WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 1075-1090. Research Article ISSN 2277 – 7105

AMELIORATIVE EFFECTS OF *ALLIUM CEPA* IN ETHANOL INDUCED NEUROPATHIC PAIN IN RATS

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Article Received on
16 June 2014,

Revised on 11 July 2014,
Accepted on 06 August 2014

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ABSTRACT

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage and is induced by noxious stimuli. The sensation of pain is a protective mechanism for the body and it initiates nociceptive reflexes. Neuropathic pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system, either central nervous system (CNS) or peripheral nervous system (PNS). As the onset of neuropathic pain may be delayed after nerve injury, pain may still be present after healing is complete. This makes proper diagnosis and early treatment difficult. Moreover, neuropathic pain commonly occurs as a secondary symptom in diseases like diabetes, cancer and herpes zoster infection; it may also occur with treatments such as chemotherapeutics or cytotoxic drugs. The various first line drugs available for neuropathic pain (such as gabapentin, pregabalin, duloxetime, tricyclic antidepressants etc.,) are not found to be fully effective in the treatment. Henceforth, alternative treatments are being researched upon. *Murraya koenigii* is one such possible alternative, which is considered in the present study and is successfully found to provide positive results towards the prevention and maintenance of peripheral neuropathic pain due to its anti-oxidant, anti-inflammatory and cellular calcium modulatory action.

KEY WORDS: - allodyna, hyperalgesia, neurological disorder, demyelination, neuropathy.

INTRODUCTION

Neuropathic pain is a chronic pain condition accompanied by significant pathological changes in the nervous system. It is caused by dysfunction in the peripheral or central nervous system. The two main symptoms of neuropathic pain are hyperalgesia, an increased response to normally painful stimuli and allodynia, a painful response to a usually non-noxious stimulus. Recent studies indicate that 2-3% of the population in the world suffer from neuropathic pain.

Alcohol is the third most globally accepted addictive substance after nicotine and caffeine. Alcohol abuse has many long-term effects which can result in early death and increases incidences for serious illness [1-2].

Alcoholic neuropathy (AN) is a neurological disorder associated with chronic alcohol abuse and is characterized by damage to the nervous system. It can be defined as the axonal degeneration in neurons of both the sensory and motor systems [3]. The morphologic basis of alcoholic neuropathy is the primary axonal damage and secondary demyelination of motor and sensitive fibers. Alcoholic Neuropathy is more common in frequent and continuous drinkers than in episodic drinkers [4] and incidence of neuropathy is higher in women as compared to men [5]. It is estimated that approximately 90% of people at some stage, consume alcohol and out of which 30% of them develop alcohol related disorders [6]. Alcohol dependence (alcoholism) is observed in 10% of men and 3–5% of women. Excessive alcohol use is one of the leading causes of preventable death in the United States [7]. Alcoholic neuropathy has a gradual onset and may take several months or even years for the symptoms to develop. Although the axonal degeneration starts even before an individual experiences any symptoms. The disease typically involves abnormalities in sensory, motor, autonomic and gait functions. The main symptom of alcoholic neuropathy is the painful sensation with or without burning quality. Other symptoms of alcoholic neuropathy may include burning pain, impaired coordination, muscle weakness, nausea with or without vomiting, numbness and pain [8-9].

Ethanol exerts a direct neurotoxic action on the central nervous system. The neurological effects of ethanol consumption are complex, encompassing both the central and peripheral nervous system [10-11]. It breaks into acetaldehyde which impairs and destroys majority of neuronal functions. As a result, signals become intensified not only from the injured neurons but also from the nearby surrounding neurons resulting in painful condition [12]. The injury

caused to a nerve or tissue results in variation in spinal excitability, leading to spontaneous firing and lowered threshold stimulus in pain sensation. This leads to an increase pain sensation [13].

In this study, the plant extract of *Allium cepa* is used in the attenuation of alcohol induced neuropathic pain. It belongs to the family of Liliaceae and is widely cultivated throughout the world [14]. It has been used as an analgesic and anti inflammatory [15], antidiabetic, antioxidant [16], antihypertensive [17], antithrombotic [18], hypoglycemic [19], antihyperlipidemic [20]. It is also proved to exhibit an antioxidant effect [16]. It contains vitamins, especially vitamin C, which has a protective function against oxidative damage and are a powerful quencher of singlet oxygen (O_2), hydroxyl (OH^-) and peroxyl (RO_2) radicals [21-22]. *Allium cepa* also inhibits lipid peroxidation. However, usefulness of *Allium cepa* in ethanol induced painful peripheral neuropathy remains to be explored.

