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Effect of Plassiflora Incarnata (Mother tincture) on Ethanol Withdrawal Induced Anxiety and Depression in Experimental Mice

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Abstract

To assess the effect *Passiflora incarnata* mother tincture on ethanol withdrawal-induced anxiety and depression in mice. The mice were divided into six different groups. Each group contains six animals. They were treated with 10% ethanol (2000mg/kg; p.o) from day 1 to 6 days, later 24hrs, ethanol withdrawal reactions were evaluated using different behavioural models like Elevated Plus Maze (EPM), Light And Dark Test (LDT), Hole Board Test (HBT), Marble Burying Test (MBT), Tail Suspension Test (TST). On last day, mice treated orally with *P. incarnata* were tested for anxiety and depression. The *P. incarnata* shows the protective effect against ethanol withdrawal anxiety and depression in mice. All the doses of *P. incarnata* employed in this study showed significantly ($p<0.05$) increased time spent in open arm in EPM, time spent in light area in LDT, head dips in HBT. Also, there was significant ($p<0.05$) decreased marbles buried and reduced immobility duration in TST as compared to ethanol withdrawal group. In conclusion *P. incarnata* showed anxiolytic and anti-depressant activity in mice with ethanol withdrawal.

Introduction

Alcohol (Ethanol) is a psychoactive substance with dependence liability. It ranks among the most widely abused drugs around the globe and its consumption contributes to 3 million deaths each year globally. Millions of people suffer from impairments and poor health as a result of alcohol misuse. 5.1% of all disease burden worldwide is attributable to alcoholism [1]. Alcohol consumption and abuse have been relevant to structural, functional, and neurochemical neuroadaptive alterations along with its two chief components i.e., dependence and withdrawal [2]. Alcohol withdrawal is the physiological alteration that occurs upon cessation of alcohol consumption after chronic alcohol intake [3]. Alcohol withdrawal is a cluster of symptoms following long-term alcohol intake result in anxiety, depression, sleep disturbance, irritability, agitation [4].

Prolonged alcohol consumption disrupts a variety of excitatory and inhibitory neurotransmitters in the brain [5,6]. The effect of alcohol on inhibitory gamma amino butyric acid (GABA) receptors was highlighted by alcohol withdrawal. Frequent alcohol consumption depletes the formation of GABA and elevates the production of excitatory neurotransmitters, prominently glutamate, dopamine, adrenaline, and serotonin, all of which raise the drinker's tolerance to alcohol [4,7].

The U.S. Food and Drug Administration has currently approved three drugs for the treatment of alcoholism: disulfiram,

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naltrexone, and acamprosate. However, they have various side effects. To overcome this, the use of herbs provides an opportunity to discover new compounds that can be used in alleviating alcohol withdrawal induced anxiety and depression. Most anti-addictive compounds include anti-anxiety, anticonvulsant, and antidepressant actions. Many studies reported that herbaceous plants can be used in the treatment of various neuropsychiatric disorders including alcohol abstinence [8,9,10]. *Passiflora Incarnata* (Passifloraceae), often called May-Pop, Maracuja, or Passionflower has been used as an anxiolytic [11]. Patel *et. al* has shown that different actions *of P. incarnata* on CNS were attributed to the benzoflavone present in the study extract [12]. The primary chemical components of the plant explored in this study include flavonoids and alkaloids possessing neuroprotective effects [13,14].

Here, we report that *P. incarnata* may prevent alcohol withdrawal induced anxiety and depression in mice and possesses anti-addictive properties.

Materials and Methods

Animals

Male albino mice (20-25 g) were purchased from Lacsmi Biofarms, Pune, India. Animals were kept in cage at a normal laboratory temperature of 25±2°C, with a relative humidity of 45–55% and a 12–12 h light/dark cycle. Food and water were restricted 2 hours prior drug administration till, completion of experiment on the day. All the experiments were carried out between 10:00 to and 16:00. The animals were maintained according to the CCSEA guidelines for the use and care of experimental animal. The Institutional Animal Ethical Committee (IAEC) approved the protocol of this study.

