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Neuroprotective and Anti-nociceptive Potential of Ambroxol in Oxaliplatin Induced Peripheral Neuropathic Pain in Rats

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Abstract

The present study was designed to investigate the neuroprotective and anti-nociceptive effects of Ambroxol in oxaliplatin induced neuropathic pain in rats. Administration of oxaliplatin (2.4 mg/kg, i.p.) for 3 weeks (5 injections per week) significantly induces neuropathic pain. The symptoms of hyperalgesia and allodynia were assessed with various behavioral models i.e., paw thermal hyperalgesia, tail-cold hyperalgesia and paw cold allodynia via hot-plate test, cold-water tail immersion test and acetone drop test at different interval of 0,1,7,14 and 21 days. Moreover, oxaliplatin administration also increases oxidative stress markers i.e., thio-barbituric acid reactive substances (TBARS), superoxide anion content and inflammatory mediators like tumor necrosis factor-alpha (TNF-α) and myeloperoxidase (MPO) were biochemically assessed from sciatic nerve tissue and surrounding muscular tissue homogenates respectively. Pharmacological co-treatments with ambroxol (1000 mg/kg, p.o.), carbamazepine (100 mg/kg, p.o.) and combination of ambroxol with pregabalin (10 mg/kg, p.o.) for 21 days (one hour prior to oxaliplatin injection), significantly ameliorate the oxaliplatin induced neuropathic pain by attenuating thermal heat hyperalgesia, tail-cold hyperalgesia and cold allodynia along with decreasing oxidative stress markers and inflammatory mediators. Therefore, on the basis of data in hand from present study, it has been concluded that ambroxol have ameliorative potential effect in oxaliplatin induced neuropathic pain.

Keywords: Hyperalgesia; Allodynia; Myeloperoxidase; TNF-alpha; Oxaliplatin; Neuropathic pain; Sciatic nerve

Introduction

Pain is defined as an unpleasant sensory and emotional experience due to actual or potential tissue damage. Pain is a protective mechanism for the body to remove the pain stimulus. Pain is always subjective and may be affected by emotional, social and spiritual components thus; it has been defined as "total pain". From a pathophysiological point of view, pain can be classified as nociceptive (somatic and visceral), neuropathic (central, peripheral, sympathetic) idiopathic or psychogenic pain [1,2].

Neuropathic pain can be classified on the basis of location of nerve-lesion in periphery or centrally [3]. Peripheral Neuropathic Pain is further classified as 1) Painful Diabetic Polyneuropathy 2) Postherpetic Neuralgia 3) Trigeminal Neuralgia 4) HIV- associated Neuropathic Pain 5) Cancer related Neuropathic Pain 6) Metabolic causes e.g. i) Alcohol Polyneuropathy ii) Nutritional Deficiency Neuropathy 7) Mechanical Nerve Compression e.g. i) Carpal Tunnel Syndrome ii) Disc Herniation 9) Post-surgical Pain e.g. i) Post-mastectomy Pain ii) Post-herniorrhaphy iii) Phantom Limb Pain iv) Failed Back Surgery Syndrome 10) Toxic Exposure Neuropathy i) Chemotherapy induced Neuropathy ii) Antiretroviral induced Neuropathy iii) Antituberculosis therapy induced Neuropathy 11) Complex Regional Pain Syndrome (CRPS) 12) Idiopathic. Central Neuropathic Pain is also further classified as 1) Post-traumatic spinal cord injury (SCI) induced Neuropathy 2) Multiple Sclerosis Related Pain 3) Transverse Myelitis 4) Post-radiation Myelopathy 5) HIV Myelopathy. Chemotherapy induced neuropathic pain is one of the peripheral neuropathic pain produced at higher doses or clinical therapeutic doses of chemotherapeutic agents used for the purpose of treating cancer.

