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SU-6656 attenuates alcohol dependence induced withdrawal syndrome in mice

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Abstract---Upregulation 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 behavioural parameters were conducted like wall climbing test, 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, wall climbing, 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.

Keywords---src kinase, SU-6656, withdrawal severity score, locomotor activity, PTZ kindling seizures, force swim test, anxiety.

Introduction

It is postulated that changes in excitatory and inhibitory neurotransmission systems contribute to pathophysiological changes and withdrawal symptoms in drug dependency (Singh et al., 2013). Several research studies have shown a connection between neurotransmission systems and withdrawal syndrome in rodent-dependent induced withdrawal syndrome (Rehni et al., 2011; Singh et al., 2013). However, the unique molecular pathways explaining observed behavioural changes in rodents are currently being investigated. Alcohol addiction is a persistent condition marked by loss of control over alcohol consumption, withdrawal symptoms following periods of abstinence, and subsequent relapse (Anton et al., 2020). Alcohol's euphoric and relaxed effects have been postulated to play a part in the onset of addictive conduct. It has been suggested that pleasurable alcohol effects become associated with environmental stimuli, which sets the scene for reward craving (Singh et al., 2020). The effects of alcohol, which subsequently cause a "relief craving," can, by comparison, play a major role in sustaining alcohol addiction (Heilig & Koob 2007; Gupta et al., 2021).

The "negative emotional state" that results from sudden cessation of chronic excessive alcohol consumption in humans involves anxiety and depression as well as somatic symptoms such as tremors and convulsions (Choi et al., 2020; Roberto et al., 2021). The current drugs used in the clinic to treat alcohol dependence have been shown to just contain the symptomatology of the withdrawal syndrome rather than affecting the pathophysiological course of the disease and various adverse effects linked with them (Sharma et al., 2020).

The src protein tyrosine kinase family is classified into nonreceptor tyrosine kinases are abundantly expressed in the central nervous system in drug addicts (Nie et al., 2021; Feng et al., 2021) and thus may be involved in mediating the effects of chronic alcohol abuse. Src is also stated to control a number of transduction mechanisms, some of which play a critical role in alcohol dependency and which, in turn, promote alcohol consumption (Wang et al., 2007). Administration of Src PTK inhibitor, PP2, in the dorsal striatum of rats attenuated operant self-administration of alcohol (Khom et al., 2020). A wide distribution of mRNA expression and protein is seen in the brain as a consequence of src kinase's essential function in alcohol addiction, which activates limbic system and reward pathway in various parts of CNS (Zhang et al., 2017). Moreover, exposure to alcohol causes a manifold increase in the transcription of c-src tyrosine kinase (Wang et al., 2019). It has also been demonstrated that pharmacological inhibition of src kinases decreases the severity of withdrawal in rodents following the cessation of chronic morphine (Rehni et al., 2008) or nicotine administration (Rehni et al., 2011). Stress response is regulated by Src kinase, which is essential for an anxiety-induced drug addiction (Keil et al., 2016). Thus, the activation of Src kinase pathway might be involved in the development of alcohol withdrawal syndrome. SU6656 is a potent and selective inhibitor of src kinase pathway (Blake et al., 2000). Therefore, the present study has been designed to investigate the effect of SU6656 on the development of alcohol withdrawal syndrome.

Materials and Methods

Swiss albino mice of either sex weighing 22 ± 5 g were employed in the present study. The protocol was approved by institutional ethical committee under reg. No. 1181/ab/08/CPCSEA. The research was performed as per CPCSEA's rules and regulations for animal well-being including free access to food and water along with exposure to natural cycle of light and dark.

Drugs and chemicals: SU-6656 (Sigma), Absolute ethanol 99.8% (Merck), Diazepam (Calmpose- Ranbaxy Research Laboratories) were purchased and used in the current study. All chemicals were of analytical quality and dissolved/diluted in sterile saline/10% dimethylsulphoxide solution in triple distilled water as appropriate.

Induction of alcohol dependence: Chronic intermittent ethanol (CIE) exposure regimen consists of repeated episodes of ethanol intoxication and withdrawal. Swiss albino mice were given 2 g/kg ethanol as 10% (v/v) solution in distilled water by oral intubation for one week on a daily basis and then the animals were withdrawn for assessing battery of various behavioral test (Sharma et al., 2020; El Mostafi et al., 2020; Bornebusch et al., 2021).

