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Effects of Quetiapine and Olazapine (D2 Antagonist) on Alcohol Dependence and Alcohol Withdrawal in Swiss Albino Mice

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ABSTRACT
Today, alcohol is widely consumed and it is also the most commonly abused drug in the world, a cause of vast medical and societal costs. Alcohol in low to moderate amounts relieves anxiety and fosters a feeling of well-being or even euphoria. The lethal dose of alcohol in humans is variable, but death generally occur when blood alcohol levels are greater than 400 to 500mg/dl. The consumption of alcohol in high doses over a long period results in tolerance and in physical and psychological dependence. Alcohol dependence increases locomotor activity through mechanism related to reward system involving mesolimbic dopaminergic pathway. A wide range of pharmacotherapy is available for the treatment of alcohol dependence and withdrawal have been introduced but none of them have shown proven efficacy and safety.
In the present study, Dopaminergic antagonist, like Quetiapine and Olanzapine were studied as new pharmacotherapy for treating alcohol dependence and withdrawal using actophotometer for alcohol induced hyperactivity and Elevated plus maze for anxiety profile. The Albino mice (wt=30 g) were divided into different groups (n=6) consisting of control(saline), alcohol (10% v/v and 20% v/v) and test (Olanzapine1.8 mg/kg and Quetiapine 36 mg/kg) groups. Alcohol was administered orally in a single dose of 2-3g/kg for actophotometer whereas for elevated plus maze, alcohol was administered orally in a dose of 3g/kg for 14 days. The test drugs, Olanzapine(1.8 mg/kg) and Quetiapine(36 mg/kg) was administered intraperitoneally in a single dose. The increase in the locomotor activity of the test group were compared with the alcohol group showed statistically significant results ($P < 0.01$) compared to alcohol group except the results obtained in Quetiapine group. The results of elevated plus maze showed that the no. of enteries in the open arm of the olanzapine and quetiapine group showed statistically significant results ($P < 0.01$) compared to alcohol group. Time spent in open arm of the test group was found to be statistically not significant when compared to alcohol group.
Keywords: *Alcohol dependence, dopamine, reward pathway, mesolimbic dopaminergic pathway, dopamine antagonists, Quetiapine, Olanzapine.*

Introduction

Alcohol, has occupied an important place in the history of mankind for at least 8000 years. Today, alcohol is widely consumed and it is also the most commonly abused drug in the world, a cause of vast medical and societal costs. The two-carbon alcohol *ethanol*, is a CNS depressant that is widely available to adults; and it's use is legal and accepted in many societies. Alcohol in low to moderate amounts relieves anxiety and fosters a feeling of well-being or even euphoria. The relevant pharmacological properties of ethanol include effects on gastrointestinal, cardiovascular and central nervous systems, and effect on

prenatal development. With more severe intoxication, CNS function generally is impaired, and a condition of general anesthesia ultimately prevails.[4] The lethal dose of alcohol in humans is variable, but death generally occur when blood alcohol levels are greater than 400 to 500mg/dl. Tolerance to ethanol develops after chronic use. The consumption of alcohol in high doses over a long period results in tolerance and in physical and psychological dependence. Alcohol dependence is "an illness marked by drinking alcoholic beverages at a level that interferes with physical health, mental health, and social, family, or occupational responsibilities.". It is actually divided into two categories: dependence and abuse. Alcohol dependence is usually more serious from a medical point of view, as it can cause a very long list of symptoms and even death. However, both alcohol dependency and abuse cause serious problems in our society.

Hypothesis for alcohol dependence is actually that drug of abuse increase locomotor activity through mechanism related to reinforcement or reward system involving mesolimbic dopaminergic pathway. Also, ethanol induced hyperactivity might provide screening model to investigate on reward pathway. Chronic drinkers, when forced to reduce or discontinue Alcohol, experience a alcohol withdrawal syndrome, which indicates the existence of physical dependence. Alcohol Withdrawal symptoms classically consist of hyper excitability in mild cases and seizures, toxic psychosis, and delirium tremens in severe ones. The dose, rate, and duration of alcohol consumption determine the intensity of the alcohol withdrawal syndrome.[8]So dopamine antagonist may decrease seizures, as a alcohol withdrawal syndrome in chronic alcoholism. Since dopamine plays an important role in alcohol dependence (positive reinforcement), D2 dopami-nergic antagonists like Quetiapine and Olanzapine provides a new role in pharmacotherapy of alcohol dependence and alcohol withdrawal.

