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BAY 11-7085 ANDSU-6656 ATTENUATES ALCOHOL DEPENDENCE INDUCED SPONTANEOUS WITHDRAWAL SYNDROME LIKE HYPERALGESIA, CONVULSION AND DEPRESSION IN MICE

Ajeet Pal Singh [*1, 2], Ashish Kumar Sharma [1] and Thakur Gurjeet Singh [3]

Nims Institute of Pharmacy [1], Nims University, Jaipur - 303121, Rajasthan, India.
Academic Dean [2], St. Soldier Institute of Pharmacy, Jalandhar - 144011, Punjab, India.
Chitkara College of Pharmacy [3], Chitkara University, Rajpura - 140401, Punjab, India.

Keywords:

Src kinase, SU-6656, nuclear factor kappa B, BAY 11-7085, Withdrawal Severity Score (WSS), Hyperalgesia, Tail flick test, Convulsion, PTZ Kindling Seizures, Depression, Force Swim Test

Correspondence to Author:
Ajeet Pal Singh

Academic Dean,
NIMS Institute of Pharmacy,
NIMS University, Jaipur - 303121,
Rajasthan, India.

E-mail: ajeetakarpuria@gmail.com

ABSTRACT: The present study has been designed to investigate the effect of SU6656, a selective inhibitor of src kinase and BAY 11-7085, a selective nuclear factor kappa B inhibitor, as potential target in a mouse model of spontaneous alcohol dependence induced withdrawal syndrome. Our experimental protocol consisted of administration of Alcohol (2 g/kg, 10%, v/v, oral), once daily for 7 days. Assessment of behavioral parameters and exploratory parameters was done on 7 day after 8 hr. of the last ethanol administration for a period of 120 minutes (90 minutes for behaviour and exploratory parameters and 30 minutes for depression and hyper responsiveness parameter). Ethanol withdrawal behaviors were hyper excitability (seizures) and this hyper excitability was behaviorally present in terms of super sensitivity to sub convulsive dose of PTZ (30 mg/kg, i.p) a convulsant. Withdrawal syndrome was quantitated in terms of a composite withdrawal severity score, exploratory behavior which was confirmed by, reflective of depression like behavior by force swim test, hyperalgesia by tail flick test. SU-6656 (1. 5 & 10 mg/kg, i.p.) and BAY 11-7085 (3. 10 & 30 mg/kg, i.p.) treatment markedly and dose dependently ($p<0.05$) attenuated spontaneous alcohol withdrawal syndrome in mice measured in terms of withdrawal severity score, PTZ Kindling Seizures, hyperalgesia, depression. Thus, it is suggested that regulator of src kinase pathway and activation of nuclear factor kappa B pathway is involved in the development of alcohol withdrawal syndrome.

INTRODUCTION:

Alcoholism and Withdrawal Syndrome: Alcoholism is a primary, psychosocial, chronic disease with genetic and environmental factors altering its development and it is an also serious medical, social and economic problem [1]. According to WHO, it is as the third major cause for premature death and disabilities in the worldwide [2]. Around 2 billion people consume alcohol in the world; with 76.3 million are affected with AUDs (alcohol use disorders).

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Recently study showed that around 4% deaths of the world's adult population (about 3.3 million deaths each year), due to alcohol addiction and approximately, 5.1 % of the total Global Burden of Disease [3, 4]. Current report has shown that alcohol addiction also a global problem regarding road traffic accidents as well as other social issues like as child negligence, violence, absenteeism in the workplace, suicides, and breakdown of the family [5].

As toxic effects of alcohol damage all organs of the body, excessive alcohol use has serious health consequences to the individual and may lead to liver cirrhosis, neuropsychiatric diseases, cancer and cardiovascular diseases among other things [6, 7]. Furthermore, chronic used alcohol lead to alcohol addiction, with acute and chronic withdrawal syndrome including as, delirium tremens, seizures,

neuropsychiatric disturbances, Wernicke-Korsakoff syndrome, and cardiovascular complications [8]. In the brain, projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), by way of the medial forebrain bundle, compose a critical component of the mesolimbic pathway [9]. The rewarding effects of alcohol due to the activation of mesolimbic dopamine (DA) system [9], in which increase in DA in the nucleus accumbens (NAc) is thought to be crucial for rewarding effect. This system also activated by other abused drugs or substance such as cocaine; morphine and nicotine which have specific targets lead to addiction. Genetic polymorphism of genes encoding neurotransmitter signaling molecules in GABA, dopamine, serotonin and opioid systems have been established [10]. The dopamine D2 receptor gene is among the stronger candidate genes involved in alcohol addiction, most likely acting via incentive salience and craving mechanisms. Other studies show that $GABA_A$ [11], receptors also major target for alcohol reward effect and the precise mechanisms of alcohol abuse and dependence are still poorly understood [12]. However, alcohol affects neuronal functions by phospholipids membranes, receptors, various ion channels, synaptic and network functions, and intracellular signaling molecules [13].

