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Original article

Sumatriptan Benzoate as a Potential Treatment for Alcohol Withdrawal Syndrome: A Preclinical Assessment Using an Animal Model

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ABSTRACT:

Alcoholism is a major public health issue, and alcoholism is still one of the most frequent drug addiction disorders globally. Alcohol is a highly addictive chemical that acts on many different neurotransmitter systems in the brain to produce a variety of different effects. These Alcohol's reinforcing effects are mediated through neurotransmitter systems. Serotonin, in particular, is a system has a critical role in regulating feelings of pleasure, liking, and addiction to alcohol. With this In this chapter, we examine the literature on the serotonin system and alcoholism. cross-talk with other neurotransmitter networks. Our focus is on serotonin. the transporter and its potential function in alcoholism, then describe many serotonin receptors and examine their role in alcoholism, and evaluate the serotonin system as a potential treatment pharmaceutical therapy, with special focus on the state of the art and future developments in the field. Effect of sumatriptan Benzoate on alcohol withdrawal is compromising evaluated by EPM, EZM, Western blot indicates no changes in GLUA1 & Sk2.Sumatriptan benzoate was reduced Ethanol withdrawal induced anxiety.

Keywords: Addiction, alcohol withdrawal, 5HT, Ethanol, Serotonin, GABA, Western Blot, Diazepam

1. INTRODUCTION

Sustained alcohol consumption promotes tolerance and addiction in both animals and humans, which is why alcohol is one of the most abused substances worldwide. Simple acyclic alcohols, with the general formula $C_nH_{2n+1}OH$, are an important class of alcohols. Ethanol (C_2H_5OH) is the form of alcohol used in the production of alcoholic drinks, and the term "alcohol" is often used to refer to ethanol alone [01-03].

The smell of alcohols is often characterised as "biting" and "hanging" in the nose. When compared to other types of alcohol, the odour of ethanol is somewhat sweeter, or more fruity [04].

Alcohol Dependence

Those who develop a physical or psychological tolerance to alcohol and continue to drink have alcohol dependency, a drug use disorder.At least three of the following seven indicators must be present throughout a 12-month period in order to meet the criteria for alcohol dependency;

• Tolerance

• Alcohol withdrawal syndrome, often known as withdrawal symptoms

Negative consequences of alcohol consumption include: drinking more than planned, drinking for longer than intended, wanting to cut down but failing to do so, spending time acquiring alcohol or recuperating from its effects, and cutting back on or giving up social, vocational, and recreational activities [05-08].

When one suddenly stops drinking, they may experience the unpleasant physical and psychological symptoms that make up the alcohol withdrawal syndrome. These signs and symptoms may be quite diverse, from a barely perceptible tremor to full-blown hallucinations and fits. The onset of alcoholism and its harmful effects on health may be aided by repeated withdrawal episodes. Treatments for withdrawal and other features of alcoholism may benefit from a deeper understanding of the genetic and physiological components of the condition. The withdrawal process is a phase of the neuronal inhibition and stimulation cycle brought on by alcohol. Acute alcohol administration has been shown to alter neuronal release of chemical messengers (i.e., neurotransmitters) and to disrupt the function of proteins in neuronal membranes, such as receptor proteins, which bind

to neurotransmitters, and ion channels, through which ions 1 (e.g., sodium or calcium) enter the cell [09]. Repeated exposure to alcohol causes the brain to make changes (called tolerance) that mitigate the beverage's initial disruptive effects. As a result of chronic alcohol use, the brain's neurons might change to the point where they no longer function correctly without alcohol. This is known as physical dependency. The rebound hyperexcitability, or withdrawal syndrome, occurs when a person abruptly stops drinking after engaging in heavy consumption because the adaptations that formed to counteract alcohol's early inhibitory activities are no longer being countered [09-11].

This article discusses the signs and symptoms of alcohol withdrawal, as well as the role of neurotransmitters, receptors, and ion channels in the illness, as well as the use of animal models to investigate its processes and genetics. [12].

The 5-HT3 receptor is a subtype of the serotonin receptor present in vagus nerve terminals and other regions of the brain, and the 5-HT3 antagonists are a family of medications that block the action of this receptor. All 5-HT3 antagonists are antiemetics, used for the prevention and treatment of nausea and vomiting; the exceptions being alosetron and cilansetron, which are used for the treatment of irritable bowel syndrome. For the treatment of cancer, they are the "gold standard" in preventing the nausea and vomiting that often accompany chemotherapy. The underlying genetics of alcoholism make it a main, chronic illness [13-16].

One and a half million Americans seek help for alcoholism or are hospitalised to a regular hospital each year as a direct result of alcohol dependence's negative health effects. Alcohol withdrawal affects these individuals and a sizable population who abstain from alcohol without medical assistance. Clinically, Alcohol Withdrawal (AW) is a condition that manifests in formerly heavy drinkers who suddenly cut down or abstain from alcohol. Constant alcohol exposure causes the CNS to adapt, mitigating the negative effects of the drug on brain function and neuronal transmission (i.e., neurons). As a result, the brain stays in a hyperactive or hyperexcited state when alcohol consumption is abruptly reduced, leading to withdrawal symptoms [17-19].

