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Research Article

ASSESSMENT OF ANTI-ADDICTIVE, ANTI-CRAVING AND ANTI-RELAPSING ACTIVITY OF LEVO-TETRAHYDROPALMATINE ON ADDICTION INDUCED RODENT MODELS

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ABSTRACT

Addiction is a chronic brain disease which include craving and relapsing response characterized by compulsive substance/drug seeking for use in despite of harmful. Addictive substance has high tendency for long lasting changes in the structure, function and working property of the brain leading to harmful and bizarre behaviours. Globally, it was accounted as an economic burden through early morbidity, crime and treatment cost. The present study was designed to assess the antiaddictive property of L-tetrahydropalmatine (*l*-THP) (20mg/kg; 10mg/kg and 3mg/kg, p.o.) on Apomorphine induced addiction (1mg/kg/ml, i.p.) rats models using- Condition Place Preference (CPP) and Drug self-administration (DSP) methods for anti-addictive study; Runway self-administration test (RST) and Elevated plus maze (EPM) methods for anti-craving activity and Cue-induced relapse method for anti-relapsing property. *l*-THP was found to be significantly ($p<0.001$) decreased the number of entries & time spent in light compartment and decrease in number of lever press in anti-addictive study. It also significantly ($p<0.001$) decreased in number of entries and time spend of rats in open arms of EPM, decreased in time taken by rats to reach goal box in Runway self-administration test for anti-craving study. But, not significantly ($p>0.05$) decreased the no. of entries and time spent in light compartment on re-exposure to CPP model for anti-relapsing study. There was a significant increase in brain Dopamine (DA) level with positive control group but also significantly decrease of DA level with treatment group. All these may be attributed to the property of *l*-THP as a DA receptor antagonist against Apomorphine- a DA receptor agonist. Hence, can be considered as effective antiaddictive drug in the treatment of addiction.

INTRODUCTION

Addiction can be defined as a chronic brain disease that induces dysfunction of several brain circuits. It also includes craving and relapsing action that is usually characterized by compulsive substance or drug seeking for use in despite of harmful consequences. The substance or drug with addiction property have high tendency to cause long lasting changes in the structure, function and working property of the brain and can lead to harmful and bizarre behaviours which is usually seen in substance induced disorder's patients[1].

Drug craving is defined as "the desire to experience the effect(s) of a previously experienced psychoactive substance" [2]. Addicted patients describe craving as a powerful feeling that drive them to relapse to drug use[3]. Craving is a prominent feature of addiction that can persist for months or years after an addict's last substance intake[4]. It was seen that craving is the crucial substrate of addiction and the driving force behind continued use of a drug in spite of increasingly severe consequences. For example, craving for alcohol is due to a true physical demand for alcohol as a result of changes in cellular metabolism[5], there is no doubt on the beliefs that the central role of craving is the cause of addiction.

Drug relapse is a part of addiction, in simple the term relapse is used when an individual is withdrawn from drugs for a period of time and following that abstinence, he/she tend to use it again. The most difficult aspect of addiction treatment is the high risk of relapse, a subject can be abstinent for months or even years but still susceptible to craving that can stimulate renewed drug seeking and taking[6]. The difficulties in finding an efficacious pharmacological treatment in preventing the relapse in drug abuse may be due to the heterogeneity of the neurobiological mechanism underlying the desire to self-administer the drug. Repeated use of drug of abuse under certain environmental condition such as sights, smell and sounds provide conditioned stimuli that can elicit drug

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relapsing. Very strong and durable conditioned responses can be established where response is not affected by usual treatment such as detoxification and rehabilitation programs[7]. After a period of brief treatment, patients often return home with well feeling and confident that they will not resume drug use. But they are usually surprised by sudden craving feeling, withdrawal or even get high when encounter people or places associated with their prior drug use[8].

Apomorphine is a non-selective dopaminergic agonist that acts on D1 and D2 receptor. It is used for diagnostic and therapeutic purposes in the treatment of Parkinson's disease (PD), male sexual dysfunction and alcoholism[9,10]. but it has been observed that the drug is reinforcing[11,12]. Tellez[13] has reported one case on a 69 years old lady with Parkinson disease, undergoing apomorphine therapy and experiences fast improvement, the patient manages to maintain herself in good condition. Two weeks after therapy the intensity and duration of drug effect is decrease, therefore with no medical indication the patient increases the dose. Finally, the patient was admitted to the hospital for acute respiratory disease, blood pressure changes drastically, profuse sweating, muscle stiffness and rest tremor of both hands. There are few more cases of dependence on apomorphine described in the literatures.Studies have done on apomorphine as an addiction inducing agent in animal models and found that apomorphine with a dose of 1mg/kg produces a significant place preference and sensitization in rats as it can precipitate and potentiate the reinforcing and rewarding effects[14]. Due to the above reason, Apomorphine with the dose of 1mg/kg was selected as an addiction inducing agent in this study.