NF-κB in Alcohol Dependency: It has been demonstrated that NMDA receptor antagonists block the development of tolerance and dependence to alcohol. Interestingly, NMDA receptor antagonists not only prevent but also reverse tolerance to alcohol after it has been established [14], indicating that NMDA receptor over activation is involved not only in the development but also in the maintenance of alcohol dependence. Moreover, protracted activation of the NMDA-type receptors has been shown to induce nuclear translocation and resultant activation of NF-κB via a calcium influx sensitive pathway. Moreover, some workers have demonstrated a potential role of NF-κB in an in vitro model of drug dependence. Therefore, it may be proposed that NF-κB over-activation might be playing a potential role in the pathogenesis of alcohol dependence [15].

Src Kinases and NF-κB: The Src Family kinases (SFKs): constitute a prominent group of non-receptor tyrosine kinases, with the Src kinase being the first identified member through research on the tumorigenic effects of retroviruses, specifically the Rous Sarcoma Virus. There are 10 known SFK members, with nine expressed in mammals (Src, Yes, Fgr, Fyn, Lyn, Hck, Lck, Blk, and Frk) and one (Yrk) exclusive to chickens [16]. These kinases play diverse roles in cellular activities such as transcription, apoptosis, differentiation, development, immunological response, and nervous system function. Dysregulation of Src kinase has been linked to cancer, sparking significant interest in this enzyme family. The structure of SFKs includes a catalytic tyrosine-protein kinase domain (SH1), two SH2 and SH3 domains, and a myristoyl group in the N-terminal region. Activation of SFKs involves complex processes influenced by cell type and extracellular cues, with the SH2/3 domains playing a key role [17]. The biological functions of SFKs encompass a wide range of cellular processes [18] and dysregulation of Src family kinases (SFKs) carries implications for various disorders, prominently cancer, as well as conditions like diabetes, hypertension, tuberculosis, and inflammation [19]. Src family of tyrosine kinases represents an important transducer system that mediates various physiological processes in the brain cells.

Src is a reported activator of NMDA receptor functioning through direct phosphorylation of tyrosine residues in the NR2A subunit of the NMDA receptors [20], which are in turn involved in effecting the causation and maintenance of alcohol dependence. Moreover, src-kinases have also shown to cause the inhibitory protein I-κB mediated activation of NF-κB [21].

Given the fact that literature indicates the role of NMDA receptor activation and the consequent stimulation of NF-κB in alcohol dependence, it might be interesting to find whether this activation of NMDA receptor-NF-κB transduction system that is aggravating alcohol dependence requires the biochemical participation of the src kinase family enzymes. Such a potential involvement of src-kinases in alcohol dependence is further supported by a study in which transcriptional profiling of alcohol treated neurons demonstrated the up regulation of c-src tyrosine kinase [22]. Therefore, src-kinases may be another good target in alcohol dependence.

MATERIAL & METHODS: Swiss albino mice of either sex weighing 25±2g were employed in the present study. They were housed in the departmental animal house and will be exposed to natural cycle of light and dark. The experimental protocol was duly approved by the institutional animal ethical committee and care of the animals will be carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 1181/PO/ab/08/CPCSEA).

Drugs and Chemicals: BAY 11-7085 (Sigma), SU-6656 (Sigma), Absolute ethanol 99.8% (Merck), diazepam (Calmpose-Ranbaxy Research Laboratories) and PTZ (Sigma, MO, USA) were purchased and used in the study. All chemicals were dissolved / diluted in sterile saline prepared in triple distilled water / 10% dimethylsulphoxide solution in triple distilled water as appropriate. The chemicals used were of analar quality and all drug solutions were freshly prepared before use.

Induction of Alcohol Dependence: The chronic intermittent ethanol exposure (CIE) regimen involves repeated periods of ethanol intoxication and withdrawal symptoms. Swiss albino mice housed in a vivarium under a 12:12 h light/dark cycle with free access to food and water were administered 2 g/kg ethanol as a 10% (v/v) solution in distilled water by oral intubation every day regimen for one week and then the animals were withdrawn for evaluation of a battery of various behavioral tests [23–25].

Experimental Protocol:

For NF-κB: Six groups were employed in the present study, with each group comprising of 6 animals out of which half were males and half females.

TABLE 1: EXPERIMENTAL DESIGN FOR NF-KB

S. no.	Groups	Treatment	Days
1.	Group I (Vehicle-vehicle control)	Vehicle (Saline, 10 ml/kg, i.p) for alcohol Vehicle (10% DMSO in water, 10 ml/kg, i.p.)	seven days
2.	Group II (Vehicle-Alcohol control)	Alcohol (2g/kg, oral) Vehicle (10% DMSO in water, 10 ml/kg, i.p.) for BAY 11-7085	seven days
3.	Group III (Alcohol–Diazepam control)	Alcohol (2 g/kg, oral) Diazepam (1 mg/kg, i.p.	seven days
4.	Group IV (BAY 11-7085 treatment +Alcohol)	Alcohol (2 g/ kg, oral) BAY 11-7085 (at a dose level of 3, mg/kg/d, i.p.)	seven days
5.	Group V(BAY 11-7085 treatment +Alcohol)	Alcohol (2 g/kg, oral) BAY 11-7085 (at a dose level of 10 mg/kg/d, i.p)	seven days
6.	Group VI(BAY 11-7085 treatment +Alcohol)	Alcohol (2 g/kg, oral) BAY 11-7085 (at a dose level of 30 mg/kg/d, i.p.)	seven days

For Src Kinase: Six groups were employed in the present study, with each group comprising of 6 animals out of which half were males and half females.