Clinical signs and severity of AW syndrome vary widely among alcoholics. Insomnia is the mildest of these manifestations1, although delirium tremens (DTs) and death are other possible outcomes. The frequency with which symptoms appear also varies considerably between different drinkers. Alcoholics who drink often may never experience withdrawal symptoms. However, some alcoholics may experience withdrawal symptoms at blood alcohol concentrations (BACs) that would be intoxicating in nonalcohol-dependent persons, but which for the dependent patients reflect a drop from their regular BACs [20-27].

Seizures and delirious tremors are common features of the withdrawal syndrome, which may progress to excite-neurotoxicity.Alcohol and other sedative-hypnotics are notorious for producing physical dependency. To put it another way, alcoholism is a disease of neuro-adaptation. When alcohol levels in the blood drop too low, a withdrawal syndrome sets in that may be treated by drinking again or using a cross-tolerant drug. In the same way that other sedative-hypnotic medications cause neuropsychiatric excitability and autonomic abnormalities, so does alcohol withdrawal. When combined with dependency on other sedative-hypnotics, withdrawal may be quite uncomfortable [28-31].

Neurochemical changes in alcohol withdrawal

Due to variations in the levels of various neurotransmitters including dopamine, adrenaline, acetylcholine, GABA, glutamate, etc., alcohol withdrawal causes a wide range of neurochemical disturbances [32].

Glutamate

When compared to the first cycle of AW, the third cycle of AW results in a substantially larger rise in glutamate levels in the brain. Subsequent withdrawal episodes were more severe because of the increased glutamate produced in the hippocampus during the first cycle of AW [33].

GABA

Both GABA and its receptors were impacted by alcohol use. As an inhibitory neurotransmitter, GABA works to reduce neural activity. Reduced focus, memory loss, emotional instability, and fatigue are all possible side effects of an overabundance of GABA. The modulation of the receptors and the catecholaminergic and GABAminergic processes that govern ethanol-induced motor impairment are both under careful control [34].

Serotonin

The neurotransmitter serotonin is originally named enteramine, but is now more often known by its more modern name. That both compounds were composed of 5-hydroxytryptamine was proven by its entire production in the early 1950s 5-hydroxytryptamine (5-HT). Subsequently, serotonin (5-HT) was found in the central nervous system (CNS) of animals and later in the mid-1950s, it was found in a wide variety of plants. The function of neurotransmitters in the brain was hypothesised. Both central and peripheral physiological responses were linked to 5-HT. Primitive data revealed it may have a role in vasoconstriction and vasodilation, control of core body temperature, regulation of sleep and hormones, and even sadness. Scientists were curious in 5-HT because of its molecular similarities to (+)-lysergic acid diethylamide (LSD), a hallucinogenic drug that had just been discovered at the time. This finding sparked

concern that 5-HT has a function in both the mechanism of action of certain psychoactive compounds and in the development of certain mental diseases [35].

Serotonin receptors

5-HT receptors were originally proposed to be the points of contact between 5-HT and its supposed targets. Serotonin (5-HT) receptors have been studied extensively and have been broken down into several subpopulations. Currently, seven families or populations of serotonergic receptors have been found (5-HT1through 5-HT7). There is more information about 5-HT1 and 5-HT2 receptors than there is about 5-HT6 and 5-HT7 receptors since their discovery roughly followed the order of their numbering. The paucity of agonists and/or antagonists with selectivity for particular populations of 5-HT receptors (such as 5-HT 1E or 5-HT5 receptors) contributes to our present inability to comprehend the function of these receptors [35].

5-HT Receptor Agonists

Although many tryptamines with a 5-HT connection bind strongly to 5-HT1A receptors, these compounds are seldom selective. The early discovery of the amino tetralin derivative 8-hydroxy- 2-(di-n-propyl amino) tetralin (8-OH DPAT), one of the most selective 5-HT1A receptor agonists, was crucial to the development of our current knowledge of these receptors. Additionally, 8-OH DPAT's activity at the 5-HT1A receptor suggested that an intact indole nucleus was not necessary for the receptor's action, which is supported by the structural similarity between 8-OH DPAT and 5-HT [36-39].

Role of 5-HT agonist

Chemical messengers known as neurotransmitters facilitate interaction and communication between neurons (i.e., neurons). Serotonin, sometimes called 5-hydroxytryptamine, is a neurotransmitter utilised by many neurons in the brain (5-HT). With a process known as neuromodulation, the signal-emitting neuron's production of serotonin affects the behaviour of the signal-receiving neurons in a subtle way. Specifically, serotonin may control the release of other neurotransmitters in certain neurons while also affecting the pace at which those neurons generate the electrical impulses (i.e., action potentials) necessary to transfer information inside the cells. Although serotonin's impact on individual neurons may be very minimal, its effect on the neurons in a specific brain region can greatly alter brain processes including learning and memory, perception of the environment, mood states, and reactions to alcohol and other drugs of abuse [40-43].