Chemotherapy induced peripheral neuropathic pain is most common and toxic neurological complication and is observed in about 30-40% patients treated with antineoplastic agents [4,5]. Oxaliplatin [(trans-1)1,2-diaminocyclohexaneoxalate-platinum (II)] is a platinum based chemotherapeutic agent used against metastatics colorectal cancer [6,7]. Approximately 65 to 87% cancer patients receiving platinum compounds such as oxaliplatin complain of chronic peripheral neuropathy [8,9]. Oxaliplatin causes damage to cell bodies, inhibition of neurite outgrowth, changes to nuclear morphology and selective atrophy of DRG neurons [10]. Although oxaliplatin have promising antineoplastic action but causes dose limiting neurotoxicity. It produces acute as well as chronic peripheral neurotoxities. Manifestations of acute neurotoxicity are pareasthesias and dysthesias, triggered by exposer to cold. Oxaliplatin induced acute as well as chronic neuropathies are reversible [10-12].

Materials and Methods

Experimental animals

Sprague Dawley rats weighing 180-250 grams, employed as experimental animal were purchased from National Institute of Pharmaceutical Education and Research (NIPER) S.A.S. Nagar Mohali (India) and maintained on standard laboratory diet (Ashirwaad Feeds Ltd., Kharar, Chandigarh, India) and having free access to tap water. They were housed in the Rayat and Bahra Institute of Pharmacy departmental animal house and were exposed to normal cycle of light and dark. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Reg. No-1380/a/10/CPCSEA).

Drugs and chemicals

Oxaliplatin injection (100 mg/ml) was purchased from Post Graduate Institute of Medical Education and Research (PGIMER) Chandigarh. Ambroxol and Pregabalin (Alembic Pharma Pvt. Ltd. Baddi (H.P.), India. All the other chemicals used in present study were of analytical grade.

Treatment schedule

The present study consists of 5 experimental groups and each group comprises of six sprague dawley rats. All the animals were acclimatized to laboratory environment for at least one week before starting experiments. After one week animals were divided into the following groups and respective treatments were given to these groups. All the behavioral testing was carried out two hours prior to any drug treatment.

Experimental protocol

Group 1: Normal group– Animals of this group were not subjected to any treatment. They were kept for 21 days. Behavioral tests were assessed on different time intervals, i.e., 0,1,7,14 and 21st day. All the animals were sacrificed and subjected to biochemical estimations of total protein, tumor necrosis factor-alpha (TNF-α), thio-barbituric acid reactive substances (TBARS), superoxide anion generation from sciatic nerve tissue and myeloperoxidase (MPO) activity in the surrounding muscular tissue sample.

Group 2: Oxaliplatin control group– Animals of this group were injected with the oxaliplatin 2.4 mg/kg i.p. 5 days/weak for total of 15 injections. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Group 3: Oxaliplatin+Ambroxol treated group– Animals of this group were received ambroxol 1000 mg/kg, p.o. for 21 consecutive days 60 minutes prior to oxaliplatin injection. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Group 4: Oxaliplatin+Carbamazepine treated group– Animals of this group were treated with carbamazepine 100 mg/kg, p.o. for consecutive 21 days 60 minutes prior to oxaliplatin injection. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Group 5: Oxaliplatin+Ambroxol+Pregabalin treated group– Animals of this group were treated with combination of ambroxol 1000 mg/kg, p.o. and pregabalin 10 mg/kg, p.o. before each oxaliplatin injections. All the behavioral tests and biochemical parameters were assessed as mentioned in group I.

Statistical analysis

All the result values were expressed as mean ± S.E.M. The data of behavioral results and biochemical parameters were statistically analyzed by two way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test and Dunnett's multiple comparison test respectively using Graph Pad Prism version - 6.0 and Graph Pad-Instat softwares. Results having value $P \leq 0.05$ were considered as statistically significant.

Induction of painful peripheral neuropathy by Oxaliplatin

Peripheral neuropathic pain was induced in rats by injecting oxaliplatin 2.4mg/kg i.p. 5 days per week (for three weeks) as described previously. Oxaliplatin injections were given daily (Monday through Friday) followed by 2 days gap per week [12].

Behavioral examinations

The experimental animals were subjected for various behavioral studies for assessment of hyperalgesia and allodynia carried out on the different day intervals of 0, 1, 7, 14 and 21 days.