Levo-tetrahydropalmatine is the alkaloid and primary active chemical constituents present in the plant *Corydolis yanhusuo* and some other plant which belongs to a species of the genera Stephania and Corydalis. *Corydolis yanhusuo* is a Chinese traditional medicine having a number of clinical indications such as sedative, hypnotic and antihypertensive property. The pharmacological activity of l-THP includes the antagonism of Dopamine receptor (D1, D2 and D3), alpha adrenergic receptors and serotonin receptors. Therefore, due to this blockade activity l-THP can be consider as an antiaddictive agent. The neurochemical mechanism of l-THP as anti-addictive property is because of its inhibiting action on Dopamine receptors and its action on nigrostriatal neuronal pathways as inhibitors of both pre and post synaptic receptors. Subsequently due to the antagonize action on dopamine receptors as well as adrenergic and serotonin receptors, l-THP may have the utility for treating opioid and other type of addiction[15].

It was recently reported that l-THP, a dopamine D1 and D2 receptor antagonist appear to be effective in attenuating cocaine self-administration, cocaine triggered reinstatement and cocaine induced conditioned place preference in animal models. Systemic administration of l-THP with the low dose of 3mg/kg, mid dose 10mg/kg and high dose 20mg/kg was found to inhibit cocaine self-administration, locomotor activity. So, the dopamine receptor antagonism action of l-THP on cocaine reward together with the previous study, support the potential use of l-THP for the treatment of cocaine addiction[16].

With the basic understanding that the circuit which is mostly associate with pleasure and reward system is the mesolimbic pathway, usually locate in the brain stem and composed of the ventral tegmental area and further project to the nucleus accumbens (reward center). Dopamine being the neurotransmitter that most commonly linked with the mesolimbic system acts as the driving force behind the pleasure seeking in human, the release of Dopamine creates a pleasurable sensation.

Considering these, the present study was designed to contrast the effect of l-THP which is the dopamine receptor antagonist with the respective dose of 3, 10 and 20 mg/kg against the Apomorphine, a dopamine receptor agonist which was considered as the addiction inducing agent at a dose of 1mg/kg body weight.

MATERIALS AND METHODS

Collection of Corydalis extract (l-THP)

Corydalis yanhusuo extract containing 98.3% of Levo-tetrahydropalmatine (*l*-THP) was procured from Lift Mode 47 W. Polk St 100-241 Chicago, IL 60605.

Location and duration of study

The study was conducted at the animal house of the Department of Pharmacology, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bangalore-560054. The study was carried out at a duration period of 6 months.

Animals

Albino rats (Wistar strain) 9-12 weeks old, weighing 150-250gm and of either sex was used for these studies. The animals were bred, reared and housed in the animal house of Department of Pharmacology, MSRUAS. Animals were maintained in established animal house under the standard hygienic conditions, at a temperature 25°-28°C and 12hrs light/ 12 hrs dark cycle, room humidity of about 60 ± 10% with food and water *ad libitum*. Cleaning was done on alternate days and paddy husk was provided as bedding material. The pharmacological study was approved by the Institutional Animal Ethics Committee of Faculty of Pharmacy, MSRUAS (IAEC certificate no. XXI/MSRFPH/M-04/12.09.2018).

Acute Oral Toxicity study

Acute oral toxicity study of test drug was carried out. Rats of either sex was grouped with 5 animals in each group. The test formulation was administered orally and observed for any sign of behavioural, neurological toxicity and mortality for a total period of 14 days.

Experimental design

The animals were randomly divided into five groups (5sets for five methods). Each group consists of six animals. The animals were grouped as following-

Group I: Sham control group
Group II: Positive control group (1mg/kg/ml Apomorphine)
Group III: T1 group - Apomorphine (1mg/kg/ml) + l-THP (3mg/kg)
Group IV: T2 group - Apomorphine (1mg/kg/ml) + l-THP (10mg/kg)

Group V: T3 group - Apomorphine (1mg/kg/ml) + l-THP (20mg/kg)

Evaluation of Anti-addictive Activity

Condition Placed Preference (CPP)

Procedure: The test was conducted in a light and dark discrimination apparatus containing three compartments with different sensory property. First compartment is a dark compartment, second is light compartment and third compartment is the start point. The experiment is conducted in three phases-

Phase I: - Pre-conditioning phase

A day before base-line value of light/dark preference of each animal was monitored. Access from one to the other compartment was open. An animal to be tested was placed in the light compartment of the box. Time spent and number of entries in the light compartment was monitored for a cut off time of 10 min.

Phase II: - Conditioning phase

In this phase access from one to the other compartment in the light or dark box was closed. Animals were exposed alternatively for twelve day i.e., (1,3,5,7,9 and 11) to Apomorphine (30mins- light compartment), (day 2,4,6,8,10 and 12) treated with saline (30min-dark compartment).

Phase III: - Post conditioning phase

Reinforcing effects of Apomorphine were monitored in a test session on day 13 of treatment. (days 13-21) weigh each rat and immediately administer drug p.o. Place the rat into the center of the box. Time spent and number of entries in the light compartment was monitored during a test session of 10 minutes.