Mechanisms of alcohol withdrawal

Multiple processes have been proposed throughout time to explain the origin (i.e. genesis) of AW. Some withdrawal effects (such seizures) were formerly assumed to be directly attributable to alcohol usage or drunkenness, for example. Overwhelming scientific and clinical data currently demonstrates that the constellation of signs and symptoms characterised as AW are induced by halting the ongoing exposure of the CNS to alcohol, even though alcoholic individuals display multiple metabolic and nutritional abnormalities.An early research of individuals who received high doses of alcohol daily supported the concept that withdrawal occurs as a consequence of "insufficient"alcohol consumption or abstinence un dependent patients, rather than due of nutritional inadequacies. Each of the well-fed individuals in the research drank the equivalent of over 30 standard drinks each day throughout the duration of the trial. These males always showed signs of withdrawal when they tried to cut down on their alcohol use. What's more, the severity of AW symptoms like hallucinations, seizures, and DT's depended on how much alcohol a person had ingested [44-49].

These results strengthen the hypothesis that alcohol use is linked to the development of withdrawal syndrome. The mechanics of withdrawal may be better understood by reviewing the basic concepts of central nervous system (CNS) neuronal transmission. In most cases, tiny chemicals termed neurotransmitters, released by the signal-emitting neuron, are responsible for the transfer of nerve impulses from one neuron to the next. Across the narrow space (synapse) between two neurons, neurotransmitter molecules travel to meet their receptor molecule counterparts on the receiving neuron. When a neurotransmitter binds to a receptor, a series of chemical and electrical events is set in motion inside the cell receiving the signal, which may either activate or inhibit the cell depending on the neurotransmitter involved [50, 51],

Consequently, the neuron receiving the signal is stimulated by excitatory neurotransmitters (such as glutamate) and inhibited by inhibitory neurotransmitters (such as gamma-aminobutyric acid [GABA]). A delicate equilibrium between excitatory and inhibitory forces is maintained under normal settings. Consistent alcohol use has a wide-ranging effect on the brain's excitatory and inhibitory neurotransmitter systems. Similarly, it's likely that numerous different neurotransmitters and pathways contribute to AW. Specifically, learn how GABA and glutamate work in the brain. Alcohol, for instance, has been shown to amplify the inhibitory effects of GABA on signal-receiving neurons, reducing neuronal activity. However, GABA receptors become less sensitive to the neurotransmitter with prolonged alcohol exposure, necessitating more alcohol concentrations to provide the same amount of suppression [51].

Pathophysiology

Long-term alcohol abuse causes changes to the brain's neurochemical systems, especially the GABAergic system.

Numerous changes take place during adaptation, such as altered gene expression and decreased GABAA receptor activation. Changes occur during acute alcohol withdrawal, such as an uptick in alpha4 containing GABAA receptor expression and a reduction in alpha1 and alpha3 containing GABAA receptor expression. It's possible that the neurochemical changes brought on by alcohol withdrawal may be mitigated with the use of drugs used in rapid detox. When alcohol and tolerant medicines are discontinued, these neurochemical changes often go back to normal. Changes in the N-mrthyl-D-aspartet receptor (NMDA) system brought on by chronic alcohol intoxication have a role in the central nervous system's hyper-excitability during alcohol withdrawal. High levels of homocysteine, which may lead to xcitoneurotoxicity, are further elevated after alcohol withdrawal. There is evidence linking quitting too soon to abnormalities in the electrocardiogram (ECG), namely an increase in interval, and the electroencephalogram (EEG). Abstaining from alcohol for any length of time causes the hypothalamic-pituitary-adrenal axis to shift, leading to increased production of corticotropin-releasing hormone and other alterations. A shortage of dopamine may underlie withdrawal's failure to provide short-term pleasure or euphoria [52].

There are hundreds of subclasses of serotonin receptors, which have been categorised from the seven main classes. We are aware of their whereabouts, activities, and origins, as well as the obstacles standing in their way. To far, several mood and behaviour diseases, as well as mental and psychological abnormalities, have been connected to certain subtypes of these serotonin receptors. Several potential medications for treating stress have been the subject of animal studies, and their results have been discussed. Some of these drugs have demonstrated promising results in human studies. In this review, we discussed the multiple functions of serotonin, its production and metabolism, the importance of serotonin receptors and ligands in the regulation of stress, and the most recent findings about these receptors and their relevance. It's quite likely that, in the not-too-distant future, appropriate drugs will be discovered to cure all illnesses induced by stress [52-54].

