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Evaluating the Significance Alleviation of Protective drugs on *Cisplatininducedneurotoxicity* on Sciatic nerves of rats

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Abstract*: Chemotherapy is one among the alternative treatment for cancer patients but still it's a unsuccessful. Chemotherapy induced peripheral neuropathy is a common adverse reaction or effect of several agents/ drugs like taxanes and vinca alkaloids etc and some synthetic prepared pharmaceutical drugs. There is a growing evidences suggesting that the physical damage by cisplatin leads to structural and functional impairment in neurons through inflammation, apoptosis and electrophysiological disturbances. The aim of the present is to study is to evaluate the neuroprotective effect of phloroglucinol in cisplatin induced toxicities in rats.Adult male rats weighing 180-220gm were used. Neuropathywas induced by cisplatin (1mg/kg,i.p, twice a week for 6 weeks) and were treated with phloroglucinol (250mg, 500mg/kg,i.p, for 6 weeks). At the end of the study body weight and heamogram were estimated even Behavioral tests including cold and hot hyperalgesia, loco motor activity, motor co-ordination were done. Rats were sacrificed and Sciatic nerve conduction velocity was determined. Histopathology of Sciatic nerve was performed and reported.Phloroglucinol treatment showed intense effects on both functional and structural abnormalities in cisplatin neuropathy with the ability tom same neurons from neurotoxic affects of cisplatin which succeeded in making space as alternate drugs for cancer treatment.*

Keywords*: cisplatin, cancer, phuloroglucinol, neuropathy.*

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I. Introduction

Peripheral neuropathy is a damage or disease affect ting nerves often causing weakness, numbness and pain, usually in hands and feet, but it may also occur in other areas of the body. It is thought to result when sensory neurons generate impulse at abnormal (ectopic) locations, for example at sites of nerves injury or demyelization. The prevalence of neuropathic pain seems to be increasing to the aging population as well as the increasing use of neurotoxic agents. Peripheral neurotoxicity is a dose limiting and disabling side effects of several important chemotherapeutic agents, in particular, vincristine, cisplatin, oxaliplatin, paclitaxel, docetaxel and other antineoplastic agents which are known causes of the peripheralneuropathy, hematological and renal toxicity. Chemotherapy induced peripheral neurotoxicity is a major clinical problem because it a dose limiting side effects of important and effective antineoplastic drugs[1]. The incidence, severity and clinical symptoms and signs of chemotherapy induced peripheral neurotoxicity depend on the drug given and its schedules. Cisplatin has had a central role in cancer chemotherapy for the last 40 years andcontinue to be among the most widely used antineoplastic drugs in clinical use. Cisplatin displays therapeutic efficacy in a broad range of solid tumors especially against testicular, ovarian and bladder cancers. Phloroglucinol(1,3,5-trihydrobenzene) is the monomeric building un it of phlorotannins known omnly for blue algae. Its reported to have neuro-regenerative, anti-inflammatory, antitumor, antimicrobial and analgesic[1,2].

II. Methodology

1.1. Experimental animals and vivarium condition

Adult male Wister rats weighing about 180-220g were used in the study. The use of animals in these experiments was authorized by IAEC (Institutional Animal Ethical Committee).Throughout the experiment, experimental rats were processed accordance with the CPCSEA guidelines. The animals were housed in grouped of three in polypropylene cages. Animal were maintained in temperature controlled at 20±2^{o}c and relative humidity of 50-60% with an alternative 12hr light/dark cycle. U. V. Sterilized clean paddy husk were use3d for bedding, they were fed with commercially available standard pellet chow (Amrut feeds, Bangalore) and filtered tap water[3,4].

Drug Preparation and groupings

Sl.no	Group name	Dose	Duration
1	Normal control	Distilled water	p.o., for 8 weeks
2	Positive control Cisplatin	1 mg/kg i.p.	Twice a week i.p., fro 6 weeks
3	Cisplatin +phluroglucinol	1mg/kg i.p.+250mg/kg p.o	Twice a week i.p., for 6 weeks+ daily p.o; fro 8 weeks
4	Cisplatin+phluroglucinol	1mg/kg i.p,+500mg/kg p.o	Twice a week i.p., for 6 weeks+ daily p.o; fro 8 weeks

2.2.Behavioral studies

2.2.1.Tail immersion test

Thermal hyperlagesia was assessed by tail immersion test. It was noted with the immersion of terminal part of the tail (1cm) in water, temperature was maintained at 46ºc and 4ºc. The duration of the tail withdrawal reflex or signs of struggle was recorded as response of heat sensation anda cutoff time of 20s was maintained.

