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Effect of Allium Sativum on the Alcohol-Induced Neuropathic Pain in Wistar Rats

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ABSTRACT

Neuropathic pain is a severe pain condition associated with pathological changes which have deleterious effects like dysfunction of the brain. Alcohol abuse is known to be the most common reason at present for developing neuropathic pain. Alcohol-induced neuropathic pain can be assessed by ache studies such as *hyperalgesia* and *allodynia* which characterizes the response severity of induced pain. In the current research, we used the Allium Sativum (Garlic) bulb extract to study the effect on alcohol-administered rodents. The behavioral and biochemical studies have been done. It has been reported in the study, that *Allium sativum* has certain protective mechanisms such as analgesic and anti-inflammatory effects, free radical scavenger, and antioxidant effects which contribute to alcohol-induced neuropathic pain. Based on the results of the current investigation, it can be concluded that *Allium sativum* bulb extract, administered at doses of 200 and 300 mg/kg in 0.9 per cent saline, is helpful in reducing the symptoms of alcoholic neuropathy. A*llium sativum* may offer a protective effect in the revocation of alcoholic-induced neuropathic pain.

Keywords: Neuropathic pain; Allium sativum; Induced pain; Anti-inflammatory effect; Alcoholic neuropathy

1. INTRODUCTION

A recurrent indication of neurological disorder is an emotional and unpleasant experience of perception of pain which leads to damage to tissues. Neuropathic pain is a severe pain condition that includes various pathological changes in the nervous system which leads to dysfunction in the brain. The major symptoms associated with ache include *hyperalgesia* and *allodynia*, *hyperalgesia* is an elevated response to pain and *allodynia* is a sore reaction toward non-noxious spurs. Data concluded over the years revealed that approximately 2-3 per cent of the population in the world suffers from neuropathic pain. After nicotine and caffeine, alcohol is the third most addictive substance[1] and it has many chronic effects which can result in early death and increase incidences of serious illness. It had been stated in the International Classification of Disease (10th revision, 1993), that alcoholism is a severe medical condition accomplished by a strong and frequent need to use alcohol. Alcoholic neuropathy (AN) is chronic alcohol abuse and damage to the nervous system due to damage to primary neurons and demyelination of secondary motor and sensitive fibers.[2] Alcoholic neuropathy symptoms take several months and years to develop and are more common in continuous drinkers than in episodic drinkers[3], women being more vulnerable than men.[4] In the United States, higher consumption of alcohol results in mortality. The condition mainly involves abnormalities in sensory, motor, and autonomic functions. The major symptom of alcoholic neuropathy is the sensation of pain.[5,6] Other symptoms associated with alcoholic neuropathy are burning pain, muscle weakness, nausea with or without vomiting, numbness, and pain.

The consumption of ethanol affects the central and peripheral nervous system and other tissues (liver), indirect metabolic changes mediated by malabsorption,

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and some molecular mechanism.[7,8] Ethanol cleaves into acetaldehyde that disrupts the sensory signaling of the brain which results in the injury to the nerves or tissues leading to the misfiring of neurons that result in painful condition.[9] The toxic effect of ethanol on the nervous system and other tissues depletes the proteins in the liver which destroys protein and lipid metabolism leading to metabolic changes which affect the action of the nervous system.

Furthermore, secretion of proinflammatory mediators couple with the protein kinase C (PKC)[10], microglia of the spinal cord and activate them after alcohol consumption[11], resulting in damage to nerves.

The plant extract of *Allium sativum* (Garlic) is used in this study for alcohol-induced neuropathic pain. It belongs to the family of Liliaceae and is widely distributed worldwide it has been used as an analgesic, antidiabetic, antioxidant, and antihyperlipidemic.[12] It also exhibits an antioxidant effect.[13] It has a protective function against oxidative damage.[14, 15] Hence, utilization of *Allium sativum* in the management of alcohol-induced peripheral neuropathy was explored in the present study.