Assessment of withdrawal severity score (WSS): Modified alcohol severity score was employed in current study to quantitate the magnitude of withdrawal syndrome in mice in terms of the earlier reported characteristic behavioral patterns seen in rodents suffering from alcohol dependence induced withdrawal syndrome. The severity of the alcohol withdrawal phenomenon was graded on a scale of 0–18 (normal score, 0; maximal severity score, 18). Behavioral observations were made for a period of 120 minute after the 8 hr of completing the dosing protocol (Sharma et al., 2020; El Mostafi et al., 2020).

Stereotypic Climbing Behaviour in Alcohol Dependent Mice: Mice were tested for stereotypic climbing behavior, as described by Protais et al., for a 10 minute test session. The amount of time engaged in a vertical posture on the cage walls was recorded with stopwatch (El Mostafi et al., 2020).

Assessment of the effect of alcohol teeth chattering, head nodding, rearing frequency: Teeth chattering, head nodding & rearing frequency observations were made for a period of 120 min after 8 hr. of last alcohol administration to quantitate the severity of the experimental withdrawal phenomenon (Karadayian et al., 2013; Bravo et al., 2020).

Assessment of anxiety like behaviour using elevated plus maze test: Anxiety like behaviour was monitored in mice using an elevated plus maze test as described by Navarro et al., 2006. Parameters like time spent in open, closed and centre arm was recorded.

Assessment of the effect of test drugs on alcohol induced anxiogenic like effect by using open field test: The experimental design analyzing the exploratory behaviour in alcohol dependent mice by locomotor activity in open field test by counting the number of lines crossed by the animals from various treatment groups were

recorded for five minutes after 8 hr. administration of the alcohol (El Mostafi et al., 2020; Barua et al., 2012; Rehni et al., 2013).

Experimental Protocol

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

7 Days Protocol	Group -I (Vehicle-vehicle Control)	Group -II (Vehicle+ Alcohol)	Group -III (Alcohol + Diazepam)	Group -IV (Low dose SU-6656 treatment+Alc)	Group -IV (Medium dose SU-6656 treatment)	Group -IV (High dose SU-6656 treatment +Alcohol)
Day 1	Vehicle (10% DMSO in water, 10 ml/kg, i.p. for SU-6656; Alcohol)	Vehicle (10% DMSO in water, 10 ml/kg, i.p. SU-6656 before 1 hr of Alcohol)	Diazepam (1 mg/kg; i.p.) once daily given before 1 hr of Alcohol (2 mg/kg; p.o.)	SU-6656 (1 mg/kg; i.p.) once daily given before 1 hr of Alcohol (2 mg/kg; p.o.)	SU-6656 (5 mg/kg; i.p.) once daily given before 1 hr of Alcohol (2 mg/kg; p.o.)	SU-6656 (10 mg/kg; i.p.) once daily given before 1 hr of Alcohol (2 mg/kg; p.o.)
Day 2- Day 7	Same procedure continue for seven days	Same procedure continue for seven days	Same procedure continue for seven days	Same procedure continue for seven days	Same procedure continue for seven days	Same procedure continue for seven days
After 8 Hr hr	After 7 th Day behaviour parameters were assessed for 120 hrs					

Statistical Analysis: All the results were analyzed using ANOVA followed by post-hoc comparison using Sheffe's multiple range test by using Sigma Stat 6.0 software (USA).

Results

Effect of SU-6656 on anxiety like behavior in alcohol dependent mice: Administration of alcohol (2 g/kg, oral) once daily for a period of 7 days, precipitated alcohol withdrawal syndrome in mice as reflected by a significant increase ($p < 0.05$) in anxiety like behaviour as measured in terms of average time spent in the open and closed arm particularly in the alcohol treated group, when compared to that of the vehicle treated control groups. Administration of SU-6656 (1, 5 and 10 mg/kg, i.p.) significantly ($p < 0.05$ each) and dose dependently attenuated alcohol induced anxiety like behaviour as measured in terms of the average time spent in the open, close and centre arms (Table 1).

Table 1: Effect of SU-6656 on increase in anxiety like behaviour in alcohol dependent/ saline treated mice.

