"EVALUATION OF NEUROPROTECTIVE POTENTIAL OF RUTIN AGAINST PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY IN RATS"

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Abstract

Background: Cancer can be stated as one of the most deadly disease all around the world. Second leading cause of death in the world. Chemotherapy and anti cancer drugs are use to kill cancerous cells in the body. Among all the different side effects of chemotherapeutic drugs peripheral neuropathy is the most common and dose-limiting side effect. **Objectives:** This study aims to assess the protective effect of the flavonoid rutin based on its anti oxidant property against paclitaxel induced peripherial neuropathy in male wister rats. Rutin has been described as cell protecting agents because of its anti-oxidant, antinociceptive and anti inflammatory actions. **Method:** Paclitaxel (1mg/kg, i.p) was injected to male wister rat on four alternate days (0,2,4 and 6). The development of sensory alterations were evaluated by various in vivo methods- muscle grip test, acetone spray test, hot plate method, and tail flick method. Rutin (30mg/kg orally) dissolved in water was administered for 14 days with a single dose of paclitaxel on the 10^{th} day. The animal's sciatic nerve were removed for histopathological evaluation and blood serum was collected for the assessment of antioxidant property. **Result**: The thermal and mechanical nociceptive response was significantly increase by paclitaxel which was prevented or reduced by rutin at all dose. Nerve degradation, axonal derangement and shrinkage, epineurium deranged, perineurium abnormality and myelin sheath damaged showed paclitaxel induced peroxidation in the scatic nerve and that rutin decreases this effect. Also the flavonoid decreases inducible nitric oxcide synthase and increase catalase in the blood serum. **Conclusion:** In this study it was observed that paclitaxel induced painful peripheral neuropathy in rats was prevented by the flavonoid rutin. The mechanism of pactitaxel- chemotherapeutic drug appears to be at least oxidative stress induced that showed neumerous neuronal damage with increase vascular edema, vascular changes etc in the sciatic nerve of the neuropathy induction group.

Keywords: Paclitaxel, Rutin, antinociceptive, scatic nerve, peripheral neuropathy.

Introduction

Damage to or dysfunction of the nervous system leads to neuropathic pain.[1] In 5%, it may be severe and 7% to 8% gets affected.[2] A single disease process or a single specific location of damage cannot explain neuropathic pain. The complete nervous system is involved here. The structure and function of peripheral, motor, sensory, and/or autonomic neurons either partially or completely involved here.[3]

In order to evaluate and, different experimental neuropathic pain models have been developed to find new therapeutic approaches which include partial transaction of sciatic nerve, partial sciatic nerve ligation and chronic constriction injury of sciatic nerve, in order the mechanisms of neuropathic pain can be evaluated. This area of neuropathy the therapeutic need is not met. Different treatment procedures are put forward. Opioids, tricyclic antidepressants, certain anticonvulsants are recommended but only shows clinical significance of (>50%) pain relief. These are also followed by several side effects.[4]

Among the anti cancer drugs, a dose-limiting painful peripheral neuropathy is produced by **Paclitaxel** when given intraperitoneally. To evaluate the effect of various drugs in neuropathic pain, this and can be used as a model of peripheral neuropathy.[5]

Rutin (3,30,40,5,7-pentahydroxyflavone-3-rhamnoglucoside,) is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea, and apple. It has demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities.[6,7,8,9,10,11,12]

In this study the flavonoids rutin is described as cell-protecting agents because of their antioxidant, antinociceptive, and anti-inflammatory actions. A preventive effect has been proposed for these agents on paclitaxel-induced painful peripheral neuropathy based on their antioxidant properties.[13]

2. Materials and methodology

2.1. Drugs used:

- Chemotherapeutic drug that will be used to induce neuropathy: **Paclitaxel** bought from "**DRUGS POINT**" Store Baihata Chariali, Assam.
- Neuroprotective agent used: **Rutin (Thomson Rutin 500mg) bought from Global Mart India via Amazon.**
- Standard drug used: **Quercitin (Healthvit Quercitin 100mg) bought from Cloudtail India via Amazon.**