Alpha-tocopherol is used as a positive control in this study which is a fat-soluble vitamin containing antioxidant properties.[16] It has also been classified as a radical-chain breaker.[17] It has been stated in three different studies that α-tocopherols act as a neuroprotective agent in an experimental model of alcoholic neuropathy.[18]

India is a vast country where Allium sativum, a perennial herb, is widely cultivated. The oral administration of the garlic extract significantly decreases serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine AST, and ALT levels.[33] Studies demonstrated garlic has antimicrobial and immune-stimulating properties, is also known to enhance fibrinolytic activity, and has a positive impact on platelet aggregation and adhesion. Standardized preparations minimise the issue caused by a strong raw garlic odour while ensuring precise dosing. Thus, a traditional folk medicine has been developed into a medical drug with relatively little adverse effects that can benefit many individuals with less severe kinds of cardiovascular disease. Due to the well-established analgesic and antinociceptive effects of Allium sativum, which have been supported by multiple prior research investigations, the idea of utilising it has become widely accepted.

The components of garlic also have anti-diabetic, antibiotic, hypocholesterolemic, fibrinolytic, and other biologically-associated properties that are related to a well-regulated biological system. Nonvolatile sulphur, which comprises peptides and proteins with activities and is present in garlic in addition to free sulfoxides, makes these veggies a significant source of medicinal agents. Thus, the present study was designed in such a way that we can investigate the role of Allium sativum bulb extract in alcohol-induced neuropathic pain in Wistar rats through this.

2. METHODOLOGY

2.1 Animals

Wistar rats weighed (200-280 g) of any sex were used in the current research. The experimental animals were acclimatized in the animal house at Central Animal House Facility, Jamia Hamdard, Delhi, and kept in cages under the standard cycle of light and dark conditions with clean drinking water and enough access to food. The Institutional Animal Ethics Committee (IAEC), Jamia Hamdard, Delhi, approved the experiment protocol, and the Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India, adhered to the rules. All the experiments for different treatment groups were performed by using similar ages of animals to avoid variability between experimental groups.

2.2 Induction of Alcoholic Neuropathy

Ethanol (10ml/kg of 35% v/v) was administered for 9 weeks to induce alcoholic neuropathy in rodents.[19] The ethanol dose was taken from studies already published.[10] The controls group was administered 10 ml/kg oral gavage of distilled water. *Allium sativum* was given orally (as an extract of 100, 200 and 300 mg/Kg of body weight in 0.9 % saline) one hour before ethanol administration. Behavioral assays were performed at the end of every week.

2.3 Drugs and Treatment Schedule

The reagents used in this experiment were freshly prepared. Ethanol, α-tocopherol, and bulb extract of *Allium sativum* (Ayush Herbs and Pharmaceuticals, Kangra, HP, India) were used in this study. To induce neuropathy, 99.9 % v/v pure ethanol was employed. The following items were purchased from Loba Chemicals: DTNB (5,5-dithiobis-2-nitrobenzoic acid), reduced glutathione (GSH), sulphanilamide, hydrochloric acid, sodium hydroxide, sodium chloride, N-(1-naphthyl) ethylenediamine dihydrochloride, and potassium dihydrogen phosphate (Mumbai, India). Magus Chemicals provided the thiobarbituric acid (TBA). Nice Chem. Pvt. Ltd. provided the trichloroacetic acid (TCA) required (Cochin, India). EDTA was purchased from Thomas baker, India.. Each dose of *Allium sativum* bulb extract was freshly prepared in normal saline (in 0.9% saline) to treat ethanol-induced neuropathy.

2.4 Behavioral Studies

2.4.1 Assessment of Thermal Hyperalgesia by Hot Plate Method

Eddy, et al., 1950 described a technique named Eddy's Hot Plate method[19] to estimate thermal hyperalgesia for evaluating the activity of an animal towards any noxious stimuli. The rats were positioned on a heated (52.5 ±0.5 °C) plate, not more than 15s to prevent any damage to the paw. The foremost indications like the licking of the paw or jumping were recorded as a marker threshold of nociceptive pain.

2.4.2 Assessment of Cold Allodynia by Tail Immersion Method (Coldwater)

The tail of the rats was engrossed in cold water (10°C ± 0.5 °C) and the withdrawal of the tail (flicking response) was recorded. Shortened tail withdrawal time indicates allodynia.

