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Research Article

Ameliorating Effects of *Nardostachys jatamansi* And Valeric Acid On Alcohol Withdrawal Induced Anxiety In Wistar Albino Rats.

Deepa B^{1*}, Shyamjith M²

^{1*} Assistant Professor, Department of Pharmacology, K S Hegde Medical Academy, Nitte (Deemed to be University), Karnataka, India. deepashyam@nitte.edu.in

² Additional Professor, Department of Pharmacology, Kanachur Institute of Medical Sciences, Karnataka, India. dr.syamjithm@kanachur.edu.in

***Corresponding Author:** Deepa B

*Assistant Professor, Department of Pharmacology, KS Hegde Medical Academy, Nitte (Deemed to be University), Karnataka, India. deepashyam@nitte.edu.in

Abstract:

Objectives: Prolonged alcohol consumption leads to dependence, and withdrawal from alcohol often results in anxiety, prompting individuals to revert to drinking. Conventional anxiolytic treatments like benzodiazepines presents challenges due to adverse effects. Therefore, alternative therapies are sought. This study investigates the anxiolytic potential of *Nardostachys jatamansi* ethanolic extract (NJEE) and its phytoconstituent, Valeric acid, in alcohol withdrawal-induced anxiety in Wistar albino rats.

Materials and Methods: Adult male Wistar rats were subjected to chronic alcohol administration (1 ml of 20% alcohol) for 30 days to induce dependence. Following alcohol withdrawal, NJEE (100 mg/kg) and Valeric acid (100 mg/kg) were administered for 30 days. Anxiety levels were assessed using the Elevated Plus Maze (EPM) and Light-Dark Arena (LDA) tests. Pro-inflammatory cytokines (TNF- α and IL-6) were measured in brain homogenates using ELISA.

Results: Findings showed significant anxiolytic effects of NJEE and Valeric acid, with treated groups spending more time in open arms of the EPM and light chambers of the LDA, indicating reduced anxiety compared to untreated alcohol-withdrawn animals. Additionally, both NJEE and Valeric acid reduced elevated TNF- α and IL-6 levels in the brain, demonstrating their anti-inflammatory properties.

Conclusion: NJEE and Valeric acid exhibit potential as therapeutic agents for managing alcohol withdrawal-induced anxiety, offering a promising alternative to conventional treatments.

Key words: *Nardostachys jatamansi*, Valeric acid, Alcohol withdrawal, Anxiolytic, Pro-inflammatory cytokines, Wistar albino rats

***Author for correspondence:** Deepa B, Email: deepashyam@nitte.edu.in

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Introduction:

Excessive alcohol consumption is a widespread concern globally, frequently resulting in alcohol dependence and presenting significant challenges to public health. Chronic alcohol consumption has harmful effects on cellular, neurochemical, and molecular processes in the body, especially the brain, resulting in behavioural abnormalities. During alcohol withdrawal, anxiety frequently emerges as a significant symptom, driving many individuals to relapse into drinking. Conventionally, anxiety during alcohol withdrawal is managed with medications like benzodiazepines, buspirone, and certain antidepressants. However, these treatments come with their own set of side effects. Improving therapeutic approaches for alcohol withdrawal is crucial for enhancing the overall well-being of individuals struggling with alcohol-related challenges. Several research works are exploring novel therapeutic interventions aimed at alleviating withdrawal induced anxiety without the complications associated with conventional drugs, striking a balance between symptom management and avoiding further dependence issues¹⁻⁶.

Considering the above facts, this research work was carried out to screen the anxiolytic activity of *Nardostachys jatamansi*, an indigenous plant of Valerianaceae family and its phytoconstituent Valeric Acid in alcohol withdrawal induced anxiety. *Nardostachys jatamansi*, also known as spikenard or jatamansi, is seen in Himalayan region of India, Nepal. This perennial herb used in traditional system of medicine like Ayurveda for its calming effects on the mind and body, alleviating conditions like stress, anxiety, insomnia, and depression. Additionally, it possesses anti-inflammatory, anti-spasmodic, and diuretic properties, expanding its therapeutic applications. Valeric Acid is one among the bioactive phytocompounds present in *Nardostachys jatamansi* roots that contribute to its therapeutic effects^{7,8}.

