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Research Paper

Trachyspermum Ammi Fruit extract: An herbal treatment for alcohol withdrawal syndrome

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ABSTRACT

The alcohol withdrawal syndrome is a cluster of symptoms observed in persons who stop drinking alcohol following continuous and heavy consumption. Milder forms of the syndrome include tremulousness, seizures, and hallucinations, typically occurring within 6-48 hours after the last drink. A more serious syndrome, delirium tremens (DTs), involves profound confusion, hallucinations, and severe autonomic nervous system over activity, typically beginning between 48 and 96 hours after the last drink. Estimates vary on the incidence of serious consequences of alcohol withdrawal. Regardless of actual incidence, recent evidence suggests that it may be important to treat everyone who is suffering from alcohol withdrawal. The objective of present study is to develop a herbal treatment for alcohol withdrawal symptoms with Trachyspermum Ammi Fruit extract. We found that the aqueous extract showing significant effect on alcohol withdrawal symptoms by evaluation of locomotor activity and forced swimming test.

Key Words- Alcohol withdrawal symptoms, *Trachyspermum Ammi*, locomotor activity and forced swimming test.

INTRODUCTION

Changes in the body that occur when a person suddenly stops drinking after a chronic alcohol use is called Alcohol withdrawal symptoms. Alcohol has a slowing effect on brain which is called sedating effect or depressant effect on the brain and over time, the brain adjusts its own chemistry to compensate for the effect of the alcohol. Withdrawing alcoholics exhibits psychiatric problems like anxiety, depression [1], sleep disturbance, neural excitation (seizures), delusion, hallucination etc.[2] Depressive symptoms often are observed in patients who are intoxicated or undergoing alcohol detoxification. As many as 15% of alcoholics are at the risk for death by suicide

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and recent consumption of alcohol appears to increase the danger of a fatal outcome from self harm.[3] Withdrawal signs and symptoms are frequently minor but can develop into a severe, even fatal, condition. Because of its medical complications, alcohol dependence is seen frequently by physicians, occurring in 15% to 20% of primary care and hospitalized patients.[4] The social, economical, psychological and physical problems associated with alcoholism may contribute to the development of depressive disorders.[3] This finding may be attributable to the release of behavioral inhibition associated with alcohol intoxication or with the depressive feeling state. Anxiety and depression is also thought to be the most important negative motivation to the revert to the alcohol use.[5] Most of the CNS acting drugs influence the locomotor activities in man and animals. The CNS depressant like barbiturates and alcohol reduce the motor activity in other words, the locomotor activity can be an index of wakefulness (alertness) of mental activity. [6]

Trachyspermum ammi popularly known as “ajowan” or “ajwain”. *Trachyspermum ammi* is a grassy, annual plant which grows in the east of India, Iran and Egypt with a white flower and small, brownish seeds. Among traditional potential herbs used as spice in day to day life, ajwain (*Trachyspermum ammi L.)* is widely used for curing various diseases in both humans and animals. Ajwan (aqueous extract) is popular remedy for diarrhea; fruits: antidiarrhoeal, antiseptic, antispasmodic, carminative, stimulant, stomachic and tonic; beneficial in bronchitis, atonic dyspepsia and flatulence; dipsomania, hysteria; sore throat; plaster or poultice applied to abdomen in colic; an important ingredient in various Ayurvedic formulations prescribed infections with worms; oil is antiseptic, carminative and expectorant, efficacious in bronchial pneumonia and other respiratory disorders; leaves: juice is anthelmintic; root: carminative, diuretic, febrifuge; useful in stomach troubles.[7] Fruit possesses stimulant and carminative properties and is regarded as antispasmodic. It is an important and remedial agent for flatulence, atonic dyspepsia, and diarrhea. [8] The seed of ajwain is bitter, pungent, and it acts as anthelmintic, carminative, laxative and stomachic. It also cures abdominal tumor, abdominal pains and piles. Ajwain seeds contain essential oil containing about 50% thymol which is strong germicide, anti-spasmodic and fungicide. Thymol is also used in toothpaste and perfumery. [9] Seeds of *Trachyspermum ammi* are used traditionally in Lebanon as antirheumatic. [10] Fixed oil from the seed contains resin acids, palmitic acid,

petroselenic acid, oleic acid and linoleic acid.[11]