TABLE 2: EXPERIMENTAL DESIGN FOR SRC KINASE

S. no.	Groups	Treatment	Days
1.	Group I (Vehicle-vehicle control)	Vehicle (Saline, 10 ml/kg, i.p) for alcohol Vehicle (10% DMSO in water, 10 ml/kg, i.p.)	seven days
2.	Group II (Vehicle-Alcohol control)	Alcohol (2g/kg, oral) Vehicle (10% DMSO in water, 10 ml/kg, i.p.) for SU-6656	seven days
3.	Group III (Alcohol–Diazepam control)	Alcohol (2 g/kg, oral) Diazepam (1 mg/kg, i.p.)	seven days
4.	Group IV (SU-6656 treatment +Alcohol)	Alcohol (2 g/ kg, oral) SU-6656(at a dose level of 1 mg/kg/d, i.p.)	seven days
5.	Group V(SU-6656 treatment +Alcohol)	Alcohol (2 g/kg, oral) SU-6656(at a dose level of 5 mg/kg/d, i.p)	seven days
6.	Group VI(SU-6656 treatment +Alcohol)	Alcohol (2 g/kg, oral) SU-6656(at a dose level of 10 mg/kg/d, i.p.)	seven days

Parameters Used In Research:
Assessment of Withdrawal Severity Score (WSS):

TABLE 3: WITHDRAWAL SEVERITY SCORE

	Points
Tail stiffness (normal = 0; maximum = 3)	3
0 No paw tremors	
1 Mild increase in paw tremors	
2 Moderate increase in paw tremors	
3 Severe increase in paw tremors	
Cage scratching (normal = 0; maximum = 3)	3
0 Normal behavior	
1 Mild increase in the cage scratching behavior	
2 Moderate increase in the cage scratching behavior	
3 Severe increase in the cage scratching behavior	
Paw itching (normal = 0; maximum = 3)	3
0 No paw itching behavior	
1 Mild increase in paw itching behavior	
2 Moderate increase in paw itching behavior	
3 Severe increase in paw itching behavior	
Chewing (normal = 0; maximum = 3)	3
0 No chewing	
1 Mild increase in chewing	
2 Moderate increase in chewing	
3 Severe increase in chewing	
Extension (normal = 0; maximum = 3)	3
0 No Extension behavior	
1 Mild increase in Extension behavior	
2 Moderate increase in Extension behavior	
3 Severe increase in Extension behavior	
Body scratching (normal = 0; maximum = 3)	3
0 No body scratching behavior	
1 Mild increase in body scratching behavior	
2 Moderate increase in body scratching behavior	
3 Severe increase in body scratching behavior	
Maximum Points	18

A set of modified withdrawal severity score was employed to quantitate the magnitude of withdrawal syndrome in mice in terms of the earlier reported characteristic behavioral patterns seen in mice suffering from experimental alcohol withdrawal syndrome *viz*. tail stiffness, Cage scratching, paw itching, extensions, body scratching, chewing. The severity of the alcohol withdrawal phenomenon was graded on a scale of 0–18 (normal score, 0; maximal severity score, 18). In the severity scores of withdrawal, 0 score point is awarded for no change in the normal behavior of mice with respect to each observation criteria, 1 score point is awarded for a mild increase in the respective observation criteria in mice, 2 score point is awarded for a moderate increase in the respective observation criteria in mice, 3 score point is awarded for a severe increase in the respective observation criteria in mice. Thus, the higher the score, the more severe is the withdrawal syndrome. Behavioral observations were made for a period of 120 minute after the 8 hr of completing the dosing protocol [23, 25].

Measurement of the Effect of Drug Treatment(s) on Alcohol-induced Hyperalgesia using Tail Flick Test: However, sub-chronic administration of alcohol is documented to cause the induction of hyperalgesia which has been quantified in terms of tail flick test [26]. Therefore, nociceptive threshold was measured by the tail flick test in mice [27]. The tail flick latency was considered as the time between tail exposure to radiant heat and tail withdrawal. Electrically heated nichrome wire was used as a source of radiant heat in the analgesiometer. The intensity of radiant heat was regulated in order to obtain pretreatment latency between 2 and 3 sec in the animals. A cut off

latency time was fixed at 10 sec. Thus, tail flick latency was observed after 8 hr. of alcohol administration [26].

Assessment of Pentylenetetrazole Induced Seizure Intensity in Alcohol Dependent Mice: 8 hr after the last ethanol administration, seizure threshold was scaled in all the groups by control on administering a convulsive drug, PTZ with a sub convulsive dose of 30 mg/kg i.p. The behavior of the mice was recorded for 30 minutes after each administration. Animals entering convulsions showed convulsive waves axially through the body, myoclonic twitching and rearing, clonic convulsions of the forelimbs, generalized tonic-clonic seizure. The number of animals that developed convulsions and the percentage of animals that went into convulsions were calculated [28, 29].