2.4.3 Assessment of Coordination of Motor Reflexes by Rotarod

The rotarod apparatus modified by Muthuraman, *et al.* (2008) was used in evaluating motor coordination (grip muscle strength) in rats.[20] The rats were placed on the rotating rod for one minute at 25 rpm and the time taken by rats to fall from the rotarod was noted down. Rats were tested for rotarod performance 1 h after oral drug administration. Results were given as mean time in seconds ± S.E.M.

2.5 Biochemical Studies

2.5.1 Collection of Blood and Tissue Samples from Rodents

For biochemical estimation, the blood was collected through retro-orbital puncture as of overnight fasted animals. The rats were then sacrificed by cervical dislocation and immediate collection of the sciatic nerve. The sciatic nerves collected were washed in sterile conditions with normal saline solution and weighed. Tissue homogenate 10 per cent (w/v) is synthesised in 0.1 M phosphate buffer (pH 7.4) followed by 15 min centrifugation at 2000 rpm to attain tidy supernatant.

2.5.2 Estimation of Total Protein

The total protein level in the sciatic nerve was assessed by the Biuret process via adding 1000 µl of Biuret reagent to 10 µl of distilled water, 10 µl of standard albumin, and 10 µl of serum to prepare a blank, standard, and test, respectively. All the ingredients were evenly shaken and incubated for 10 min at 37 ^{0}C. The absorbance of the standard and test sample was recorded against a blank solution at 555 nm spectrophotometrically after 10 minutes.

2.5.3 Estimation of Lipid Peroxidation

The measurement of the concentration of thiobarbituric acid reactive substances (TBARS) as depicted by[21] Niehaus and Samuelsson (1968) is the commonly used method for estimating the index of peroxidation of lipid. In this procedure, 0.1 ml of supernatant is combined with 2 ml of the TBA-TCA-HCL (thiobarbituric acid, trichloroacetic acid, and hydrochloric acid) reagent in a 1:1:1 ratio. The TBARS reagent was synthesized by combining identical quantities of 15 per cent TCA, 37 per cent TBA, and 0.25N HCL. The mixture formed is kept for 15 min in a hot water bath, cooled, and centrifuged for 10 min at 1000 rpm. The absorbance of the clear supernatant solution against blank solution was determined at 532 nm (UV-1700 Spectrophotometer) and the values obtained were expressed in nmol/mg of protein.

2.5.4 Estimation of Reduced Glutathione

1.0 ml of 10 per cent issue homogenate of sciatic nerve remained precipitated with 1.0 ml of 4 per cent sulpho-salicylic acid. The prepared solution was stored at 4°C for one hour and later centrifuged for 15 min. The absorbance at 412nm was evaluated after a few minutes with an expression of decreased levels of GSH level.

2.5.5 Estimation of Nitrite

Griess reaction is used in the estimation of the concentration of nitrites in the serum.[22] In this procedure, 0.1 mL of nerve homogenate supernatant is combined with 0.25 mL of 0.1 percent N-(1-naphthyl) ethylenediamine dihydrochloride and 0.25 mL of 1 percent sulfanilamide (made in 3 N HCL). The absorbance was determined at 545 nm.

2.6 Statistical Analysis

The data were evaluated using one-way analysis of variance (ANOVA) and Tukey's Test for multiple comparisons, and the findings are shown as mean SEM. There was statistical significance at the p-value of 0.05.

3. RESULTS

3.1 Behavioral Observations

3.1.1 Assessment of Thermal Hyperalgesia

The threshold of nociception was lowered in alcohol-treated rodents compared to rodents in the normal control group tested in Eddy's Hot Plate, indicating hyperalgesia. On day 0 the mean paw licking response in ethanol administered rodents was not varied from normal control rats. Throughout 9 weeks, there was no alteration in the mean paw licking response threshold of the control group. Administration of ethanol, two times a day, for 9 weeks reduced ($p< 0.05$) the threshold of mean response of licking paw in comparison to a normal control group of rats. On persistent treatment with a moderate and high dose of *Allium sativum* (200 and 300 mg/kg of body weight in 0.9% saline) for 9 weeks increased the threshold of licking paw in rats treated with ethanol (Fig. 1). Furthermore, a low dose of *Allium sativum* (100 mg/kg) was not shown any effect. Treatment with α-tocopherol (100 mg/kg, *p.o.*) appreciably alleviated ethanol persuaded neuropathic pain in comparison to the ethanol control group. *Allium sativum per se* did not show effect.