2.2. Animals:

Wistar rats weighing between 160-220 g were maintained in standard laboratory conditions at room temperature (25±2 °C) with 12:12 h L:D. The animals were given pellet chow and water ad libitum except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) at Karnataka College of Pharmacy, Bangalore. Studies were performed in accordance with the CPCSEA guidelines **Reg No. 1564/ PO/ Re/ S/ 11/CPCSEA.**

2.3. Induction of peripheral Neuropathy:

Paclitaxel

In the case of paclitaxel, it is consistently prepared with ethanol and diluted with saline. In this model, Wistar rats were administered single intraperitoneal (i.p.) injection of 1 mg/kg of paclitaxel on four alternate days (0, 2, 4, and 6). The volume of injection was kept constant at 1 mL/kg. This model typically presents sensory neuropathy. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages. Neuropathy animals were validated for peripheral nerve damage by nerve sensitivity by analgesic screening model such as Hot plate Method, Tail flick method and Acetone spray method and the assessments were done on days 0 (before first dose of paclitaxel) and on days 7, 14, 21, and 28.[14,15]

2.4. Experimental Protocol

Paclitaxel induced peripheral neuropathy model:

Animals was divided into four groups each having 6 rats. Non neuropathic animals are grouped for normal control. Peripheral neuropathy was induced in rats by administration of paclitaxel (1mg/kg) by intraperitoneal injection for four alternate days (0, 2, 4, and 6). The volume of injection was kept constant at 1mL/kg. The animals were weighed and nerve damage test are performed by nerve sensitivity by analgesic screening test. Blood was collected of neuropathic rats for the estimation of serum antioxidant using assay methods and the animals were weighed sacrificed at the end of the experiments for histopathological examination of each group. [14,15]

Grouping of animals (n = 6):

Group 1: Normal control – rats injected with 2.5 ml/kg saline (i.p)

Group 2: PIPN-control – rats injected with paclitaxel (1 mg/kg; i.p.) for four alternate days (0, 2, 4, and 6).[15]

Group 3: Treatment group – rats orally administrated with rutin (30 mg/kg) dissolved in water for 14 days with a single dose of paclitaxel (1 mg/kg; i.p.) on the 10th day.[16]

Group 4: Standard group - rats orally administrated with quercetin (50 mg/kg) dissolved in water for 14 days with a single dose of paclitaxel (1 mg/kg; i.p.) on the 10th day.[16]

2.5. *In-vivo* models for Chemotherapy induced Peripheral Neuropathy:

a) <u>Muscular grip strength test using Rota rod method</u>:

The loss of muscle grip is an indication of muscle relaxation. This effect can be easily studied in animals using inclined plane or rotating rods. The difference in the fall off time from the rotating rod between the

control and treated animal was taken as an index of muscle relaxation. The angle of the slope of the inclined plane or the rate of rotation of the rod was adjusted such that a normal animal can stay on the plane or on the rod for an appreciable period (3-5min) of time. The animals underwent a protest on the apparatus. Only those animals, which demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min, were used for the test. [17]

b) Cold Allodynia -Acetone spray test:

Cold Allodynia was assessed using acetone. Acetone was applied to the plantar surfaces of the left hind paw and withdrawal responses was noted. Testing was repeated 5 times with 3–5 min between each test. The intensity of cold allodynia was expressed as withdrawal frequency: (n paw withdrawal responses/n trials)×100.[18]

c) Analgesic Activity by Hot-plate Method:

Animals was individually placed on a hot plate maintained at constant temperature (55^0C) and the reaction of animals such as paw licking or jump response was taken as the end point. Normally animals showed response in 6-8sec. A cut off period of 15 sec observed to avoid damage to the paws. Prior to any treatment, the animals were allowed to familiarize with the test procedure and apparatus, and baseline values was obtained.[19]

d) Pain sensation test using Tail flick method:

Before initiating the test, the tail of each animal was dipped in water at 29 °C for 30 mins. Then the whole tail was submerged water at 49 °C. The time taken for the animal to show a characteristic tail flick response was recorded. The test was repeated three times for each animal and the average was considered as the withdrawal latency for each animal.[20]

e) Sciatic nerve Morphometry & Histopathology

Histopathology is an important tool to evaluate the protective effect of the drugs acting on the damaged or necrotic cells produced by the induction of neuropathy. Crucially, axonal degeneration has been reported due to these events, suggesting that cellular oxidant and inflammatory mediators play a key role in the pathogenesis of painful neuropathy under in vivo conditions. Histopathological examination of sciatic nerves gave the evidence of the neuropathy induced neuronal damage with increase in edema, vacuolar changes and necrosis significantly in neuropathy induction group.

The animals was euthanized using high dose of anaesthetic and then sacrificed. The bilateral sciatic nerves was isolated and 1 cm of sciatic nerve tissue was fixed with 10% formaldehyde. Longitudinal and transverse sections (5µm) were prepared with semi-automatic microtome and placed on glass slide coated with Meyer's egg albumin. Then the sections were covered with DPX (SRL, India) mounting medium with cover glass and observed under light microscope (Nikon, Japan) to study the histopathological changes.[22]

2.6. *In-vitro* assessment for paclitaxel induced peripheral neuropathy

Assessment of Anti-oxidant effect in blood serum:

4.6. a) Nitric oxide scavenging activity

Nitrite (NO_2) and nitrate (NO_3) are stable final products of NO metabolism and may be used as indirect markers of NO presence. Total NO concentration is commonly determined as a sum of nitrite and nitrate concentrations. NO concentration was determined using an indirect method based on measurement of nitrite concentration in serum according to Griess's reaction. To 20µL Griess reagent, Add 100ul deionized water and 100 µL sample were added in test tube. Add 100ul deionized water + 100ul Griess reagent is use as a blank. Allow the sample/reagent mixture and blank to develop in the dark for 30 min. (The mixture will continue to develop past 30 minutes although at a relatively slow rate. Measure the absorbance of sample mixture and blank solutions at 548nm using the spectrophotometer. The values were expressed as units/ milligram protein.[21]

4.6. b) Estimation of Catalase:

To 1.9 mL phosphate buffered saline (pH 7.0), 100µl of supernatant was added. To this 1 mL of H_2O_2 was added and the change in the absorbance was recorded at 240 nm for 3 min. The values was expressed as units/milligram protein.[23]

3. RESULT

On 14th day Paclitaxel-induced neuropathy was seen. In tail flick and cold allodynia (Acetone spray) a significant decreased in latency was observed, and it was persisted till 28 days. Apart from this other abnormal behavioural response was not seen. Data presented in Tables 5.2 and 5.4 shows the effect of **Rutin** at various doses on tail flick and cold allodynia latency. In PIPN (Paclitaxel induced peripheral neuropathy) disease controlled rats, the muscular grip strength was significantly ($p < 0.001$) decreased as compared to normal control rats. The treatment of CIPN rats showed a significant ($p< 0.001$; $p < 0.05$) improvement in the muscular grip strength as compared to PIPN control rats mentioned in table 5.1. The hot plate response time also showed significant increase in case of treated PIPN (Paclitaxel as chemotherapeutic agent) group, mentioned in table 5.3.

The thermal and mechanical nociceptive response was significantly increase by paclitaxel which was prevented or reduced by rutin at all dose. Myelin sheath damaged, axonal derangement and shrinkage, nerve degradation, epineurium deranged, perineurium abnormality and showed that the scatic nerve is peroxidised by paclitaxel and that rutin decreases this effect. Also the flavonoid decreases inducible nitric oxcide synthase and increase catalase in the blood serum.