Alcohol Withdrawal Syndrome

The alcohol withdrawal syndrome is a set of signs and symptoms that typically develops in alcohol dependent people within 6–24 h of their last drink. It may occur unintentionally if abstinence is enforced by illness or injury, or deliberately if the person voluntarily stops drinking because of an alcohol-related illness, or as a prelude to becoming and remaining abstinent. Tremor of extended hands, tongue or eyelids, sweating, Nausea and/or vomiting, Sinus tachycardia.. Psychomotor agitation, Insomnia, Anxiety, Headache, Fever, Decreased attention, Disorientation, clouding of consciousness, Hallucinations (which may be visual, tactile or auditory, Withdrawal seizures).The severity of alcohol withdrawal syndrome is therefore a major risk factor of early relapse.[4]

The aims of detoxification or treatment are to provide safe withdrawal from alcohol and enable the patient to become alcohol free. Prepare the patient for ongoing treatment for their dependence on alcohol [**12]** The objective of this study is to evaluation behavioral modification specially CNS depression after inducing Ethanol to mice and evaluation of *Trachyspermum ammi* fruit extract activity after alcohol withdrawal in mice using actophotometer .

MATERIALS AND METHODS

Plant material

Fruit of *Trachyspermum Ammi* was purchased from local market of Raipur, Chhattisgarh and authentified by Dr. S. K. Mahajan, Professor of Botany, Govt. P. G. College, Khargone, M.P. The Voucher Specimen was deposited for future reference.

Preparation of aqueous extract

The powder drug was dried and packed well in Clevenger apparatus (12 cycles) and extracted with water as a solvent. After extraction the aqueous ajwain extract was collected. The aqueous ajwain extract were subjected for further studies.

Experimental animals

Healthy adult Swiss albino mice (25-40 g) of either sex were procured from Ghosh enterprises, Kolkata. Male and female animals were housed in separate polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25 ± 30°C, 35–60 % relative humidity). The animals had free access to food and water throughout the course of the study. The animals were kept fasted 2 h before and 2 h after drug administration. The experimental protocol was approved by

Institutional Animals Ethics Committee IAEC (Regd. No. 1321/ac /10 /CPCSEA 28-01-2010) and approval no. CIP/IAEC/004/ 12 and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and Chemicals

Imipramine hydrochloride (Sigma-Aldrich, St Louis,USA) and Ethanol (95%) were procured from E. Merck Ltd (India), Mumbai. All drugs were dissolved in distilled water and administered either intraperitoneally (i.p.) or orally (p.o.). Distilled water was used as the vehicle.

EXPERIMENTAL PROCEDURE

Acute Oral Toxicity Studies

Acute toxicity was performed according to the fixed dose procedure of OECD Guideline No. 420. The suspension of aqueous extract of fruits in tween 80 was administrated orally to overnight fasted Swiss Albino mice at two different doses 1000 and 2000 mg/kg b.w. respectively. The animals were observed continuously for the initial 4hrs for behavioral changes and mortality and intermittently for the next 6 h and then again at 24 h and 48 h after dosing for a total of 14 days. The behavior parameters observed were convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased respiration. In this study dose of 2000 mg kg^{-1} was found to be safe, so dose of 200 mg kg^{-1} was chosen for the experimentation.[13]

EXPERIMENTAL DESIGN

Evaluation of antidepressant activity

Evaluation of locomotor activity

CNS depressant activity was performed according to the method reported by S.K. Kulkarni, (2009). The alcohol dependence was induced in mice by the oral, once daily administration of 10% v/v ethanol (2g/Kg) for approximately one week. The animals were then withdrawn from alcohol. Th e spontaneous locomotors activity of each rat was recorded individually for 10 min using an actophotometer. The movement of the animal cuts off a beam of light falling on the photocell, and the count is recorded digitally.

Overnight fasting normal rats were divided into five groups of six animals per group (n=6). Group I served as normal control received water as vehicle. Group II served as positive control received 10% v/v alcohol only. Group III served as standard group and received Imipramine (15mg kg^{-1} p.o.) suspended in vehicle, Group IV and V served as test group received 100 mg $kg^{-1}$and 200 mg kg^{-1} dose of aqueous extract of fruits of ajwain suspended in vehicle was administrated orally to the animals respectively.

