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Possible Modulation of Dopamine (D2) Signalling in Alcohol Induced Neuropathic Pain in Rats

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Abstract: ***The present study has been designed to investigate the possible modulation of Dopamine (D2) signalling in alcohol induced Neuropathic Pain in rats. Wistar albino rats of either sex (180-220g) (n=6) were employed in present study. Rats were pair fed with isocaloric liquid diets containing 30% (2.5ml each) of ethanol for 4 weeks. Chronic alcohol administration was reported to induce increased pain perception. Two weeks prior to initiating the experiment, rats were adapted to the liquid diet by incrementing the ethanol content 0% to 11.8%, 23.6% and then 37% of caloric content model followed by daily drug treatment of Metoclopramide (1.25-2.5mg/kg, i. p. /day) which is d2 receptor antagonist and Bromocriptine (2.5mg/kg, i. p. /day) which is d2 receptor agonist. The periodic checkup of neuropathic pain was carried out by using hot plate, hot immersion and cold allodynia test. Serum biochemical lipid peroxidation, NO and GSH level was assessed on 28th day. Chronic ethanol exposure induced Neuropathy showed elevated hyperalgesia, allodynia response, serum biochemical lipid peroxidation and NO, decreases GSH level. Treatment with bromocriptine results in increased sensation of pain, metoclopramide antagonize D2 receptor results in lowering of neuropathic pain perception, serum biochemical lipid peroxidation and NO measurement, finds increase in GSH level. The finding from the present study may conclude that the down regulation of Dopamine D2 cascade in amelioration of nervous sensitization on chronic intake of alcohol in rats and thereby reducing pain perception in the condition of alcoholic Neuropathy.***

Keywords: Ethanol; Neuropathy; D2 receptor; Metoclopramide; Bromocriptine

1. Introduction

Neuropathic Pain is the disease of somatosensory system which is the part of sensory system and concerned with the conscious of pain, Touch, Position, Movement, Temperature and vibration and produced lesion in Central Nervous System and also in peripherally (1) Hence the damage evoked two forms of pain:

Allodynia- Pain to a stimulus that does not normally provoke pain. It may be provoked by non-noxioustouch stimulation (mechanical allodynia) or cooling (cold allodynia) (2)

Hyperalgesia- An abnormally increased sensitivity to pain (2). It is typically evoked by noxious stimuli like heat stimulation (1). International Association described the Neuropathic pain as caused or initiated by a dysfunction or by primary lesion in Nervous system (3).

Chronic pain is of two types as inflammatory nociceptive pain and neuropathic pain. Neuropathic pain is produced by damage to the neurons in the peripheral and CNS and involves sensitization of these system. In central sensitization, neurons that originate in the spinal cord become hyper stimulated, increasing pain signals and thereby increasing pain sensation. It is most commonly associated with chronic allodynia and hyperalgesia. Based on the nervous system Lesion location Pain Perception divided into groups. Peripheral neuropathic pain is associated with metabolic Disorders (e. g. Diabetic induced peripheral neuropathy), Autoimmune disease which affects CNS (Multiple Sclerosis), Vascular Disease (e. g. Stroke), Trauma and cancer. (4).

Neuropathic pain is experienced due to sensitization of neurons and neuronal damage. In neuropathic pain, there is an accumulation of sodium channels appears at the site of nerve injury and dorsal root ganglion. (5). An enhanced activation of immune cells induces acute and long-term functional and structural changes in dorsal root ganglia of injured dermatoms (6). Hyperalgesia is the initial nociceptive sensitivity i. e. is a form of peripheral sensitization involving neuronal plasticity in primary afferent nociceptors (7). Central sensitization is a pathological pain states and involve the propagated recruitment of central neurons in nociceptive response which results amplification of pain process and broadening of nociceptive fields (8). Alcoholism. Back pain, Hip Problems, Chemotherapy, Diabetes, AIDS and HIV infection, Multiple Sclerosis and spine surgery are the common cause of Neuropathic pain. Symptoms of Neuropathic Pain includes: shooting, burning pain, Tingling and numbness. The treatment of chronic neuropathic pain is challenging and the response to most treatments is generally modest (9). Motor cortex stimulation has also served as a treatment for chronic pain in patients who are resistant to other treatments (10, 11). The mechanisms of motor cortex are still poorly understood but several hypothesis exist.

Dopamine is most often associated with motivation, including reward-seeking behaviour, drug abuse and addiction in brain. Past researches implicated the mesolimbic dopamine system in reward and drug-seeking behaviour (12). In alcohol abuse and substance abuse, receptors of Dopamine play very significant role (13). Dopamine d2 Receptors are mostly associated with the reinforcing alcoholic effects among the various subtype of dopamine receptors (14). In Pain processing Dopamine play a significant role in various parts including Periaqueductal Grey (PAG), Basal Ganglia. Thalamus and spinal cord

centrally (15). Alcohol dependence and chronic pain share common neural circuits giving rise to the possibility that chronic pain states could significantly affect alcohol use patterns and alcohol dependence could influence pain sensitivity (16). Alcohol-induced central nervous system disorders have since the ancient times been in the spotlight of medical sciences because of disturbances of psychic and motor functions. The epidemiological data indicate that the chronic abuse of alcohol reaches 10% (17) and that the frequency of alcoholic neuropathy may range 12-30%. The electronic microscopy proved clearly the presence of primary axonal lesion in alcoholic neuropathy (18). In people with alcoholic neuropathy, the peripheral nerves have been sensitized and even damaged on chronic alcoholic usage.

When chronic alcoholic suddenly stops drinking, withdrawal of alcohol leads to a syndrome of increased excitability of the CNS called "delirium tremens" (DTs) (19). Alcohol is thought to exert a direct neurotoxic action on the peripheral nervous system, resulting in a neuropathy that mostly involves small diameter fibres (20).