4. DISCUSSION

The treatment used to kill or destroy cancer cell is called Chemotherapy. However these treatments may cause different side effects and chemotherapeutic based regimens appear to reached a therapeutic plateau. A very common side effect of many chemotherapeutic drugs is **Peripheral neuropathy.** It is dose-limiting.

About 19% to over 85% patients suffers from **Chemotherapy-induced peripheral neuropathy (CIPN**). The peripheral sensory, motor and autonomic neurons are damaged by CIPN. A total 6 substance group are responsible for the same. These are : immunomodulatory drugs (thalidomide), vinca alkaloids, platinum-based antineoplastic agents, proteasome inhibitors (bortezomib) , epothilones (ixabepilone) and taxanes. Among these, the

less neurotoxic drugs are bortezomib and vinca alkaloids and the most neurotoxic are thalidomide, platinum-based agents, ixabepilone and taxanes.[24]

After chemotherapy when measured in the first month ,CIPN is seen at approximately 68.1%, at 3 months it is 60.0% and after 6 months its 30.0%. [25]

A flavonol, Rutin which is abundantly found in plants, such as passion flower, apple, buckwheat and tea. It includes the pharmacological activities: neuroprotective, anticarcinogenic, antioxidant, vasoprotective, and cardioprotective activities and cytoprotective. [6,7,8,9,10,11,12]

In this study the flavonoids rutin is described as cell-protecting agents because of their antioxidant, antinociceptive, and anti-inflammatory actions. Based on their antioxidant properties, a preventive effect has been proposed for these agents on paclitaxel-induced painful peripheral neuropathy.[8]

The paclitaxel induced peripheral neuropathy study was conducted by assessing the muscular grip strength, pain sensation test, analgesic activity by Hot-plate Method, anti-oxidant effect in blood serum (Nitric oxide scavenging activity and Estimation of Catalase) and Histopathology of Sciatic nerve.

The muscular grip strength test was conducted and the time of residence/fall of normal control, PIPN control, test group (Rutin) and standard group (Quercitin) was found to be 153.3 ± 0.7 sec, 53 ± 0.8 sec, 124 ± 0.8 sec, and 144.2 ± 0.8 sec respectively. **(Fig no. 1)**