Alpha tocopherol is taken to serve as positive control in this study. It is a fat soluble vitamin having antioxidant properties. It has also been classified as a radical-chain breaker [23]. Many studies have described that the antioxidant vitamins like α-tocopherol (Vitamin E), prevent neurotoxicity in neurons exposed to alcohol [24-25]. Studies also indicate that tocotrienols exhibit neuroprotective effects [26-27]. It has been reported that tocotrienols and α-tocopherols play a neuroprotective role in an experimental model of alcoholic neuropathy [28].

MATERIALS AND METHODS

Experimental Animals

Wistar rats (200-280 g) of either sex were used for behavioral paradigm of alcoholic neuropathy. Animals were housed under standard conditions of light and dark cycle in the Animal House of Chandigarh College of Pharmacy, Landran, Mohali, India with a balanced rat feed and clean drinking water *ad libitum.* Animals were acclimatized to laboratory conditions before the behavioral tests. All experiments were carried between 08:00AM and 04:00 PM. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. All the experiments for different treatment groups were performed using age matched animals to avoid variability between experimental groups.

Plant Material

The bulb of *Allium cepa* was collected, extracted and authenticated by Ayush Herbs and Pharmaceuticals, Kangra, Himachal Pradesh, India.

Drugs and Chemicals

All regents and chemicals used in this study were of highest analytical grade and were freshly prepared. Ethanol, α-tocopherol and bulb extract of *Allium cepa* (Ayush Herbs and Pharmaceuticals, Kangra, HP, India) were used in this study. Ethanol 99.9% v/v was used to induce neuropathy. DTNB (5,5-dithiobis-2-nitrobenzoic acid), reduced glutathione (GSH), sulphanilamide, hydrochloric acid, sodium hydroxide, sodium chloride, N-(1-naphthyl) ethylene diamine dihydrochloride, potassium dihydrogen phosphate, were purchased from Loba Chemicals (Mumbai, India). Thiobarbituric acid (TBA) was purchased from Magus Chemicals. Trichloroacetic acid (TCA) was purchased from Nice Chem. Pvt. Ltd. (Cochin, India). EDTA was purchased from Thomas baker, India. Each dose of *Allium cepa* bulb extract was freshly prepared in normal saline (0.9 %) to treat ethanol induced neuropathy.

Induction of Alcoholic Neuropathy

Alcoholic Neuropathy was induced by administration of 10g/kg of 35% v/v ethanol [29] for 9 weeks. The dose of ethanol was decided on the basis of the existing literature [30]. The rats serving as controls were given 10 g/kg oral gavages of distilled water. α-tocopherol and *Allium cepa* were administered by oral route one hour before ethanol dosing daily for 9 weeks. All the behavioral assays were done at the end of every week.

Density of absolute ethanol = 99.9% (v/v).

Desired Concentration =35% (v/v)

So to make 35% v/v ethanol, 35ml of absolute ethanol was added to 65ml of distilled water to make the volume up to 100 ml.

Calculation of Dosing Volume

Density of final mixture (35% v/v ethanol) = 0.97gm/ml.

Therefore, Administered Volume $= \frac{\text{Dose (10 g/kg) x Rat Body Weight}}{\text{1000 x Density (0.97 g/ml)}}$

Experimental Animals

Experimental Groups	Treatment (mg/kg)
Group I	Normal control
Group II	Alcohol control
Group III	AC(300) *per se*
Group IV	Ethanol + AC (100)
Group V	Ethanol + AC (200)
Group VI	Ethanol + AC (300)
Group VII	Ethanol + α-tocopherol (100)

AC: *Allium cepa* bulb extract

GROUP I- Normal control group comprised of animals given distilled water in place of ethanol by oral gavages.

GROUP II- Alcohol control group rats were administered ethanol by oral route for 9 weeks.

GROUP III- Animals received high dose of *Allium cepa* (300mg/kg, *p.o.*) in Normal control rats.

