	5.22±0.69	5.5±0.625	7.60±0.45$^{\#\#\#}$

Results were expressed as Mean±SEM, n=6 rats per group. Two- way ANOVA was done followed by Bonferonni's test, ***, ** * indicates $p<0.001$, $p<0.01$, $p<0.05$ respectively in comparison of normal group and Vincristine control.###, ##,# indicates $p<0.001$, $p<0.01$, $p<0.05$ respectively when compared with vincristine group.

Effect of Methanolic extract of *Clerodendrum viscosum* on Cold plate test

The administration of Vincristine sulphate as neuropathy inducer, reflected thermal allodynia, with significance ($p<0.001$) development, this was indicated with increased paw licking, lifting or jumping from cold plate surface.

Table 2: Effect of Methanolic extract of *Clerodendrum viscosum* on Cold plate test

	Group I	Group II	Group III	Group IV	Group V
Days					
0 day	18.44±0.26	18.14±0.34	16.8±0.36	18.01±0.08	16.92±0.77
7th day	18.25±0.43	12.65±1.40***	14.34±0.56	14.98±0.93	17.81±0.54$^{\#\#\#}$
10th day	17.94±0.57	09±0.74***	12.27±.53$^{\#}$	17.08±0.57$^{\#\#\#}$	18.54±0.73$^{\#\#\#}$
14th day	17.99±0.36	14.30±2.13**	12.52±0.74$^{\#}$	18.36±0.46$^{\#\#\#}$	19.35±0.55$^{\#\#\#}$

Results were expressed as mean± SEM, n=6 rats per group. Two- way ANOVA was done followed by Bonferonni's test, ***, ** * indicates $p<0.001$, $p<0.01$,$p<0.05$ respectively in comparison of normal group and Vincristine control.###, ##,# indicates $p<0.001$, $p<0.01$,$p<0.05$ respectively when compared with vincristine group.

Effect of Methanolic extract of *Cleodendrunm Viscosum* on Sciatic functional index

Vincristine administered rats resulted in sciatic functional loss as reflected by a significant rise (p< 0.001) in sciatic functional index level. Administration of methanolic extract of *Cleodendrunm Viscosum* (400 and 200mg/kg) significantly attenuated (p<0.001) Vincristine induced rise in sciatic functional index in a dose dependent manner. However, treatment groups at all the dose level were unable to restore the sciatic functional index to a baseline score. Similar effect was seen with pregabalin administration.

Table 3: Effect of Methanolic extract of *Cleodendrunm Viscosum* on Sciatic functional index

Group	Sciatic Nerve Index		
	0^{th}day vs 7^{th}	7^{th} vs 10 day	10^{th} vs 14 day
Vincristine	-62.1224	-29.7827###	-23.967##
Pregabalin	-41.5676	-11.8742##	-3.7160###
Methanolic extract of *cleodendrum viscosum* (200mg/kg)+ vincristine	-48.4804	-21.4903##	-10.6196##
Methanolic extract of *cleodendrum viscosum* (400mg/kg)+vincristine	-47.2397	-13.6793###	-8.2724###

The values are expressed in mean ± SEM (n=6), ## indicates P<0.01, ### indicates P<0.001 compared to control.

Effect of Methanolic extract of *Cleodendrunm Viscosum* on Formalin test

Vincristine induced peripheral neuropathy, the administration of methanolic extract of *Cleodendrum Viscosum* at 200mg/kg and 400mg/kg respectively showed a significance (P<0.001) reduction in paw licking and paw elevation in acute phase(0-10min), while in delayed phase(30-40min) it showed a significant reduction in paw licking and elevation as shown in (figure 1) and (Figure 2) respectively.

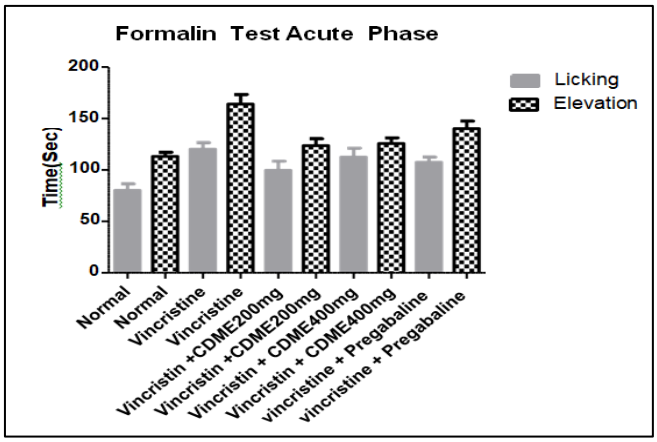

Figure 1: **Effect of Methanolic extract of Cleodendrum Viscosum on Acute phase of Formalin test**

Results were expressed as mean±SEM, n=6 rats per group. Two- way ANOVA was done followed by Bonferonni's test, ***, ** * indicates p<0.001, p<0.01,p<0.05 respectively in comparison of normal group and Vincristine control.###, ##,# indicates p<0.001, p<0.01,p<0.05 respectively when compared with vincristine group. CDME – Methanolic extract of *Cleodendrum Viscosum* Leaves.