Group	Duration (s)		
	Open	Closed	Center
VEH-VEH	60.5 ± 2.3	165.2 ± 2.8	74.1 ± 2.3
ALC-VEH	5.1 ± 2.2*	248.6 ± 6.8	44.2 ± 3.9
ALC- DIA	68.2 ± 3.3 **	159.4 ± 3.8 **	72.3 ± 2.6

ALC- SU-6656 (1 mg/kg, i.p.)	28.1 ±2.7**	231.9 ± 4.1	41.0 ± 2.5
ALC- SU-6656 (5 mg/kg, i.p.)	42.1 ±2.7**	200.1 ± 3.4	57.8 ± 2.2
ALC- SU-6656 (10 mg/kg, i.p.)	55.9 ±2.1**	172.9 ± 3.4 **	71.2 ± 1.9

Doses employed in the study were as follows: Vehicle (normal saline, 10 ml/kg & 10 % dimethylsulphoxide solution i.p.), Alcohol (2.0 g/kg, oral) was administered once daily, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are MEAN ± S.E.M.] * =P<0.05 vs. ALC-VEH; ** =P<0.05 vs. ALC- SU-6656.

Effect of SU-6656 on alcohol induced alteration in withdrawal severity score (WSS) in mice Administration of alcohol (2 g/kg, oral) once daily for 7 days resulted in substantial increases (P<0.05) in withdrawal severity scores in the alcohol control group as compared to the vehicle-treated control group. Significantly and dose-dependent SU-6656 (1, 5, and 10 mg/kg, i.p.) administration suppressed alcohol-induced withdrawal in mice, as calculated by WSS (Figure 1).

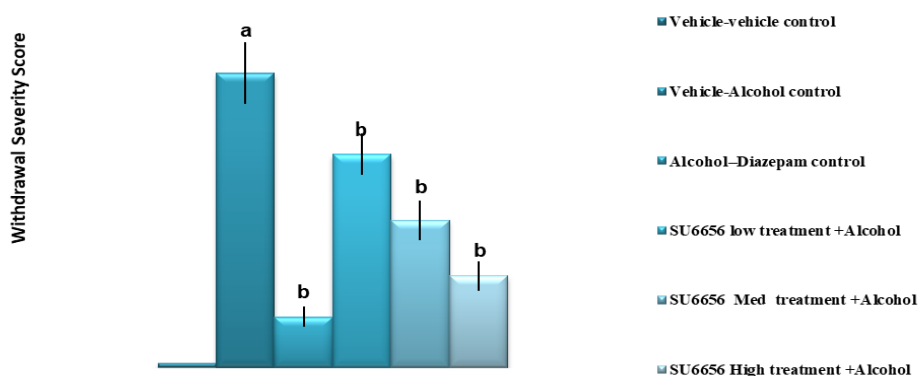


Figure 1: Effect of SU-6656 on withdrawal severity score in alcohol dependent/ vehicle treated mice. Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are mean ± S.E.M.] a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

Effect of SU-6656 on alcohol induced alteration in teeth chattering& rearing frequency:Administration of alcohol (2 g/kg, oral) once daily for 7 days elicited a substantial increase (P<0.05) in teeth chattering and rearing frequency in the alcohol group, as compared to the vehicle-treated control group. When the assessment is for teeth chattering activity and rearing frequency, administration of SU-6656 (1, 5 & 10 mg/kg, i.p.) substantially and dose dependently decreases the frequency of teeth chattering and rearing in alcohol dependent mice (Figure 2& 3).

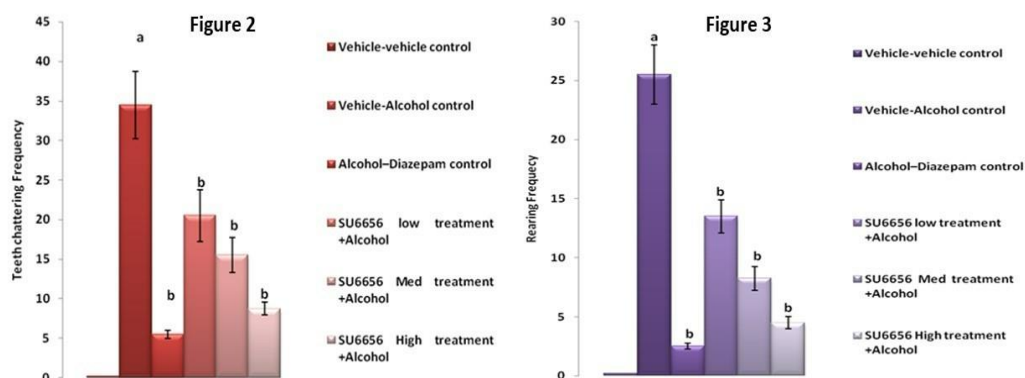


Figure 2& 3: Effect of SU-6656 on teeth chattering and rearing frequency in alcohol dependent/vehicle treated mice. Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are mean \pm S.E.M.] a = P <0.05 vs. VEH-VEH control; b = P <0.05 vs. ALC-VEH.