Drugs and Chemicals

P. incarnata was purchased from SBL, Pvt. Ltd, India. Ethanol was purchased from Changshu Hong sheng Fine chemical Co. Ltd China. Diazepam was purchased from Neon laboratory limited Palghar, Thane, India.

Preparation of doses of *P. incarnata* mother tincture

P. incarnata (PI) mother tincture was prepared in double distilled water (DDW) in a three different dilutions as follows:

PI-1 (1ml of *P. incarnata* was diluted in 20ml DDW).

PI-2 (1ml of *P. incarnata* was diluted in 10ml DDW).

PI-3 (1ml of *P. incarnata* was diluted in 5ml DDW).

All the above dilutions were administered orally to the animals in the respective group at a dose of 0.3 ml/animal.

Study Design

The mice (n=6 per group) were divided into 6 groups. Group I: Saline, Group II: Ethanol (2 g/kg, i.p. 10% v/v) till day 6 and saline on day 7 [15], Group III: Ethanol (2 g/kg, i.p. 10% v/v) till day 6 followed by PI 1 (0.3 ml/animal, p.o.) on day 7. Group IV: Ethanol (2 g/kg, i.p. 10% v/v) till day 6 followed by PI 2 (0.3 ml/animal, p.o.) on day 7. Group V: Ethanol (2 g/kg, i.p. 10% v/v) till day 6 followed by PI 3 (0.3 ml/animal, p.o.) on day 7. Group VI: Animals administered Ethanol + diazepam (1mg/kg, i.p.) [16].

Assessment of *P. incarnata* for anti-anxiety and anti-depression effects

Elevated plus maze

EPM is widely used for behavioral tests for research on anxiety initially developed for mice and rats [17]. The EPM is the form of a plus with two open arms and two closed arms facing opposite to each other and separated by a central square with two arms of the same dimension and enclosed by walls. The maze was made of a wood and was located 25 cm above the floor[17]. After 30 min. of drug treatment, mice were individually placed at center of plus maze. During the 5 min test period, the number of entries and time spent on the open arm were recorded [18].

Light and Dark Test

The light/dark test is employed to test the anxiogenic and anxiolytic behavior in rodents [19]. The apparatus consists of two 25x25x25 cm boxes that were linked together. One box had plywood over the top, making it dark serving as dark box. The other box i.e., light box, is open and painted white [18].The time spent in the light box and the transitions from the light to the dark box both were recorded for five minutes [20].

Hole board test

The apparatus made up of a wooden sheet (40 X 40 cm), placed 3.5 cm above the apparatus's base and has 16 holes spaced at an equal distance 1.5 cm apart. The apparatus was raised to a 25 cm height (Lister, 1987). The number of head dips was counted over the period of five minutes while treated mice were placed in a corner [21].

Marble Burying Test

This test refers to the inhibition of the natural behaviour of burying novel objects in rodents as a measure of anxiolytic activity of the test compounds [22]. Marble burying test was carried using a propylene cage with 12 clean glass marbles placed equidistance i.e. 4 cm apart on the husk. Mice were administered test drug 30 minutes prior to the test. Then mice were placed in the cage containing marbles. The numbers of marble buried at least two-third of the area were counted.

Tail Suspension test

The tail suspension test is widely used method to evaluate antidepressant-like effects in mice. The test is based on the duration of immobility after the short-term, unavoidable stress of being suspended by their tail [23]. Animal were suspended 50 cm away from the ground and placed 1cm apart from the tip of the tail. During a test period of 6 minutes, the amount of time the mice were immobile was measured [24].

Statistical Analysis

Each group data is presented as the mean ±S.E.M. Statistical analyses was performed by One-way ANOVA followed by Dunnet 't's test using Graph-pad Prism version 5.0 software. p value at < 0.05 was considered as statistically significant.

Results

Effect of *P. incarnata* on behavior of Ethanol withdrawal mice in Elevated plus maze test.