GROUP IV- Animals received low dose of *Allium cepa* (100mg/kg, *p.o.*) (1 hr priorethanol dosing)

GROUP V- Animals received intermediate dose of *Allium cepa* (200mg/kg, *p.o.*) (1 hr before ethanol dosing)

GROUP VI- Animals received high dose of *Allium cepa* (300mg/kg, *p.o.*) (1 hr before ethanol dosing)

GROUP VII- α-tocopherol (100mg/kg, *p.o.*) was administered (1 hr before ethanol dosing)

Treatment Schedule

All the animals were acclimatized to laboratory environment for at least 2 hr before testing. The animals were randomly divided into 7 experimental groups with 5 animals in each group. Control group was given distilled water (DW) in place of ethanol by oral gavages. Rats were administered alcohol for a total period of nine weeks. Treatment with *Allium cepa* bulb extract (100, 200 and 300 mg/kg, *p.o.*, once daily) and α-tocopherol (100mg/kg, *p.o.*, once daily) (positive control) were given one hour before ethanol administration [15]. All the rats were subjected to behavioral parameters, which are thermal hyperalgesia, cold allodynia and motor coordination, before the start of the experiment and at the end of every week till 9 weeks to study the changes with alcohol consumption as well as treatment drugs. On the 10th week animals will be sacrificed to study the various biochemical parameters which include assessment of reduced glutathione level, measurement of lipid peroxidation, estimation of protein level and nitrite level.

Alcohol administration and Induction of Neuropathy

Alcohol was administered by oral gavages twice a day for a period of nine weeks. Animals were sacrificed on the 10th week for studying biochemical parameters.

Behavioral Studies

Assessment of Thermal Hyperalgesia

Hot Plate Method

Thermal hyperalgesia of was assessed by using Eddy's hot plate as described method of [31], for assessing the reactivity against noxious thermal stimuli. The rats were placed on the top of a controlled preheated (52.5 ±0.5°C) and maintained hot plate surface. The latency to the first sign of paw licking or jump response was noted as an index of nociceptive pain threshold. The cut-off time of 15 s was maintained to avoid damage to the paw.

Assessment of Cold Allodynia

Tail Immersion Method (Cold water)

Allodynia will be measured using Tail immersion method in cold water [32]. In this method, tail of rats will be immersed in cold water (10°C ± 0.5°C) until tail withdrawal (flicking response) or signs of struggle will be observed. Cut off time will be 15 seconds. Shortening of the tail withdrawal time indicates allodynia.

Biochemical Studies

Collection of blood and tissue samples in rats

For biochemical assessment, at the end of treatment schedule, blood was collected from overnight fasted rats through retro-orbital puncture and the animals were euthanized by cervical dislocation immediately after behavioral assays, followed by collection of sciatic nerve. Sciatic nerves were rapidly removed, washed with sterile in normal saline and weighed. A 10% (w/v) tissue homogenate is prepared in 0.1 M phosphate buffer (pH 7.4) and centrifuged for 15 min at 2000 g to obtain the clear supernatant.

Estimation of total protein

The total protein level in sciatic nerve was estimated by Biuret method using commercially available kit. 1000 µl of Biuret reagent was added to 10 µl of distilled water, 10 µl of standard albumin and 10 µl of serum to prepare blank, standard and test, respectively. Mixed well and incubated at 37^0 C for 10 minutes. The absorbance of test and standard samples were noted against blank at 555 nm spectophotometrically after 10 minutes.

Estimation of lipid peroxidation

Concentration of thiobarbituric acid reactive substances (TBARS) was determined as an index of lipid peroxidation as described by the method of Niehius and Samuelsson (1968) [33]. In this method, 0.1 ml of supernatant of sciatic nerve homogenate was treated with 2 ml of (1:1:1 ratio) thiobarbituric acid-trichloroacetic acid-hydrochloric acid (TBA-TCA-HCL) reagent. TBARS reagent was prepared by mixing equal volumes of TBA (37%), TCA (15%) and HCL (0.25 N).Then the mixture was placed in hot water bath for 15 min, cooled and centrifuged at 1000 rpm for 10 mins. The absorbance of the clear supernatant was measured at 532 nm (UV-1700 Spectrophotometer) against blank. Finally, the values are expressed as nmole/mg of protein.

Estimation of reduced glutathione

The concentration of endogenous antioxidant reduced glutathione (GSH) level in the alcoholic neuropathy will be estimated by the method of Jollow *et al.*, 1974 [34].In this method, 1.0 ml of sciatic nerve homogenate (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 hour and then subjected to centrifugation for 15 min. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1M, pH 7.4) and 0.2 ml 5,5, dithiobis (2- nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm and the reduced GSH levels were expressed as µmole/mg of protein in sciatic nerve.