Animal models of alcohol withdrawal

To further describe and quantify the withdrawal syndrome, scientists have experimented with inducing physical reliance in animals via a variety of means. The most often used methods are ingesting alcohol via a nutritionally balanced liquid diet, inhaling alcohol vapour, or receiving repeated daily doses of alcohol through injection or a tube placed down the throat (i.e., intubation). While there are pros and cons to each method, they all share the goal of maintaining blood alcohol concentrations (BACs) within a very narrow range over time.Animals suffering from alcohol withdrawal may exhibit tremors, motor dysfunction, and autonomic overactivity. Seizures are a well researched symptom. Convulsions may occur on their own or be triggered by human interaction, depending on the intensity of the withdrawal.

2. METHODOLOGY

Animals may be become physically dependent on alcohol by a variety of methods, including a balanced diet. We'll start with Gavage and we'll lock up the bottle for good measure. Seen the signs of alcoholism directly.

Animal

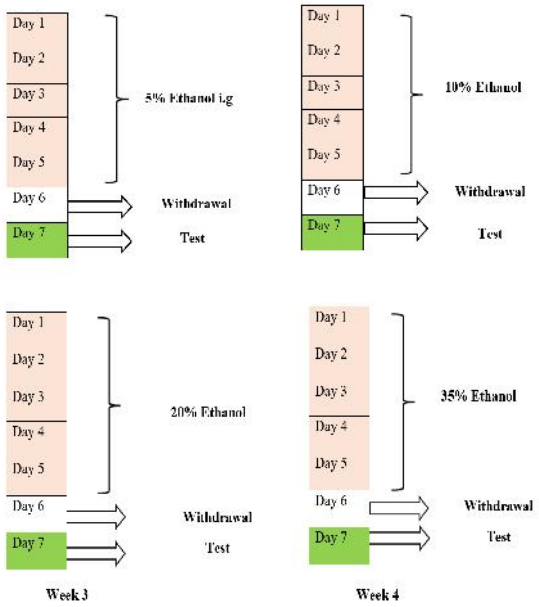

Fig 1: Mice between the ages of 6 and 8 weeks old were housed in a room with a constant temperature of 21–23 degrees Celsius and a light/dark cycle of 12 hours.

Mice were provided with all of their nutritional needs, with the exception of the brief period that they were taken from their cages for the experiments (Figure 1). All animal procedures were reviewed and authorised by the IAEC and followed the CPSCEA's criteria for the use and care of live animals. Ethical standards for animal research were strictly adhered to throughout all processes and animal handlings.

Grouping of animals

Table 1: Each model will include 8 separate groups, each with 6 animals.

MethodGroup 1: Vehicle control.	(Normal Drinking Water in bottle along with 0.2ml water i.g for day 1-5 followed by normal drinking water in water bottle till day 28)
Group 2: Standard per se.	(Normal Drinking Water in bottle along with 0.2ml water i.g for day 1-5 followed by normal drinking water in water bottle till day 28)
Group 3: Test drug per se.	(Normal Drinking Water in bottle along with 0.2ml water i.g for day 1-5 followed by normal

	drinking water in water bottle till day 28)
Group4:Negative Control	(5% v/v ethanol in water bottle along with 0.2 ml of 5% v/v ethanol i.g. for day 1 to 5 followed by 10% v/v ethanol in water bottle for day 8 to 12, 20% v/v ethanol in water bottle for day 15 to 19 and 35% v/v ethanol in water bottle for day 22 to 26. On day 6, 7, 13, 14, 20, 21, 27 and 28, normal drinking water in water bottle will be presented to animals.)
Group 5: Positive Control	(5% v/v ethanol in water bottle along with 0.2 ml of 5% v/v ethanol i.g. for day 1 to 5 followed by 10% v/v ethanol in water bottle for day 8 to 12, 20% v/v ethanol in water bottle for day 15 to 19 and 35% v/v ethanol in water bottle for day 22 to 26. On day 6, 7, 13, 14, 20, 21, 27 and 28, normal drinking water in water bottle will be presented to animals.)
Group 6: ALCW + Sumatriptan Benzoate 20 mg/kg	(5% v/v ethanol in water bottle along with 0.2 ml of 5% v/v ethanol i.g. for day 1 to 5 followed by 10% v/v ethanol in water bottle for day 8 to 12, 20% v/v ethanol in water bottle for day 15 to 19 and 35% v/v ethanol in water bottle for day 22 to 26. On day 6, 7, 13, 14, 20, 21, 27 and 28, normal drinking water in water bottle will be presented to animals.)
Group 7: ALCW + Sumatriptan 40 mg/kg	(5% v/v ethanol in water bottle along with 0.2 ml of 5% v/v ethanol i.g. for day 1 to 5 followed by 10% v/v ethanol in water bottle for day 8 to 12, 20% v/v ethanol in water bottle for day 15 to 19 and 35% v/v ethanol in water bottle for day 22 to 26. On day 6, 7, 13, 14, 20, 21, 27 and 28, normal drinking water in water bottle will be presented to animals.)
Group 8: ALC + Sumatriptan 20 mg/kg + Diazepam 1mg/kg	(5% v/v ethanol in water bottle along with 0.2 ml of 5% v/v ethanol i.g. for day 1 to 5 followed by 10% v/v ethanol in water bottle for day 8 to 12, 20% v/v ethanol in water bottle for day 15 to 19 and 35% v/v ethanol in water bottle for day 22 to 26. On day 6, 7, 13, 14, 20, 21, 27 and 28, normal drinking water in water bottle will be presented to animals.)