As Shown **figure 1**, chronic ethanol consumption led to significant ($p< 0.05$) decreased in time spent in open arm of EPM in mice due to anxiogenic action of ethanol. In contrast, administration of *P. incarnata* was found to relieve ethanol abstinence-induced anxiety in mice as indicated by significantly ($p< 0.05$)

increased open arm time in EMP in comparison to the group II.

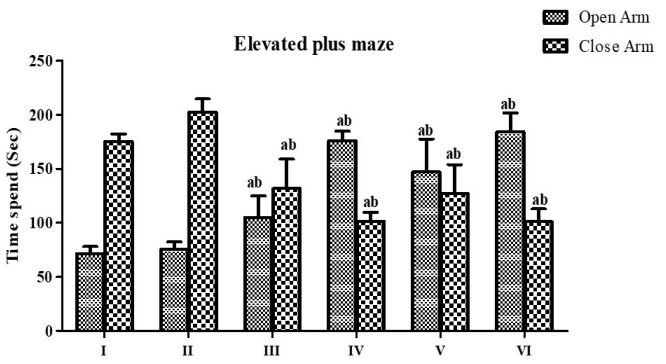

Figure 1: Effect of P. incarnata on behavior of Ethanol withdrawal mice in Elevated plus maze test.

Values expressed mean ± SEM (n=6), analyses by one-way ANOVA followed by Dunnett's test. [a]p< 0.05 compared to group I (vehicle-treated) and [b]p< 0.05 compared to group II (ethanol treated) group.

Effect of *P. incarnata* on behavior of Ethanol withdrawal mice in Light and Dark test

Figure 2 shows the effect of *P. incarnata* on parameters tested in light-dark apparatus. The vehicle treated and ethanol withdrawal mice showed significant (p< 0.05) increase in time spent in dark compartment than the light compartment indicating anxiogenic behavior due to abstinence. Mice treated with *P. incarnata* showed significant (p< 0.05) rise in the time spent in light compartment pointing anxiolytic activity of the tincture. More significant effect was shown in group IV that received PI 2 when compared to vehicle treated and ethanol withdrawal groups.

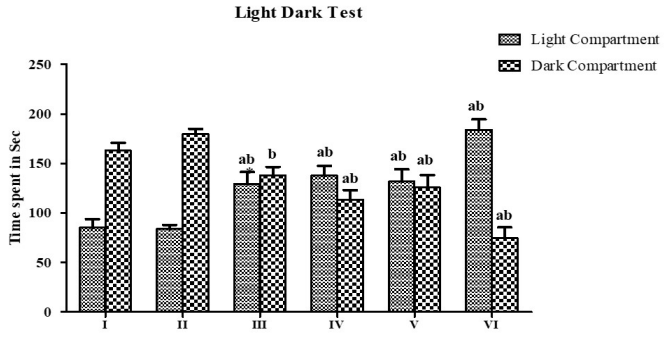

Figure 2: Effect of P. incarnata on light-dark exploration in Ethanol withdrawal mice.

Values expressed mean ± SEM (n=6), analyses by one-way ANOVA followed by Dunnett's test. [a]p< 0.05 compared to group I (vehicle-treated) and [b]p< 0.05 compared to group II (ethanol treated) group.

Effect of *P. incarnata* on behaviour of Ethanol withdrawal mice in Hole board test.

Chronic ethanol administration resulted in decreased no. of head dips in HBT in mice in comparison to the group I. Animals dosed with *P. incarnata* (Groups III, IV and V) shown significant (p< 0.05) increase in head dips in comparison to the group II. More significant effect was obtained with mice treated with PI 2 indicating anti-anxiety activity of the *P. Incarnata* in ethanol withdrawal mice **(Figure 3)**.