Estimation of nitrite

The nitrite concentration in the serum will be measured by Griess reaction [35]. In this method, 0.1 mL of supernatant of the nerve homogenate was mixed with 0.25 mL of 1% sulfanilamide (prepared in 3 N HCL) and 0.25 mL of 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride with shaking. After 10 min, absorbance was measured at 545 nm and the values of nitrite concentration were obtained from sodium nitrite standard curve and were expressed in µg/ml of protein.

Stastical Analysis

The results are expressed as Mean ± SEM. The behavioral and biochemical data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Test for multiple comparison. The p value < 0.05 was considered to be statistically significant.

RESULTS

Behavioral studies

Assessment of Thermal Hyperalgesia

The nociceptive threshold was significantly lower in alcohol control rats as compared with the normal control rats tested in Eddy's Hot Plate Test, indicating the development of hyperalgesia. The mean paw licking response threshold in ethanol treated rats on day 0 was not significantly different from normal control rats. There was no significant change in the mean paw licking response threshold of normal control rats during the time period of 9 weeks. Ethanol administration, twice a day, for 9 weeks significantly decreased ($p< 0.05$) mean paw licking response threshold compared to normal control rats. Chronic treatment with moderate and high dose of *Allium cepa* (200 and 300 mg/kg) for 9 weeks significantly and dose dependently increased paw licking threshold in ethanol treated rats (Fig. 1). However, low dose of *Allium cepa* (100 mg/kg) was not showing any significant effect. Treatment with α-tocopherol (100mg/kg, *p.o.*) significantly attenuated ethanol induced neuropathic pain as compared to ethanol control group. *Allium cepa per se* did not show any significant effect.

Assessment of Cold Allodynia

The mean tail withdrawal latency in ethanol treated rats on day 0 was not significantly different from normal control rats.There was no significant change in the mean tail withdrawal latency of normal control rats during the time period of 9 weeks. Ethanol administration for 9 weeks significantly decreased ($p< 0.05$) mean tail withdrawal latency as compared to normalcontrol rats. Significant and dose dependent restoration of decreased mean tail withdrawal latency was observed in rats treated with *Allium cepa* (200 and 300 mg/kg) as compared to ethanol treated rats. However, low dose of *Allium cepa* did not show any significant effect (Fig. 2). Treatment with α-tocopherol (100mg/kg, *p.o.*) significantly attenuated ethanol induced neuropathic pain as compared to ethanol control group. Furthermore, *Allium cepa per se* did not show any significant effect on cold allodynia.

Biochemical studies

Estimation of total protein

Total protein level was not affected significantly as a result of alcohol induced neuropathy as compared to normal control rats (Table 1).

Estimation of lipid peroxidation

Rats receiving ethanol had significantly increased level of TBARS, an index of lipid peroxidation, in sciatic nerve as comparison to normal control rats. Lipid peroxide (TBARS) level in the sciatic nerve of alcohol treated rats was significantly ($p< 0.05$) elevated as compared to normal rats. Administration of moderate and high dose of *Allium cepa* (200 and 300 mg/kg) significantly decreasedTBARS level as compared to alcohol control rats. However, low dose of *Allium cepa* was not effective in decreasing the increased TBARS level. Treatment with α-tocopherol (100mg/kg, *p.o.*) significantly reduced the elevated level of TBARS as compared to ethanol control group. Furthermore, *Allium cepa per se* did not show any significant effect (Table 1).

Estimation of reduced glutathione

Nerve injury causes marked reduction in GSH level in the sciatic nerve of ethanol treated rats. The reduced glutamate level was significantly decreased as compared to normal control rats. This GSH reduction was significantly ($p< 0.05$) and dose-dependently reversed by the administration of moderate and high dose of *Allium cepa* (200 and 300 mg/kg) in ethanol treated rats. However, low dose of *Allium cepa* (100 mg/kg, *p.o.*) did not show any statistically significant effect in restoring the depleted level of GSH. Treatment with α-tocopherol (100mg/kg, *p.o.*) significantly restored the depleted level of GSH as compared to ethanol control group. Furthermore, *Allium cepa per se* did not show any significant effect (Table 2).