Paw thermal hyperalgesia (Hot Plate Test)

Thermal hyperalgesia (noxious thermal stimuli) of the hind paw was assessed using Eddy's hot plate as described by method of Eddy et al. [13]. The temperature of the plate was maintained 52.5 ± 0.5°C. The rats were placed on the top of a controlled preheated plate to assess the withdrawal response of hind paw to the nociceptive threshold. The cut-off times of 20 seconds was maintained for thermal hyperalgesia.

Paw cold allodynia (acetone drop method)

Cold allodynia were assessed using the acetone drop method as described by Choi et al. and de la Calle et al. [14,15]. Rats were placed on wire mesh grid and 0.1 ml (100 µl) of acetone was sprayed with the help of plastic syringe on plantar surface of hind paw. Normal rats either ignored the stimulus or occasionally responded with a small and brief withdrawal of hind paw. Responses with respect to either paw licking, shaking or rubbing the hind paw was observed and recorded as paw lifting duration. The cut off time was 20 seconds.

Tail-cold hyperalgesia (cold-tail immersion test)

The cold hyperalgesia was assessed by using tail immersion in cold water as described by Necker et al. [16]. The terminal tip 1 cm of tail was immersed in cold water (temperature maintained 0-4°C). The withdrawal latency of tail from cold water was recorded. The cut off time for cold water tail immersion test was taken as 20 sec.

Biochemical estimations

All experimental animals were sacrificed at the end of experiment by cervical dislocation. The sciatic nerve and the muscular tissue beneath the sciatic nerve were located and isolated immediately. Isolated sciatic nerve was excised into small pieces and the uniform

sciatic nerve homogenates were prepared with 0.1 M Tris- HCl buffer (pH 7.4) and phosphate buffer (pH 7.4). The test tubes with homogenates were kept in ice water for 30 minutes and centrifuged at 4°C (2500 rpm, 10 min). The supernatants of respective group homogenates were separated, and employed to estimate superoxide anion generation, total protein, thio-barbituric acid reactive substances (TBARS), and tumor necrosis factor-alpha (TNF-α) respectively. Also, homogenate of surrounding muscular tissue was prepared with phosphate buffer (pH 7.4) for measuring myeloperoxidase (MPO) activity.

Estimation of tissue total protein

Total protein content in sciatic nerve was determined by Lowry's method using bovine serum albumin (BSA) as a standard [17]. The protein content was determined spectrophotometrically at 750 nm and expressed as mg per ml of 10% sciatic nerve homogenate.

Estimation of superoxide anion generation

The superoxide anion generation was measured by Wang method [18]. A weighed amount of tissue was taken in 5 ml phosphate buffered saline containing 100 µM of nitro-blue tetrazolium (NBT) and incubated at 37°C for 1.5 hours. The reduction of NBT was stopped by adding 5 ml of 0.5 M HCl. Then, the tissue was taken out and was minced and homogenized in a mixture of 0.1 M sodium hydroxide and 0.1% sodium dodecyl sulphate in water containing 40 mg/L diethylene triamine penta acetic acid. Then mixture was centrifuged at 20,000 rpm for 20 min and the resultant pellet was suspended in 1.5 ml of pyridine and kept at 80°C for 1.5 hours to extract formazan (an adduct formed after reaction of NBT with superoxide anions). The mixture was again centrifuged at 10,000 rpm for 10 min and absorbance of formazan was determined spectrophotometrically at 540 nm. The amount of reduced NBT was calculated using the formula

$$Amount\ of\ reduced\ NBT = \frac{A \times V}{T \times Wt \times \varepsilon \times 1}$$

Where A is absorbance, V is volume of pyridine (1.5 ml), T is time for which the tissue was incubated with NBT (1.5 hours), Wt is blotted wet weight of tissue (mg), εis extinction coefficient (0.72 L/mmol/mm) and l is length of light path (1 cm). Results were expressed as reduced NBT picomole per min per mg of wet tissue.