3.1.2 Assessment of Cold Allodynia

The mean tail withdrawal latency in ethanol administered animals on day 0 was not varied from the normal group. No alteration in mean tail withdrawal latency of normal control rats throughout the study. Consumption of

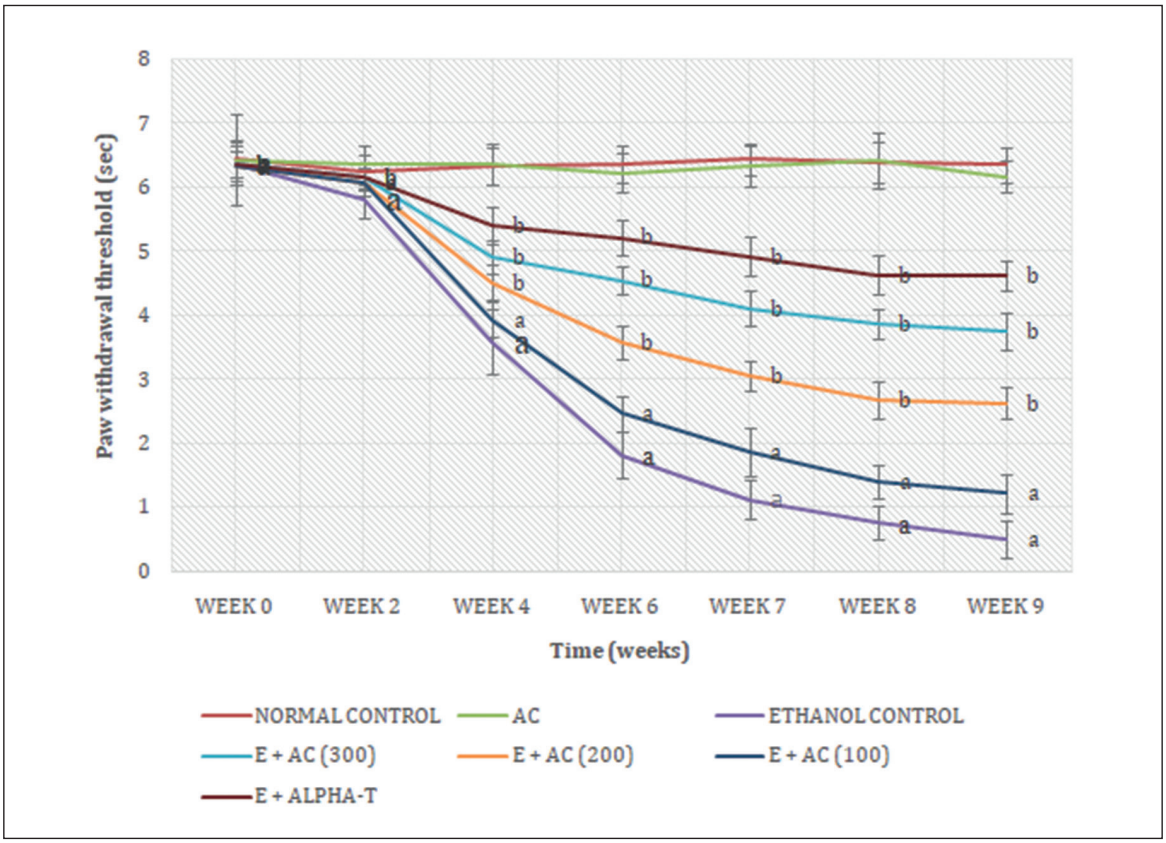

Figure 1. Effect of *Allium sativum* (100, 200, and 300 mg/kg) on Thermal Hyperalgesia in ethanol-treated rats. Results are expressed as Mean ± S.E.M., n=5.
[a]p<0.05 vs. Normal control group
[b]p< 0.05 vs. Ethanol treated group