Estimation of nitrite

The biochemical changes observed in the ethanol treated rats were associated with increase nitrite level in sciatic nerve. Nitrite level in ethanol treated rats was significantlyincreaseas compared to normal control rats. Chronic administration of *Allium cepa* (200 and 300 mg/kg) significantly ($p< 0.05$) and dose dependently reduced these increased nitrite level as compared to ethanol treated rats. However, low dose of *Allium cepa* (100 mg/kg) was not effective (Table 2). Furthermore, *Allium cepa per se* did not show any significant effect.

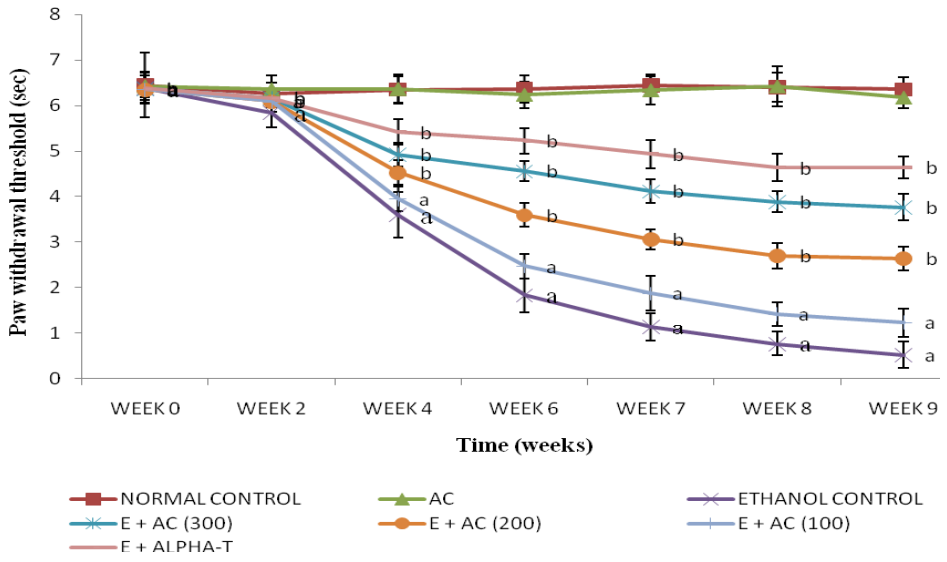

Fig 1: Effect of *Allium cepa* (100, 200 and 300 mg/kg) on Thermal Hyperalgesia in ethanol treated rats. Results are expressed as Mean ± S.E.M., n=5.

[a]$p<0.05$ vs. Normal control group

[b]$p< 0.05$ vs. Ethanol treated group

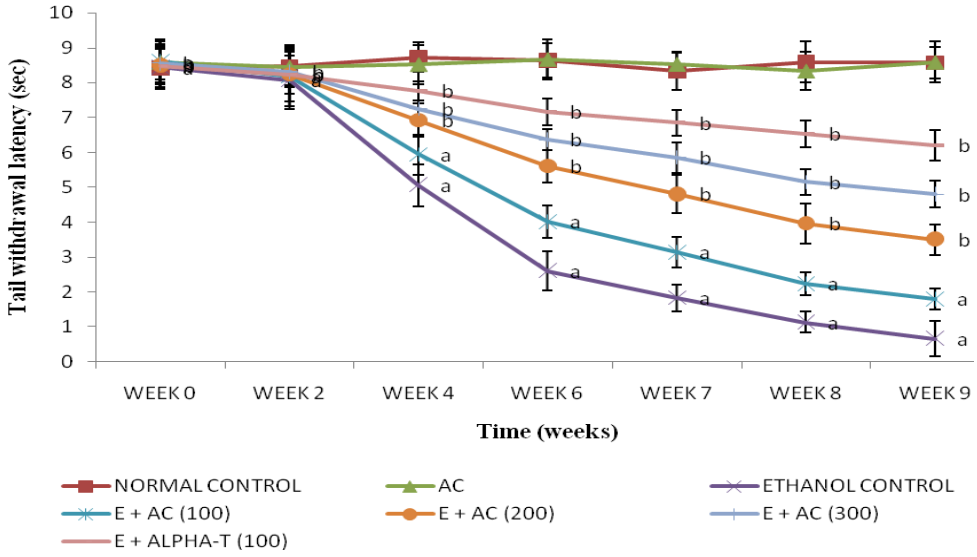

Fig 2: Effect of *Allium cepa* (100, 200 and 300 mg/kg) on Cold Allodynia in ethanol treated rats. Results are expressed as Mean ± S.E.M., n=5.