2.2.2. Motor co-ordination assessment

The rat-rod (rotating rod) test is widely used in rodents to assess their "minimal neurological deceit" such as impairment motor function (e.g. ataxia) and coordination. The rat rod unit consistsof rotating rod, 75mm in diameter, which was divided into four parts b y compartmentalization to permit the testing of four rats at a time. Briefly, in a training session, the rats were placed on the rod that was set to 25rpm and the performance time that each rat was able to remain on the rota-rod was recorded[5,6].

2.2.4. Grip force assays

To evaluate muscle hyperalgesia, forelimb forces were measured using a hand-made grip force analyzer similar to the one described by. This apparatus measured the amount of tensile force each rat exerted against a wire mesh grid attached to as a force transducer (model). Forelimb grip force measurements were obtained. For measuring forelimb grip force, the apparatus was placed in front of the rat facing thewire mesh grids. Duration of the force each animal applies to the mesh grid is self dependent. Therefore, the level of force exerted is subject to evaluate factors, such as muscle hyperalgesia, that influence the behavioral performance of the animal[7].

2.2.5. Mechanical hyperalgesia

The nociceptive flexion was quantified using as fabricated Randall-Selitto paw pressure device which applies a linearly increasing mechanical force (in g) to the dorsum of the rats hind paw. Nociceptive threshold, expressed in grams, was applied by increasing pressure to the hind paw until a squeak (vocalization threshold) was elicited. As this test involves animal handling, the rats were handled, as follows: 3 days before the experiment, rats were handled without escaping from the hand for 20s, 2 or 3 times depending on their capacity to quiet. On the day of the experiment, rats were again handled 2-3 times for 20s. No rats should show aversive reaction during handling. Then, the paw of the rat was placed under the tip, and the progressive pressure applied until the rats vocalized[8].

2.2.6. Electrophysiology

Measurement Sciatic of nerve conduction velocity

Ascsiatic nerve conduct ion velocity test is an electrical test that is used todetermine the adequacy of the conduction of the nerve impulse as it courses down a nerve. This test is used tom detect signs of nerve e injury. In this test, the nerve is electricity stimulated, and the electrical impulses 'down steam' from the stimulus is measured. One electrode stimulates the nerve with a very mild electrical impulse. The resulting activity is recorded by the other electrodes. The distance between stimulating and recording electrodes and the time it takes for impulse transmission (nerve conduction velocity)[8,9-12].

Procedure: Rats were anesthetized. The left sciatic/ tibia nerve was dissected rapidly and placed in an insulate box and stimulate at the distal end. The nerve was stimulate by biphasic pulse (duration:0.1s, intensity: 200Mv). The average of 10 potential traces was measured andthe nerve length between stimulant and recording electrode was recorded. The time interval between stimulus artifact andtake off first negative deflection was taken as latency.

Velocity = d / latency (m/s) or (mm/ms)

Latency = latency at electrode R 1(ms)

d = distance (mm) from stimulating electrode tp recording electrode R1[14].

2.2.7. Heamogram

Method of collection of samples

After completion of experimental period, approximately 0.5ml of blood was drawn from the retro-orbital plexus of the rats under light anesthesia. Each blood sample was collected in clean, labeled and dry micro-centrifuge

tubes containing 10% EDTA solution (0.5 ml blood). The blood samples were analyzed within 6 hours for the hemoglobin, WBC, RBC and platelet count.

2.2.8. Haemoglobin estimation

The apparatus consists of two verticals sealed tubes with standard brow color. Between the two tubes is a compartment to hold a graduated tube. Blood is transfused into the central compartment and diluted with N/10 HCL until the color matches that of standard tubes .haemoglobin values are read as % Hb.

2.2.9. Total WBC Estimation

Blood with drawn from retro orbital puncture was taken in the WBC pipette up to the 0.5 mark and diluted with WBC diluting solution exactly up to the 11.0 mark. WBC's are counted using the WBC chamber of Neubauer's counting chamber.