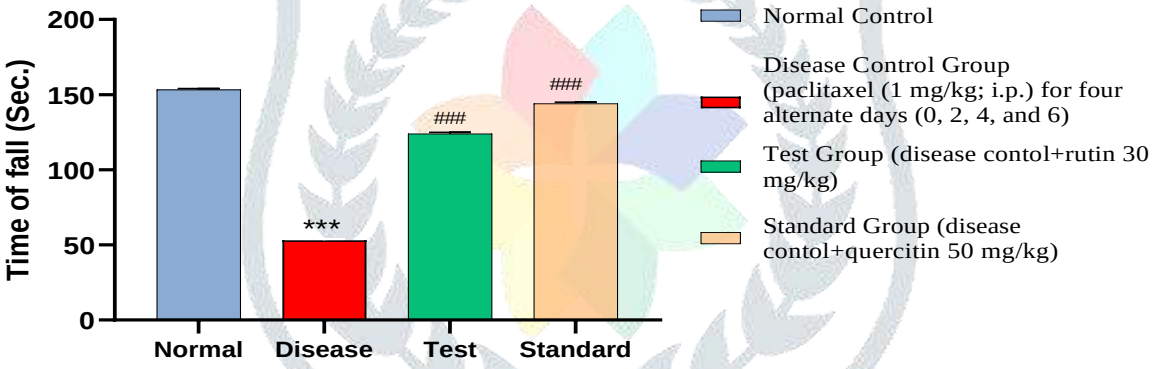


Fig. no. 1: Effect of Rutin on Time of residence/fall

The **pain sensation test** was conducted and the **tail flick latency** for normal control, PIPN control, test group (Rutin) and standard (Quercitin) was found to have significant difference when compared between PIPN control group , test group and the standard group. For pain sensation test, cold Allodynia was assessed using acetone. And the tail withdrawl response rate (%) of normal control, PIPN control, test group (Rutin) and standard group (Quercitin) was found to be 42.83 ± 0.8 %, 73.33 ± 0.8 %, 57.67 ± 0.5% and 51.83 ± 0.6 % respectively **(Fig no. 2)** And conducting tail flick method, the tail flick latency for normal control, PIPN control, test group (Rutin) and standard group (Quercitin) was found to be 3.3 ± 0.1 sec, 13 ± 0.5 **sec,** 6.8 ± 0.4 sec and 5.7 ± 0.4 sec respectively **(Fig no. 4)**

Similarly the **hot plate response time** for normal control, PIPN control, test group (Rutin) and standard (Quercitin) was found to be 7.1 ± 0.4 sec, 22 ± 0.6 sec, 16.5 ± 0.5 sec and 13.3 ± 0.5 sec respectively. **(Fig no. 3)**

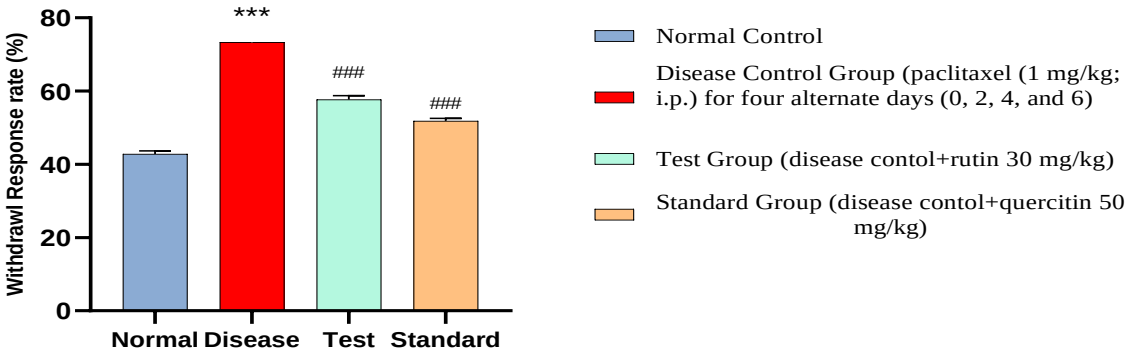


Fig. no.2: Effect of Rutin on withdrawal response rate (%)