Estimation of thio-barbituric acid reactive substances

The lipid peroxidation in the sciatic nerve was estimated by measuring the thio-barbituric acid reactive substances by the method of Okhawa et al. [19]. The reaction mixture was prepared by mixing 0.2 ml of sciatic nerve homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of thio-barbituric acid (TBA). The reaction mixture was made up to 4.0 ml with distilled water, and then heated in water bath at 95°C for 60 min. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) were added to reaction mixture and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was separated out and its absorbance at 532 nm was measured. TBARS level (MDA concentrations) were calculated from the standard curve of 1,1,3,3-tetramethoxy propane (TMP).

Estimation of myeloperoxidase activity

Myeloperoxidase (MPO) activity was measured by Grisham et al. [20]. The presence of myeloperoxidase was measured at 460 nm for 3 minutes. Myeloperoxidase activity is expressed as Unit per gram (U/g) tissue. One unit of MPO activity is defined as that degrading 1 µmol peroxide per min at 25°C. The results were expressed as myeloperoxidase activity units per milligram of protein at one minute.

Estimation of tumor necrosis factor-alpha (TNF-α) level

Sciatic nerve samples were utilized for determination of TNF-α level. TNF-α levels (sensitivity: 25 pg/ml) were determined by using rat TNF-alpha ELISA kit and procedure was followed according to the manufacturer instructions. Sciatic nerve homogenate was prepared with phosphate buffer (pH 7.4). Recombinant anti-Rat TNF-alpha was used as a standard to generate a standard curve. The absorbance was measured spectrophotometrically at 450 nm. The results were expressed as pictograms of TNF-α per mg of protein.

Results

Effect of ambroxol, carbamazepine and combination of ambroxol with pregabalin on paw thermal hyperalgesia

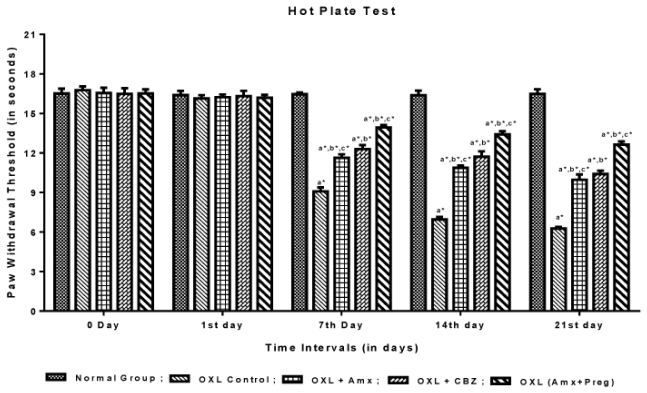

Figure 1: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on paw thermal hyperalgesia assessed by hot plate method in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n = 5 rats per group. Two-way ANOVA followed by Bonferroni's test, a^*=p<0.05 vs normal control, b^*=p<0.05 vs oxaliplatin control group, c^*=p<0.05 vs carbamazepine treated group, ns=p >0.05 represent no significance.

Oxaliplatin administration significantly resulted into development of thermal heat hyperalgesia as reflected by decrease in paw withdrawal threshold, when compared to normal group. Pharmacological co-treatments with ambroxol (1000 mg/kg, p.o.) and carbamazepine (100 mg/kg, p.o.) significantly attenuated oxaliplatin induced decrease in the noxious nociceptive threshold for thermal conduction heat hyperalgesia. Effect of ambroxol in attenuating oxaliplatin induced decrease in paw withdrawal threshold was

significantly comparable to the carbamazepine. Similarly, a safe additive effect in attenuating paw thermal hyperalgesia was observed in ambroxol with pregabalin (10 mg/kg, p.o.) treated group (Figure 1).