Calculation:

Number of cells in 1 cumm of blood =

$$= \frac{\text{Cells counted X dilution factor X chamber depth}}{\text{Area of chamber counted}}$$

$$= \frac{\text{Cells counted X 20 X 10}}{4}$$

= Cells counted X 50

2.2.10. Platelet count

Pipette 5µl of the well mixed (at least 8 complete, gentle inversions of the specimen tube) blood specimen into 100µl of the filtered PBS-BSA diluents. Add 5µl of the anti-CD41 and 5µl of the anti-CD61 staining solution and incubate for 15minutes, in the dark, at ambient temperature (18ºC - 22ºC).After 15minutes, prepare a final 1:1000 dilution from counting by adding 4.85 ml of the PNS-BSA diluents. Mix well by gentle inversions to ensure proper and equal distribution of RBC's and platelets[15].

2.2.3Biochemical estimation

Sciatic nerve homogenate preparation

Sciatic nerve samples were rinsed with ice cold saline (0.9% w/v sodium chloride) and homogenized in chilled phosphate buffer (pH 7.4). The homogenate thus obtained was used to measurement of lipid per oxidation, reduced glutathione, SOD, catalase and nitric oxide.

Procedure

0.2, 0.4, 0.6, 0.8 and1ml of working standard were pipette in to the series of labeled test tube. 1 ml of the sample was pipette in another test tube. Volume was made up to 1ml in all the test tubes. A tube with 1 ml of distilled water served as theblank.5ml of reagent C was added to all the best tubes including the test tubes labeled 'blank' and 'unknown'. Contents of the tubes were mixed by shaking the tubes and allow standing for 10minutes. Then 0.5ml of reagent D was added rapidly with immediate mixing well and incubated at room temperature in the dark for 30min. Then absorbance was recorded at 660 nm against blanks[5,16].

III. Results

3.1. Behavioral studies

3.1.1 Cold hyperalgesia

Effect of oral administration of phuroglucinol (250, 500 mg/kg oral) on cold hyperalgesia (4ºC) in rats administered cisplatin(1mg, /kg i.p.) tail withdrawal latency for normal rats was found to be 14.816±0.047sec. Tail withdrawal latency for cisplatin treated rats was found to be 12.000±0.435sec which was significantly lower compared to normal rats. Tail withdrawal latency in rats treated with phluroglucinol (250, 500 mg/kg oral) was found to be (14.816±0.060,12.933±0.638sec) is significantly higher the cisplatin treated rats.

Table 1: Effect of oral administration of phluroglucinol on cold hyperalgesia in cisplatin treated rats.

Groups	Treatment	Latency in seconds
Group 1	Normal Control	14.816 ± 0.047
Group 2	Cisplatin (1mg/kg i.p.)	12.000 ± 0.4345
Group 3	Cisplatin (1mg/kg.i.p) + Phluroglucinol (250mg/kg.p.o)	14.816 ± 0.060
Group 4	Cisplatin (1mg/kg i.p) + Phluroglucinol (500mg/kg p.o)	12.933 ± 0.638

3.1.2 Thermal hyperalgesia

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on cold hyperalgesia (4ºC) in rats administrated cisplatin (1mg/kg i.p.).Tail with drawn latency for normal rats was found to be 14.850 ±0.042sec. Tail withdrawn latency for cisplatin treated rats was found to be 8.566±0.943sec which was significantly lower compared to normal rats. Tail withdrawal latency rats treated with phlurogluvcinol (250, 500mg/kg i.p.) was found to (12.050±0.5670, 9.318±0.2650).

Table 2: Effect of oral administered of phluroglucinol on thermal hyperalgesia in cisplatin treated rats.

Groups	Treatment	Latency in seconds
Groups 1	Normal Control	14.850±0.042
Groups 2	Cisplatin (1mg/kg i.p.)	8.566±0.943
Groups 3	Cisplatin (1mg/kg i.p)+ phluroglucinol(250mg/kg .p.o)	12.050±0.570
Groups 4	Cisplatin (1mg/kg i.p)+ phluroglucinol(500mg/kg .p.o)	9.318±0.265

3.1.3 Motor Corodination

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on neuromuscular cordination (4ºC) in rats administrated cisplatin (1mg/kg i.p.).Fall of time for normal rats was found to be 136.033 ±8.684sec. Fall of time for cisplatin treated rats was found to be 248.166±0.477sec which was significantly lower compared to normal rats. Tail withdrawal latency rats treated with phluroglucinol (250, 500mg/kg i.p.) was found to (60.516±3.452, 46.900±1.945).