[a]$p<0.05$ vs. Normal control group

[b]$p< 0.05$ vs. Ethanol treated group

Table 1. Effect of *Allium cepa* in Ethanol induced alterations in total protein level and TBARS

Groups	Protein (mg/ml)	TBARS (nmole/mg of protein)
Normal Control	4.37 ± 0.39	2.686 ± 0.62
Ethanol Control	4.53 ± 0.28[a]	8.460 ± 0.62[a]
E + α-T (100)	4.39 ± 0.27[b]	3.220 ± 0.55[b]
E + AC (100)	4.54 ± 0.38[a]	6.824 ± 0.36[a]
E + AC (200)	4.48 ± 0.29[b]	5.090 ± 0.70[b]
E + AC (300)	4.42 ± 0.31[b]	4.374 ± 0.73[b]
AC	4.40 ± 0.21	2.558 ± 0.66

AC: *Allium cepa* bulb extract; E: Ethanol; α-T: Alpha tocopherol

Results are expressed as Mean ± S.E.M., n=5.

[a]$p<0.05$ vs. Normal control group

[b]$p< 0.05$ vs. Ethanol treated group

Table 2. Effect of *Allium cepa* in ethanol induced alterations in reduced GSH and nitrite level

Groups	NO (µg/ml of protein)	GSH (µmol/mg of protein)
Normal Control	96.782 ± 6.330	0.104 ± 0.012
Ethanol Control	330.382 ± 13.019[a]	0.031 ± 0.006[a]
E + α-T (100)	151.226 ± 12.261[b]	0.085 ± 0.004[b]
E + AC (100)	291.002 ± 12.237[a]	0.054 ± 0.009[a]
E + AC (200)	249.178 ± 13.235[b]	0.069 ± 0.005[b]
E + AC (300)	192.138 ± 19.289[b]	0.077 ± 0.003[b]
AC	95.604 ± 7.316	0.108 ± 0.013

AC: *Allium cepa* bulb extract; E: Ethanol; α-T: Alpha tocopherol

Results are expressed as Mean ± S.E.M., n=5.

[a]$p<0.05$ vs. Normal control group

[b]$p< 0.05$ vs. Ethanol treated group

DISCUSSION

Alcoholic Neuropathy was induced by administration of 10g/kg of 35% v/v ethanol twice a day [29]. The development of neuropathic pain following chronic ethanol consumption is well reported [36-37]. Alcoholic Neuropathy was developed in a total period of nine weeks and biochemical parameters were assessed on the 10th week. The concentration of alcohol was selected on the basis of previously reported studies [38]. *Allium cepa* (100, 200 and 300 mg/kg) was given one hour before ethanol administration for 9 weeks [22]. α-tocopherol (100 mg/kg) was used as a positive control [29].

In the present study, the chronic administration of ethanol for 9 weeks resulted in marked reduction in thermal hyperalgesia and mechanical allodynia [29]. These results were reliable when compared with the previous reports demonstrating neuropathic pain like state in the rats following chronic alcohol consumption [30, 36-37].

According to some studies, *Allium cepa* exhibits an analgesic and anti inflammatory effect by bringing about a reduction in pain, inflammation and the signal transduction pathway which results in a decline in plasticity at dorsal root of spinal cord through deprivation in P substance [39]. In addition, some studies also proposed that onion inhibits the pain receptors at dorsal root of spinal cord, thus resulting in an inhibition of pain signal transduction [15, 40]. Therefore, in this study, the continuous administration of *Allium cepa* decreased pain perception and treated thermal hyperalgesia and mechanical allodynia.

Oxidative injury has been implicated in pathophysiology of alcoholic neuropathy. ROS triggers second messengers which are involved in central sensitization of dorsal horn cells [41]. It also activates spinal glial cells which play an important role in chronic pain. Nitric oxide is also implicated in neuropathic pain. It sensitizes spinal neurons and also contributes to the sensitization of central neurons by disinhibition [42-43]. Reduced glutathione is a major low molecular weight scavenger of free radicals in cytoplasm. Depletion of glutathione increases the susceptibility of neurons to oxidative stress and hyperalgesia [44-45]. A significant increase in TBAR levels, a decline of reduced glutathione and increase in nitric oxide level were observed in the sciatic nerve of ethanol treated rats.