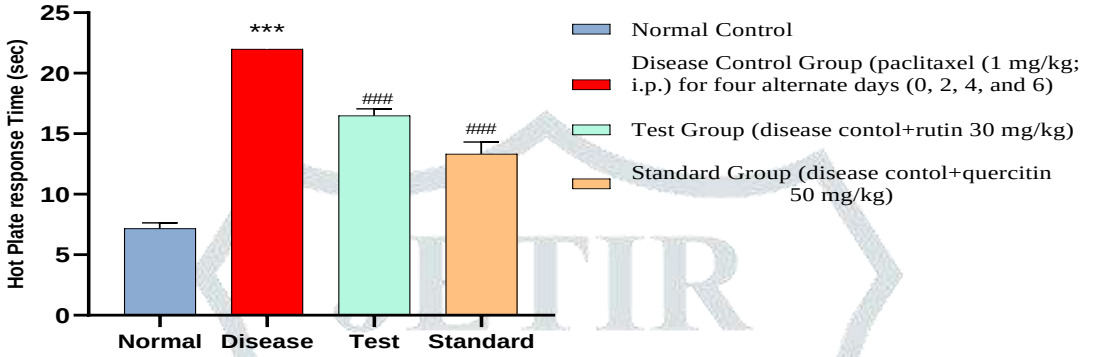


Fig no. 3: Effect of Rutin on Response time

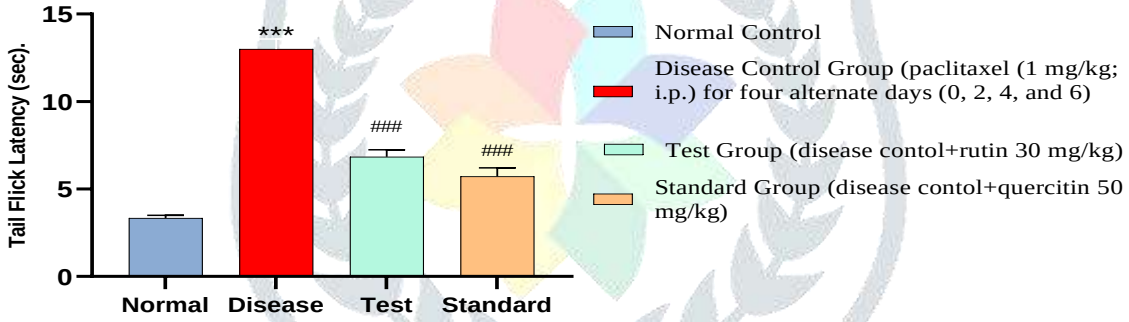


Fig no. 4: Effect of Rutin on Tail Flick Latency (sec)

The **nitric oxide scavenging activity** of normal control, PIPN control, test group (Rutin) and standard (Quercitin) group was found to be 13.37 ± 0.1, 65.1 ± 0.2, 39.77 ± 0.1 and 42.1 ± 01 units/mg protein respectively (**Fig no. 5**). Similarly, the **catalase activity** of normal control, PIPN control, test group (Rutin) and standard (Quercitin) group was found to be 10.17 ± 0.2, 4.9 ± 0.3, 13.05 ± 0.3 and 10.97 ± 0.2 units/mg protein respectively (**Fig no. 6)**

Serum NO (µmoles/g protein)

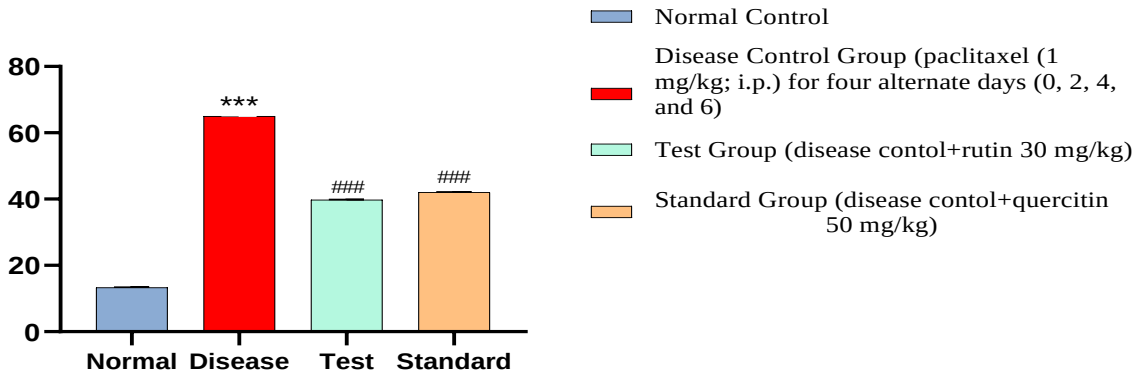


Fig No. 5: Nitric oxide scavenging activity in PIPN rats. (paclitaxel as inducing agent)