Drug self-administration paradigm (DSP/DST)
Procedure

The study follows the non-operant conditioning - lever press method:

One week daily prior to experiment, the grouped animals are allowed to become acclimated to the environmental condition. Ad libitum access to food and water was provided except during the training phase. Initially, four hours prior to the operant condition training the food was withdrawn from the animals. After four hours the animals are exposed to the equipment and train for lever pressing based on the reward system i.e. a single lever press will release one or more (based on the dose) drug content food pellet(s) (food pellets contain addiction inducing agent) as the reward for lever pressing. This step is repeated until the animals can do it independently.

The numbers of lever press are noted (increase in the number of lever press indicate the addictive activity of the drug). Once the training cum induction phase is done, test drug with respective dose is administered orally and leave aside for 30 mins. After 30mins the animals are re-expose to and allow to freely access the equipment. The numbers of lever press are noted for overall statistical calculation. Decrease in the number of lever press indicate the effectiveness of test drug or vice versa[17].

Evaluation of Anti-craving Activity

Runway self-administration test (RST).

The reward pathway- Dopaminergic/opioidergic dysregulation characterized by reward seeking and hedonism (pleasure seeking)

Procedure

Runway setup is composed of three segments with a start box and goal box in the opposite end of the alley**.**

The training procedure is consisting of six phases:

Phase 1- Habituation phase

This phase is done in one day where each animal is individually placed in the start box for about 90 seconds, the door is then open and allow the subject to freely roam the straight runway path for 5 minutes.

Phase 2- Pre-conditioning phase

During this phase, each animal is allowed to run from start box to the goal box. As soon as the animal enter into the goal box the door of the goal box will be close. The time interval between the opening of the start box and the closing of the goal box is recorded. The initial run time reading is considered as the baseline reading for pre-conditioning phase. Animals are returned to the home cage after recording of the run times.

Phase 3- Conditioning phase

In this phase, each animal is allowed to run from start box to goal box via runway. Run times are recorded for each trial. As the animal enters into the goal box (kept with food pellets) the addiction inducing agent is administered via single peritoneal injection each day for a period of five days (Day 1-5). The animals are supposed to be kept in the goal box for 5 mins before returning them to their home cage.

Phase 4- Post-conditioning phase

During the post-conditioning phase, the animal is placed in the start box for 90 seconds after which the start box door will be open, Animals are then allowed to run freely to the goal box without the administration of any substance (Day 6).

Phase 5- Extinction test

The two days extinction period (Day 7-8) follows 24 hours after post-conditioning phase. No treatment or testing should be conducted but the run times should be recording each day.

Phase 6- Reinstatement phase

In the reinstatement phase (Day 9-13). Each animal was administered with a priming dose of the test drug. Fifteen minutes after the administration, animals are placed individually in the start box for a single runway trial as described in the post conditioning phase[18].

Elevated Plus Maze (EPM)

The relief pathway- GAB Aergic/glutamatergic dysregulation characterized by stress reactivity, anxiety sensitivity, and hyper-arousability.

Procedure

Elevated Plus Maze (EPM) is a widely model used for behavioral assay in the assessment of anxiety and anxiety related disorders in preclinical addictive study. EPM is a four-armed maze with two open arm and two enclosed arms. The principle of this model is based on the tendency of rodents to avoid exposure to open areas and their distinctive behavior to search for food, to test for their fear and anxiety behaviour. The subject preference for open arm was observed and the performances of the treated group and the disease group are compared with that of the control group. Rodents have a distinctive preference for closed or enclosed arms where they feel safe. Hence, they tend to avoid exploring to the exposed arms.

Animals treated with anxiolytic drug showed reduced anxiety-like behaviour and are more likely to explore the exposed arms of the maze. On the other hand, animals treated with anxiogenic drugs spend less time in the open spaces due to the heightened anxiety-like feeling. The time duration where animal spends on either of the arms provide information regarding the anxious state of the subject. Arms entries are recorded only when all four paws of the subject are placed inside the arms. After the set time is elapsed the subjects is removed from the maze and placed back into its home cage[19].

In the case of this study, Addiction inducing agent (Apomorphine) is consider as the anxiolytic agent and the test drug (l-THP) is considered as the anxiogenic agent.

Evaluation of Anti-relapsing Activity

Cue-induced relapse (CIR)

Cue is a neutral environment stimulus such as light, sound, visual appearance and foot shock that is associated with drug use and can initiates relapse[20]. Presenting cues which is previously paired with drug use will initiate drug craving and seeking, as it is associated with the activation of glutamatergic projection from prefrontal cortex to the nucleus accumbens resulting in relapse[21,22].

Procedure

The test was conducted in a light and dark discrimination apparatus containing three compartments with different sensory property. First compartment is a dark compartment, second is light compartment and third compartment is the start point. The light condition was considered as the cue for initiating the relapse. The experiment is conducted in six phases-

Phase I: - Pre-conditioning phase

A day before base-line value of light/dark preference of each animal was monitored. Access from one to the other compartment was open. An animal to be tested was placed in the light compartment of the box. Time spent and number of entries in the light compartment was monitored for a cut off time of 10 min.