Therefore in Neuropathy the primary axonal damage and secondary demyelinisation of motor and sensitive fibres (18) are considered to constitute the damage to the nerve tissue and due to the chronic consumptionof Alcohol there is activation of dopamine receptor (21) and leads to increase in oxidative stress they activate the glial cell which leads to neuronal damage and cause Neuropathic Pain (22).

2. Material and Methods

Animals: Wistar albino rats (180-200 g) of either sex procured from Animal house of Haryana agricultural university, Hisar and kept in animal house of Shoolini University, Solan (H. P.) were employed in present study. Animals was acclimatized to standard laboratory condition 5 days prior to experimentation, maintained at 12-12 hrs light/dark cycles and food and water *ad libitum* polypropylene cages. The present study was conducted at School of Pharmaceutical sciences, Shoolini University (H. P.) during September, 2013 to July, 2014. The experimental protocol was undertaken on getting approval from the Institutional Animal Ethical Committee (IAEC) vides Experimental Protocol No. IAEC/SU-PHARMA/13/020.

Drug/ Reagents/ Chemicals: Metoclopramide specific (d2) receptor antagonist was used in the study of suppression of dopamine d2 receptor on chronic alcohol induced neuropathy and procured from Ipca Laboratories Ltd Naroda, Ahmedabad (Gujarat) and Bromocriptine, specific d2 receptor agonist procured from Novartis was used. The chemicals EDTA, Trichloroacetic acid (TCA), Sulphanilamide, Orthophosphoric acid, sodium citrate were procured from M/S Nice chemicals (Cochin, India). (N-(1-napthyl) ethylene diaminedihydrochloride, 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) and Triss buffer were procured from M/s Himedia laboratories, Mumbai (India). Thiobarbituric acid (TBA) was procured from M/s Molychem, Mumbai (India). Sodium nitrite was procured from M/s Finar chemicals, Ahmedabad.

2.1 Experimental Protocol Chronic alcohol exposure model of Neuropathic Pain

Samson in 1986 has been developed this model for neuropathic pain by giving the isocaloric liquid diet of alcohol to the animals. Long term alcohol consumption has been documented to induce small-fibers painful neuropathy characterised by distal axonal degeneration (23) Adult Wistar rats (180-200 g) were pair fed with isocaloric liquid diets containing 30% ethanol for 4 weeks (i. e.2.5 ml each) (24). Controls were adapted to ethanol free liquid diet over the same period. Behavioural assessments were made on 7th day, 14th, 21st, 28th day. At the end of the experiment, blood was collected from retro-orbital plexuses. Bromocriptine specific (d2) agonist (1.25-2.5 mg/kg) (25) and metoclopramide specific (d2) antagonist (2.5 mg/kg) (26) were given intraperitoneal started from the first day.

Table 1: Experimental protocol (n=6)

Sr. No.	Group	Treatment
1.	Normal control	Chow diet+ vehicle
2.	Neuropathic control	Isocaloric diet containing (30% alcohol)
3.	Metoclopramide	Alcohol + Metoclopramide (2.5mg/kg)
4.	Bromocriptine low	Alcohol + Bromocriptine (1.25mg/kg)
5.	Bromocriptine high	Alcohol + Bromocriptine (2.5mg/kg)
6.	Metoclopramide+ Bromocriptine	Alcohol + Met. (2.5mg/kg) +Brom. (2.5mg/kg)

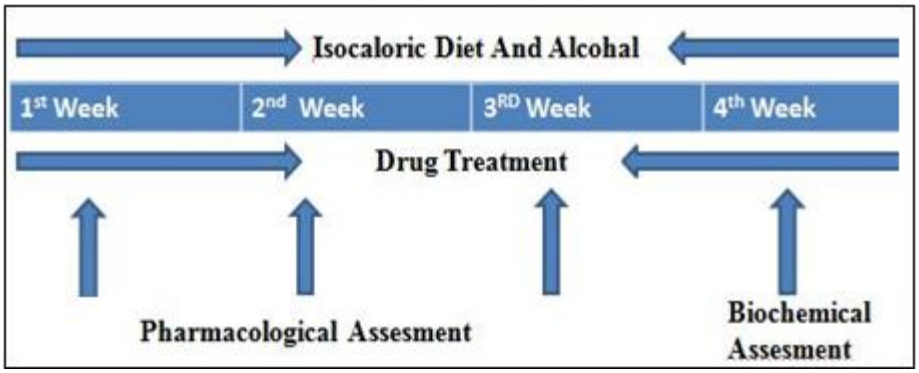

Figure 1: Figure form of 4 weeks protocol

2.2 Measurement of Antinociceptive Activity

2.2.1 Eddy's Hot plate method

The hot plate test, initially described by N. B. Eddy and D. Leimbach (1953), evaluates that when a footpad contact with heated surface their is generation of thermal pain reflexes. During the experiments the animals is confined in a removable clear acrylic cylinder where the latency time to the first hind paw and jumping responses are measured. Thermal hyperalgesia was assessed by placing individual animal on a hot plate (Eddy's Hot Plate) maintained at 55°C on 7^{th}, 14^{th}, 21^{st} and 28^{th} after chronic administration of alcohol. To avoid the thermal pain, the latency to first sign of jumping or paw licking response was taken as an index of pain threshold. A cut off time of 15 sec was maintained throughout the experimental protocol (27).

2.2.2 Cold Allodynia

Cold allodynia an increased sensitivity to normally non-painful cool temperature, is a characteristic feature of clinical neuropathic pain (28). Cold allodynia was assessed after 2 hours of assessment of hyperalgesia by measuring paw (ipsilateral) withdrawal latency (PWL), ice-cold water (4°C±2°C) was taken in a beaker. The paws of rats were submerged gently in water and the withdrawal time was assessed on 7^{th}, 14^{th}, 21^{st} and 28^{th} day after chronic administration of alcohol. A cut off time of 15 sec was maintained throughout the experimental protocol (29).