Table 3. Effect of rats administered of Phluroglucinol on motor co-ordination in cisplatin treated rats.

Groups	Treatment	Fall of time(s)
Groups 1	Normal Control	248.166±0.477
Groups 2	Cisplatin (1mg/kg i.p)	136.033±8.684
Groups 3	Cisplatin (1mg/kg i.p) + Phluroglucinol (250mg/kg o.p)	60.516±3.452
Groups 4	Cisplatin (1mg/kg i.p) + Phluroglucinol (500mg/kg o.p)	46.900±1.945

3.1.4 Locomotor activity

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on locomotor activity in rats administrated cisplatin (1mg/kg i.p.). Normal of counts for normal rats was found to be 423.333 ±8.950 no of counts. No of counts for cisplatin treated rats was found to be 289.833±16.205 no of counts which was significantly lower compared to normal rats. No of counts rats treated with phluroglucinol (250, 500mg/kg i.p.) was found to (370.000±23.974, 368.500±24.504).

Table 4 . Effect of oral administration of phluroglucinol on locomotor activity in cisplatin treated rats.

Groups	Treatment	No of counts
Groups 1	Normal control	423.333±8.950
Groups 2	Cisplatin (1mg/kg i.p)	289.833±16.205
Groups 3	Cisplatin (1mg/kg i.p)+ phluroglucinol (250mg/kg p.o)	370.000±23.974
Groups 4	Cisplatin (1mg/kg i.p)+ phluroglucinol (500mg/kg p.o)	368.500±24.504

3.1.5.Mechanical hyperalgesia

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on mechanical hyperalgesia in rats administrated cisplatin (1mg/kg i.p.). Mechanical threshold for normal rats was found to be 158.427±5.584 no of counts. Mechanical threshold for cisplatin treated rats was found to be 75.137±4.826g which was significantly lower compared to normal rats. Mechanical threshold rats treated with phluroglucinol (250, 500mg/kg i.p.) was found to (109.902±6.364, 79.060±2.656).

Table 4 . Effect of oral administration of phluroglucinol on Mechanical hyperalgesia in cisplatin treated rats.

Groups	Treatment	Mechanical threshold
Groups 1	Normal Control	158.427±5.584
Groups 2	Cisplatin (1mg/kg i.p)	75.137±4.826
Groups 3	Cisplatin (1mg/kg i.p) + phluroglucinol (250mg/kg o.p)	109.902±6.364
Groups 4	Cisplatin (1mg/kg i.p) + phluroglucinol (500mg/kg o.p)	79.060±2.656

3.1.6.Electrophysiology

Measurement of sciatic nerve conduction velocity

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on sciatic nerve conduction velocity in rats administrated cisplatin (1mg/kg i.p.). Sciatic nerve conduction velocity for normal rats was found to be 42.833±1.492m/s. Sciatic nerve conduction velocity for cisplatin treated rats was found to be 20.666±0.881 m/s which was significantly lower compared to normal rats. Mechanical threshold rats treated with phluroglucinol (250, 500mg/kg i.p.) was found to (30.666±1.9954 m/s, 28.666±0.843 m/s).

Table 5. Effect of oral administration of phluroglucinol on Sciatic nerve conduction velocity in cisplatin treated rats.

Groups	**Treatment**	**NCV (m/s)**
Groups 1	Normal Control	42.833±1.492
Groups 2	Cisplatin (1mg/kg i.p)	20.666±0.881
Groups 3	Cisplatin (1mg/kg i.p) + phluroglucinol (250mg/kg o.p)	30.666±1.944
Groups 4	Cisplatin (1mg/kg i.p) + phluroglucinol (500mg/kg o.p)	28.666±0.843

3.1.7. Heamogram

WBC count

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on WBC count in rats administrated cisplatin (1mg/kg i.p.). WBC count for normal rats was found to be 6.733 ±0.313x10^3µl . WBC count for cisplatin treated rats was found to be 5.133±0.301x10^3µl which was significantly lower compared to normal rats.WBC count in rats treated with phluroglucinol (250, 500mg/kg i.p.) was found to (5.600±0.396x10^3µl, 5.500±0.352 x10^3µl).