ethanol for 9 weeks considerably reduced ($p< 0.05$) the latency of mean tail removal in comparison to normal control rats. The rats administered with *Allium sativum* (200 and 300 mg/kg) restored the reduced latency of mean tail removal as compared to rats treated with ethanol. However, a low dose of *Allium sativum* did not show any significant effect (Fig. 2). Treatment with α-tocopherol (100mg/kg, *p.o.*) appreciably alleviated ethanol-induced neuropathic pain in comparison to the ethanol control group. Furthermore, *Allium sativum* did not exhibit an effect on cold allodynia.

3.1.3 Assessment of Motor Co-ordination

The motor activity was ($p< 0.05$) lowered in alcohol-treated rats in comparison to the normal group. Ingestion of moderate and large doses of *Allium sativum* (200 and 300 mg/kg) increased motor performance as observed from time spent on revolving rods in a dose-dependent manner (Fig. 3). The lower dose of *Allium sativum* (100 mg/kg of body weight in 0.9% saline) was not effective in increasing motor performance. Treatment with α-tocopherol (100mg/kg, *p.o.*) appreciably alleviated ethanol-induced neuropathic pain in contrast to ethanol control rat. Further, *Allium sativum per se* did not exhibit a significant effect on motor performance.

3.2 BIOCHEMICAL STUDIES

3.2.1 Allium Sativam Increased Lipid Peroxidation, Decreased Glutathione

In order to study the biochemical effects of allium sativum, we examined the effects of allium sativum injested rats. The total protein was not disturbed in alcohol-induced neuropathy when compared with rodents in the normal group (Table 1). Rats receiving ethanol had an increased level of TBARS, an index of lipid peroxidation, in the sciatic nerve in contrast to the normal group. The lipid peroxide (TBARS) level in the sciatic nerve of alcohol ingested animals was significantly ($p< 0.05$) high in comparison to normal rodents. Treatment with a moderate and elevated dose of *Allium sativum* (200 and 300 mg/kg of body weight in 0.9% saline) drastically modified the levels of TBARS in contrast to animals in the alcohol-treated animal while a lowered dose of *Allium sativum* was not effective in decreasing the increased TBARS level. Treatment with α-tocopherol (100 mg/kg, *p.o.*) extensively reduced the augmented TBARS levels compared to the alcohol control group. Furthermore, *Allium sativum per se* did not show any effect (Table 1).

The levels of GSH were highly reduced in the sciatic nerve of rats treated with ethanol when compared