Forced swim test (FST)

The development of immobility when the rats are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. The cylindrical container (diameter 10 cm, height 25 cm) was filled to a 19-cm depth with water at (25°C). The duration of immobility during the 6-min test was scored. Each rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. [14]

RESULT

Evaluation of locomotor activity

The antidepressant effects of ***Trachyspermum Ammi* Fruit extract** (100 and 200 mg/kg) and imipramine were studied by observing its effect on actophotometer. (100 mg/kg only, p.o.) which exhibited slight increase in locomotor activity when compared with the positive control animals that received the ethanol only. But the results were not much significant statistically. The (100 mg/kg only, p.o.) and imipramine-treated group exhibited statistically significant (p<0.05) increase in locomotor activity when compared with the positive control group. The results are tabulated in Table 3.

Antidepressant Activity Forced swim test (FST)

The antidepressant effects of ***Trachyspermum Ammi* Fruit extract** (100 and 200 mg/kg) and imipramine were studied by observing the changes in the duration of immobility by Forced swim test (FST). In FST, ***Trachyspermum Ammi* Fruit extract** 100 and 200 mg/kg, p.o. produced significant reduction (p<0.01 and p<0.001 respectively) in the immobility period when compared with that of positive control group animals that received only ethanol. The extract (200 mg/kg) was found to be effective and it exhibited activity similar to that of the conventional drug imipramine (p<0.001). The results are tabulated in Table 4.

DISCUSSION

Chronic and excessive ethanol consumption followed by withdrawal results in the development of abstinence syndrome. The most common and prominent feature of alcohol withdrawal is anxiety, which is also considered to the most important negative motivation to revert to alcohol use. These signs and symptoms of alcohol withdrawal have been attributed to the perturbation of central neurotransmitters and ion channel activity.

Evidence indicates that during ethanol withdrawal there is an up regulation of excitatory NMDA receptors and a down regulation of inhibitory GABA-A receptors.

Therefore, a drug that either facilitates the action of GABA or decreases glutamate activity may be effective in EW induced anxiety behavior. Since the extent of anxiety is subjective and often difficult to quantify, objective animal models have been used to study the behavioral measures of anxiety during alcohol withdrawal. Locomotors activity and muscle coordination are an index of alertness. Increase in motor activity is an indication of CNS antidepressant property. At a higher dose (200 mg/kg), the extract exhibits a stimulating effect. The antidepressant action of the extract at the higher dose (i.e. 200mg/kg) may be attributed to the presence of some constituents at an optimal concentration to reduce sedation and depression. Thus the aqueous extract of *Trachyspermun Ammi* showed positive result in the treatment of alcohol withdrawal syndrome in the case of locomotors activity. There are many neurotransmitters in CNS which may be responsible for alcohol withdrawal syndrome. Mainly the up-regulation of NMDA receptor and down regulation of GABA receptors. With the use ethanol in mice there may be regulation of GABA receptors due to which the animal spend much more time in the open arm in elevated pulse maize test and similarly showed more locomotion in case of locomotors activity. With the administration of the various doses of the aqueous extract there was once again down regulation of the GABA receptors and the animals showed less locomotion in the actophotometer.

CONCLUSION

Since ancient times, people have been using plants in various ways as a source of medicine. From the above preclinical study, we can conclude that aqueous fruits extract of *Trachyspermun Ammi* show a significant antidepressant activity in alcohol withdrawal syndrome models of depression. We believe that *Trachyspermun Ammi* has the potential to be used as an adjuvant in the treatment of depression and other mood disorders. Further research is required to gain closer insights into the exact mechanism of its action.

FUTURE SCOPE

As in today's world most of the populations are suffering from alcohol withdrawal syndrome There are very few drugs available for the treatment of this syndrome. Disulphiram, a well known drug is available in the market for the treatment of alcohol withdrawal syndrome, but the main side effect it causes include vomiting, thus reducing the patient compliance. Trachyspermun ammi is commonly used as spices and thus is well known to most of population. It can be used for the treatment of

alcohol withdrawal syndrome as it is sweet in taste and has not any side effect as it is used as spices in Indian subcontinent**.**

REFERENCE

1. F. M. Theo, H. Heidi, Repeated characterization of alcohol withdrawal reactions in rats chronically exposed to an alcohol liquid diet, *Prog Neuropsychopharmacol Biol Psychiatry* 1994; 18: 947-60,.

2. Smith, J.W. Medical manifestations of alcoholism in the elderly. International Journal of Addiction, 1995; 30: 1749-1798.

3. Madden, J.S. Alcohol and depression. British Journal of Hospital Medicine 1993; 50:261–264.

4. E. Benlhabib, J. I. Baker, J. E. Keyler, and A. K. Singh, Kudzu root extract suppresses voluntary alcohol intake and alcohol withdrawal symptoms in P rats receiving free access to water and alcohol, *J Med Food*, 2004; 7: 168–179.