Table 6 . Effect of oral administration of phluroglucinol on WBC count in cisplatin treated rats.

Groups	**Treatment**	**WBC Count (x10^3µl)**
Groups 1	Normal Control	6.733±0.313
Groups 2	Cisplatin (1mg/kg i.p)	5.133±0.301
Groups 3	Cisplatin (1mg/kg i.p) + phluroglucinol (250mg/kg o.p)	5.600±0.396
Groups 4	Cisplatin (1mg/kg i.p) + phluroglucinol (500mg/kg o.p)	5.500±0.352

3.1.8. Platelet Count

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on platelet count in rats administrated cisplatin (1mg/kg i.p.). Platelet count for normal rats was found to be 403.333 ±20.956x10^3 µl . Platelet count for cisplatin treated rats was found to be 403.333±25.489x10^3µl which was significantly lower compared to normal rats.Platelet count in rats treated with phluroglucinol (250, 500mg/kg i.p.) was found to (508.166±10.749x10^3µl, 506.833±19.652 x10^3µl).

Table 7 . Effect of oral administration of phluroglucinol on Platelet count in cisplatin treated rats.

Groups	**Treatment**	**Platelet count(x10^3/µl)**
Groups 1	Normal Control	538.833±20.956
Groups 2	Cisplatin (1mg/kg i.p)	403.333±25.489
Groups 3	Cisplatin (1mg/kg i.p) + phluroglucinol (250mg/kg o.p)	508.166±10.749
Groups 4	Cisplatin (1mg/kg i.p) + phluroglucinol (500mg/kg o.p)	506.833±19.652

3.1.9. Biochemical Estimation

Catalase

Effects of oral administration of phluroglucinol (250, 500mg/kg oral) on catalase activity in rats administrated cisplatin (1mg/kg i.p.). Catalase activity for normal rats was found to be 68.757 ±2.888 µmol/min/mg. Catalase activity for cisplatin treated rats was found to be 42.111±1.509 µmol/min/mg which was significantly lower compared to normal rats. Platelet count in rats treated with phluroglucinol (250, 500mg/kg i.p.) was found to (52.776±1.308, 45.296±0.881µmol/min/mg).

Table 8. Effect of oral administration of phluroglucinol on Catalase activity in sciatic nerve homogenate in cisplatin treated rats.

Groups	**Treatment**	**Catalase activity (µmol/min/mg of protein)**
Groups 1	Normal Control	68.757 ± 2.888
Groups 2	Cisplatin (1mg/kg i.p)	42.111 ± 1.509
Groups 3	Cisplatin (1mg/kg i.p) + phluroglucinol (250mg/kg o.p)	52.776 ± 1,308
Groups 4	Cisplatin (1mg/kg i.p) + phluroglucinol (500mg/kg o.p)	42.296 ± 0.881

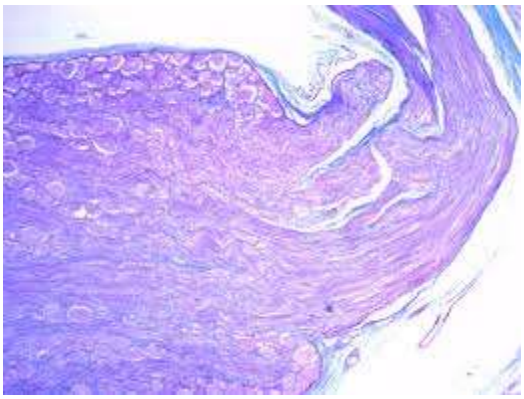

Fig .Histopathology of Dorsal root ganglion.

Fig. Histopathology of dorsal root ganglion of normal control group.

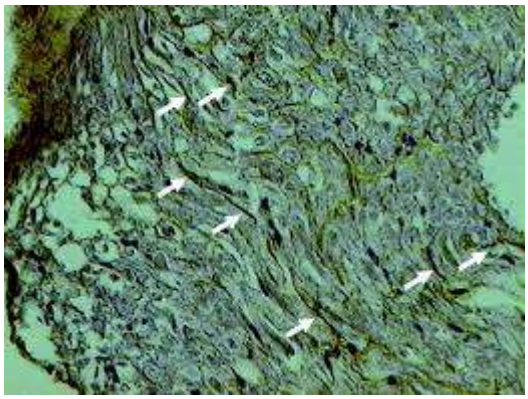

Fig. Histopathology of dorsal root ganglion of cisplatin control group.