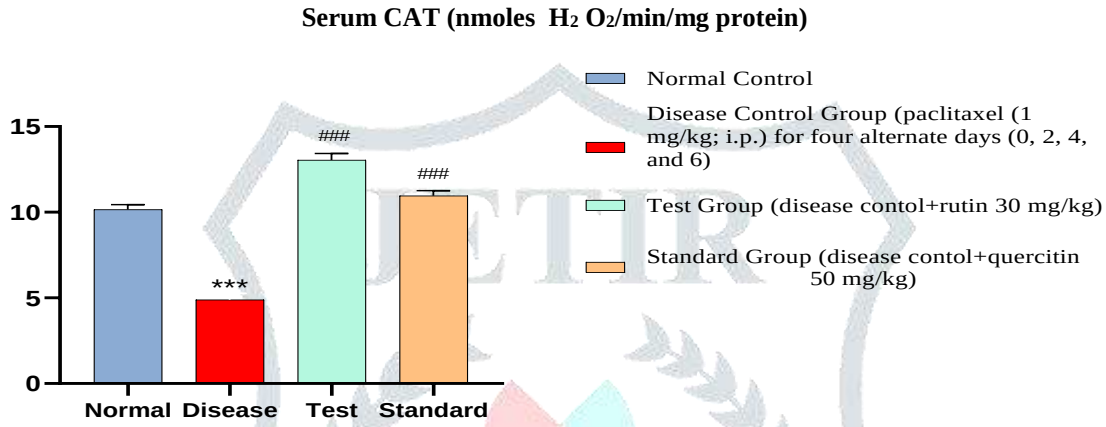
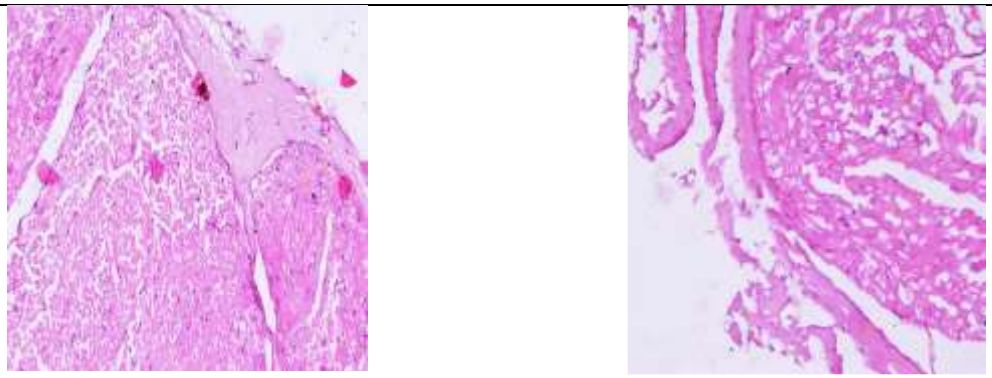


Fig No. 6: Catalase activity in PIPN rats. (paclitaxel as inducing agent)

Histopathological study of sciatic nerve reveled that the axonal degeneration level of those rats treated with **rutin (Group 3)**were found to be significantly lower when compared to those rats in the **PIPN control group (Group 2)**. In the group treated with **rutin (30mg/kg)**, the degeneration of myelin was found to be significantly lower as compared the rats in the PIPN control group. Also aggressive axonal shrinkage and damage of the oligofascicular nerve fibre was seen in PIPN control group but very mild in the **rutin** treated group. Deranged epinerium and some abnormalities in the perineurium were found to be significantly highest in the PIPN control group compared to the **rutin** treated groups. (**Fig No. 7**)



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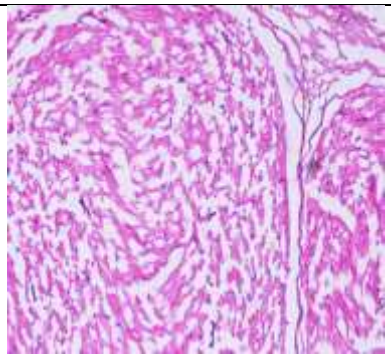