A dopamine antagonist is a drug which blocks dopamine receptors by receptor antagonism. There are five known types of dopamine receptors in the human body; they are found in the brain, peripheral nervous system, blood vessels, and the kidney. The atypical antipsychotics (also known as second generation antipsychotics) are a group of antipsychotic drugs used to treat psychiatric conditions. Some atypical antipsychotics are FDA approved for use in the treatment of schizop-hrenia. The first atypical anti-psychotic medication, clozapine, was discovered in the 1950s, and introduced into clinical practice in the 1970s. During the 1990s, olanzapine, risperidone and quetiapine were introduced, with ziprasidone and aripiprazole following in the early 2000s. The latest atypical anti-psychotic, paliperidone, was approved by the FDA in late 2006.

The mechanism of action of these agents is that they modulate the dopamine neurotransmitter system which is the most important mechanism by which anti-psychotics exert their benefits. The side effects reportedly associated with the various atypical antipsychotics vary and are medication-specific. Atypical antipsychotics have a lower likelihood for the development of tardive dyskinesia than the typical antipsychotics. Akathisia is more likely to be less intense with these drugs than the typical antipsychotics. Sometimes atypical antipsychotics can cause abnormal shifts in sleep patterns, and extreme tiredness and weakness.

Olanzapine is a atypical antipsychotic resembles Clozapine in blocking multiple monoaminergic (D_2, 5-HT_2) muscarinic and H_1 receptors. Both positive and negative symptoms of schizophrenia appeared to be benefitted. Monotherapy with Olanzapine may be as effective as a combination of lithium/valproate and benzodiazepines. Olanzapine is a potent antimuscarinic, produces dry mouth and constipation. It has few extrapyrimidal side efeects: causes weight gain and carries a higher risk of worsening diabetes. Incidence of stroke may be increased in the elderly. Olanzapine is metabolized by CYP1A2 and glucuronyl transferase. The $t_{1/2}$ is 24-30 hours.

Quetiapine is a new short acting atypical antipsychotic requires twice daily dosing. It blocks D_2, 5-HT_1 and H_1 receptors in the brain. It had minimal extrapyrimidal side effects. However, it is quiet sedating and postural hypotension can occur. Weight gain and rise in blood sugar are infrequent. It is metabolized mainly by CYP3A4. The $t_{1/2}$ is 6 hours.[9]

Methodology and Results

1) Effect of Quetiapine and Olanzapine on Alcohol Dependence

1.1) Ethanol Induced Hyperactivity in Mice

Increases in spontaneous locomotor activity in rodents are produced by many drugs of abuse including alcohol through mechanism related to reinforcement, i.e. the mesolimbic dopamine (DA) system. It is, therefore, possible that ethanol-induced hyperactivity provides a screening model to investigate the effect of ethanol on mesolimbic dopamine neurotransmission and, thus, the efficacy of new compounds in the treatment of alcohol dependence. Ethanol had bidirectional effects on locomotion in mice: hyperactivity at low doses (2-3 g/kg) and sedation at high doses (4-5 g/kg). In the studies in which rats or mice have access to ethanol, a variety of dopamine antagonist have been reported to decrease ethanol intake. The effect of D_2 antagonists, Quetiapine (36 mg/kg) and Olanzapine (1.8 mg/kg),on alcohol induced hyperactivity was measured using actophotometer.

Animals: Swiss male albino mice of *Mus musculus* species belonging to the age group of 8-10 weeks with average body weight 30 g were used as experimental animals. The animals were housed in an animal facility for at least 4 days prior to behavioural testing with food and water available *ad libitium.* Then animals were allocated to different treatment groups (control and test groups).

Drugs: The drugs used were Quetiapine (36 mg/kg) and Olanzapine (1.8 mg/kg) obtained as a gift sample from Mepromax Pharmaceuticals, Deharadun. Drugs were injected orally as solution or suspension in saline containing 0.6% Carboxymethly cellulose. Ethanol was diluted to a 20% v/v solution in saline and administered in a dose of 19 ml/kg corresponding to dose of ethanol 3 g/kg.