2.2.3 Tail Immersion method

The tail immersion test, initially described by Turner (1971) evaluate Nociception by Deeping tail in hot water. In this method, rats were divided in five groups and were placed into individual restraining cages leaving the tail hanging out freely. To adapt the cages animals were allowed for 30 minutes before testing. The distal 2-3 cm portion of rat tail was immersed in hot water maintained at 55±1°C (30). Within a few seconds the rats reacts by withdrawing the tail. The reaction time was recorded in 0.5 sec units by a stopwatch. The cut off time was considered 13-15 sec. (31).

2.3 Biochemical Estimation

2.3.1 Estimation of Glutathione (GSH)

The estimation of GSH was done by the method of Ellman (1959). Briefly, 1ml of serum was mixed with 1ml of 20% (w/v) TCA reagent containing 1mM EDTA and kept for 10 minutes at room temperature. The proteins were precipitated and centrifuge at 2000 rpm for 10 minutes.0.20 ml of supernant was taken and 1.8 ml of Ellman's reagent (0.1 nM of DTNB in 0.3 M phosphate buffer with 1% sodium citrate). Reduced glutathione was expressed as µM of GSH/ml of serum. A blank sample was prepared by using double distilled water in place of the filtrate. At 412 nm the optical density was measured and pale yellow color was developed.

2.3.2 Estimation of Lipid Peroxidation

The quantitative measurement of lipid peroxidation was performed according to the method of Wills (1996). The amount of malondialdehyde (MDA), a measure of lipid peroxidation was assayed in the form of thiobarbituric acid reacting substances (TBARS). Briefly, 0.5 ml of blood serum and 0.5 ml of TRIS HCL were incubated at 37°C for 2h. After incubation, 1ml of 10% trichloroacetic acid was added and centrifuged at 1000 rpm for 10 min. For 10 minutes the tubes were kept in boiling water after adding of 1ml of 0.67% thiobarbituric acid in the 1ml of supernatant. At 532 nm the absorbance was measured after cooling and adding of 1ml double distilled water. Thiobarbituric acid reactive substances were quantified using extinction coefficient of chrome (1.56×10M cm) and expressed as n mol of malondialdehyde per nM/ml of serum.

2.3.3 Estimation of Nitric oxide (NO)

The estimation of nitric oxide in terms of stable nitrite/nitrate products was determined with a colorimeters assay using Greiss reaction (Green et al., 1982). Equal volumes of sample and Greiss reagent (0.1% N-(1-napthyl) ethylenediaminedihydrochloride, 1% sulphanilamide and 2.5% phosphoric acid) were mixed, the mixture was incubated for 10 min at room temperature and the absorbance was measured at 540 nm. The concentration of nitrite/nitrate was determined from standard curve prepared with 0.1 ml of 100 µM sodium nitrite (32).

Table 2: ConcentrationVs Absorbance of GSH using rat serum

Sr. No.	Concentration (µM)	Absorbance (540 nm)
1.	10	0.045
2.	20	0.058
3.	30	0.079
4.	40	0.103
5.	50	0.103
6.	60	0.131
7.	70	0.157
8.	80	0.154
9.	90	0.192
10.	100	0.205

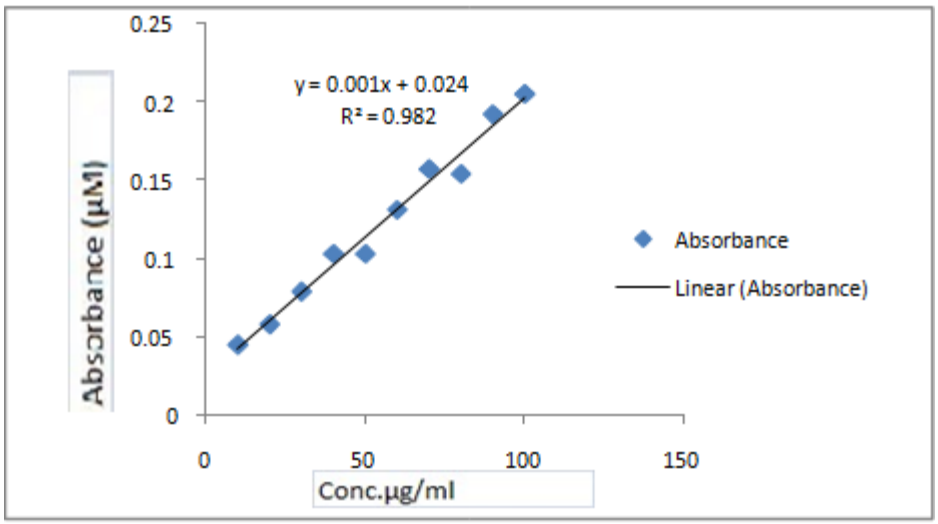

Figure 2: Standard curve for serum glutathione using Glutathione (GSH)