Assessment of Depression using Force Swim Test: The Forced Swim test (FST) was performed according to the method described by Porsolt *et al.,* (1977) [30]. Animals were prescreened on the previous day by placing the animals individually in the five liter glass beakers, filled to a height of 15 cm with water (room temperature). After 5-6 min., immobility reaches a plateau where the rats remained immobile for approximately 80% of the time. After 15 minutes in the water, the mice were removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages. On the day of experiment, mice were again placed individually in the five liter glass beakers, filled to a height of 15 cm with water and the duration of immobility is recorded during the last four minutes of a six minutes test. A mouse is considered immobile when floating motionless or makes only the movements necessary to maintain the head above water surface. The water is changed after each test. Antidepressants decrease the immobility time [30–32].

Statistical Analysis: All the results were expressed as mean ± standard error of mean (S.E.M.). Data of the results was analyzed using ANOVA followed by post-hoc comparison using Sheffe's multiple range test. For elevated plus maze, the numbers of entries and time (in seconds) spent in both arms was compared in saline- and nicotine-treated mice. A value of $P<0.05$ was considered to be statistically significant. The statistical analysis was done using Sigma Stat 6.0 software.

RESULTS:

Results of NF-κB:

Effect of BAY 11-7085 on alcohol induced Hyperalgesia in Mice: Sub-chronic administration of alcohol is documented to cause the induction of hyperalgesia which has been quantified in terms of tail flick test (Dina *et al.,* 2007). Therefore, nociceptive threshold was measured by the tail flick test in mice (D'Amour and Smith, 1941). After chronic intermittent administration of alcohol (2 g/kg, oral) once daily for a period of 7 days, produced significant hyperalgesia, as indicated by decreased tail-flick latencies ($P <0.05$), when compared to vehicle control group. Moreover, pair-wise comparisons confirmed that administration of BAY 11-7085 (3, 10 & 30 mg/kg, i.p.) significantly ($p<0.05$ each) and dose dependently attenuated alcohol induced hyperalgesia measured in terms reversal of withdrawal induced decrease in the tail flick latency (Table 3 & Fig. 1).

Effect of BAY 11-7085 on PTZ kindled seizures in Ethanol Withdrawal Mice: In normal mice a sub convulsive dose of PTZ (30 mg/kg i.p) has not shown any convulsions (0% convulsion). Whereas PTZ administration after 7 days of Ethanol treatment in mice has shown convulsion in the Ethanol treated animals. Ethanol + Diazepam (1mg/Kg) and Ethanol + BAY 11-7085 (3, 10 & 30 mg/kg, i.p.) showed significantly ($P <0.05$) and dose dependently increase in seizure threshold. Administration of BAY 11-7085 (3, 10 & 30 mg/kg, i.p.) significantly ($P <0.05$) and dose dependently attenuated PTZ Kindled seizures in alcohol dependent mice (Table 4 & Fig. 2).

Effect of BAY 11-7085 on force swim test in Ethanol Withdrawal Mice: Compared to control, Ethanol + Diazepam (1mg/kg) ($P <0.05$) and Ethanol + BAY 11-7085 (3, 10 & 30 mg/kg, i.p.) ($P<0.01$) have produced significantly ($p<0.01$) and dose dependently decrease in immobility time in the FST. But compared to Ethanol group, all the other groups (Ethanol + Diazepam (1 mg/Kg), Ethanol + BAY 11-7085 (3, 10 & 30 mg/kg, i.p.) have decreased the immobility time ($P<0.001$) (Table 5 & Fig. 3).

TABLE 3: EFFECT OF BAY 11-7085 ON TAIL FLICK LATENCY (SEC.) IN ALCOHOL DEPENDENT/VEHICLE TREATED MICE

Group	Treatment	TFL
1	VEH-VEH	7.6 ±
2	ALC-VEH	2.2 ± 2.2
3	ALC-DIA	7.8 ± 3.3[a]
4	ALC-BAY11-7085 (3mg/kg, i.p.)	3.4 ±2.9[b]
5	ALC-BAY11-7085 (10mg/kg, i.p.)	5.2 ±3.2[b]
6	ALC-BAY11-7085 (30mg/kg, i.p.)	6.6 ±2.8[b]

Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, BAY 11-7085(3, 10 & 30 mg/kg, i.p.) [Values are mean ± S.E.M.] a =*P*<0.05 vs. VEH-VEH control; b =*P*<0.05 vs. ALC-VEH.