Spontaneous locomotor activity: Locomotor activity was measured using an actophotometer with squared arena activity cages that operates on photoelectric cells, which are connected in circuit with a counter. Mice were injected with calculated doses of one of the test drugs or it's vehicle and placed individually in holding cage for 30 minutes before testing. They were then injected i.p. with saline or ethanol (20% v/v) and placed immed-iately in activity cages. Each animal was tested once. Photocell interrupts were automatically recorded for 10 minutes.

Statistical Analysis: Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's t-test.

Table 1: Effect on Ethanol induced hyperactivity in mice using Actophotometer

Gp. No.	Treatment	Locomotor activity			Average ± SEM
		30 mins	60 mins	90 mins	
1	Control	839±23.45*	837.2±21.24*	844.2±20.98*	840.1±20.98**
2	Alcohol treated	963±30.90*	951.2±29.42*	959.6±28.75*	957.9±3.50*
3	Olanzapine treated	778±23.78**	797.6±23.77**	791.0±24.44**	788.9±5.75**
4	Quetiapine treated	801.8±24.60**	895.2±25.81^{NS}	904.6±19.11^{NS}	867.2±32.81^{NS}

- Values are mean± SD, n=5 for each group, *P<0.05 compared to control; **P<0.01 compared to control; ***P<0.0001 compared to control: NS= statistically not significant (P>0.05);
- Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's t-test.

In the present study, Dopaminergic antagonist, like Quetiapine and Olanzapine were studied as new pharmacotherapy for treating alcohol dependence and withdrawal using actophotometer for alcohol induced hyperactivity. Ethanol had bidirectional effects on locomotion in mice: hyperactivity at low doses (2-3 g/kg) and sedation at high doses (4-5 g/kg). In the studies in which rats or mice have access to ethanol, a variety of dopamine antagonisthave been reported to decrease ethanol intake. The effect of D2 antagonists, Quetiapine (36 mg/kg) and Olanzapine (1.8 mg/kg), on alcohol induced hyperactivity was measured using actophotometer at 30, 60 and 90 minutes interval. The olanzapine group showed statistically significant results 788.9±5.75 ($P < 0.01$) as compared to alcohol group whereas the results obtained in Quetiapine group at 60 and 90 minutes are statistically not significant.

2.0) Effect of Quetiapine and Olanzapine on Alcohol Withdrawal

2.1) Ethanol Withdrawal Induced Anxiety Using Elevated Plus Maze

Ethanol has anti-anxiety and anti-convulsant effects, it's withdrawal in animals can be monitored by determining their anxiety profile. The anxiety profile was determined using Elevated plus maze which consisted of two open arms and two enclosed arms with an open roof and is elevated to a height of 25 cm (for mice). Rodents have aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. When animal entered open arm, they freezed, became immobile and defecate and show fear like movements. The test drug, Quetiapine (36 mg/kg) and Olanzapine (1.8 mg/kg) increased per cent prefrence of the animals to open arm and increased number of enteries and average time spent by the animal in open arm. Favorable modulation of the anxiety profile by a test drug, indicates it's potential usefulness in combating alcohol withdrawal clinically.

Animals:Swiss male albino mice of *Mus musculus* species belonging to the age group of 8-10 weeks with average body weight 30 g were used as experimental animals. The animals were housed in an animal facility for at least 4 days prior to testing with food and water available *ad libitium.* Animals were given 10% v/v ethanol in a dose of 3 g/kg for 14 days. 24 hour after last dose animals were allocated to different treatment groups (control and test groups).

Drugs: The drugs used were Quetiapine (36 mg/kg) and Olanzapine (1.8 mg/kg) obtained as a gift sample from Mepromax Pharmaceuticals, Deharadun. Drugs were injected orally as solution or suspension in saline containing 0.6% Carboxy-methly cellulose. Ethanol was diluted to a 10% v/v solution in saline and administered in a dose of 19 ml/kg corresponding to dose of ethanol 3 g/kg.

Anti-anxiety profile: Elevated plus maze for mice which consisted of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm) with an open roof and is elevated to a height of 25 cm which was constructed in college laboratory using hard card board. The control group received saline and the test group received test drug orally i.e. Quetiapine (36 mg/kg) and Olanzapine (1.8 mg/kg). Half an hour after drug administration animals were placed on the elevated plus maze, with head facing towards the open arm and then stopwatch was started to study the effects of the drug on anxiety profile for 5 minutes (number of entries and average time spent in open arm).