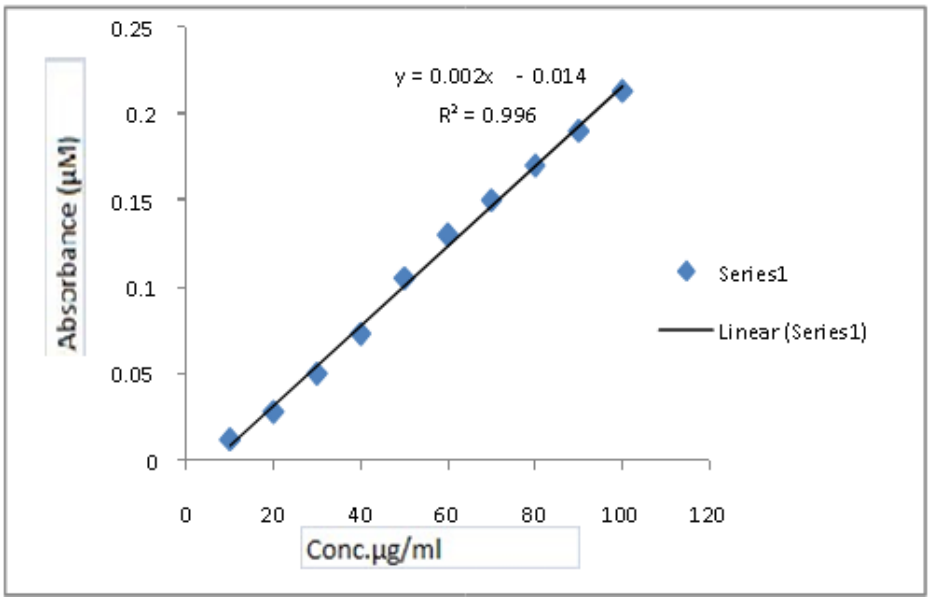

Table 3: Concentration Vs absorbance of TBARS using rat serum

Sr. No.	Concentration (µM)	Absorbance (532)
1.	0	0
2.	12.5	0.07
3.	25	0.145
4.	50	0.28
5.	100	0.55

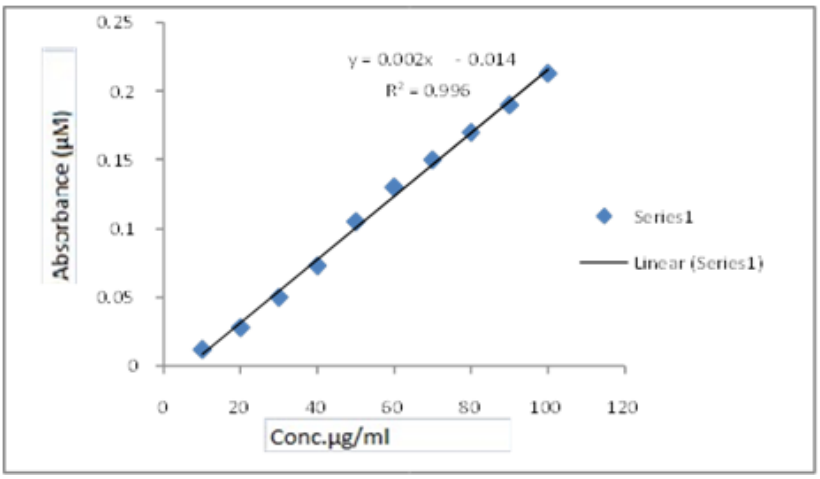

Figure 3: Standard curve for serum TBARS using Malondialdehyde (MDA)

Table 4: Concentration Vs Absorbance of NO using rat serum

Sr. No.	Concentration (uM)	Absorbance (412)
1.	10	0.012
2.	20	0.028
3.	30	0.05
4.	40	0.073
5.	50	0.105
6.	60	0.13
7.	70	0.15
8.	80	0.17
9.	90	0.19
10.	100	0.213

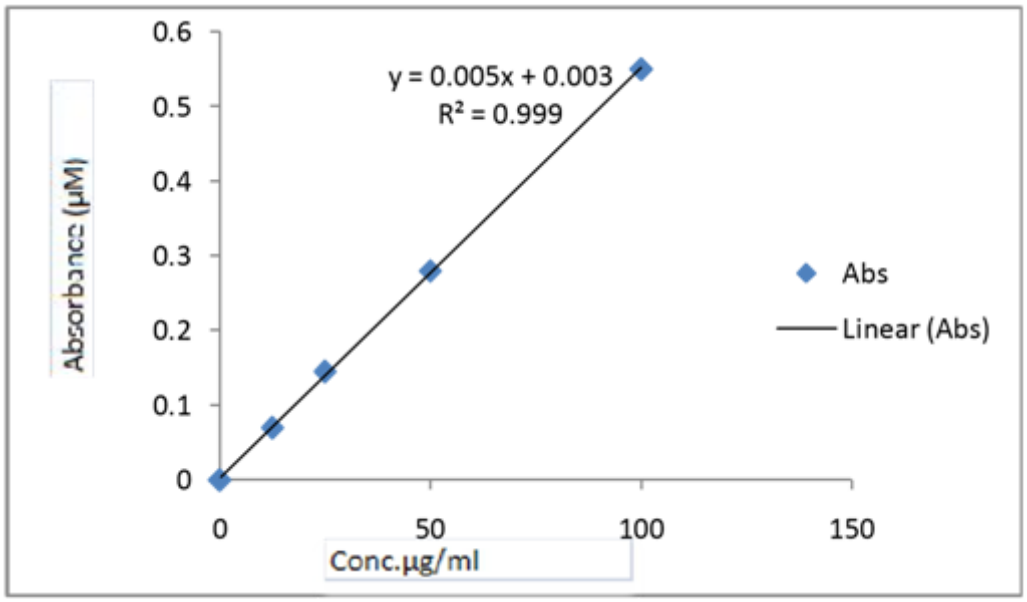

Figure 4: Standard Curve for Nitrite/ Nitrate level using Sodium nitrite

Linear equation is Y= mx + c where, m is slope, c is displacement and x, y is intercept

$Y = mx + c$

$X = y - c / m$

We can find out the value of x intercept by plugging the value of y-intercept.

3. Statistical Analysis

Results were expressed as mean ± Standard deviation (SD), analysed by one-way and two-way ANOVA followed by Bonferroni's multiple comparison analysis as *post-hoc* test. p value< 0.05 was considered to be statistically significant, Graph pad Prism Instate software was used as statistical tool.