Phase II: - Conditioning phase

In this phase access from one to the other compartment in the light or dark box was closed. Animals were exposed alternatively for twelve day i.e., (1,3,5,7,9 and 11) to inducing agent (30mins- light compartment), (day 2,4,6,8,10 and 12) treated with saline (30min-dark compartment).

Phase III: - Preference test

Reinforcing effects of apomorphine were monitored in a test session on day 13 of treatment. The test activity was recorded. After preference test was completed subjects are removed from the apparatus and returned to their home cage.

Phase IV: - Post-conditioning phase (Days 13-21)

Weigh each rat and immediately administer test drug p.o. and wait for 30minutes. After 30 minutes place the animal into the center of the box. Time spent and number of entries in the light compartment was monitored during a test session for 10 minutes.

Phase V: - Extinction phase

During this phase animals are return to their home cage with free access to food and water for a period of two weeks. No treatment was given during this period.

Phase VI: - Reinstatement test

In this phase the animals are subjected for re-exposure to the apparatus under stimuli (light). The numbers and time spent in the dark compartment was noted. Decreased in the number and time spent in the dark light indicating the positive effect of the test drug against relapse or vice versa[23].

Estimation of Dopamine (DA)

Preparation of tissue extract

The animals were sacrificed, whole brain was dissected out and the subcortical region including striatum was separated. The tissue was weight and homogenized in 5ml HCl-Butanol solution for 1min. The sample was then centrifuged for 10min at 2000 rpm. 1ml of the supernatant phase was removed and added to centrifuge tube containing 2.5ml heptane and 0.31ml HCl of 0.1M. shake the tube vigorously for 10 min and then centrifuged under the same conditioned as above in order to separate the two phases, the overlaying organic phase was discarded. 0.2ml was then taken for estimation of Dopamine [all steps are carried out at 0°C].

Estimation of Dopamine

To the 0.2ml of aqueous phase, 0.05ml of 0.4M HCl and 0.1ml of Sodium acetate buffer was added, followed by 0.1ml of Iodine solution for oxidation. The reaction was stopped after 2min by addition of 0.1ml Na_2SO_4 solution. 0.1ml Acetic acid was added after 1.5min. the solution was then heated to 100°C for 6min, the sample is then cooled at room temperature, excitation and emission spectra was read from the fluorimeter at 330-375nm [24].

The dopamine level is calculated using the following formula

$$X_{express} = \frac{\text{Sample O.D - Blank O.D}}{\text{Standard O.D – Blank O.D}} \times \text{Conc. of standard (500µg/ml)}$$

Histopathological evaluation of brain

At the end of the treatment period, brain was isolated and stored in 10% Formalin solution, Haematoxylin and Eosin stain was used as a staining agent. The section was observed under

light microscope and the morphological changes in the subcortical region of the brain were analysed.

RESULTS

Acute Oral Toxicity study

The body weight of the rats before and after administration was noted that there are no changes in the skin, fur, eye, respiratory, circulatory, autonomic and central nervous system, motor activity and behavioural pattern was observed. No sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted. The sign and onset of toxicity are not shown.

Evaluation of Anti-addictive Activity

Condition Placed Preference (CPP)

Table 1 Effect of test drug on number of entries and time spent in light compartment

Behavioral parameter	No. of entries in the Light compartment		Time spent in the light compartment (sec)	
Groups	Pre-conditioning	Post- conditioning	Pre-conditioning	Post- conditioning
Sham control	0.3333±0.5164	0.5±0.5477	1±0.0524	1±0.0940
Positive control	2±0.4944	5±0.4650	452.4±0.0580	576±0.596
T1 (3mg/kg)	2±0.4944	3±0.4650*	450±0.5155	324±0.5589***
T2 (10mg/kg)	2±0.4944	2.3±0.5165***	453.6±0.4310	234±0.5187***
T3 (20mg/kg)	2±0.4944	2.3±0.5165***	451.8±0.5650	168±0.5155***

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where ***p<0.001 of T2 and T3 is highly significant in comparison with Positive control. *p<0.05 of T1 is less significant in comparison with positive control in number of entries in light compartment. ***p<0.001 of T1, T2 and T3 is highly significant in comparison with positive control in case of time spent in light compartment.