Procurements of Chemicals & Drug

Absolute alcohol from Sigma Aldrich, Sumatriptan Benzoate Procured from Carbanio Diazepam Procured from Carbanio.

Various Parameters Used to Study Alcohol dependency as in Table 1.

Elevated plus maze (EPM)

The testing environment is plus-shaped equipment with two open arms and two enclosed arms, all of which have open roofs and are raised between 40 and 70 centimetres from the ground. The fear of open areas among rodents serves as the basis for this paradigm [55].

Open field test (OFT)

A Circular Open Field Developed by Calvin S. Hall to examine the emotionality of rats, the open field test (OFT) is a regularly used qualitative and quantitative measure of general locomotors activity and readiness to explore in rodents.

Elevated zero maze (EZM)

The EZM is a raised circular apparatus in which two quadrants of a circular arena are surrounded by elevated arms, while the other two quadrants remain open. Rats will be put at the entrance to a closed quadrant of the raised zero maze (wall height 20.5 cm, outside circumference 119.4 cm, platform width 10.2 cm, floor to apparatus 63.5 cm) (wall height 20.5 cm, outer diameter 119.4 cm, platform width 10.2 cm, floor to apparatus 63.5 cm). Room lights will switch off and the labyrinth will be lighted from above. Time spent in the closed or open quadrants had to measure above 5 min. Anxiety-like behaviour is connected with greater time in the closed quadrants.

Western blot analysis

Protein will be extracted from dorsal or ventral hippocampi (vHC) from Swiss albino mices. Animals will be selected to represent the greater and lower ethanol self-administration people from each group. dHC and vHC were homogenised in homogenization buffer (50mM Tris-Base, pH 7.35; 20 mM HEPES, pH 7.4; 5 mM EDTA, pH 8.0; protease and phosphatase inhibitor cocktail [Halt™, Thermo Fisher]). Synaptoneurosomes prepare as previously describe in. Samples were dissolved in Sodium Dodecyl sulphate (SDS) sample buffer and resolve on a 4e20% gradient SDS polyacrylamide gel (Bio-Rad) and transferred to nitrocellulose for western blot analysis. Antibodies use for western blot analysis will mouse anti-actin, and fluorescent secondary antibodies. Blots will image using LICOR Odyssey CLx infrared imaging system.

3. RESULTS AND DISCUSSION

Elevated Plus Maze

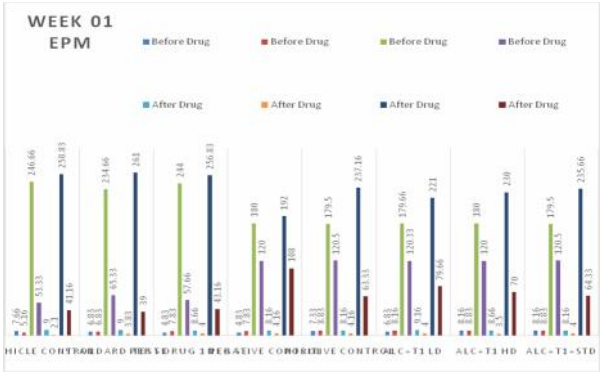

Fig 2: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate

treatment at withdrawal close arm time is more as compare to open arm and vice versa after sumatriptan benzoate.

First Three groups are not showing any differences after that the anxiety decreases. All the values calculated by using ANOVA (Figure 2).

Value are mean± SD (n=6)

^{ns}P>0.05, *P<0.05, **P<0.01 when compared to negative control group.

$^{@}$P<0.001 when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant (p<0.001) increase in the Open arm time in negative control group as compared to positive control group. Whereas in ALC+T, LD treated group 6, 7 and 8 the number of Close arm entries and time spent in close arm get significantly decreased (p<0.01) compared to negative control group.

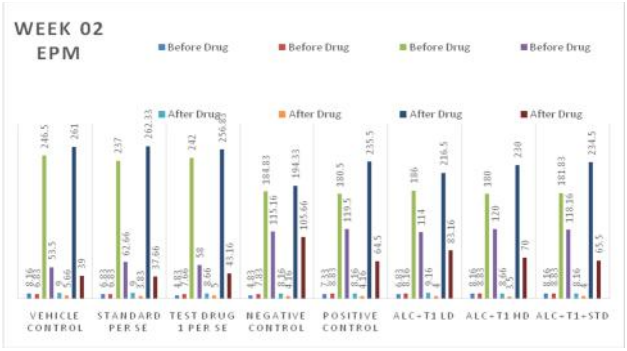

Fig 3: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to open arm and vice versa after sumatriptan benzoate.