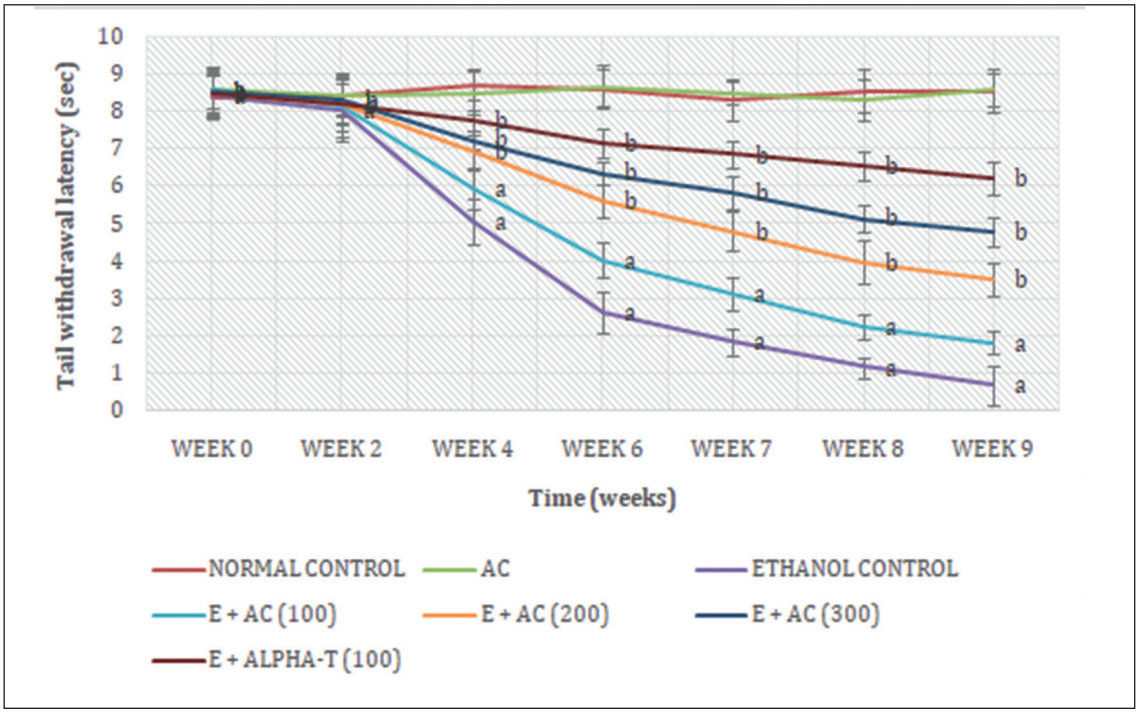

Figure 2. Effect of *Allium sativum* (100, 200, and 300 mg/kg) on Cold Allodynia in ethanol-treated rats. The results are shown as Mean S.E.M., with n = 5.
[a]p<0.05 vs. Normal control group
[b]p< 0.05 vs. Ethanol treated group