5. Smith, M.F., "Pharmacological Management of Alcohol Withdrawal", *A Meta-analysis and Evidence-Based Practice Guideline*1997; 2: 278.

6. Kulkarni S.K., Practical Pharmacology and clinical pharmacy, I edition 2007 (reprint 2009), Vallabh publication, SU-221, Pitampura, Delhi: 2009

7. Chatterjee Asima and Chandraprakashi Satyesh, The Treatise of Indian Medicinal Plants, *CSIR* 1995; 4: 45.

8. A. Absar Qureshi1 and K..E. Swar Kumar "phytochemical constituent and pharmacological activities of trachyspermum ammi", *Plant Archives* 2003;10(2) 955-959,.

9. Bentely, L. S. and H. Wrimen. Medicinal Plants. Asiatic Publishing House, New Delhi, India, 1999.

10. V. Krishnamoorthy, and M. B. Madalageri, "Bishop weeds (Trachyspermum ammi): an essential crop for north Karnataka. J. Med. And Aromat. Plants Sci.1999; 21(4): 996-998.

11. Marc, El Beyrouthy, A. Nelly, D. D. Annick and D. Frederic, "Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon", *J. Ethnopharmacol*. 2008;120: 315-334.

12. Hugh Myrick and Raymond F. Anton, "Treatment of withdrawa",l *Alcohol Health and Research* 1998; 22(1): 56-89.

13. OECD Guideline, (2004), Guideline For Testing of Chemicals, Acute Oral Toxicity – Fixed Dose Procedure, No. 420, anx-2d.

14. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther. 1977Oct; 229(2):327-336.

Table 1: Qualitative and temporal differences between early and late alcohol Withdrawal symptoms

Clinical feature	Early/minor Withdrawal	Late / minor Withdrawal
Symptoms	Mild Agitation	Extreme psychomotor and autonomic activity
	Anxiety	Disorientation
	Restlessness	Confusion
	Tremor	Sensory- perceptual
	Anorexia	Impairment
	Insomnia	
Time Post Alcohol	0 -48 hr	24-150 hr
Peak of withdrawal severity	24-36 hr mild	72-96 hr potentially life threatening
Seizures	Yes, 6-48 hr post alcohol	Yes, 24-48 hr post alcohol

Table 2: Putative relationships between neurotransmitters disturbances and alcohol withdrawal symptoms

Neurotransmitters	Chronic alcohol	Withdrawal	Withdrawal symptoms
Glutamate	↘	↗	seizures
GABA	↘	↘	Anxiety
Noradrenaline	↘	↗	Sympathetic overdrive
Dopamine	↘	↘ or ↗	Depression or, hallucination, deliriums tremens
Serotonin	↗ or ↘	↘	Anxiety, depression

↘ -Decrease, ↗ - Increase

Table 3: Effect of aqueous extract on animals in locomotors activity

Group	Locomotors Activity For 10 Min
Normal Control	333±1.690
Positive Control	86.2±1.96
Standard Group	320±1.38
Test Group 100mg/kg	281±1.02
Test Group 200mg/kg	306±2.06

The data were expressed as mean standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). $n = 6$ test was followed by Dunnett's't'-test, ***p<0.001 vs control were considered as significance.