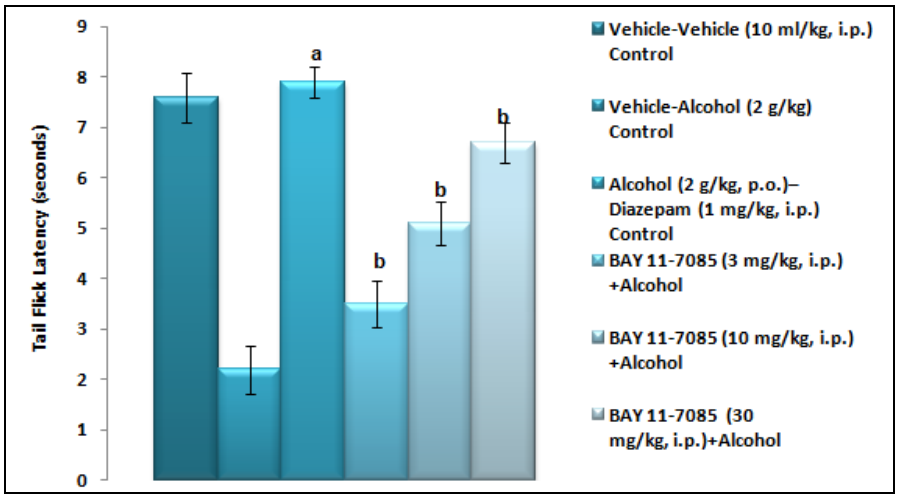

FIG. 1: EFFECT OF BAY 11-7085 ON TAIL FLICK LATENCY (SEC.) IN ALCOHOL DEPENDENT/VEHICLE TREATED MICE

TABLE 4: EFFECT OF BAY 11-7085 ON PERCENTAGE OF ANIMALS CONVULSED UPON ADMINISTRATION OF PENTYLENETRAZOL (30 mg/kg) TO CONTROL, ETHANOL (2 g/kg)

Group	Treatment	% Convulsed
1	VEH-VEH	-
2	ALC-VEH	99.8 ± 2.2
3	ALC-DIA	-[a]
4	ALC-BAY11-7085 (3mg/kg, i.p.)	48.2 ±2.9[b]
5	ALC-BAY11-7085 (10mg/kg, i.p.)	32.2 ±3.2[b]
6	ALC-BAY11-7085 (30mg/kg, i.p.)	14.2 ±2.8[b]

Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, BAY 11-7085(3, 10 & 30 mg/kg, i.p.) [Values are mean ± S.E.M.] a =*P*<0.05 vs. VEH-VEH control; b =*P*<0.05 vs. ALC-VEH.

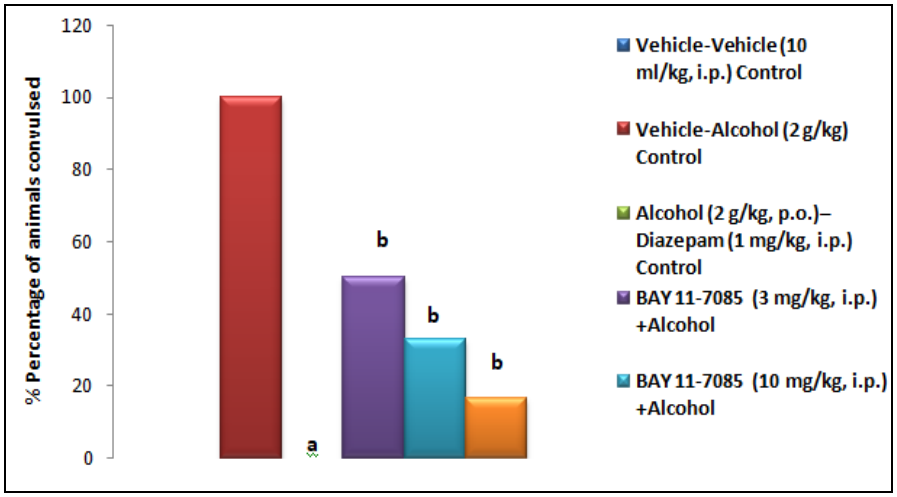

FIG. 2: EFFECT OF BAY 11-7085 ON PERCENTAGE OF ANIMALS CONVULSED UPON ADMINISTRATION OF PENTYLENETRAZOL (30 mg/kg) TO CONTROL, ETHANOL (2 g/kg)

TABLE 5: EFFECT OF BAY 11-7085 ON IMMOBILITY TIME (SEC.) IN ALCOHOL DEPENDENT/ VEHICLE TREATED MICE

Group	Group	Immobility
1	VEH-VEH	121.8±3.4
2	ALC-VEH	82.8 ± 2.2
3	ALC-DIA	54.4 ± 3.3[a]
4	ALC-BAY11-7085 (3mg/kg, i.p.)	98.8 ±2.9[b]
5	ALC-BAY11-7085 (10mg/kg, i.p.)	92.2 ±3.2 [b]
6	ALC-BAY11-7085 (30mg/kg, i.p.)	62.8 ±2.8 [b]

Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, BAY 11-7085(3, 10 & 30 mg/kg, i.p.) [Values are mean ± S.E.M.] a =*P*<0.05 vs. VEH-VEH control; b =*P*<0.05 vs. ALC-VEH.