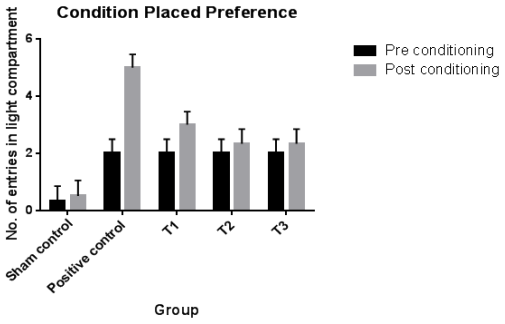


Figure 1a No. of entries in light compartment

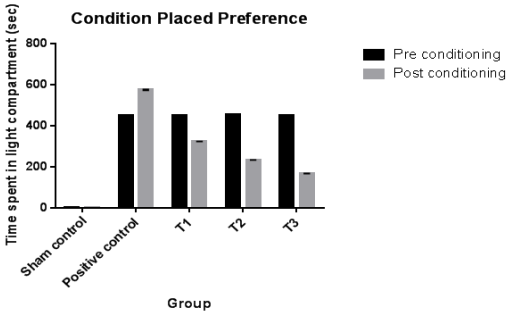


Figure 1b Time spent in light compartment

The effect of test drug on the no. of entries in the light compartment was found to be highly significant ***p<0.001 with T1 and T2 and less significant *p<0.05 with T1 when compared the positive control group. Sham control group was found to discriminate the light compartment whereas the positive control group and the pre-conditioning treatment (T1, T2, T3) was found to frequently enters with more numbers of time in the light compartment that considered as the addicted group. In the post-conditioning, the no. of entries of treatment group in the light compartment was found to be decrease when compared with the positive control group, indicating that the drug may be useful in the treatment of addiction. Similarly, the time spent in light compartment was also found to be decrease in treatment group in comparison to positive control group with ***p<0.001 which is highly significant.

Table 2 Effect of test drug on percentage food intake

S. No	Groups	Percentage food intake (%)
1	Sham control	97.6±0.5420
2	Positive control	102.3±0.4147
3	T1 (3mg/kg)	98.83±0.4967***
4	T2 (10mg/kg)	97.09±0.2458***
5	T3 (20mg/kg)	97.005±0.1768***

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where ***p<0.001 of T1, T2 and T3 are highly significant in comparison with positive control.

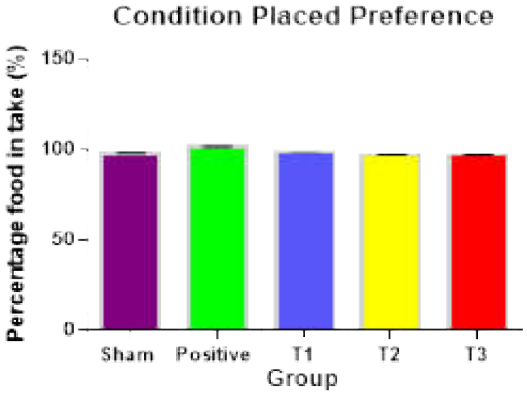


Figure 2 Percentage food intake

The percentage food intake was found to be more (above the normal range) with positive group when compare with the normal group. Where as in test group (T1, T2 and T3) it was found to be highly significant ***p<0.001, as the percentage food intake came down closer to a normal range (Sham control group range) in comparison with the positive control group. With this it was conclude that the test drug is having an anti-addictive and anti-craving property.

Table 3 Effect of test drug on percentage growth rate

S. No.	Groups	Percentage growth rate (%)
1	Sham control	66.05±8.36
2	Positive control	101.97±12.21
3	T1 (3mg/kg)	75.27±3.016***
4	T2 (10mg/kg)	66.92±3.351***
5	T3 (20mg/kg)	62.56±3.016***

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where ***p<0.001 of T1, T2 and T3 are highly significant in comparison with positive control.

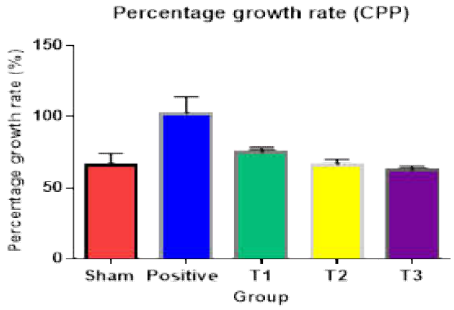


Figure 3 Percentage growth rate

The percentage growth rate of positive control group was found to be highly increased when compared with Sham control group. The effect of test drug (T1, T2 and T3) in rats growth rate was found to be highly significant (***p<0.001) and normalize the growth rate pattern of the test animals in comparison with positive control group. In the graph each column represents the mean percentage growth rate, the error bar indicates SEM.

Drug self-administration paradigm (DSP/DST)

Table 4 Number of Responses (DSP)

Behavioural parameter/ Groups	No. of response	
	Pre-conditioning	Post-conditioning
Sham control	1.16±0.166	1.33±0.21
Positive control	2.5±0.3416	2.5±0.22
T1 (3mg/kg)	2±0.2582	1.33±0.21*
T2 (10mg/kg)	1.66±0.2108	0.9±0.22**
T3 (20mg/kg)	1.83±0.3073	0.8±0.22**

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where **p<0.01 of T2 and T3 is moderately significant and *p<0.05 of T1 is less significant in comparison with positive control.