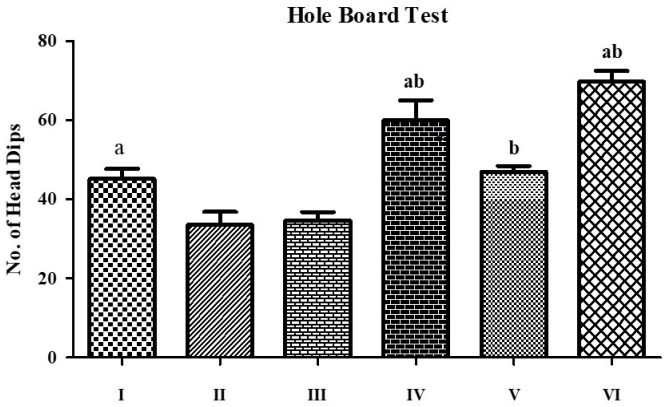

Figure 3: Effect of P. incarnata on behaviour of Ethanol withdrawal mice in Hole board test.

Values expressed mean ± SEM (n=6), analyses by one-way ANOVA followed by Dunnett's test. [a]p< 0.05 compared to group I (vehicle-treated) and [b]p< 0.05 compared to group II (ethanol treated) group.

Effect of *P. incarnata* on behaviour of Ethanol withdrawal mice in Marble burying test.

Figure 4 shows the effect of *P. incarnata* on parameters tested in marble burying apparatus. The vehicle treated and ethanol withdrawal mice showed significant (p< 0.05) increase marble burying indicating anxiogenic behavior due to abstinence. Mice treated with *P. incarnata* showed significant (p< 0.05) decreased marble burying in mice shows anxiolytic activity of the tincture. More significant effect was shown in group IV that received PI 2 when compared to vehicle treated and ethanol withdrawal groups.

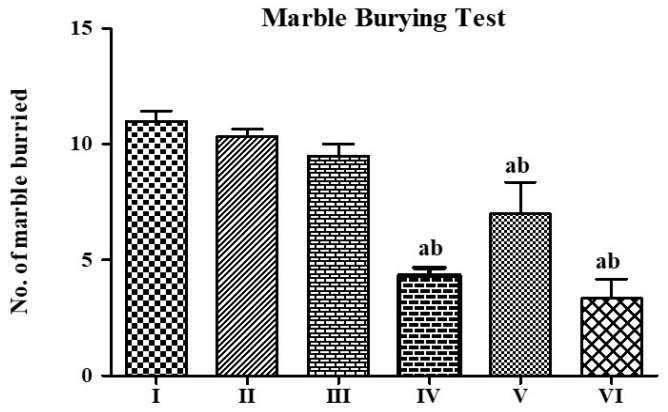

Figure 4: Effect of P. incarnata on Marble burying behavior in Ethanol withdrawal.

Mice

Values expressed mean±SEM (n=6), analyses by one-way ANOVA followed by Dunnett's test. [a]p< 0.05 compared to group I (vehicle-treated) and [b]p< 0.05 compared to group II (ethanol treated) group.

Effect of *P. incarnata* on behavior of Ethanol withdrawal mice in Tail suspension test.

Chronic ethanol administration resulted in increased immobility time in TST in mice in comparison to the group I. Animals dosed with *P. incarnata* (Groups , IV and V) shown significant (p< 0.05) decrease in duration of immobility as compared to the group II. More significant (p< 0.05) effect was obtained with mice treated with PI 2 indicating anti-depressant potential of

the *P. incarnata* in ethanol withdrawal mice **(Figure 5)**.

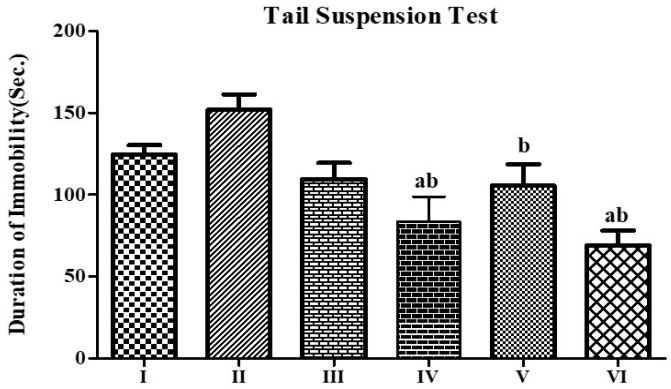

Figure 5: Effect of P. incarnata on behavior of Ethanol withdrawal mice in Tail suspension test.