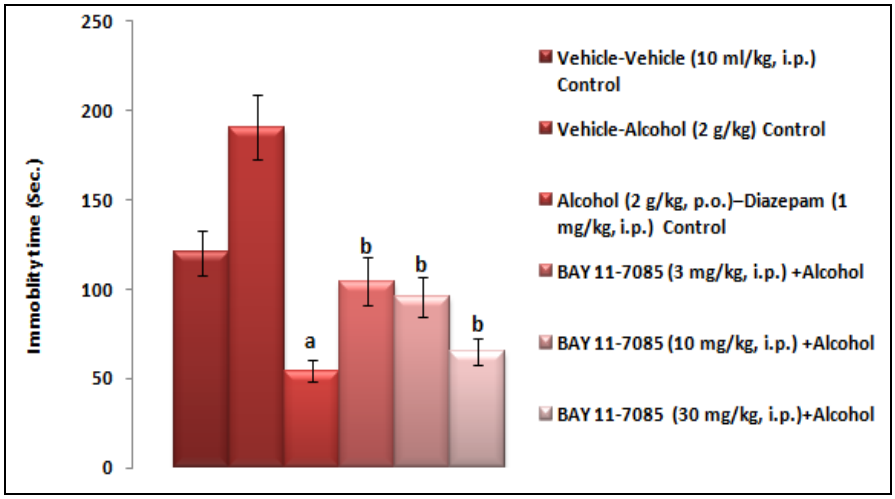

FIG. 3: EFFECT OF BAY 11-7085 ON IMMOBILITY TIME (SEC.) IN ALCOHOL DEPENDENT/ VEHICLE TREATED MICE

Results of SU-6656 SRC kinase:

Effect of Various Treatment(s) on Alcohol Induced Hyperalgesia in Mice: Sub-chronic administration of alcohol is documented to cause the induction of hyperalgesia which has been quantified in terms of tail flick test (Dina *et al.*, 2007). Therefore, nociceptive threshold was measured by the tail flick test in mice (D'Amour and Smith, 1941). After chronic intermittent administration of alcohol (2 g/kg, oral) once daily for a period of 7 days, produced significant hyperalgesia, as indicated by decreased tail-flick latencies (P <0.05), when compared to vehicle control group. Moreover, pair-wise comparisons confirmed that administration of SU-6656 (1, 5 & 10 mg/kg, i.p.) significantly (p<0.05 each) and dose dependently attenuated alcohol induced hyperalgesia measured in terms reversal of withdrawal induced decrease in the tail flick latency (Table 6 & Graph 4).

Effect of su-6656 on ptz kindled seizures in Ethanol Withdrawal Mice: In normal mice a subconvulsive dose of PTZ (30 mg/kg i.p) has not shown any convulsions (0% convulsion). Whereas PTZ administration after 7 days of Ethanol treatment in mice has shown convulsion in the Ethanol treated animals. Ethanol + Diazepam (1mg/Kg) and Ethanol + SU-6656 (1, 5 & 10 mg/kg, i.p.) showed significantly (P<0.05) and dose dependently increase in seizure threshold. Administration of SU-6656 (1, 5 & 10 mg/kg, i.p.) significantly (P<0.05) and dose dependently attenuated PTZ Kindled seizures in alcohol dependent mice (Table 7 & Graph 5).

Effect of su-6656 on force Swim Test in Ethanol Withdrawal Mice: Compared to control, Ethanol + Diazepam (1 mg/Kg) (P<0.001) and Ethanol + SU-6656 (1, 5, 10 mg/kg, i.p.) (P<0.05) have produced significantly (P<0.05) and dose dependently decrease in immobility time in the FST. But compared to Ethanol group, all the other groups (Ethanol + Diazepam (1 mg/Kg), Ethanol + SU-6656 (1, 5 & 10 mg/kg, i.p.) have decreased the immobility time (P<0.001) (Table 8 & Graph 6).

TABLE 6: EFFECT OF SU-6656 ON TAIL FLICK LATENCY (SEC.) IN ALCOHOL DEPENDENT/VEHICLE TREATED MICE

Group	Treatment	TFL
1	VEH-VEH	6.0±2.2
2	ALC-VEH	2.2±2.8
3	ALC- DIA	5.8±2.6[a]
4	ALC- SU-6656 (1 mg/kg, i.p.)	3.8±3.3[b]
5	ALC- SU-6656 (5 mg/kg, i.p.)	4.4±2.2[b]
6	ALC- SU-6656 (10 mg/kg, i.p.)	5.6±1.8[b]

Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are mean ± S.E.M.] a =*P*<0.05 vs. VEH-VEH control; b =*P*<0.05 vs. ALC-VEH.

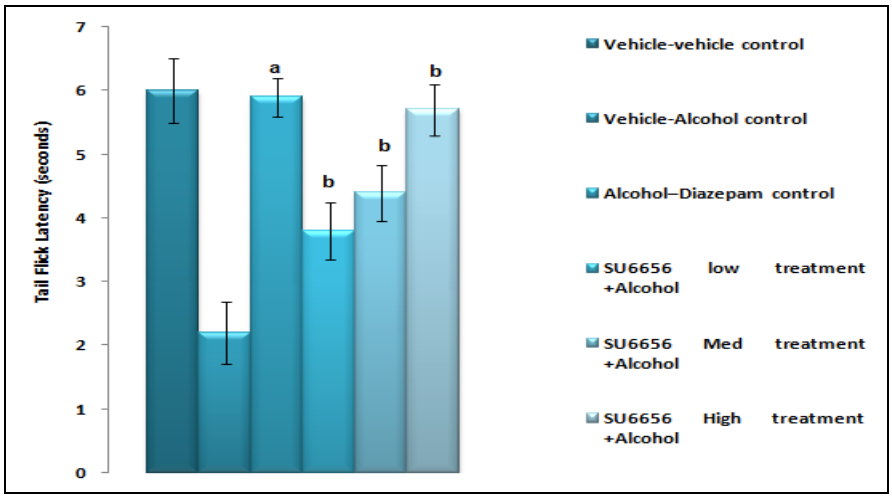

FIG. 4: EFFECT OF SU-6656 ON TAIL FLICK LATENCY (SEC.) IN ALCOHOL DEPENDENT/VEHICLE TREATED MICE

TABLE 7: EFFECT OF SU-6656 ON PERCENTAGE OF ANIMALS CONVULSED UPON ADMINISTRATION OF PENTYLENETRAZOL (30 mg/kg) TO CONTROL, ETHANOL (2 g/kg)