4. Result

4.1 Serum Biochemical Estimation

4.1.1 Effect of drug treatments on GSH (Reduced Glutathione) reaction:

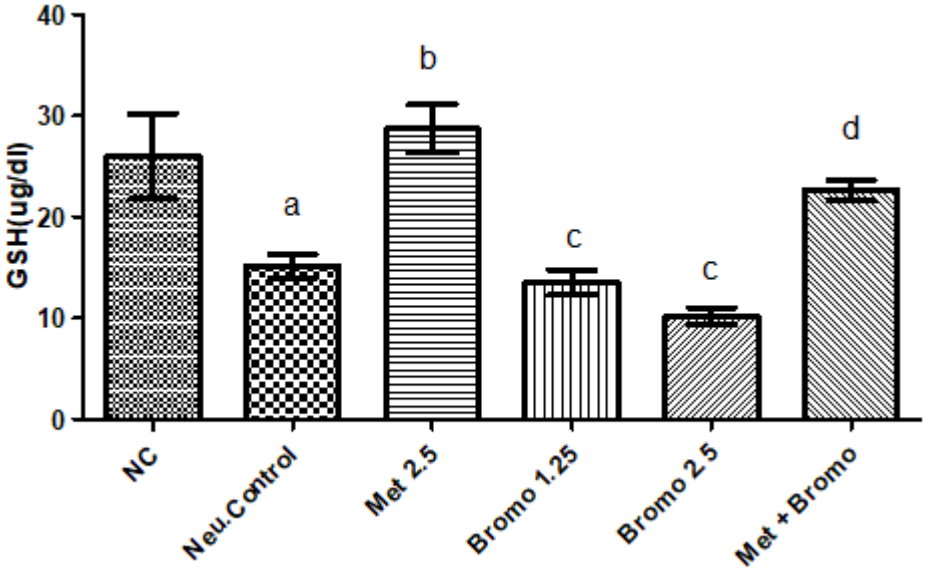

Figure 5: Results are expressed in Mean with ± S. D analysed by one way ANOVA followed by Bonferroni Multiple Comparison; a [p<0.05 vs Normal Control], b [p<0.05 vsNeu. Control], c [p<0.05 vsNeu. control], d [p<0.05 vsBromo.2.5]

Chronic intake of ethanol in isocaloricdiet caused significant (p<0.05) decrease in GSH level in neuropathic control animals, as compared to normal control. However, administration of metoclopramide (2.5mg/kg, i. p/day) to met.2.5 group, showed significant increase in GSH level, as compared with Neu. Control. Administration of bromocriptine 2.5 mg/kg (i. p.) produced significant decrease in GSH level, as compared to Neuropathic control. Administration of Met.2.5 and Bromo 2.5 mg/kg i. p. /day showed significant increase in GSH level, as compared with Bromo.2.5.

4.1.2 Effect of drug treatments on lipid Peroxidation reaction:

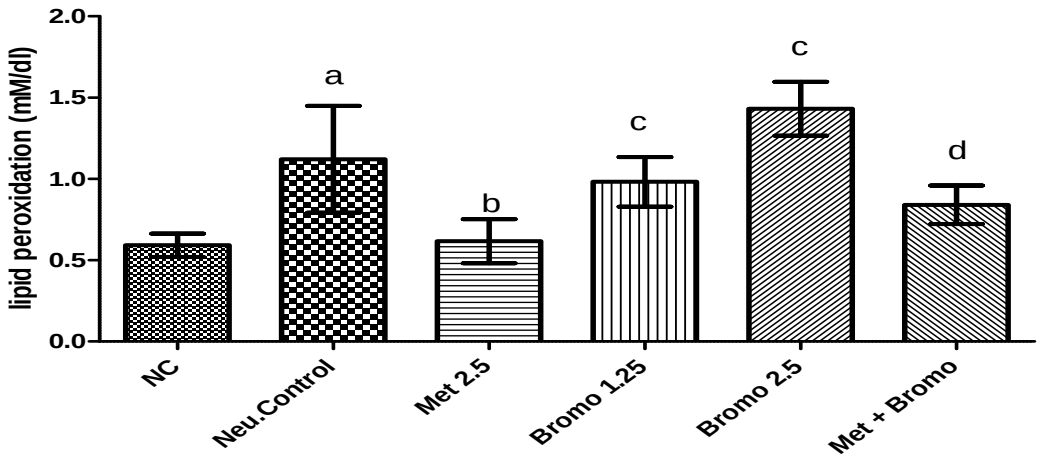

Figure 6: Results are expressed in Mean with ± S. D analysed by one way ANOVA followed by Bonferroni Multiple Comparison; a [p<0.05 vs Normal Control], b [p<0.05 vsNeu. Control], c [p<0.05 vsNeu. control], d [p<0.05 vsBromo.2.5]

Chronic intake of ethanol inisocaloric diet caused significant (p<0.05) increase in MDA level in neuropathic control animals, as compared to normal untreated rats. However, Treatment with metoclopramide 2.5 mg/kg (i. p. /day) in met 2.5 group showed decrease in MDA level as compared to neuropathic control. Administration of bromocriptine 2.5 mg/kg (i. p. /day) produced significant (p<0.05) increase in MDA level as compared to Neuropathic control. Administration of Met 2.5 and Bromo 2.5mg/kg i. p. /day showed significant decrease in MDA level as compared with Bromocriptine2.5 mg/kg i. p. /day.