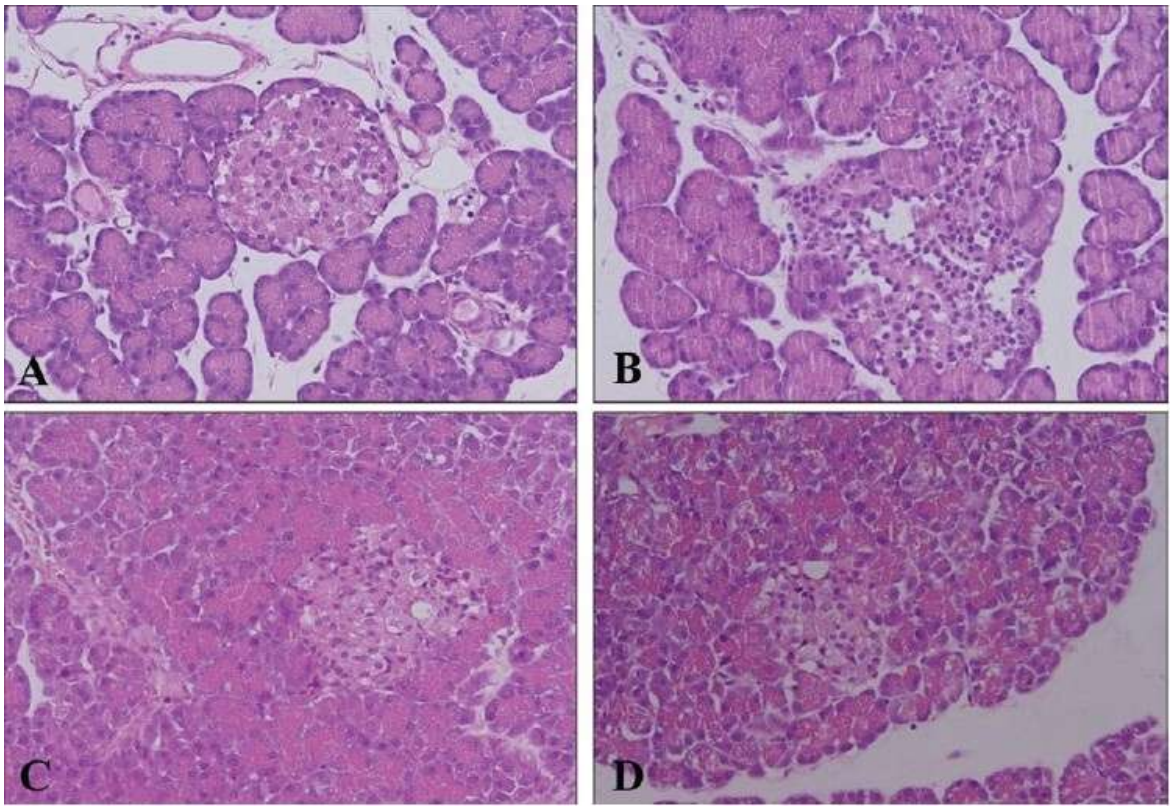

Fig. Hispathology of dorsal root ganglion of cisplatin control treated with phluroglucinol (250mg/kg p.o) group.