Effect of SU-6656 on alcohol induced alteration in head nodding in mice: The spontaneous withdrawal syndrome in the mice was stated to be the reflection of a substantial increase (P <0.05) in head nodding of alcohol group once daily for a duration of 7 days relative to a vehicle control group. Administration of SU-6656 substantially attenuated alcohol withdrawal syndrome and dose dependently (1, 5, and 10 mg/kg, ip) attenuated alcohol in mice when measured in head nodding (Figure 4).



Figure 4: Effect of SU-6656 on head nodding frequency in alcohol dependent/vehicle treated mice. 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 \pm S.E.M.] a = P <0.05 vs. VEH-VEH control; b = P <0.05 vs. ALC-VEH.

Effect of SU-6656 on alcohol induced climbing behaviour in mice: Oral administration of alcohol (2 g/kg, i.p.) once daily for a duration of 7 days triggered spontaneous alcohol withdrawal syndrome in mice as shown by a substantial

increase ($P < 0.05$) in climbing activity as compared to vehicle control group. SU-6656 (1, 5 & 10 mg/kg, i.p.) administration substantially and dose dependently diminished alcohol-induced withdrawal syndrome in mice as measured by climbing behavior (Figure 5 & 6).

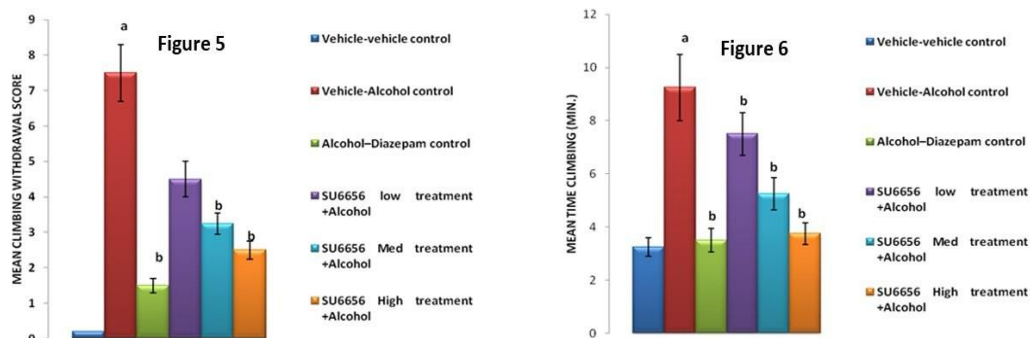


Figure 5& 6: Effect of SU-6656 on climbing withdrawal score& mean climbing time in alcohol dependent/ vehicle treated mice. Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are mean \pm S.E.M.] a = $P < 0.05$ vs. VEH-VEH control; b = $P < 0.05$ vs. ALC-VEH.

Effect of SU-6656 on open field test in ethanol withdrawal mice: In alcohol dependent group number of line crossed by mice significantly decreased ($P < 0.05$) as compared to vehicle control group. Compared to the alcoholic control group, the amount of lines crossed during open field experiments was substantially ($P < 0.05$) increased in positive control group i.e diazepam and dose dependently in SU-6656 treatment (1, 5 & 10 mg/kg, i.p.) groups (Figure 7).