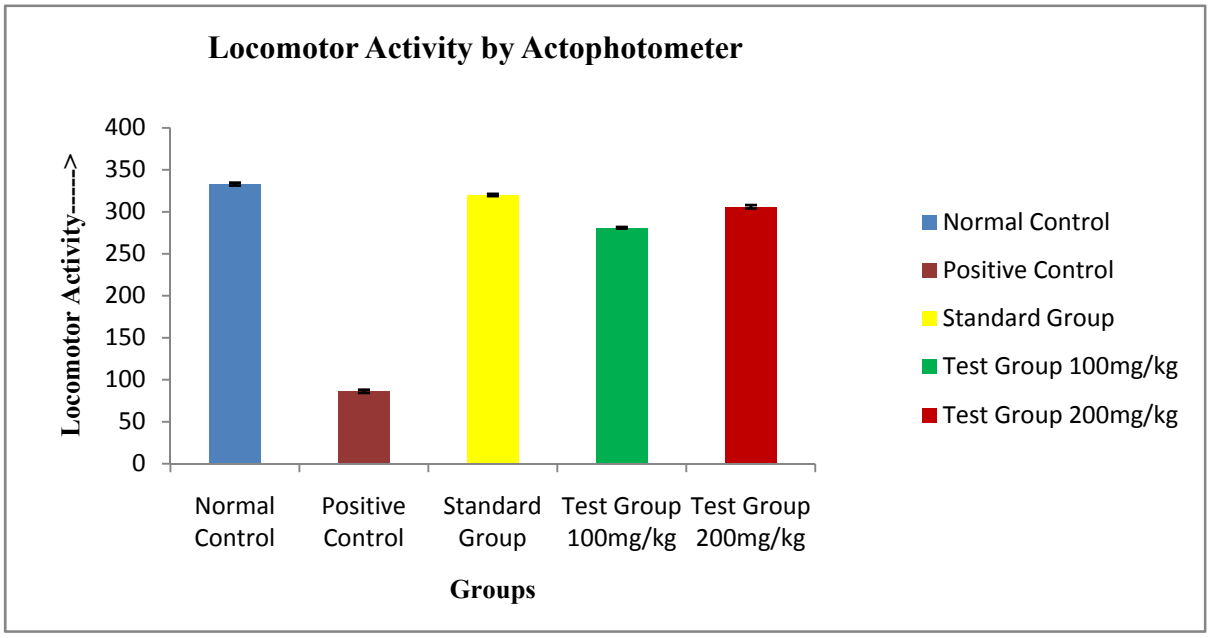

Figure 1: Graph showing effect of extract on locomotors activity in groups

Table 4: Effect of aqueous extract on animals in **Forced swim test (FST)**

Group	**Forced swim test Duration of immobility (in sec)**
Normal Control	133.55±9.38
Positive Control	145.55±9.39
Standard Group	58.63±10.46***
Test Group 100mg/kg	75.22±8.19**
Test Group 200mg/kg	60.84±5.82***

The data were expressed as mean standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). $n = 5$ test was followed by Dunnett's't'-test, ***p<0.001 vs control were considered as significance.

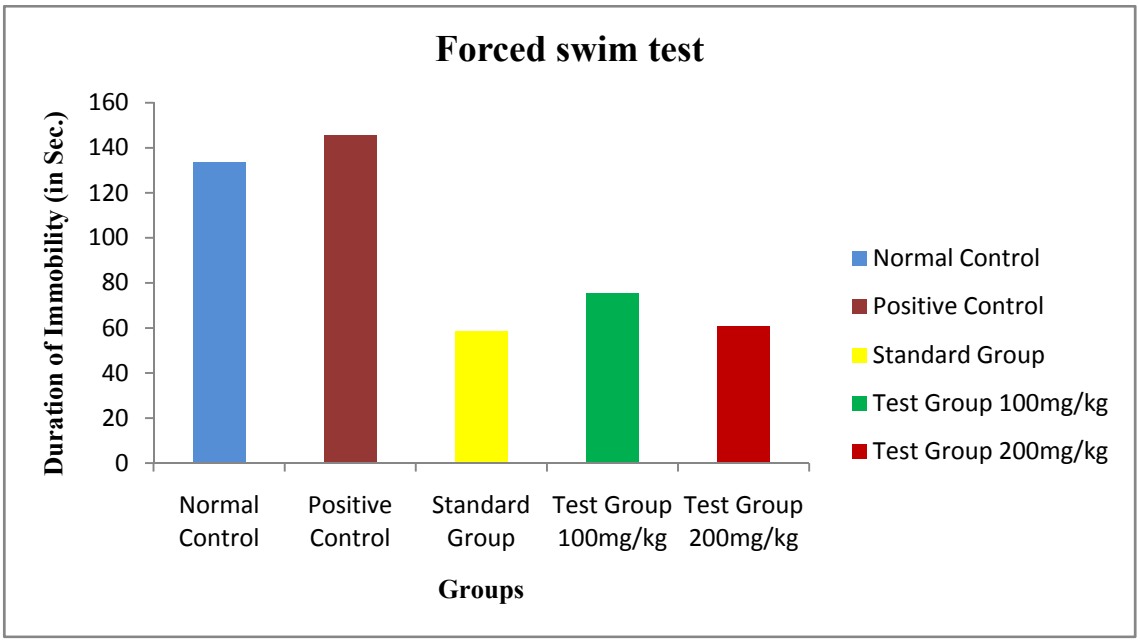

Figure 2: Graph showing effect of extract on Duration of immobility activity in groups