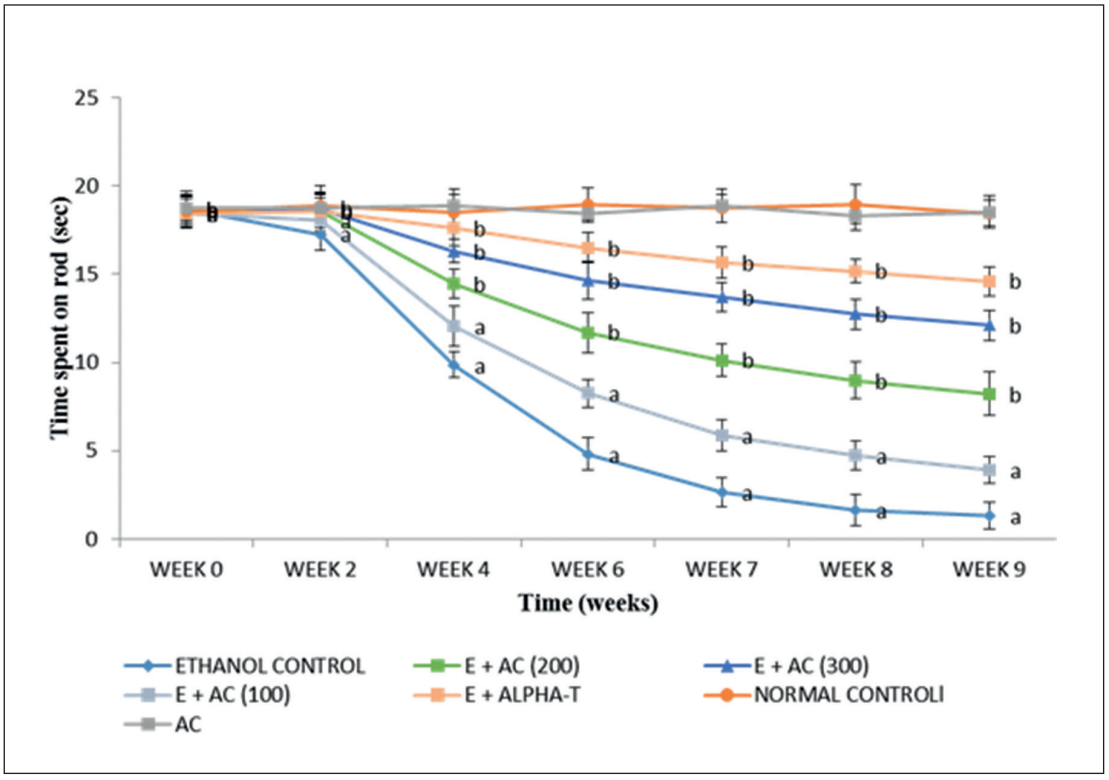

Figure 3. Effect of *Allium sativum* (100, 200, and 300 mg/kg) on Rota Rod in ethanol-treated rats. Results are shown as Mean SEM, where n=5.
[a]p<0.05 vs. Normal control group
[b]p< 0.05 vs. Ethanol treated group

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

Groups	Protein (mg/ml)	TBARS (nmol/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 + AS (100)	4.54 ± 0.38[a]	6.824 ± 0.36[a]
E + AS (200)	4.48 ± 0.29[b]	5.090 ± 0.70[b]
E + AS (300)	4.42 ± 0.31[b]	4.374 ± 0.73[b]
AS	4.40 ± 0.21	2.558 ± 0.66

AS: Allium sativum bulb extract; E: Ethanol; α-T: Alpha-tocopherol
Results are presented as Mean S.E.M., n=5.
[a]p<0.05 vs. Normal control group
[b]p< 0.05 vs. Ethanol treated group

to normal rats on inducing nerve injury. Administration of moderate and high doses of *Allium sativum* (200 and 300 mg/kg) in ethanol-treated rats reversed the altered levels of GSH. Furthermore, a lowered dose of *Allium sativum* (100 mg/kg, *p.o.*) did not show any statistical effect in restoring the depleted level of GSH. The exhausted GSH levels were appreciably reestablished on treatment with α-tocopherol (100mg/kg, *p.o.*) in contrast to ethanol-treated rats. *Allium sativum per se* did not show any effect (Table 2).

Table 2. Effect of *Allium sativum* 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 + AS (100)	291.002 ± 12.237[a]	0.054 ± 0.009[a]
E + AS (200)	249.178 ± 13.235[b]	0.069 ± 0.005[b]
E + AS (300)	192.138 ± 19.289[b]	0.077 ± 0.003[b]
AS	95.604 ± 7.316	0.108 ± 0.013

AS: Allium sativum bulb extract; E: Ethanol;
α-T: Alpha-tocopherol
The results are shown as Mean S.E.M., with n = 5.
[a]p<0.05 vs. Normal control group
[b]p< 0.05 vs. Ethanol treated group

The increased level of nitrite in the sciatic nerve is associated with biochemical changes in ethanol-treated rats. Nitrite level in ethanol-administered animals was increased in comparison to normal control rodents. Ingestion of *Allium sativum* (200 and 300 mg/kg of body weight in 0.9% saline) was reduced in comparison to the ethanol group while a smaller dose of *Allium sativum* (100 mg/kg) was not effective (Table 2). However, *Allium sativum* did not show any effect.

4. DISCUSSION

Alpha-tocopherol is a vitamin that helps in generating the neurodegenerative effect of alcohol ingestion.[19] It has been stated that α-tocopherol can inhibit the excess formation of nitric oxide in the brains of diabetic rodents[19], thus α-tocopherol exhibits anti-nociceptive activity by increasing oxidative stress levels that lead to the downregulating of the effect of protein kinase C. From the above, it may be concluded that repeated oral administration of *Allium sativum* bulb extracts modified biochemical parameters and behavioral symptoms like analgesia, inflammation, antioxidant effect, and highly diminished levels of TBARS in alcohol-induced neuropathic pain.

Alcoholic Neuropathy is a chronic disease produced by ingestion of 10ml/kg body weight of 35 per cent v/v ethanol two times a day.[19] The consumption of ethanol was responsible for the development of neuropathic pain and it was developed in nine weeks with an estimation of biochemical parameters on the 10th week. Before an hour of administering ethanol, *Allium sativum* was given orally (100, 200, and 300 mg/kg) throughout the study. α-tocopherol (100 mg/kg of body weight in 0.9% saline) was used as a positive control.[19] From the present research, it has been revealed that inadequate intake of ethanol for 9 weeks leads to a marked decline in mechanical allodynia[19] and the result obtained were reliable when compared to previous reports[10] indicating neuropathic pain.