Effect of ambroxol, carbamazepine and combination of ambroxol with pregabalin on tail cold hyperalgesia

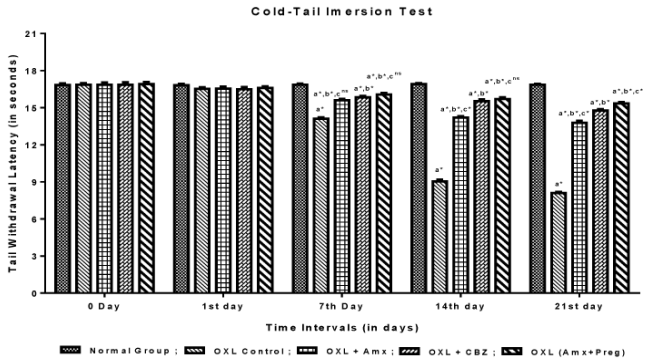

Figure 2: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on tail-cold hyperalgesia assessed by cold water tail immersion test in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n=5 rats per group. Two-way ANOVA followed by Bonferroni's test, a^*=p<0.05 vs normal control, b^*=p<0.05 vs oxaliplatin control group, c^*=p<0.05 vs carbamazepine treated group, ns=p>0.05 represent no significance.

Tail cold hyperalgesia was assessed by cold water tail-immersion test (0-4°C). Oxaliplatin administration was associated with the development of tail cold hyperalgesia that was reflected as tail withdrawal latency to cold water (0–4°C), when compared to normal group. Co-treatments with ambroxol (1000 mg/kg, p.o.) and carbamazepine (100 mg/kg, p.o.) significantly attenuated the tail cold hyperalgesia to cold stimuli. Pharmacological effect of ambroxol in attenuating tail withdrawal duration was significantly comparable to the carbamazepine. Similar effects were observed in ambroxol with pregabalin (10 mg/kg, p.o.) treated rats (Figure 2).

Effect of ambroxol, carbamazepine and combination of ambroxol with pregabalin on paw cold allodynia

Oxaliplatin administration was associated with the development of paw cold allodynia that was reflected as decrease in hind paw withdrawal duration on application of acetone to plantar surface of paw, when compared to normal group. Co-treatment with ambroxol (1000 mg/kg p.o.) and carbamazepine (100 mg/kg p.o.) significantly attenuated oxaliplatin induced hind paw cold allodynia to a non-noxious nociceptive threshold (acetone drop) and the effect of ambroxol was significantly comparable to carbamazepine. Similar results were observed in ambroxol with pregabalin (10 mg/kg, p.o.) treated rats (Figure 3).

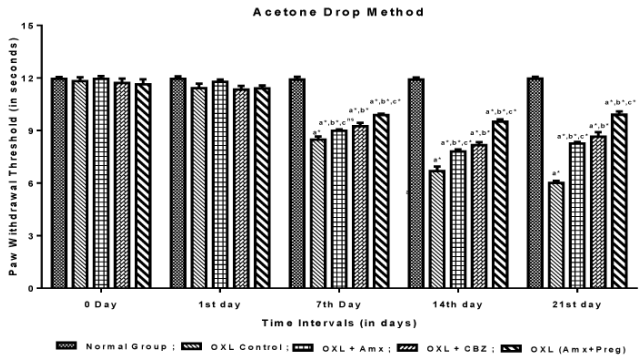

Figure 3: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on paw-cold allodynia assessed by acetone drop method in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n = 5 rats per group. Two-way ANOVA followed by Bonferroni's posthoc test, a^*=p<0.05 vs normal control and b^*= p<0.05 vs oxaliplatin control group, c^*= p<0.05 vs carbamazepine treated group, ns=p>0.05 represent no significance.

Effect of ambroxol, carbamazepine and combination of ambroxol with pregabalin on biochemical parameters

Oxaliplatin administration was associated with an increase in the oxidative stress markers i.e., thio-barbituric acid reactive substances (TBARS), superoxide anion generation and inflammatory mediators like myeloperoxidase (MPO) and tumor necrosis factor-alpha in the sciatic nerve tissue (TNF-α), when compared to the normal control group (Figures 4-7).