4.1.3 Effect of drug treatments on NO (Nitric Oxide) reaction:

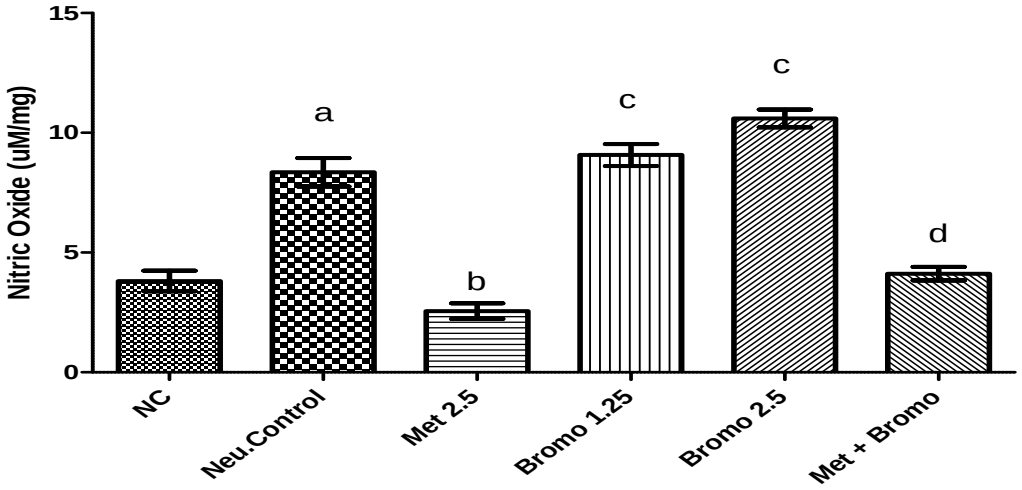

Figure 7: Results are expressed in Mean with ± S. D analysed by one way ANOVA followed by Bonferroni Multiple Comparison; a [p<0.05 vs Normal Control], b [p<0.05 vsNeu. Control], c [p<0.05 vsNeu. control], d [p<0.05 vsBromo.2.5]

Chronic intake of ethanol in isocaloric diet caused significant (p<0.05) increase in Nitric oxide (NO) level in neuropathic control animals, as compared to normal untreated rats. However, Treatment with metoclopramide 2.5 mg/kg (i. p. /day) in met 2.5 group showed decrease in Nitric oxide (NO) level as compared to neuropathic control. Administration of bromocriptine 2.5 mg/kg (i. p. /day) produced significant increase in Nitric oxide (NO) level as compared to Neuropathic control. Administration of Met 2.5 and Bromo 2.5mg/kg i. p. /day showed significant decrease in Nitric oxide (NO) level as compared with Bromocriptine 2.5 mg/kg i. p. /day.

4.2. Antinociceptive Activity Estimation

4.2.1 Hot Plate Test

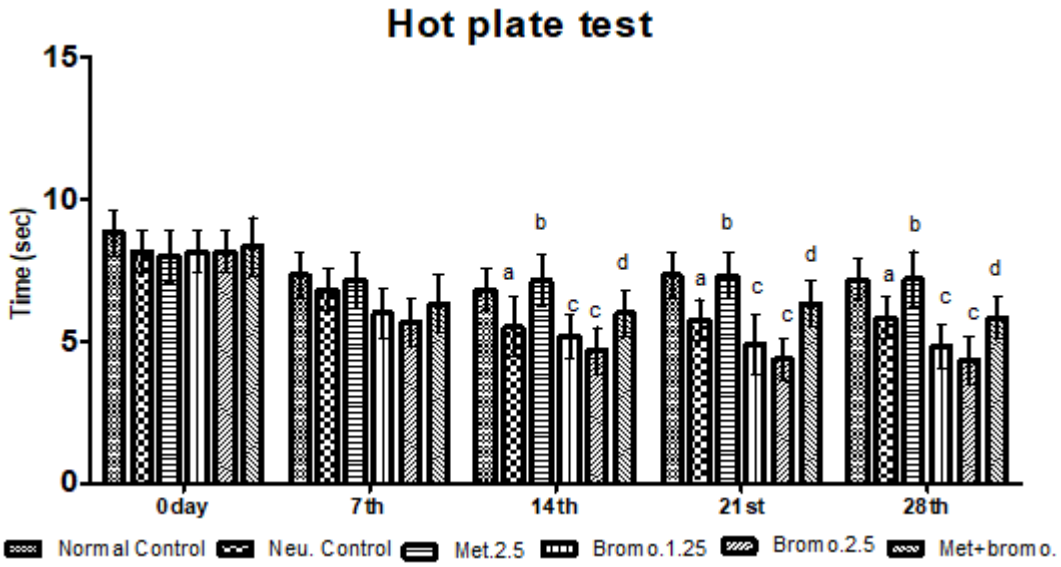

Figure 8: Results are expressed in Mean with ± S. D analysed by Two way analysis of variance (ANOVA) followed by Bonferroni Multiple Comparison; a [p<0.05 vs Normal Control], b [p<0.05 vsNeu. Control], c [p<0.05 vsNeu. control], d [p<0.05 vsBromo.2.5].

Chronic intake of ethanol in diet caused significant pain perception in the form of decrease in nociceptive reaction time on 14th.21st, 28th days in neuropathic control animals, as compared to normal untreated rats. However, Treatment with metoclopramide 2.5 mg/kg (i. p.) reversed the effect of alcoholic nociception as reflected by increased reaction time, as compared to neuropathic control on 14th, 21st, 28th days. Administration of bromocriptine 1.25 and 2.5 mg/kg (i. p.) produced significant pain response in the form of decreased reaction time as compared to neuropathic control. Treatment with metoclopramide 2.5 and bromocriptine 2.5mg/kg (i. p.) produced the increased reaction time, as compared with bromocriptine 2.5 mg/kg (i. p.).