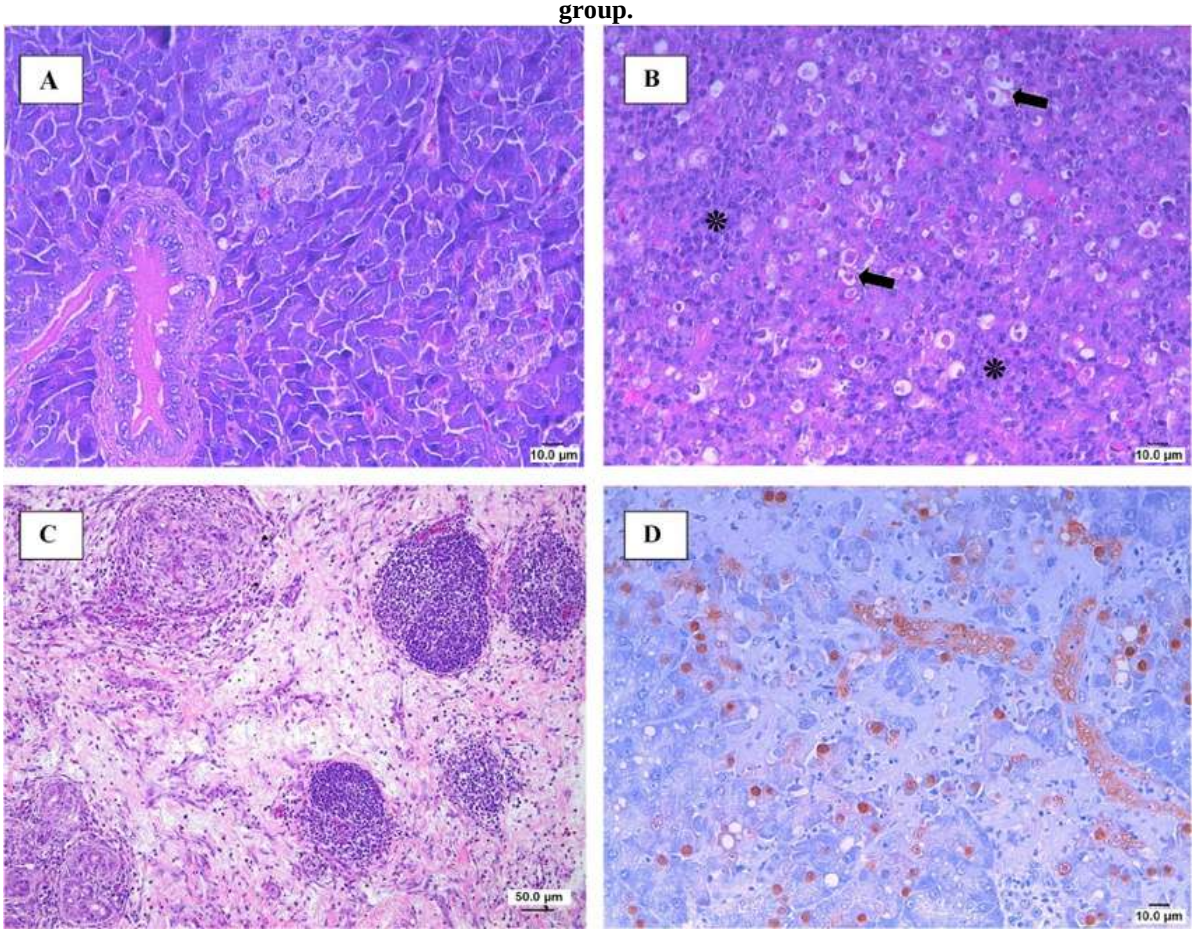

Fig. Hispathology of dorsal root ganglion of cisplatin control treated with phluroglucinol (500mg/kg p.o) group.

IV. Discussion

Cisplatin is an important component of chemotherapy used against various tumor types such as pancreatic cancer, breast cancer, osteosarcoma and metastatic melanoma however their clinical use is severe by dose limiting neurotoxicity.In the present treatment with phluroglucinol in two doses each (250, 500mg/kg, i.p) significantly alleviated cisplatin induced peripheral neuropathy in rats. Rats treated with these drugs exhibited increased cold andthermal hyperalgesia, improved nerve conduction velocity, mechanical hyperalgesia and higher level of antioxidant enzymes compared with cisplatin treated grouped rats. The neuroprotective role of phluroglucoinol in the study could probably be due to its antioxidant activity and improvement in the nerve blood flow therefore restored motor nerve conduction velocity deficits and improvement in cold and thermal hyperalgesia, mechanical hyperalgesia and histological changes as compared to cisplatin treated rats. This study results provide evidence that phluroglucinol improves the hematological status by showing significant increase in heamoglobin , platelet and RBC as compared to cisplatin treated rats and the WBC count in treated groups remained normal without any significant difference.

V. Conclusion

The research involved in the evaluation of neuroprotective activity of phluroglucinol in cisplatin induced peripheral neuropathy rat model with the characterization of behavioral, haematology, electrophysiology, biochemical and histopathology parameters. Our findings suggest the favorable effects of phluroglucinol on both functional and structural abnormalities in cisplatinneuropathy. They have the ability to save neurons from neurotoxic insults from cisplatin. They show promise and can be used as adjuvant drugs in cancer treatment.

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