Groups

I	Normal
II	Alcohol (1 ml of 20% alcohol) orally for 30 days
III	NJEE (100mg/Kg) orally for 30 days
IV	Valeric acid 100mg/Kg orally for 30 days
V	Alcohol (1 ml of 20% alcohol) orally for 30 days + NJEE (100mg/Kg) orally for next 30 days
VI	Alcohol (1 ml of 20% alcohol) orally for 30 days + Valeric acid 100mg/Kg orally for next 30 days

On 31st day, alcohol was withdrawn and substituted with drinking water. On 32nd day, after confirming the development of withdrawal symptoms⁹, drugs/vehicle was administered to the respective animals for next thirty days. On 61st day, the animals were taken for the following pharmacological experiments for screening the role of drugs on anxiety.

a. Elevated plus maze (EPM)

The EPM test is a commonly used behavioural assay in rodents to assess anxiety-like behaviour. It involves placing a rodent on a plus-shaped maze elevated above the ground, with two open arms and two enclosed arms. This test has been widely validated to measure anxiety

Materials and Methods:

Animals:

Healthy adult male Wistar albino rats (175-200g), 6 months old age, were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC) bearing number KSEMA/IAEC/01/2017. The animals were maintained under standard conditions in the Animal House, department of Pharmacology, K S Hegde Medical Academy, Nitte (Deemed to be University).

Chemicals:

Absolute alcohol (Sigma Aldrich Chemicals, Bangalore)
Valeric Acid (Rajesh Chemicals)
Elisa Kits to estimate IL6 & TNF α (Krishgen Biosystems)

Instruments:

Soxhlet apparatus to prepare the plant extract. Elevated plus maze and Light Dark Arena for screening anxiolytic activity.

Plant material: *Nardostachys jatamansi* ethanolic extract (NJEE)

Nardostachys jatamansi root powder was obtained from Rajesh chemicals, Mumbai. For preparing the ethanolic extract, a weighed quantity (500 g) of *Nardostachys jatamansi* root powder was used and extracted with ethanol (90 %) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60°C.

Research Design:

For studying the therapeutic role of *Nardostachys jatamansi* and Valeric Acid on alcohol withdrawal induced anxiety in Wistar albino rats, animals were grouped as follows;

in rodents. The plus-maze combines three potential anxiogenic factors – novelty, height and open space. Briefly, the cross-shaped maze consists of four arms that are interconnected by a central platform. Two opposing arms are surrounded by side- and end-walls (closed arms), whereas the remaining two arms are unprotected (open arms). The set-up consists of a maze of two open arms (25 cm $\times$ 5 cm), crossed with walls (35 cm high) and central platform (5 cm $\times$ 5 cm). The maze is suspended 50 cm above the room floor. The animal will be usually placed on the central platform, facing one of the enclosed arms and observed for 5 minutes. During the 5-min test period, the time spent in open and enclosed arms, was recorded. Increased time spent in

open arms or increased entries into open arms suggest reduced anxiety-like behaviour, while the opposite indicates increased anxiety-like behaviour¹⁰.

b. Light Dark Arena (LDA)

Light-dark exploration test is one of the few tests specifically designed for use in rats. LDA test is a behavioural assay used in rodents to evaluate anxiety-like behaviour and exploration. In this test, the animal will be placed in an arena with two chambers; 2/3rd is open and illuminated (the light chamber) and the other 1/3rd with opaque walls and a cover (the dark chamber). The door between the two compartments permits rat to move from one side to another. The rat will be released in the light compartment and observed for 5 minutes. During that time the time spent in light and dark compartment, was recorded¹⁰.

After the pharmacological experiments, animals were euthanized, brains were dissected out. IL-6 and TNF- α levels were estimated with commercially available ELISA kits by following the instructions given by the manufacturer.

Statistical analysis:

Results are expressed as mean $\pm$ SD. One-way analysis of variance (ANOVA) is carried out using InStat-GraphPad software (Graph Pad software Inc., CA, USA) and the statistical comparisons among the groups are performed with Tukey Kramer test. $p < 0.05$ is considered significant.

Results:

a. NJEE & Valeric acid exhibited anxiolytic activity in Elevated Plus Maze test

From the results [Table:1], it was observed that alcohol withdrawn animals (Group II) have significantly ($p < 0.001$) spend less time in open arms and more time in the closed arms of EPM on comparing with the normal (Group I); clearly indicating anxiogenic behaviour in alcohol withdrawn animals.