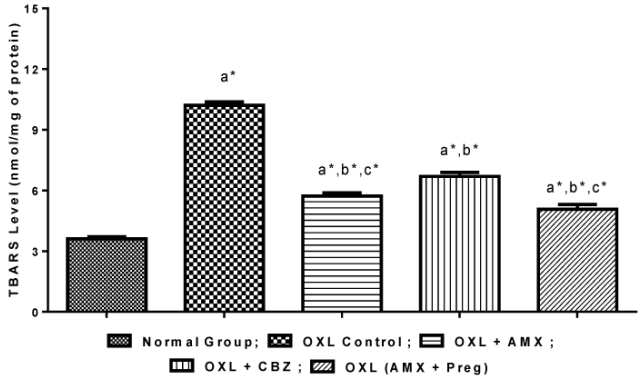

Figure 4: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on thio-barbituric acid substances (TBARS) in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n=5 rats per group. One-way ANOVA followed by Tukey's multiple range test, a^*=p≤0.05 vs normal control, b^*=p≤0.05 vs oxaliplatin control group and c^*=p≤0.05 vs Carbamazepine treated group.

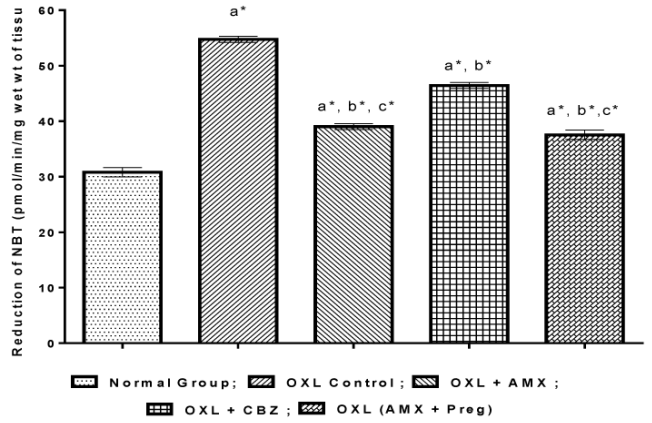

Figure 5: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on reduction of NBT in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n=5 rats per group. One-way ANOVA followed by Tukey's multiple range test, a*=p<0.05 vs normal control and b*=p<0.05 vs oxaliplatin control group c*=p<0.05 vs carbamazepine treated group.

Pharmacological co-treatments with ambroxol (1000 mg/kg p.o.) and carbamazepine (100 mg/kg p.o.) significantly (p≤0.05) attenuated the oxaliplatin induced increase in oxidative stress markers and inflammatory mediators. Similar effects were observed in ambroxol with pregabalin treated rats (Table 1).

Experimental Groups	TBARS (nmol/mg of protein	Superoxide Anion Content (pmol/min/mg of wet tissue)	Myeloperoxidase (MPO) activity (U/min/mg of protein)	Tumor Necrosis Factor-α (TNF- α) (pg/mg of protein)
Normal Group	3.61 ± 0.05	30.80 ± 0.35	10.48 ± 0.27	3.40 ± 0.19
OXL Control	10.21 ± 0.07 a*	54.76 ± 0.23 a*	128.12 ± 1.59 a*	10.95 ± 0.13 a*
OXL + Ambroxol	5.73 ± 0.06 a*,b*,c*	39.03 ± 0.22 a*,b*,c*	24.49 ± 0.25 a*,b*,c*	6.16 ± 0.09 a*,b*,c*
OXL + Carbamazepine	6.69 ± 0.08 a*,b*	46.43 ± 0.21 a*,b*	36.87 ± 2.56 a*,b*	8.67 ± 0.14 ans, b*
OXL (Amb + Preg)	5.07 ± 0.10 a*,b*,c*	37.55 ± 0.35 a*,b*,c*	16.04 ± 0.41ans,b*,c*	5.79 ± 0.14 a*,b*,c*

Table 1: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on thio-barbituric acid substances (TBARS), superoxide anion content, myeloperoxidase (MPO) activity and tumor necrosis factor-alpha (TNF-α) in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n=5 rats per group. One-way ANOVA followed by Tukey's multiple range test, a*=p<0.05 vs normal control, b*=p<0.05 vs oxaliplatin control group and c*=p≤ 0.05 vs Carbamazepine treated group. Value ns=p>0.05 represent no significance.