Statistical Analysis: Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's t-test.

Table 02: Ethanol withdrawal induced anxiety using Elevated Plus Maze (EPM)

Grp No.	Treatment groups	Open arm					Closed arm				Total ± SEM
		No. of entries	Attempt to enter	Time spent		Rears	No. of entries	Time spent	Rears	Doubling	
				OA	CA						
1	Control	17.51±1.92**	14.96±1.63**	11.67±1.49*	13.78±1.65**	4.66±0.88*	19.16±1.95**	13.78±1.65**	5.16±0.68**	2.07±0.8*	102.7±12.65**
2	Alcohol treated	26.33±2.45**	15.0±1.94NS	10.83±1.49*	14.23±1.89*	7.50±0.67*	22.32±2.0**	14.23±1.89*	6.52±0.61*	6.32±0.21NS	120.28±13.18*
3	Olanzapine treated	16.75±1.53**	13.67±1.30**	19.17±0.83NS	16.33±0.75**	5.66±1.05*	18.05±1.96**	16.33±0.75**	6.79±1.0*	4.19±1.03**	116.94±10.2**
4	Quetiapine treated	20.8±1.85**	16.3±2.01NS	11.12±1.56NS	12.56±1.3*	3.66±0.61**	21.60±2.0**	12.56±1.35**	8.66±0.11NS	4.06±0.63NS	117.52±11.47*

- OA= Open arm ; CA=Closed arm
- Values are mean± SD, n=5 for each group, *P<0.05 compared to control; **P<0.01 compared to control; ***P<0.0001 compared to control: NS= statistically not significant (P>0.05);
- Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's t-test.

Ethanol has anti-anxiety and anti-convulsant effects, it's withdrawal in animals can be monitored by determining their anxiety profile. The anxiety profile (no. of entries in open arm, time spent in both arms, rears and doubling) was determined using elevated plus maze. Rodents have aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. The test drug, Quetiapine (36 mg/kg) and Olanzapine (1.8 mg/kg) increased per cent prefrence of the animals to open arm and increased number of enteries and average time spent by the animal in open arm. The no. of enteries in the open and closed arm of the olanzapine group and quetiapine group showed statistically significant results 16.75±1.53(P < 0.01) and 20.8±1.85 (P<0.01) respectively compared to alcohol group. Time spent in open arm of the test group was found to be statistically not significant when compared to alcohol group. Rears in open and closed arm of the olanzapine group and quetiapine group was found to be statistically significant 5.66±1.05 and 3.66±0.61(P < 0.01) respectively when compared to alcohol group. Doubling in the open and closed arm of the test group showed statistically significant results 4.19±1.03(P < 0.01) compared to alcohol group except quetiapine group which showed statistic-ally not significant. Favorable modulation of the anxiety profile by a test drug, indicates it's potential usefulness in combating alcohol withdrawal clinically.

Conclusions

Drug dependence is defined as compulsive craving that develops as a result of repeated administration of the drug. Dependence occurs with a wide range of psychotropic drugs, by many different mechanisms. Today, alcohol is widely consumed. Like other sedative-hypnotic drugs, alcohol in low to moderate amounts relieves anxiety and fosters a feeling of well-being or even euphoria. However, alcohol is also the most commonly abused drug in the world, and a cause of vast medical and societal costs. Abrupt alcohol withdrawal leads to a characteristic syndrome of motor agitation, anxiety, insomnia, and reduction of seizure threshold. In its mildest form, the alcohol withdrawal syndrome of tremor, anxiety, and insomnia occurs 6-8 hours after alcohol consumption is stopped. These effects usually abate in 1-2 days. In some patients, more severe withdrawal reactions occur in which visual hallucinations, total disorientation, and marked abnormalities of vital signs occur. Alcohol withdrawal is one of the most common causes of seizures in adults.