First Three groups are not showing any differences after that the anxiety decreases. All the values calculated by using ANOVA (Figure 3).

Value are mean± SD (n=6)

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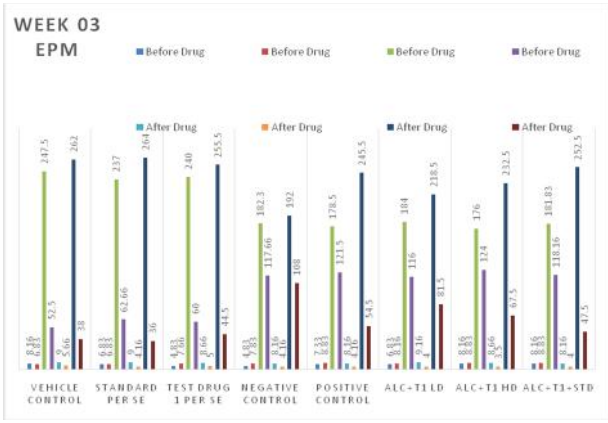

Fig 4: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to open arm and vice versa after sumatriptan benzoate.

First Three groups are not showing any differences after that the anxiety decreases. All the values calculated by using ANOVA (Figure 4).

Value are mean± SD (n=6)

^{ns}P>0.05, *P<0.05, **P<0.01 when compared to negative control group.

$^{@}$P<0.001 when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant (p<0.001) increase in the Open arm time in negative control group as compared to positive control group. Whereas in ALC+T, LD treated group 6, 7 and 8 the number of Close

arm entries and time spent in close arm get significantly decreased (p<0.01) compared to negative control group.

Elevated Zero Maze

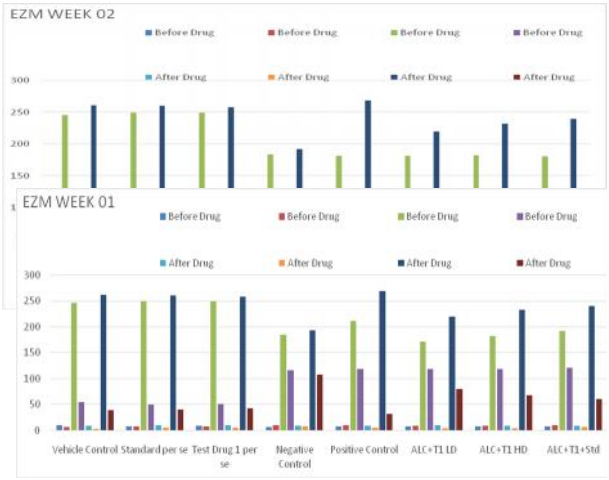

Fig 5: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to open arm and vice versa after sumatriptan benzoate.

First Three groups are not showing any differences after that the anxiety decreases. All the values calculated by using ANOVA (Figure 5).

Value are mean± SD (n=6)

^{ns}P>0.05, *P<0.05, **P<0.01 when compared to negative control group.

$^{@}$P<0.001 when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant (p<0.001) increase in the Open arm time in negative control group as compared to positive control group. Whereas in ALC+T, Low dose and High dose treated group 6, 7 and 8 the number of Close arm entries and time spent in close arm get significantly decreased (p<0.01) compared to negative control group.

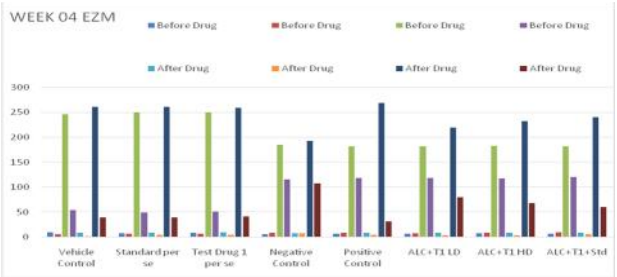

Fig 6: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to open arm and vice versa after sumatriptan benzoate. First Three groups are not showing any differences after that the anxiety decreases. All the values calculated by using ANOVA.

Value are mean± SD (n=6)

^{ns}P>0.05, *P<0.05, **P<0.01 when compared to negative control group (Figure 6).

$^{@}$P<0.001 when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant (p<0.001) increase in the Open arm time in negative control group as compared to positive control group. Whereas in ALC+T, Low dose and High dose treated group 6, 7 and 8 the number of Close arm entries and time spent in close arm get significantly decreased (p<0.01) compared to negative control group.