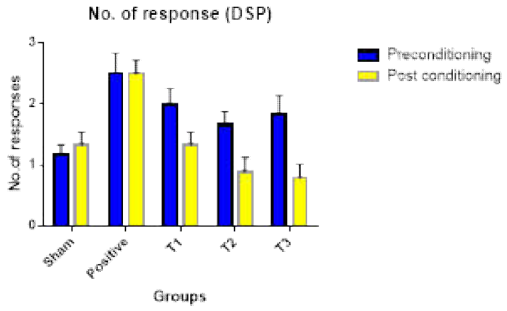


Figure 4 Number of responses

The number of responses was found to be increase in positive control group when compared to Sham control group. The effect of test drug on the treatment group (T1, T2 and T3) was found to be significant with the *p<0.05 in T1 and **p<0.01 in T2 and T3 in comparison with positive control group. Indicating that the test drug is having some anti-addictive property.

Evaluation of Anti-craving Activity

Runway self-administration test (RST)

Table 5 Runtimes during pre and post-conditioning in rats traversing runway

Behavioural parameter/ Groups	Runtimes (Sec)	
	Pre-conditioning	Post-conditioning
Sham control	141.40±0.50	141.60±0.50
Positive control	83.20±0.58	83.80±0.58
T1 (3mg/kg)	82.66±0.49	98.33±0.33***
T2 (10mg/kg)	83.16±0.47	120.83±2.49***
T3 (20mg/kg)	83.16±0.47	136.33±0.66***

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where ***p<0.001 of T1, T2 and T3 are highly significant in comparison with positive control.

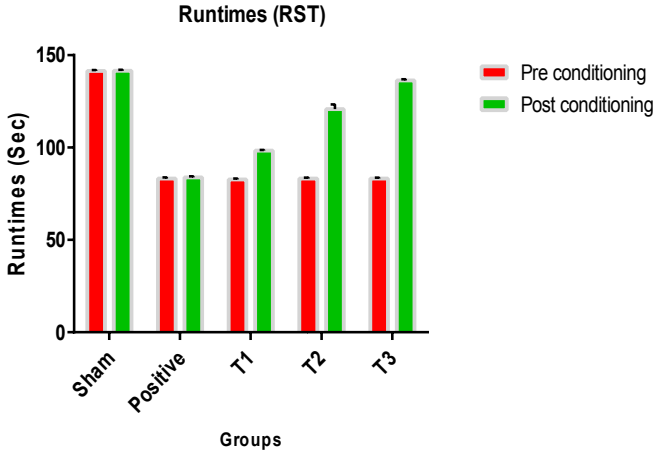


Figure 5 Runtimes during pre and post-conditioning in rats traversing runway

The runtime or time taken in seconds by rats to run from start box to goal box was found to be less with positive control group when compared to sham control group. The effect of test drug on treatment group (T1, T2 and T3) was found to be highly significant ***p<0.001 as it increases the time taken by rats to reach the goal box where addictive substance was given and behaving normally as those in the sham control when compared to positive control group. From this it can be assessed that rats are paying least attention in finding the addictive substance which is available in the goal box. Hence, it may have anti-craving property.

Elevated plus maze (EPM)

Table 6 Percentage number of entries and time spent in open arms

Behavioural parameter	Sham Control	Positive control	T1 (3mg/kg)	T2 (10mg/kg)	T3 (20mg/kg)
Percentage number of entries in open arms	29.80±0.44	80.55±0.43	68.23±0.27***	56.73±0.29***	49.07±0.66***
Percentage time spent in open arms	34.17±0.24	67.29±0.42	58.24±0.26***	54.17±0.35***	46.83±0.17***

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where ***p<0.001 of T1, T2 and T3 are highly significant in comparison with positive control.