Specific drug treatment for detoxification in severe cases involves two basic principles: substituting a long-acting sedative-hypnotic drug for alcohol and then gradually reducing the dose

of the long-acting drug. Because of their wide margin of safety, benzodiazepines are preferred, including long acting benzodiazepines like chlordiazepoxide, clorazepate, and diazepam, have the advantage of requiring less frequent dosing. A disadvantage of the long-acting drugs is that they accumulate, especially in patients with compromised liver function. Short-acting drugs such as lorazepam and oxazepam are rapidly converted to inactive water-soluble metabolites that will not accumulate, and for this reason the short-acting drugs are especially useful in alcoholic patients with liver disease. After the alcohol withdrawal syndrome has been treated acutely, sedative-hypnotic medications must be tapered slowly over several weeks. Complete detoxification is not achieved with just a few days of alcohol abstinence. Several months may be required for restoration of normal nervous system function, especially sleep.

The major objective of drug therapy in the alcohol withdrawal period is prevention of seizures, delir-ium, and arrhythmias. Potassium, magnesium, and phosphate balance should be restored as rapidly as is consistent with renal function. Thiamine therapy is initiated in all cases. Dopaminergic antagonist, like Quetiapine and Olanzapine were studied as new pharmacotherapy for treating alcohol dependence using actophotometer for alcohol induced hyperactivity. The increase in the spontaneouslocomotor activity purely indicate that alcohol induced hyperactivity at a dose of 2-3g/kg was decreased by Olanzapine but Quetiapine did not show any such decreased in hyperactivity.

A wide range of pharmacotherapy is available for the treatment of alcohol dependence and withdrawal has been introduced but none of them have proven efficacy and safety. Hence there is a new pharmacotherapy which is quite effective and safe. Dopaminergic antagonist, like Olanzapine and Quetiapine can be used as pharmacotherapy for alcohol dependence. Thus, this approach may provide a good pharmacotherapy for alcohol dependence and alcohol withdrawal.

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- **Articles related to alcohol dependence**

  Mia Ericson et al., reported that the nicotinic acetylcholine receptor antagonist mecamylamine perfused via reversed microdialysis in the ventral tegmental area antagonizes the increase of accumbal extracellular dopamine levels and lowers ethanol intake in the rat.

  Mannelli P et al., reported that craving is a multidimensional symptom considered as a cardi-nal feature of drug dependence and also reported the effects on craving for alcohol of drugs acting in different manners at the dopamine receptors.

  Srisurapanont M et al., demonstrated that opioid antagonists can decrease alcohol consumption in animals. Also the effect-tiveeness of opioid antagonists in attenu-ating or preventing the recommencement of alcohol consumption in patients with alcohol dependence was reported. In addition, discontinuation rate, death, patient satisfaction, functioning, health-related quality of life and economic outcomes were also evaluated.

- **Polycarpou A et al.,** focused on the evidence of anticonvulsants can be used in the treatment of alcohol withdrawal symptoms and also evaluated the effectiveness and safety of anticonvulsants in the treatment of alcohol withdrawal.

- **Articles related to drugs Olanzapine and Quetiapine**

  Vacheron M et al., reported the use of novel antipsychotic agents as alternative therapy to a lithium therapy and/or the use of conventional antipsychotics. Bipolar disorder consists of alternating depressive and manic episodes. It mainly affects

younger subjects, and is often associated with alcohol and drug addictions. Conventional antipsychotics are effective but they may induce late dyskinesia, weight gain, sedation, sexual dysfunction and depression. The combination of valproate with antipsychotics provides greater improvement in mania than antipsychotic medication alone and results in lower dosage of the antipsychotic medication. The effects of olanzapine in the treatment of mania have also been demostrated The olanzapine treatment group had significantly greater improvement of mania. Significantly more weight gain and cases of dry mouth, increased appetite and somnolence were reported with olanzapine.

- **Deeks E et al.,** reported that combination therapy of olanzapine and fluoxetine is effective in the treatment of patients with acute bipolar depression. The combination improves depressive symptoms and symptom severity in this patient population, with an efficacy greater than that of olanzapine alone or lamotrigine. Furthermore, olanzapine and fluoxetine is generally well tolerated. Although associated with weight gain and potential elevations in glucose, lipid and prolactin levels.
- **Federica Locchi et al.,** reported thatatypical antipsychotics, such as olanzapine, have been reported to display anxiolytic properties as shown in several preclinical and clinical studies. Furthermore, several experimental evidences have shown that olanzapine reduces fear and anxiety in activated anxiety-like behavior test such as Geller-Seifter test, ultrasonic vocalization test and stress-induced ethanol consumption.