Garlic possesses COX inhibitory effect in its therapeutic range. It inhibits the production of arachidonic acid[23] and prevents the synthesis of various proinflammatory cytokines like leukotrienes, and thromboxane via inhibition of COX and LOX (lipoxygenase) acting as an anti-inflammatory agent. Flavonoids inhibit the proliferation and functioning of lymphocytes exhibiting anti-inflammatory activity. Hence, elevated concentrations of flavonoids like the quercetin in the extract and juice of garlic report their potent effects in managing inflammation.[24]

Various studies showed that *Allium sativum* exhibits certain analgesic and anti-inflammatory properties as it reduces pain and inflammation and the pathways of signal transduction leading to deficiency of substance P due to deteriorated plasticity at the dorsal root of the spinal cord.[25,26] Moreover, both onion and garlic comprise a substance named ajoene that inhibits receptors that induce pain in the dorsal root of the spinal cord hence inhibiting pathways and signals that induce pain. Therefore, the present study depicts that continuous administration of *Allium sativum* decreases the perception of pain and

successfully manages mechanical allodynia and thermal hyperalgesia.

The etiology of alcohol-induced neuropathy implicated that ROS sensitizes dorsal horn cells centrally by acting on secondary messengers[27] and it also stimulates spinal glial cells that induce chronic pain.[28] Nitric oxide sensitizes central and spinal neurons and plays a vital role in pain due to alcohol-induced neuropathy.[25,29,30] The reduced levels of glutathione make neurons more vulnerable and sensitive to hyperalgesia and oxidative stress.[31,32] On examining the sciatic nerve of alcoholic rats, decreased levels of glutathione and augmented levels of TBAR and nitric oxide were observed.

5. CONCLUSION

The study has been done to enumerate the ameliorative effect of *Allium sativum* bulb extract in alcohol-administered neuropathy in rodents. Alcoholic Neuropathy was induced by ingesting ethanol orally two times a day for nine weeks. Furthermore, allodynia, thermal hyperalgesia, and motor coordination have been used as behavioral markers of Alcoholic Neuropathy whereas an increase in lipid peroxidation, increase in nitrite level and decrease in reduced glutathione is considered biochemical markers of Alcoholic Neuropathy.

It has been concluded with the study that in a dose-dependent manner Allium sativum could be a potential preventive agent for alcohol-induced neuropathies. *Allium sativum* bulb extract in a dose of 200 and 300 mg/kg has a beneficial role in the attenuation of Alcoholic Neuropathy. It has been observed that Motor function, mechanical allodynia, thermal hyperalgesia, and changes in oxidative stress, reduced GSH levels, and increased lipid peroxidation were reduced after oral administration of ethanol twice a day. Treatment with a low dose of *Allium sativum* (100 mg/kg of body weight in 0.9% saline) did not improve pain in ethanol-treated rats.

Administration of moderate and high doses of *Allium sativum* (200 and 300 mg/kg of body weight in 0.9% saline, respectively) for 9 weeks, one hour before ethanol administration, significantly improved ethanol-induced motor function, mechanical allodynia, and thermal hyperalgesia. It can also be stated from the biochemical analysis that administration of a significant dose of *Allium sativum* produces oxidative stress. However, a low dose of *Allium sativum* (100 mg/kg of body weight in 0.9% saline) did not produce any statistical effect on various behavioral and biochemical parameters. It has been reported that *Allium sativum* has certain protective mechanisms such as analgesic and anti-inflammatory effects, free radical scavenger, and antioxidant effects which contribute to alcohol-induced neuropathic pain. According to the aforementioned study, *Allium sativum* bulb extract, administered at doses of 200 and 300 mg/kg of body weight in 0.9 per cent saline, is useful in the reduction of alcohol neuropathy. *Allium sativum* may provide a protective effect in the relief of neuropathic pain brought on by alcohol.

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