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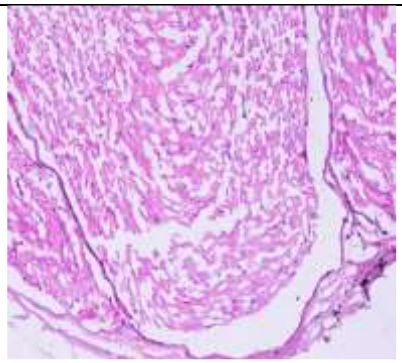

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Fig. no.7: Histological findings. a) **Normal Control Group** showing histological structures of monofacicular nerve fibre, myelin sheath and Epinerium with no degradation. b) **Disease control Group** showing axonal derangement, Myelin sheath damage and deranged epinerium and some abnormalities in the perineurium. c) **Test Group (Rutin)** showed very mild axonal shrinkage and damaged Oligofascicular nerve and myelin sheath. d) **Standard Group (Quercitin)** where myelin loosening through the wavy peripherial line surrounding the axon. Damaged oligofascicular nerve fibre and axonal shrinkage.

5. Conclusion

In the present study the neuroprotective potential of **rutin** against **paclitaxel induced peripheral neuropathy** in rats, was scientifically evaluated and validated. Here, induction of peripheral neuropathy was done by the chemotherapeutic agent Paclitaxel. And because of their anti-inflammatory , antinociceptive and antioxidant actions rutin was stated as cell-protecting agents. An effect of protection has been proposed for these agents on paclitaxel-induced painful peripheral neuropathy (PIPN) grounded on their antioxidant properties. Therefore, the flavonoids rutin was evaluated for neuroprotective activity and was subjected to various analysis to ascertain the presence of its activity. In this study it was observed that paclitaxel induced painful peripheral neuropathy in rats was prevented by the flavonoid rutin. The mechanism of pactitaxel- chemotherapeutic drug appears to be at least oxidative stress induced that showed neumerous neuronal damage with increase vascular edema, vascular changes etc in the sciatic nerve of the neuropathy induction group. Hence, the present study validates the use of the flavonoids rutin as a neuroprotective agent.

6. Summary

The present study deals with the neuroprotective activity of flavonoid **rutin**, in paclitaxel induced peripherial neuropathic (PIPN) rats. The present study indicates the significant increase in muscular grip strength, decrease in pain response time compared to that of the PIPN control. The effect of **rutin** in PIPN rats showed a significant difference in cold allodynia induced by acetone. The treated group with **rutin** significantly inhibit acute cold allodynia compared to the PIPN induced rats. Treatment of **rutin (30mg/kg)** in PIPN rats also showed decrease in nitric oxide and increase

in the catalase activity which shows the anti-oxidant property of the **Rutin.** Histopathological study of sciatic nerve revealed that the axonal degeneration level of those rats treated with **rutin** were found to be significantly lower when compared to those rats in the PIPN control group. In the group treated with rutin (30mg/kg), the degeneration of myelin was found to be significantly lower as compared the rats in the PIPN control group. Also aggressive axonal shrinkage and damage of the oligofascicular nerve fibre was seen in PIPN control group but very mild in the **rutin** treated group. Deranged epinerium and some abnormalities in the perineurium were found to be significantly highest in the PIPN control group compared to the **rutin** treated groups. The thermal and mechanical nociceptive response was significantly increase by paclitaxel which was prevented or reduced by rutin at all dose. Nerve degradation, axonal derangement and shrinkage, epineurium deranged, perineurium abnormality and myelin sheath damaged showed paclitaxel induced peroxidation in the scatic nerve and that rutin decreases this effect. Also the flavonoid decreases inducible nitric oxide synthase and increase catalase in the blood serum. Hence, on the basis of biochemical parameters, *in-vivo* and *in-vitro* experimental models, **rutin** possess anti-oxidant and neuroprotective activity.

7. Conflicts of Interest

No potential conflicts of interest were there as declared by the authors.

8. Acknowledgments

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