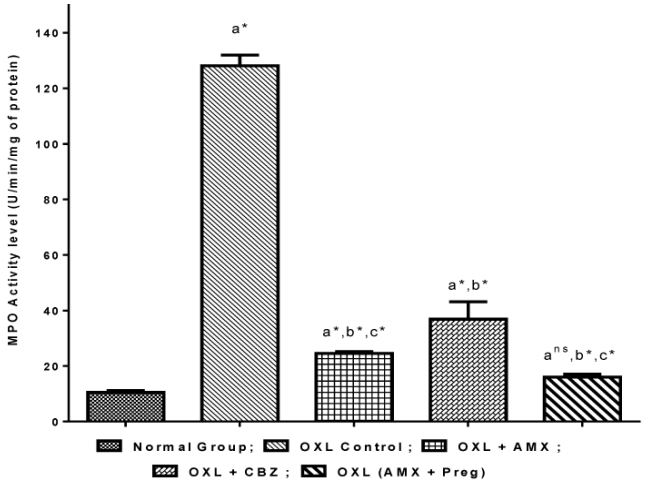

Figure 6: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on Myeloperoxidase (MPO) activity in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n = 5 rats per group. One-way ANOVA followed by Tukey's multiple range test, a*=p<0.05 vs normal control, b*=p<0.05 vs oxaliplatin control group, c*=p< 0.05 vs Carbamazepine treated group and ns p>0.05 represent having no significance.

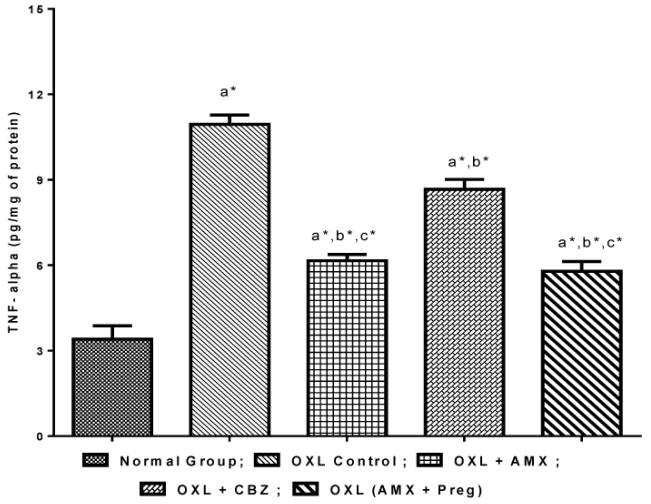

Figure 7: Effect of Ambroxol, Carbamazepine and combination of Ambroxol with Pregabalin on Tissue Necrosis Factor – alpha (TNF-α) level in oxaliplatin induced peripheral neuropathic pain. Values were expressed as mean ± S.E.M; n=5 rats per group. One-way ANOVA followed by Tukey's multiple range test, a*=p<0.05 vs normal control, b*=p<0.05 vs oxaliplatin control group and c*=p< 0.05 vs Carbamazepine treated group.

Discussion

In the present study, administration of oxaliplatin (2.4 mg/kg, i.p.) for consecutive 21 days was associated with significant development of

neuropathic pain behaviors such as thermal-heat hyperalgesia, tail-thermal allodynia and paw-cold allodynia assessed with hot-plate test, cold-tail immersion test and acetone drop test. Also, oxaliplatin administration led to significant increase in oxidative stress markers and inflammatory mediators as reflected by an increase in the sciatic nerve tissue thio-barbituric acid substances (TBARS), superoxide anion content, enhanced tumor necrosis factor-alpha (TNF-α) level and increased activity of myeloperoxidase (MPO). All these behavioral and biochemical alterations, in present study are in consistent with the earlier researches [7,10].