Values expressed mean ± SEM (n=6), analyses by one-way ANOVA followed by Dunnett's test. [a]p< 0.05 compared to group I (vehicle-treated) and [b]p< 0.05 compared to group II (ethanol treated) group.

Discussion

In present study, we assessed the effect of *P. incarnata* in ethanol withdrawal induced anxiety and depression in mice. After the cessation of excessive or prolonged alcohol use, a set of symptoms known as ethanol withdrawal occur in alcohol-dependent people [25]. Ethanol withdrawal is characterized by the development of anxiety, the most significant negative motivational factor to revert to alcohol use. It has been reported that, anxiety following ethanol withdrawal is due to the malfunction of array of neurotransmitters and their receptors. These include GABA, 5-hydroxytrptamine (5-HT), and adrenergic receptors [26]. Alcohol interferes with a neurotransmitter systems, which affects brain function by disturbing the balance between inhibitory and excitatory (N-methyl-D-Aspartate) neurotransmitters [27]. Excitatory (NMDA) receptors are upregulated during ethanol withdrawal, while inhibitory $GABA_A$ receptors are downregulated [7,28]. Thus, the drugs either modulating the GABA or inhibiting glutamate activity may be effective in ethanol withdrawal-induced anxiety.

Passiflora have been well known and widely used species to treat anxiety since earlier times and *P. incarnata* is the extensively studied species of the genus Passiflora. Also, it is widely used as a natural anxiolytic [29].

The elevated plus maze is the most validated test for assessing anxiety-like behavior in rodents. Ethanol withdrawal-induced anxiety is regarded as the measure of psychologic dependence to ethanol. In the EPM test, typical anxiolytic drugs show increased number of entries and time spent in the open arms [30]. In the study undertaken, *P. incarnata* showed increased entries and time spent in the open arms in EPM test thus, reveling ethanol withdrawal induced anxiety.

The light-dark test is the exploration-driven test that rely on the voluntary locomotion of rodents. In general, rodents present a tendency to stay in a relatively safe area (the dark chamber of the box) versus a more aversive area (the light chamber of the box) Ethanol withdrawal mice showed decreased exploration time in the light chamber, indicating anxiogenic-like behavior. These results are consistent with others showing that after 2 weeks of 14 days ethanol binge-drinking increase anxiety-like behavior in mice following ethanol withdrawal across various paradigms including the light-dark test [31]. *P. incarnata* treatment showed increased entries and time spent in the open chamber in light-dark test thus, attenuating the ethanol withdrawal induced anxiety in mice.

Upon facing an unfamiliar environment or object as in case of hole board test and marble burying test, animals can exhibit the exploration like locomoting around the environment, orientating towards novelty, and touching or sniffing novel objects [32]. In the study envisaged, treatment with *P. incarnata* increased the nose poking of mice as an indication of enhanced curiosity whereas, the burying behavior is reduced pointing towards anxiolytic activity in ethanol withdrawal mice.

Ethanol-induced depression occurs during and at the time of ethanol intoxication or abstinence and is usually associated with distress and impairment. The model of depression chosen for the present study was tail suspension test which highlights anhedonia [33]. It was observed that mice treated with *P. incarnata* after chronic ethanol administration showed reduction in immobility in tail suspension test in contrast to animals those received ethanol only. This indicated the anti-depressive activity of *P. incarnata* for overcoming withdrawal induced depression.

In conclusion, we report that acute administration of *P. incarnata* mother tincture may prevent ethanol withdrawal-induced anxiety and depression due to its anti-addiction potential. *P. incarnata* attenuated anxiety and depression-like behavior produced after abstinence. Further studies are needed to find the mechanisms underlying the *P. incarnata* effects following ethanol withdrawal.

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