Whereas, the animals which were treated with NJEE (Group V) and Valeric acid (Group VI) for 30 days after alcohol was withdrawn, has significantly ($p < 0.001$) spend more time in open arms and less time in the closed arms of EPM on comparing with the alcohol withdrawn animals, which were not treated by any drug (Group II); clearly indicating anxiolytic action of NJEE and valeric acid.

b. NJEE & Valeric acid exhibited anxiolytic activity in Light Dark Arena test

From the results [Table:2], it was observed that alcohol withdrawn animals (Group II) have significantly ($p < 0.001$) spend less time in open area and more time in the dark area of LDA on comparing with the normal (Group I); clearly indicating anxiogenic behaviour in alcohol withdrawn animals.

Whereas, the animals which were treated with NJEE (Group V) and Valeric acid (Group VI) for 30 days after alcohol was withdrawn, has significantly ($p < 0.001$, $p < 0.01$) spend more time in bright arena and less time ($p < 0.001$) in the dark arena of LDA on comparing with the untreated alcohol withdrawn animals (Group II); clearly indicating anxiolytic action of the test drugs.

Table -1: Time spend in the arms of Elevated Plus Maze

Groups		Time spends (Sec) in the open arm	Time spends (Sec) in closed arm
I	Normal	100.16 $\pm$ 7.96	188.33 $\pm$ 6.154
II	Alcohol (1 ml of 20% alcohol)	35.6 $\pm$ 1.92 ^a	239.33 $\pm$ 8.38 ^g
III	NJEE (100mg/Kg)	140.83 $\pm$ 6.43 ^{b, d}	154.3 $\pm$ 3.88 ^h
IV	Valeric acid(100mg/Kg)	117.3 $\pm$ 5.5 ^c	167.7 $\pm$ 4.96 ^{i, j}
V	Alcohol (1 ml of 20% alcohol) +NJEE (100mg/Kg)	91.01 $\pm$ 5.79 ^e	175.6 $\pm$ 3.09 ^k
VI	Alcohol (1 ml of 20% alcohol) + Valeric acid(100mg/Kg)	81.17 $\pm$ 5.67 ^f	182 $\pm$ 7.23 ^l

N=6, One way ANOVA with Tukey Kramer
a :P<0.001 comparing Group II with Group I
b: P<0.001 comparing Group III with Group I
c:P<0.05 comparing Group IV with Group I
d:P<0.001 comparing Group III with Group IV
e:P<0.001 comparing Group V with Group II
f:P<0.001 comparing Group VI with Group II
g:P<0.001 comparing Group II with Group I
h:P<0.001 comparing Group III with Group I
i: P<0.01 comparing Group IV with Group I
j: P>0.05 comparing Group III with Group IV
k:P<0.001 comparing Group V with Group II
l:P<0.001 comparing Group VI with Group II

Table -2: Time spend in the arena of Light Dark Arena

Groups		Time spends in light arena	Time spends in dark arena
I	Normal	124.8 ± 6.6 ^a	175.66 ± 9.832
II	Alcohol (1 ml of 20% alcohol)	70.6 ± 8.82	218.33 ± 3.983 ^g
III	NJEE (100mg/Kg)	150.3 ± 9.44 ^{b, d}	140.83 ± 9.10 ^{h, j}
IV	Valeric acid(100mg/Kg)	139.33 ± 4.88 ^c	156.33 ± 3.03 ⁱ
V	Alcohol (1 ml of 20% alcohol) + NJEE (100mg/Kg)	140.6 ± 6.57 ^e	154.83 ± 6.70 ^k
VI	Alcohol (1 ml of 20% alcohol) + Valeric acid(100mg/Kg)	114.5 ± 7.79 ^f	175.16 ± 3.869 ^l

N=6, One way ANOVA with Tukey Krammer
a: P<0.001 comparing Group II with Group I
b: P<0.01 comparing Group III with Group I
c: P>0.05 comparing Group IV with Group I
d: P>0.05 comparing Group III with Group IV
e: P<0.001 comparing Group V with Group II
f: P<0.01 comparing Group VI with Group II
g: P<0.001 comparing Group II with Group I
h: P<0.001 comparing Group III with Group I
i: P<0.01 comparing Group IV with Group I
j: P>0.05 comparing Group III with Group IV
k: P<0.001 comparing Group V with Group II
l: P<0.001 comparing Group VI with Group II

c. NJEE & Valeric acid reduced TNF- α level in brain homogenate

From the results [Table:3], it is observed that brain TNF- α values are significantly ($p<0.001$) increased in untreated alcohol withdrawn animals (Group II) comparing with the normal ones (Group I).