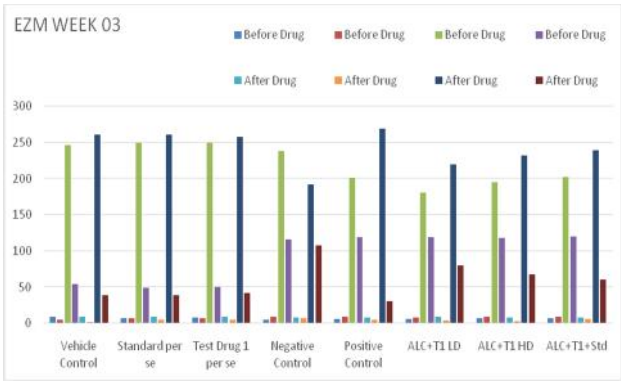

Fig 7: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to open arm and vice versa after sumatriptan benzoate.

First Three groups are not showing any differences after that the anxiety decreases. All the values calculated by using ANOVA (Figure 7).

Value are mean± SD (n=6)

^{ns}P>0.05, *P<0.05, **P<0.01 when compared to negative control group.

$^{@}$P<0.001 when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant (p<0.001) increase in the Open arm time in negative control group as compared to positive control group. Whereas in ALC+T, Low dose and High dose treated group 6, 7 and 8 the number of Close arm entries and time spent in close arm get significantly decreased (p<0.01) compared to negative control group.

The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare

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Value are mean± SD (n=6)

$^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$ when compared to negative control group.

$^{@}P<0.001$ when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant ($p<0.001$) increase in the Open arm time in negative control group as compared to positive control group. Whereas in ALC+T, Low dose and High dose treated group 6, 7 and 8 the number of Close arm entries and time spent in close arm get significantly decreased ($p<0.01$) compared to negative control group.

Open Field Test

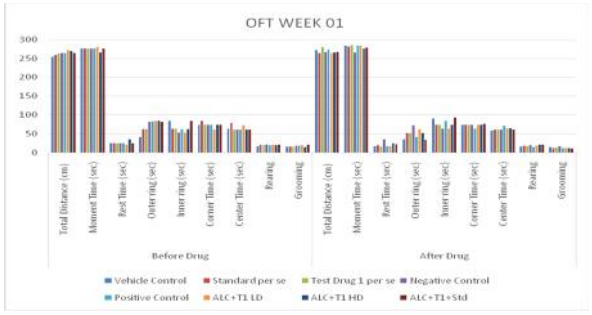

Fig 8: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to after sumatriptan benzoate.

First Three groups are not showing any differences like the time they spent before & after drug are same after that in sumatriptan at low doses show less effects on alcohol induced anxiety and with combination of diazepam it shows compromising effects on the anxiety. All the values calculated by using ANOVA (Figure 8).

Value are mean± SD (n=6)

$^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$ when compared to negative control group.

$^{@}P<0.001$ when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant ($p<0.001$) increase in the Inner ring time in negative control group as compared to positive control group. Whereas in ALC+SMT, Low dose and High dose treated group 6, 7 and 8 the Inner zone time increases and outer zone time get significantly decreased ($p<0.01$) compared to negative control group.

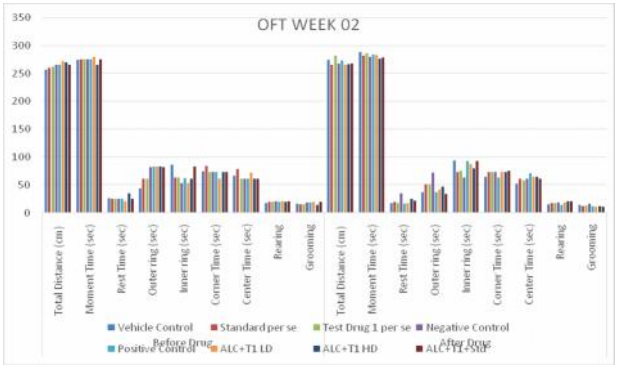

Fig 9: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to after sumatriptan benzoate.

First Three groups are not showing any differences like the time they spent before & after drug are same after that in sumatriptan at low doses show less effects on alcohol induced anxiety and with combination of diazepam it shows compromising effects on the anxiety. All the values calculated by using ANOVA (Figure 9).

Value are mean± SD (n=6)

$^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$ when compared to negative control group.

$^{@}P<0.001$ when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant ($p<0.001$) increase in the Inner ring time in negative control group as compared to positive control group. Whereas in ALC+SMT, Low dose and High dose treated group 6, 7 and 8 the Inner zone time increases and outer zone time get significantly decreased ($p<0.01$) compared to negative control group.

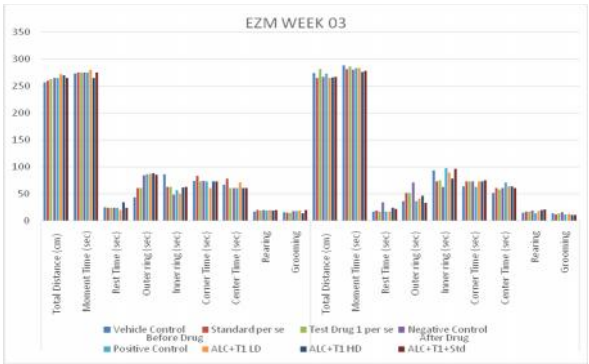

Fig 10: The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to after sumatriptan benzoate.