Group	Treatment	% Convulsed
1	VEH-VEH	0.0±0.0
2	ALC-VEH	100.2±2.8
3	ALC- DIA	0.0±0.0[a]
4	ALC- SU-6656 (1 mg/kg, i.p.)	50.2±3.3[b]
5	ALC- SU-6656 (5 mg/kg, i.p.)	30.2±2.2[b]
6	ALC- SU-6656 (10 mg/kg, i.p.)	8.2±1.8[b]

Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are mean ± S.E.M.] a =*P*<0.05 vs. VEH-VEH control; b =*P*<0.05 vs. ALC-VEH.

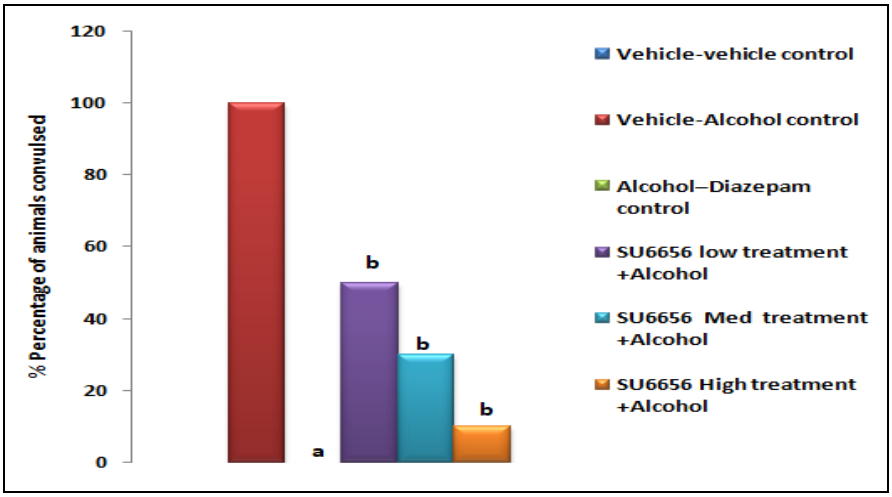

FIG. 5: EFFECT OF SU-6656 ON PERCENTAGE OF ANIMALS CONVULSED UPON ADMINISTRATION OF PENTYLENETRAZOL (30 mg/kg) TO CONTROL, ETHANOL (2 g/kg)

TABLE 8: EFFECT OF SU-6656 ON IMMOBILITY TIME (SEC.) IN ALCOHOL DEPENDENT/ VEHICLE TREATED MICE

Group	Treatment	Immobility
1	VEH-VEH	120.2±2.2
2	ALC-VEH	185.2±2.8
3	ALC- DIA	68.7±2.6[a]
4	ALC- SU-6656 (1 mg/kg, i.p.)	132.2±3.3[b]
5	ALC- SU-6656 (5 mg/kg, i.p.)	105.2±2.2[b]
6	ALC- SU-6656 (10 mg/kg, i.p.)	83.8±1.8[b]

Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are mean ± S.E.M.] a =*P*<0.05 vs. VEH-VEH control; b =*P*<0.05 vs. ALC-VEH.