In alcohol withdrawn animals which were treated with NJEE (Group V) and Valeric acid (Group VI) TNF- α values are significantly ($p<0.001$) reduced on comparing with untreated alcohol withdrawn animals (Group II)

Table 3: Brain homogenate TNF- α value

Groups		Brain TNF- α in pg/ml
I	Normal	51.008 ± 3.565
II	Alcohol (1 ml of 20% alcohol)	174.84 ± 5.129 ^a
III	NJEE (100mg/Kg)	50.258 ± 2.90 ^b
IV	Valeric acid(100mg/Kg)	53.46 ± 4.37 ^c
V	Alcohol (1 ml of 20% alcohol) + NJEE (100mg/Kg)	88.13 ± 5.44 ^d
VI	Alcohol (1 ml of 20% alcohol) + Valeric acid (100mg/Kg)	92.27 ± 3.58 ^e

N=6, One way ANOVA with Tukey Krammer
a: P<0.001 comparing Group II with Group I
b: P<0.001 comparing Group III with Group I
c: P<0.001 comparing Group IV with Group I
d: P<0.001 comparing Group V with Group II
e: P<0.001 comparing Group VI with Group II

d. NJEE & Valeric acid reduced IL-6 level in brain

From the results [Table:4], it is observed that brain IL-6 values are significantly ($p<0.001$) increased in untreated Alcohol withdrawn animals (Group II) comparing with the normal ones (Group I).

In alcohol withdrawn animals which were treated with NJEE (Group V) and Valeric acid (Group VI) IL-6 values are significantly ($p<0.001$) reduced on comparing with untreated alcohol withdrawn animals (Group II)

Table 4: Brain IL-6 values

Groups		Brain IL-6 in pg/ml
I	Normal	7.01 ± 9.571
II	Alcohol (1 ml of 20% alcohol)	155.33 ± 5.033 ^a
III	NJEE (100mg/Kg)	8.166 ± 1.197 ^b
IV	Valeric acid(100mg/Kg)	11.166 ± 1.64 ^c
V	Alcohol (1 ml of 20% alcohol) + NJEE (100mg/Kg)	59.5±2.8 ^d
VI	Alcohol (1 ml of 20% alcohol) + Valeric acid(100mg/Kg)	64.16±4.45 ^e
N=6, One way ANOVA with Tukey Kramer a: P<0.001 comparing Group II with Group I b: P>0.05 comparing Group III with Group I c: P>0.05 comparing Group IV with Group I d: P<0.001 comparing Group V with Group II e: P<0.001 comparing Group VI with Group II		

Discussion

The study highlights that alcohol withdrawal can trigger anxiety and elevate levels of pro-inflammatory cytokines, specifically TNF- α and IL-6, in the brain.

Alcohol consumption alters the delicate balance of neurotransmitters in the brain, initially enhancing inhibitory neurotransmitters like GABA, glycine, and adenosine, while suppressing excitatory neurotransmitters such as glutamate and aspartate. However, with chronic alcohol use, the brain compensates by decreasing inhibitory signalling and increasing excitatory signalling to maintain homeostasis. When alcohol use is suddenly stopped, this balance is disrupted, resulting in excessive excitatory activity, a phenomenon known as alcohol withdrawal syndrome. This syndrome manifests with symptoms like seizures, delirium, and anxiety.^{1-8, 11-13}

Beyond neurotransmitter changes, research has established a link between anxiety disorders and cytokine level alterations in the pathophysiology of several neurological and psychiatric conditions, including alcoholism. Chronic alcohol consumption activates microglia, causing the release of pro-inflammatory cytokines like IL-6 and TNF- α , which increase inflammation and contribute to neuronal damage. These cytokines may also play a role in anxiety symptoms by promoting neuroinflammation and disrupting neurotransmitter balance.¹⁴

The study has provided vital information regarding the therapeutic role of *Nardostachys jatamansi* and Valeric acid on alcohol withdrawal anxiety. The pharmacological and biochemical findings of this study indicate that administration of NJEE and valeric acid effectively mitigated the anxiety induced by alcohol withdrawal in experimental animals.

The pharmacological screening results reveal that abrupt alcohol withdrawal induces anxiety in experimental animals, a behaviour effectively ameliorated by administration of NJEE and Valeric acid. Biochemical analysis further demonstrates a significant increase in the levels of pro-inflammatory cytokines IL-6 and TNF- α in animals subjected to sudden alcohol withdrawal. These cytokine level alterations may underlie the observed anxiogenic activity. Notably, treatment with NJEE and Valeric acid

reverses this cytokine level changes, thereby attenuating anxiogenic behaviour during alcohol withdrawal.

Conclusion

NJEE and Valeric acid can be utilized as an agent for mitigating ethanol withdrawal induced anxiety.

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