Chemotherapy induced neuropathic pain is acute as well as chronic. The primary focus is to prevent or treat the painful condition associated with the chemotherapy induced neuropathic pain. Though, there are some effective drugs such as antidepressants (SSRIs, SNRIs and TCAs), anticonvulsants (carbamazepine, phenytoin, lamotriagine, topiramate, gabapentine and pregabalin), anti-arrhythmic (lignocaine), NMDA Antagonists, NK receptor antagonists (Lanepitant), opioids and other analgesics that are used clinically for the symptomatic management of neuropathic pain, but their full clinical exploitation is limited due to their life threatening adverse effects associated with their clinical use [8,21,22]. Moreover, none of the medication has been found clinically effective in chemotherapy induced neuropathic pain. Therefore, there has been an urgent need of alternative medicines for the management of chemotherapy induced neuropathic pain. The present study has been designed to explore the possible therapeutic strategy in the management and to prevent chemotherapy induced neuropathic pain in terms of behavioral, oxidative markers and inflammatory mediators.

Moreover, data in hand from present research and literature support from previous studies revealed that oxidative stress is the major target for the management of oxaliplatin induced neuropathic pain. Various studies has been conducted to target the oxidative stress in oxaliplatin induced neuropathic pain. All these studies focused on oxidative stress as a major pathophysiological marker in the pathogenesis of oxaliplatin induced neuropathic pain [7]. Also, many antioxidants therapies ameliorate oxaliplatin induced oxidative stress in the development of neuropathic pain. Beside, oxidative stress, present study also showed that neuro-inflammation is also an important pathophysiological marker in the pathogenesis of oxaliplatin induced neuropathic pain. Data from previous studies also confirmed that pharmacological treatment with ambroxol has been proved that ambroxol exhibits good antioxidative and anti-inflammatory potentials [23-26].

In present study, pharmacological co-treatment with ambroxol (1000 mg/kg, p.o.) has been reported to ameliorate the oxaliplatin induced neuropathic pain as ambroxol significantly alleviates hyperalgesia and allodynic related pain behaviors in rats. This contention is supported by the evidences that ambroxol possesses anti-nociceptive anti-oxidative and anti-inflammatory actions [23-27].

Also, some previous studies revealed the role of voltage gated sodium channels in the pathogenesis of oxaliplatin induced neuropathic pain, as oxaliplatin inside the body is metabolized to oxalate and dichloro (1,2-diaminocyclohexane) platinum metabolites that cause the morphological damage to the nerves [10,28]. Oxalate interfere with the normal functioning of voltage gated sodium channels and chelate the neuronal calcium and magnesium ions which play a vital role in the neuronal normal functioning [11,29]. Some previous studies revealed that ambroxol have voltage gated sodium channels 1.8 (VGSCs) inhibitory potential [23,30]. Treatment with ambroxol (1000 mg/kg, p.o.) has also been shown its anti-nociceptive effect in rat model of sciatic nerve ligation induced neuropathic pain [27]. Therefore, it may be hypothesized from the present study that antinociceptive effect of ambroxol in oxaliplatin induced neuropathic pain may be due to inhibition of neuronal voltage gated sodium channels 1.8 (VGSCs 1.8).

Pharmacological co-treatments with ambroxol (1000 mg/kg, p.o.), carbamazepine (100 mg/kg, p.o.) and combination of ambroxol with pregabalin (10 mg/kg, p.o.) significantly ameliorate oxaliplatin induced peripheral neuropathic pain. The ameliorative effect of ambroxol may be due neuroprotective and antinociceptive potentials. Neuroprotective and antinociceptive potential of ambroxol may supposed via decreasing oxidative stress markers, inflammatory mediators and inhibition of voltage gated sodium channels in chemotherapy induced peripheral neuropathic pain. Present results also reveals, that ambroxol exhibits overall good antioxidant and anti-inflammatory potentials when compared to positive standard carbamazepine. Also, combination of ambroxol with pregabalin shows a safe additive effect in terms of reducing oxidative stress markers and inflammatory mediators in oxaliplatin induced neuropathic pain.

Conflict of Interest

There is no conflict of interest in present study.

Acknowledgement

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