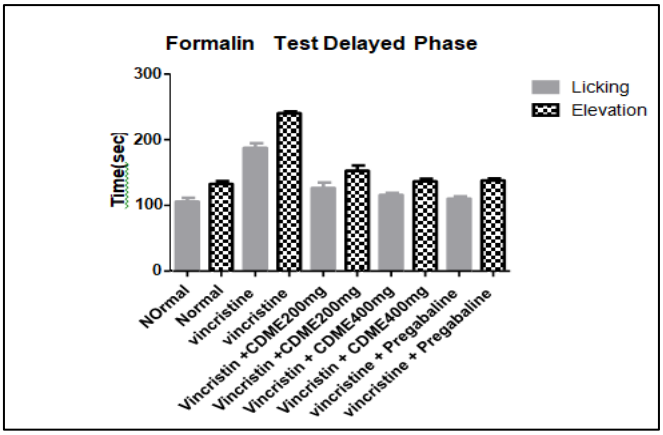

Figure 2: Effect of Methanolic extract of Cleodendrum Viscosum on Delayed phase of Formalin test

Results were expressed as mean±SEM, n=6 rats per group. Two- way ANOVA was done followed by Bonferonni's test, ***, ** * indicates p<0.001, p<0.01, p<0.05 respectively in comparison of normal group and Vincristine control.###, ##,# indicates p<0.001, p<0.01,p<0.05 respectively when compared with vincristine group.
CDME – Methanolic extract of *Cleodendrum Viscosum* Leaves

Histopathological Examination

The histopathological studies revealed that the sciatic nerve exhibited axonal degeneration and fibers derangements. The administration of Methanolic extract of *Cleodendrum Viscosum* (CDME) significantly attenuated Vincristine induced axonal degeneration in 200mg/kg and 400mg/kg. Pregabalin groups exhibited similar attenuation of sciatic nerve degeneration. However, the study concluded that the aforementioned plant extract exhibited significant protective effect against Vincristine induced peripheral neuropathy. A-Exhibited normal sciatic nerve, B-Exhibited sciatic nerve degeneration in Vincristine, C-shows sciatic nerve axonal recovery in pregabalin treated rat, D-shows sciatic nerve axonal regeneration in 200mg/kg CDME treated rat and E- shows more Sciatic nerve axonal regeneration in 400mg/kg in CDME treated rat.

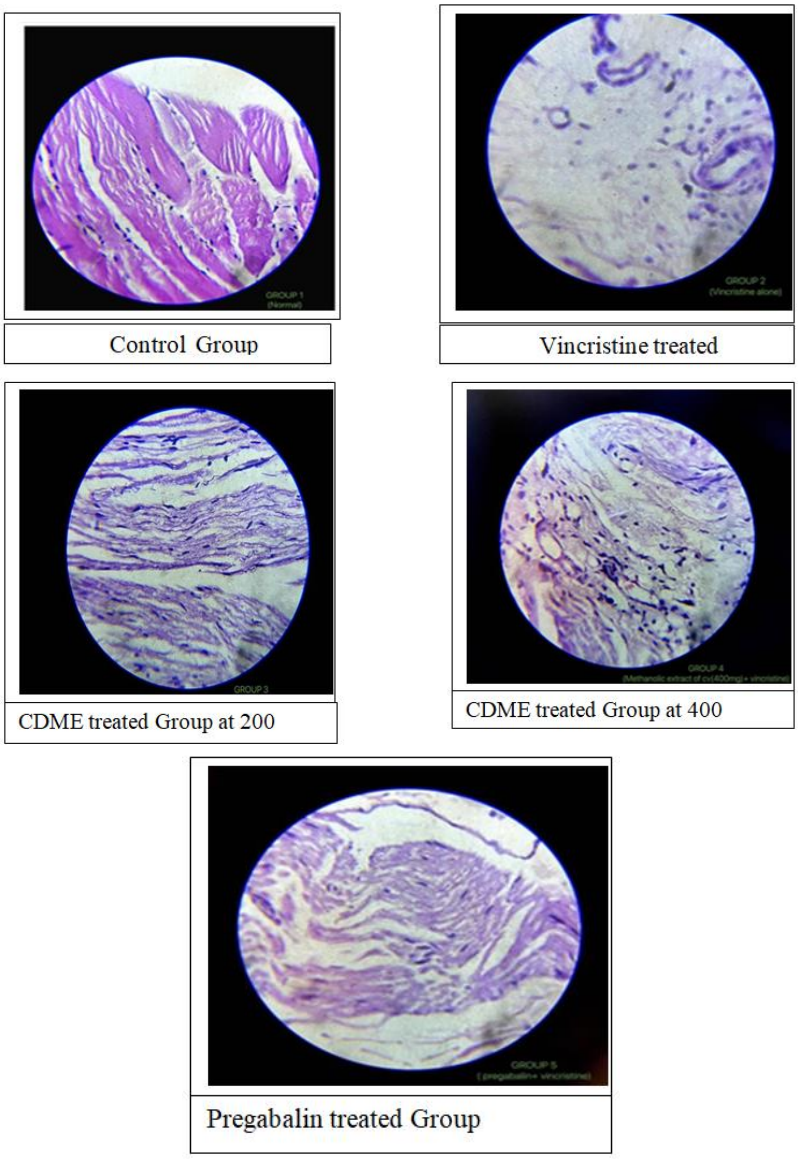

Figure 3: Effect of Methanolic extract of *Clerodendrum viscosum* on histopathological changes in vincristine induced neuropathy rats

CONCLUSION

Vincristine induced neuropathy is consider as a serious consequence which leads to the discontinuation of therapy in cancer patients. Based on the finding methanolic extract of *Cleodendrum Viscosum* leaves able to prevent the vincristine induced neuropathy by different mechanism. It attenuate neuronal damage by exhibiting alteration in glutamate signaling pathway. The entire study concluded that our plant extract exhibited protective effect against Vincristine induced neuropathy by reducing axonal swelling and nerve degeneration.

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Conflict of Interest: The Authors declare there is no potential conflict of interest with respect to the research.

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