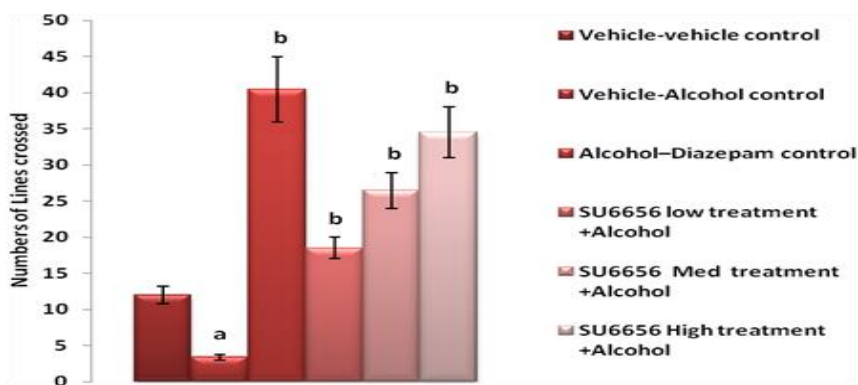


Figure 7: Effect of SU-6656 on number of lines crossed in open filed test in alcohol dependent/ vehicle treated mice. Doses employed in the study were as follows: vehicle (DMSO, 10 ml/kg), alcohol (2.0 g/kg, oral) was administered once daily for seven days, SU-6656 (1, 5 & 10 mg/kg, i.p.) [Values are mean \pm S.E.M.] a = $P < 0.05$ vs. VEH-VEH control; b = $P < 0.05$ vs. ALC-VEH.

Discussion

In the present investigation, administration of SU6656, a selective inhibitor of src kinase (Rehni et al., 2011; Blake et al., 2000) produced a significant attenuation of the development of alcohol dependence induced withdrawal syndrome in alcohol dependent mice, particularly in terms of withdrawal severity score, Climbing behaviour, teeth chattering, exploratory behavior, head nodding, rearing frequency, anxiety like behavior and Locomotor sensitization by using Open Field Test in mice as reported earlier (Sharma et al., 2020; El Mostafi et al., 2020). Conformity to findings from other research is borne out by the results from these findings, which indicate the highest levels of withdrawal syndrome at eight hours following abstinence in rodents (Sharma et al., 2020; Assis et al., 2020).

Non-receptor tyrosine kinases, Src extensively expressed in neuronal cells and its activation leads to pathophysiologic progression of neurodegenerative diseases (Fowler et al., 2019). In cocaine addiction src-PTKs implicated via an NMDA receptor activation-linked mechanism. Furthermore, chronic alcohol administration stimulates Src kinases, which have been implicated in mediating opioid dependence-induced withdrawal actions (Rehni et al., 2008). It is also noted that Src kinase activation mediates the stimulation of various excitatory neurotransmitters, which play an important role in alcohol addiction and dependence induced withdrawal syndrome (Xie et al., 2013).

Interaction between Src kinase signaling pathway and glutamate-mediated synaptic activity effect on alcohol-seeking behaviour in rodents. Src family kinases (SFKs) are involved in both NMDA-mediated activation of TrkB and TrkB-mediated tyrosine phosphorylation of NMDA receptors (Barry & McGinty 2017). The N-methyl-D-aspartate receptor is involved in the neurobehavioral effects of alcohol (Jury et al., 2018). Activation of glutamatergic systems has been implicated to have a role in ethanol-induced withdrawal syndrome in mice (Hansen et al., 2020). Src kinases are important regulators of synaptic plasticity and behavioral responses to drug abuse (Sgobbi et al., 2020). Therefore, it was postulated that pharmacological manipulation of Src kinase might exert a beneficial effect on alcohol dependent mice.

Src kinase controls stress responses, is crucial to anxiety-related condition pathogenesis. It also modulates and controls the levels of various neurotransmitters that plays prominent role in mood disorders and anxiety (Keil et al., 2016). Chronic administration of alcohol to rodents activates various pro-inflammatory molecules in neuronal tissue that are associated with neuroinflammation. It is due to interactive tyrosine phosphorylation of Src kinase (Luster et al., 2020). In similar line by inhibiting the activity of Src kinase with SU-6656 in current study attenuates the anxiety like behaviors in mice. Therefore, it may be inferred that the src kinase is involved in the precipitation alcohol withdrawal syndrome. On the basis of the above discussion, it may be concluded that the selective inhibition of src kinase attenuates the propagation of alcohol dependence and thereby reduce withdrawal signs in vivo, as observed in the withdrawal symptoms in alcohol dependent mice.

Conclusion

In current study selective pharmacological modulation of Src kinase attenuates the spontaneous 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.

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Conflicts of Interest

There are no conflicts of interest.

Credit Author Statement

Conceptualization: Conceived and designed the experiments: Ajeet Pal Singh, Ashish Kumar Sharma, Thakur Gurjeet Singh. Analyzed the data: Thakur Gurjeet Singh Wrote the manuscript: Ajeet Pal Singh, Thakur Gurjeet Singh, Editing of the Manuscript: Ashish Kumar Sharma & Thakur Gurjeet Singh Critically reviewed the article: Ashish Kumar Sharma, Thakur Gurjeet Singh All authors read and approved the final manuscript.

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