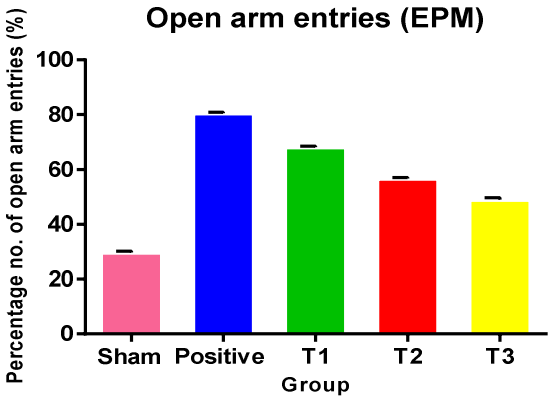


Figure 6 a Percentage number of entries in open arms

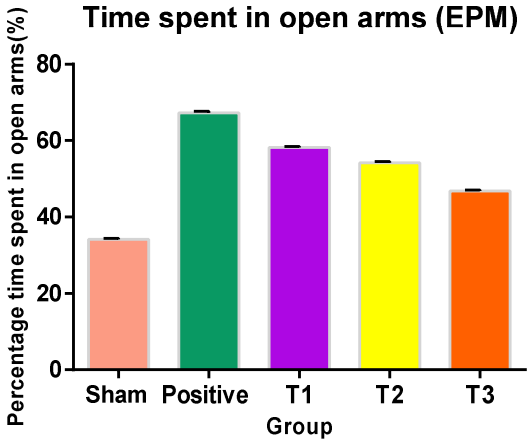


Figure 6 b Percentage time spent in open arms

The percentage number of entries in open arms was found to be more with positive control group where addictive substance was induced when compared to sham control group. The effect of test drug on T1, T2 and T3 group were found to be highly significant (***p<0.001) in reducing the number of entries into the open arms, bringing the normal behaviour in animals. This indicated that the drug is having an anxiogenic activity by acting against the anxiolytic (addictive) agent, the mechanism on which the drug acts is not known but suspected to be due to GABAergic/glutamatergic dysregulation. By reduction in the number of entries in light arms (addiction inducing arms) it may be considered that the test drug is having anti-craving activity. In the similar way, the percentage time spent in the open arms of the treatment groups (T1, T2 and T3) was also found to be highly significant (***p<0.001) in comparison with positive control group.

Evaluation of Anti-relapsing Activity

Cue-induced relapse (CIR)

Table 7 Effect of test drug on numbers of entries and time spent in light chamber

Behavioural parameter/ Groups	No. of entries in the Light chamber	Time spent in the light chamber (sec)
Sham control	0.333±0.5164	1±0.0941
Positive control	4.666±1.211	576±0.6693
T1 (3mg/kg)	4.166±1.472^{ns}	573.4±3.622^{ns}
T2 (10mg/kg)	4.166±1.169^{ns}	569.5±4.805^{ns}
T3 (20mg/kg)	4.333±1.211^{ns}	569.5±5.525^{ns}

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where ^{ns}p>0.05 of T1, T2 and T3 are non-significant in comparison with positive control.

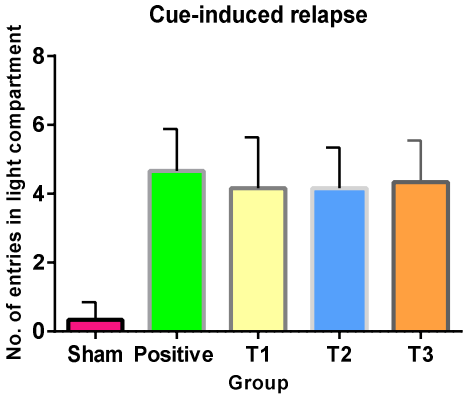


Figure7a No. of entries in light compartment (CIR)

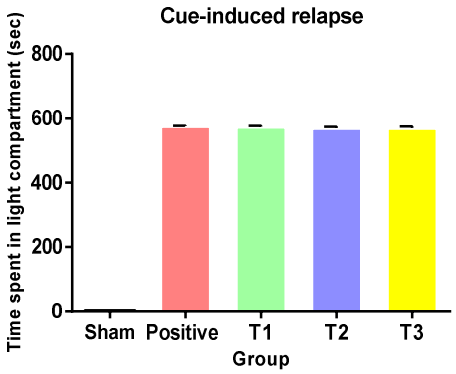


Figure7b Time spent in light compartment (CIR)

The effect of test drug on the number of entries and time spent of treatment groups (T1, T2 and T3) in light compartment on re-exposure after treatment was found to be non-significant ^{ns}p>0.05. This indicated that the test drug does not have anti-relapsing property.

Estimation of Dopamine (DA)

Table 8 Estimation of Dopamine (DA)

Group/ Parameter	Sham control	Positive control	T1 (3mg/kg)	T2 (10mg/kg)	T3 (20mg/kg)
DA (µmole/g of tissue)	31.28±0.33	54.26±0.20	42.08±0.06***	36.16±0.08***	33.07±0.05***

Values are expressed as Mean ± SEM, n=6, One-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where ***p<0.001 in comparison with Sham control. ***p<0.001 is highly significant in comparison with positive control.

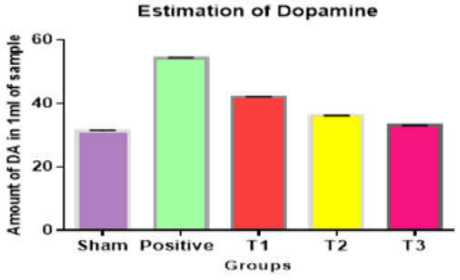
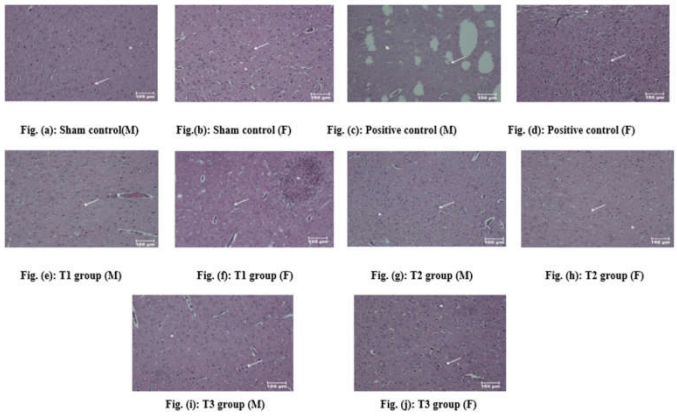