4.2.2 Cold Allodynia

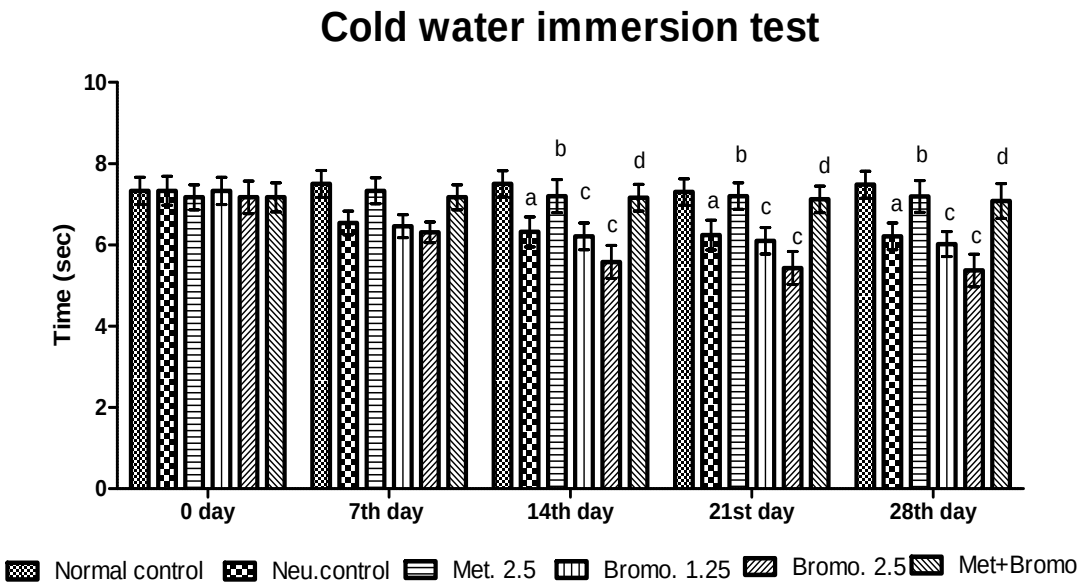

Figure 9: Results are expressed in Mean with ± S. D analysed by Two Way ANOVA followed by Bonferroni Multiple Comparison; a [p<0.05 vs Normal Control], b [p<0.05 vsNeu. Control], c [p<0.05 vsNeu. control], d [p<0.05 vsBromo.2.5].

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4.2.3 Hot Water Immersion

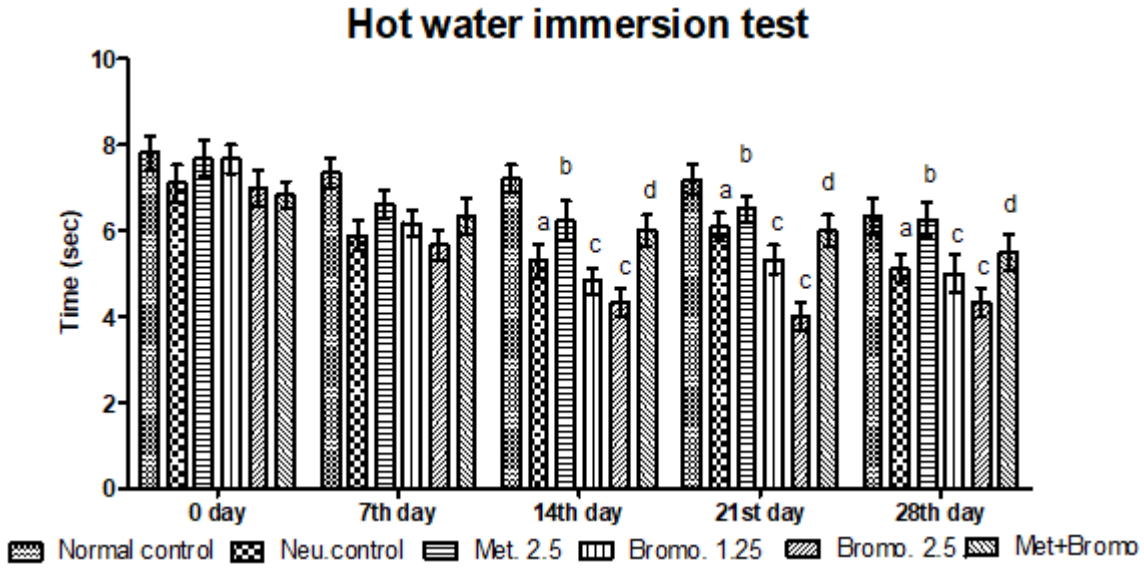

Figure 10: Results are expressed in Mean with ± S. D analysed by Two Way ANOVA followed by Bonferroni Multiple Comparison a [p<0.05 vs Normal Control], b [p<0.05 vsNeu. Control], c [p<0.05 vsNeu. control], d [p<0.05 vsBromo.2.5].

Chronic intake of ethanol in diet caused significant pain perception in the form of decrease in nociceptive reaction time on 14th.21st, 28th days in neuropathic control animals, as compared to normal untreated rats. However, Treatment with metoclopramide 2.5 mg/kg (i. p.) reversed the effect of alcoholic nociception as reflected by increased reaction time, as compared to neuropathic control on 14th, 21st, 28th days. Administration of bromocriptine 1.25 and 2.5 mg/kg (i. p.) produced significant pain response in the form of decreased reaction time as compared to neuropathic control. Treatment with metoclopramide 2.5 and bromocriptine 2.5mg/kg (i. p.) produced the increased reaction time, as compared with bromocriptine 2.5 mg/kg (i. p.).

5. Discussion

The findings of present study demonstrated the down regulation of dopamine d2 cascade in amelioration of

nervous sensitization on chronic intake of alcohol in rats and thereby reducing pain perception in the condition of alcoholic neuropathy.