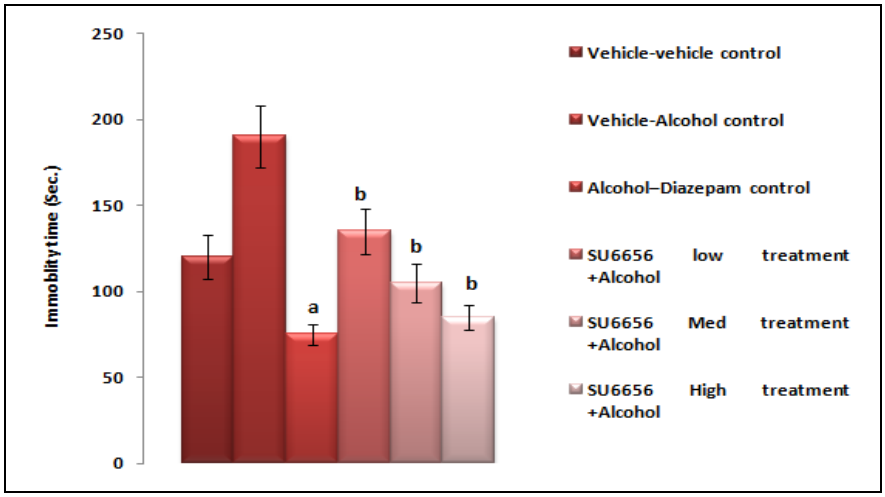

FIG. 6: EFFECT OF SU-6656 ON IMMOBILITY TIME (SEC.) IN ALCOHOL DEPENDENT/ VEHICLE TREATED MICE

SUMMARY: The present study has been designed to explore about the effect of SU-6656, a potent & selective inhibitor of src kinase and BAY 11-7085, a selective nuclear factor kappa B inhibitoron the development of alcohol withdrawal syndrome in mice. Sub-acute administration of alcohol (2 g/kg, oral) for 7 days was used to induce spontaneous alcohol withdrawal in mice. Behavioral observations exploratory parameters were done on 7 day after 8 hr. of the last ethanol administration for 120 minutes. Withdrawal syndrome was quantitatively assessed in terms of a composite withdrawal severity score, Wall climbing test and exploratory behavior which was confirmed by reflective of depression-like behavior by force swim test and withdrawal syndrome related anxiety was assessed according to the results of the elevated plus maze test. The key findings listed below may be summarized based on the outcomes of the current investigation.

Administration of Alcohol (2 g/kg, 20%, v/v, oral), once daily for 7 days. Assessment of behavioral parameters and exploratory parameters was done on 7 day after 8 hr. the last ethanol administration for a period of 120 minutes. Ethanol withdrawal behaviors were hyper excitability (seizures) and this hyper excitability was behaviorally present in terms of super sensitivity to sub convulsive doses of PTZ a convulsant. Precipitated withdrawal syndrome in mice which is reflected in a significant increase in withdrawal severity scores, body tremor, wall climbing, alcohol induced hyperalgesia by tail flick method and withdrawal syndrome related anxiety.

Administration of SU-6656, selective inhibitor of src kinaseand BAY 11-7085, a selective nuclear factor kappa B inhibitorsignificantly and dose dependently attenuated spontaneous ethanol withdrawal syndrome in alcohol dependent mice.

It is possible to derive the conclusion that Src kinase and nuclear factor kappa B inhibition slows the onset of alcoholism based on the spontaneous alcohol withdrawal symptoms seen in alcohol-dependent animals. Therefore, inhibitor of src kinase and nuclear factor kappa B may be

investigated as potential pharmaceutical treatments for the treatment of alcohol withdrawal syndrome.

CONCLUSION: Up regulation of Src kinase plays a important role in alcohol addiction, so pharmacological modulation of Src kinase with SU6656 a selective inhibitor, may play a prominent in pharmacotherapy in alcohol dependence induced withdrawal syndrome. Experimental approach: Administration of Alcohol (2 g/kg, 10%, v/v, oral), once daily for 7 days. Assessment of behavioral parameters and exploratory parameters was done on 7 day after 8 hr. of the last ethanol administration for a period of 120 minutes. Various behavioral parameters were conducted like composite withdrawal severity score, anxiety like behaviour assessed in open field and elevated plus test. Treatment with SU-6656 (1, 5 & 10 mg/kg, i.p.) markedly and dose dependently ($p<0.05$) attenuated spontaneous alcohol withdrawal syndrome in mice measured in terms of withdrawal severity score, locomotor sensitization by open field test and anxiety. Thus, it is suggested that activation of Src kinase pathway is involved in the development of alcohol dependence induced withdrawal syndrome. Modulation of src kinase may be used as therapeutic agent to overcome the problems related with alcohol dependence. In current study selective pharmacological modulation of Src kinase attenuates the spontaous withdrawal syndrome in rodent. Therefore, it may be assumed that src kinase pathway activation might be involved in mediating the precipitation of alcohol withdrawal syndrome, Convulsions, Hyperalgesia and depression in mice.

Up regulation of nuclear factor kappa B plays a prominent role in drug addiction. The current study explored the neuroprotective effect of BAY 11-7085, a selective nuclear factor kappa B inhibitor, on the spontaneous alcohol withdrawal syndrome in mouse model of alcohol addiction. Administration of Alcohol (2g/kg, 10%, v/v, oral), once daily for 7 days. Assessment of behavioral parameters and exploratory parameters was done on 7 day after 8 hr. of the last ethanol administration for a period of 120 minutes. Various behavioral parameters were conducted like wall climbing test, composite withdrawal severity score, anxiety like behaviour assessed in open field and elevated plus test. Treatment with BAY 11-7085 markedly and dose dependently ($p<0.05$) attenuated spontaneous alcohol withdrawal syndrome in mice measured in terms of withdrawal severity score, wall climbing, locomotor sensitization by open field test and anxiety. Thus, it is suggested that activation of nuclear factor kappa B pathway is involved in the development of alcohol dependence induced withdrawal syndrome. Modulation of NFK-β may be used as therapeutic agent to overcome the problems related with alcohol dependence. Therefore, it may be inferred that the nuclear factor kappa B inhibitor is involved in the precipitation alcohol withdrawal syndrome. On the basis of the above discussion, it may be concluded that the inhibition of nuclear factor kappa B inhibitor attenuates the propagation of alcohol dependence and thereby reduce withdrawal signs in vivo, as observed in the withdrawal symptoms in alcohol dependent mice. Nevertheless, further studies are required to elucidate the involvement of nuclear factor kappa B inhibitor related transduction systems in the alcohol dependence.

Therefore, it may be assumed that src kinase and nuclear factor kappa Bpathway activation might be involved in mediating the precipitation of alcohol withdrawal syndrome, Convulsions, Hyperalgesia and depression in mice.

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