The administration of *Allium cepa* also exhibited an anti oxidant effect due to presence of phenolic compounds, namely flavonoids, anthocyanins, quercitin and kaemferol [46]. The mechanisms by which *Allium cepa* brings about its anti oxidant effect is by free radical scavenging, chelation of transition metal ions, and inhibition of oxidases [47]. *Allium cepa* also inhibits lipid peroxidation. Henceforth, treatment with *Allium cepa* produced significant protection in alcoholic neuropathy as evident from improvement in the reduction in nociception.

In the present investigation, α-tocopherol was used as a standard drug. α-tocopherol is an essential fat soluble vitamin contributing to chain breaking antioxidant effect and helps in maintaining the permeability and integrity of membrane, thus shielding neurodegenerative effect of chronic alcohol administration. Very recently it has been found that α-tocopherol

can prevent the overproduction of nitric oxide in the brains of diabetic rats having cognitive dysfunctions [29]. Thus, α-tocopherol exerts anti-nociceptive action by attenuating the increased oxidative stress level which may result in further inhibition of protein kinase C, an important mediator of neuropathic pain. This assumption gets further strengthened from a study suggesting that at the posttranslational level, α-tocopherol inhibits protein kinase C, 5-lipoxygenase and phospholipase A2 [48]. In addition to potent antioxidant activity, the suppression of nitrosative stress level both in serum and in sciatic nerve also contributes significantly to prevent the alcoholic neuropathy in rats.

On the basis of above, it may be concluded that abrogation in Alcoholic Neuropathy with repeated oral administration of *Allium cepa* bulb extract may alleviate established behavioral and biochemical symptoms of neuropathic pain, possibly through analgesic, anti inflammatory, antioxidant effect, reduction in TBARS and nitric oxide level and analgesic effect in alcohol induced neuropathic pain.

CONCLUSION

The present study was designed to investigate the ameliorative effect of *Allium cepa* bulb extract in alcohol induced neuropathy in rats. In the present study, Alcoholic Neuropathy was induced by administration of ethanol by oral gavages twice a day for a period of nine weeks. Mechanical allodynia and thermal hyperalgesia have been used as behavioral markers of Alcoholic Neuropathy whereas increase in lipid peroxidation, increase in nitrite level and decrease in reduced glutathione are considered as biochemical markers of Alcoholic Neuropathy. On the basis of results obtained in present study, the following salient findings may be summarized

1. Oral administration of ethanol twice a day led to mechanical allodynia, thermal hyperalgesia and changes in oxidative and nitrosative stress, reduced GSH level and increase lipid peroxidation when compared with normal control rats.
2. Administration of a low dose of *Allium cepa* (100 mg/kg) did not improve pain nociception in ethanol treated rats.
3. Administration of moderate and high doses of *Allium cepa* (200 and 300 mg/kg, respectively) for a period of 9 weeks, one hour prior to ethanol administration, significantly improved ethanol induced mechanical allodynia and thermal hyperalgesia.

4. Biochemical analysis revealed that administration of moderate and high dose of *Allium cepa* (200 and 300 mg/kg respectively) significantly attenuated ethanol induced oxidative and nitrosative stress, reduced GSH level and increase lipid peroxidation.

   However, low dose of *Allium cepa* (100 mg/kg) did not produce any statistically significant effect in various behavioral and biochemical parameters.
5. The present study reports that *Allium cepa* has multiple protective mechanisms such as analgesic and anti inflammatory effect, free radical scavenger and antioxidant effect through which it attenuates alcohol induced neuropathic pain.

   On the basis of above study it may be concluded that *Allium cepa* bulb extract in a dose of 200 and 300 mg/kg has a beneficial role in attenuation of Alcoholic Neuropathy. Hence, *Allium cepa* may offer a novel and a protective approach in abrogation of alcoholic induced neuropathic pain.

ACKNOWLEDGEMENT

The authors would like to extend their gratitude towards Dr. Anita Kotwani, Associate Professor, Vallabhbhai Patel Chest Institute, University of Delhi, for her motivation, support and sharing some extremely valuable ideas and suggestions without which this article would have been incomplete.

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