Neuropathic pain is a chronic disease, affecting wider population now a days. In most cases neuropathic pain occurs by inducing or causing injuries to the spinal cord and peripheral nerves. Peripheral nerve injury produces a persistent neuropathic painful state that is characterized by spontaneous pain in the form of allodynia and hyperalgesia (33). Several experimental models of peripheral mononeuropathy such as a chronic constriction injury (CCI) (34) or partial lesion (35) of the sciatic nerve or its roots and chronic administration of alcohol have been developed.

Alcoholic neuropathy may occur by a combinations of the direct toxic effects of ethanol or its metabolites and nutritional deficiency especially thiamine, deficiencies seen in alcoholics (36). Alcohol diminishes thiamine absorption in the intestine, reduces hepatic stores of the thiamine and affects the phosphorylation of thiamine which convert it to its active form. Alcohol exerts its effects through its metabolism and the key role of degradation of ethanol is played by ethanol dehydrogenase and acetaldehyde dehydrogenase in which ethanol is converted to acetate and reach to the organs in blood flow (37). Both the metabolic enzymes: ethanol and acetaldehyde dehydrogenase require the availability of NAD+ (Nicotine amide adenine dinucleotide) and reduce it to NADH. Formation of acetaldehyde products increase oxidative stress and also promote nutritional deficiency which further results neuronal damage (38). The electronic microscopy proved clearly the presence of primary axonal lesion in AN (39). At present the secondary demyelinisation of motor and sensitive fibers and axonal damage primarily are considered to constitute the morphological basis of alcoholic damage to nerve tissues. The demyelinisation is explained as the result of a slowing-down (deceleration) of axoplasmic flow and a degradation of the quality of biological properties of axonal enzymes and proteins. This type of degeneration so called "dying-back"

Alcohol-induced DNA strand breaks might cause neuronal death (16). Alcohol neurotoxicity may have several mechanisms, including glutamate excitotoxicity and oxidative stress. Oxidative stress is known to play a role in neuropathic pain. The ROS are importantly involved in the development and maintenance of pain (22), particularly in the process of central sensitization of dorsal horn cells or they activate spinal glial cells which play a role in chronic pain.

Nervous system is capable of significant plasticity with various peripheral and central changes occurring in response to injury or experience (40), altering function, chemistry and structure of the neurons. These changes further underlie with the altered sensitivity characteristics of neuropathic pain. The Peripheral sensitization reveals as the nociception and central sensitization takes place at various level ranging from the dorsal horn to the brain (4).

Dopamine play a role in pain processing in multiple levels of the CNS including the spinal cord, thalamus, periaqueductal gray (PAG), thalamus, basal ganglia and cingulate cortex (41). Dopamine has both a pronociceptive and an antinociceptive role in pain modulation (42). Dopamine receptor activation attenuate pain via activation of neurons involved in descending inhibition (43). The ascending nigrostriatal pathway and the descending fibers from the hypothalamic nucleus (A11) to the dorsal horn are involved in dopaminergic pain modulation (44). Dopaminergic neurons in the A11 nucleus send ipsilateral projections to the spinal cord (45).

Chronic intake of alcohol produced significant changes in dopamine levels. Cell bodies of dopamine-releasing neurons located in the ventral tegmental area (VTA) are activated by alcohol (21) and leads to release of dopamine in the nucleus accumbens (NAc), resulting in rewarding and reinforcing experience. Rewarding and addictive invoke the G-protein coupled receptors (GPCRs) to influence intracellular cAMP and affect protein kinase activities which results in neuronal damage and causes neuropathic pain.

The present study showed that the chronic intake of alcohol caused significant thermal hyperalgesia as well as cold allodynia in response to non-noxious stimulus responses of alcoholic neuropathy on hot plate, hot immersion and cold immersion tests in control animals on 7^{th}, 14^{th}, 21^{st} and 28^{th}days. These findings are in confirmation with the earlier literature base on alcohol induced nervous sensitization and neuropathic syndrome (46).

In present study treatment with Metoclopramide (2.5mg/kg i. p. /day) a specific dopamine d2 receptor antagonist (47) showed significant decrease in hyperalgesia and allodynia neuropathic responses on 7^{th}, 14^{th}, 21^{st} and 28^{th} day periodically by hot plate, hot immersion and cold immersion test as compared to neuropathic control. Decreased hyperalgesia and allodynia responses are due to the alteration of dopamine cascade thereby reduced availability of dopamine. Endogenous effect of dopamine is modified with the use of antagonist which was traced in the form of decreased pain response. In further study on 28^{th} day serum biochemical estimation was done, which found significant decrease in lipid peroxidation level and NO level, and increase in GSH level.

Whereas treatment with bromocriptine a dopamine d2 receptor agonist mobilizes the production and release of dopamine and have potentiated the nociception response of alcohol induced neuropathy which was assessed in the form of increased pain perception signed hyperalgesia and allodynia in bromocriptine (1.25 and 2.5mg/kg i. p. /day) group as compared to the neuropathy control. In addition to serum biochemical estimation was done, which found increase in lipid peroxidation and NO level, and decrease in GSH level.

Administration of dopamine antagonist prior to the treatment of bromocriptine dopamine receptor agonistsignificantly ameliorated the nociceptive response as compared to bromocriptine (2.5mg/kg i. p. /day) high dose treated rats. In further study on 28^{th} day serum biochemical estimation was done, which found decrease level of lipid peroxidation and NO level and increased in GSH level. Thus, we do suggest

the mechanism just mentioned may contribute the role in the nociceptive effect of dopamine in neuropathic pain.

6. Conclusion

The finding from the present study may conclude that the down regulation of dopamine d2 cascade in amelioration of nervous sensitization on chronic intake of alcohol in rats and thereby reducing pain perception in the condition of alcoholic Neuropathy.

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