First Three groups are not showing any differences like the time they spent before & after drug are same after that in sumatriptan at low doses show less effects on alcohol induced anxiety and with combination of diazepam it shows compromising effects on the anxiety (Figure 10). All the values calculated by using ANOVA.

Value are mean± SD (n=6)

$^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$ when compared to negative control group.

$^{@}P<0.001$ when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant ($p<0.001$) increase in the Inner ring time in negative control group as compared to positive control group. Whereas in ALC+SMT, Low dose and High dose treated group 6, 7 and 8 the Inner zone time increases and outer zone time get significantly decreased ($p<0.01$) compared to negative control group.

The graph is indicating clear difference between before treatment at withdrawal phase and after Sumtriptan benzoate treatment at withdrawal close arm time is more as compare to after sumatriptan benzoate. First Three groups are not showing any differences like the time they spent before & after drug are same after that in sumatriptan at low doses show less effects on alcohol induced anxiety and with combination of diazepam it shows compromising effects on the anxiety. All the values calculated by using ANOVA.

Value are mean± SD (n=6)

$^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$ when compared to negative control group.

$^{@}P<0.001$ when compared to control

Above Graphs show the effect of sumatriptan on the alcohol withdrawal syndrome was found that there were significant ($p<0.001$) increase in the Inner ring time in negative control group as compared to positive control group. Whereas in ALC+SMT, Low dose and High dose treated group 6, 7 and 8 the Inner zone time increases and outer zone time get significantly decreased ($p<0.01$) compared to negative control group (Figure 11 and 12).

Western Blot

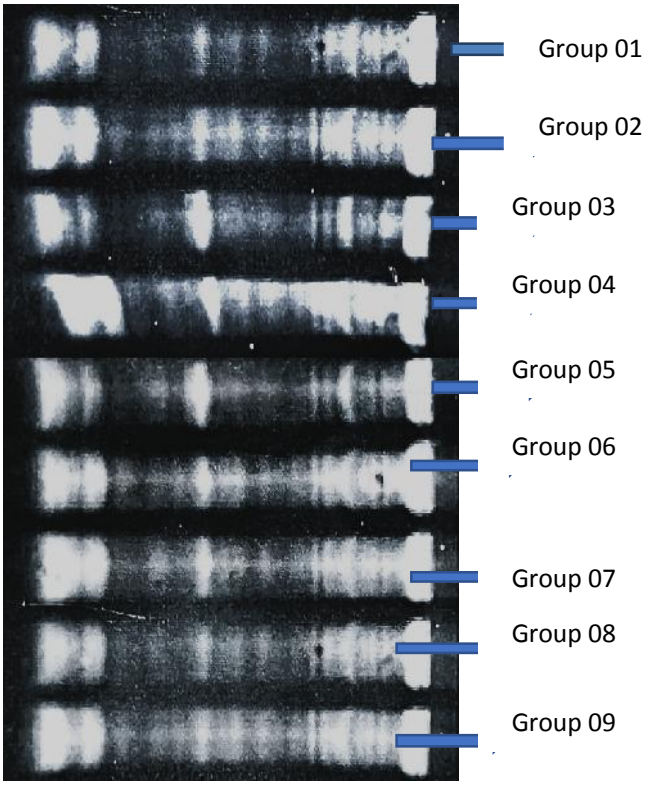

Fig 11: Determination of protein concentration of GluA1(Glutamate A1) and SK2 using Western blots.

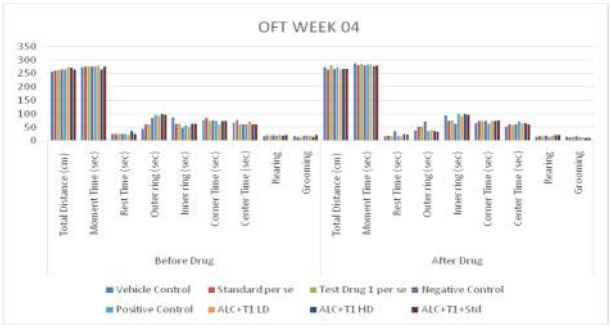

Fig 12: As per Data obtain after western blotting there is no change in Proteins.

4. CONCLUSION

As per the observation of findings suggested that Sumatriptan benzoate in functioning Fine with the Diazepam. The following graphs demonstrates that in EPM before drug and after drug it helps to lessen the alcohol withdrawal produced anxiety because animals spend more time on open after drug provided. On the other hand EZM is also displaying similar. In case of Open field test animals are spending less time in outside ring than the inside ring thus concluded that Sumatriptan benzoate performing well with alcohol induced anxiety. Western blot demonstrates that there is no any significant changes in the protein (Glu A1 & SK2) (Glu A1 & SK2).

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