Figure 8 Estimation of Dopamine

Dopamine is a neurotransmitter that plays a key role in reward, signalling and addiction. In particular the nigro-mesolimbic dopaminergic pathway has implicated in the addictive properties of many drug of abuse i.e., by increasing the intracellular dopamine levels in the nucleus accumbens via blockade of specific DA reuptake channel. The DA which are highly enriched in NAc plays an important role in reinforcement and reward. Measurement of the levels of dopamine release in the apomorphine treated subjects was found to be increase when compared with the test drug treated group, this is based on DA agonist-antagonist action.

In Figure8 the result showed that DA level in positive control group were significantly increased on comparing with the sham control group. On the other hand, DA level is significantly (***p<0.001) reduces in test drug treated animals (T1, T2 and T3) when compared with the positive control group.



Histopathological evaluation of brain

Figure (a): Sham control Male- Brain cerebral cortex showing normal architecture of neurons with pyramidal cells (arrows) and few blood vessels. The molecular layer and external pyramidal layer were observed (asterisk). Haematoxylin and Eosin stain, scale bar=100µm.
Figure (b): Sham control Female- Brain cerebral cortex showing normal architecture of neurons with pyramidal cells (arrows) and few blood vessels. The molecular layer and external pyramidal layer were observed (asterisk). Haematoxylin and Eosin stain, scale bar=100µm.
Figure (c): Positive control male- Brain cerebral cortex showing distortion of normal architecture and degeneration of neurons with perineuronal vacuolation (arrows) and pyramidal cell degeneration. The cortex showing damage to cortical layer of brain with numerous vacuoles (asterisk). Haematoxylin and Eosin stain, scale bar=100µm.
Figure (d): Positive control female-Brain cerebral cortex showing distortion of normal architecture and degeneration of neurons with perineuronal vacuolation (arrows) and pyramidal cell degeneration. The cortex showing damage to cortical layer of brain with numerous vacuoles (asterisk). Haematoxylin and Eosin stain, scale bar=100µm
Figure (e): T1 male-Brain cerebral cortex showing normal architecture with mild degeneration of neurons with perineuronal vacuolation (arrows) and congestion of blood vessels with perivascular cuffing with mononuclear cells (asterisk). The molecular layer and external pyramidal layer were observed. Haematoxylin and Eosin stain, scale bar=100µm.
Figure (f): T1 female-Brain cerebral cortex showing normal architecture with mild degeneration of neurons with perineuronal vacuolation (arrows) and pyramidal cells. The molecular layer and external pyramidal layer were observed (asterisk). Haematoxylin and Eosin stain, scale bar=100µm.
Figure (g): T2 male-Brain cerebral cortex showing normal architecture of neurons and pyramidal cells (arrow). The molecular layer and external pyramidal layer were observed (asterisk). Haematoxylin and Eosin stain, scale bar=100µm.
Figure (h): T2 female-Brain cerebral cortex showing normal architecture of neurons and pyramidal cells (arrow) and focal area of infiltration with mononuclear cells (asterisk) indicating neuronal degeneration. The molecular layer and external pyramidal layer were observed. Haematoxylin and Eosin stain, scale bar=100µm.
Figure (i): T3 male-Brain cerebral cortex showing normal architecture of neurons (arrows) and pyramidal cells (asterisk). The molecular layer and external pyramidal layer were observed. Haematoxylin and Eosin stain, scale bar=100µm.
Figure (j): T3 female-Brain cerebral cortex showing normal architecture of neurons (arrows) and pyramidal cells (asterisk). The molecular layer and external pyramidal layer were observed. Haematoxylin and Eosin stain, scale bar=100µm.

The section from Sham control group of both male and female showed normal appearance of the cerebral cortex. Exposure of Positive control group to Apomorphine causes changes in the brain cerebral cortex with distortion of normal architecture and degeneration of neurons with perineuronal vacuolation and pyramidal cell degeneration. The cortex showing damage to cortical layer of brain with numerous vacuoles. Rats treated with different dose of test drug (*l*-THP) shows normal architecture of neurons and pyramidal cells hence, marked improvement in the affected brain.

DISCUSSION

Previous study reported that *l*-THP attenuated cocaine self-administration and suggested that it may have potential pharmacotherapeutic agent for use in drug dependent populations. The present study was designed and performed to assess the antiaddictive, anticraving and antirelapsing property of *l*-THP on Apomorphine addiction. The effect of *l*-THP on Apomorphine discriminative stimulus and re-inforcing effect most likely rises from its action on DA receptors. DA receptor through agonism activity of Apomorphinegets increases and hence with the antagonism activity of *l*-THP can attenuate discriminative stimulus properties towards apomorphine.

In antiaddictive study, administration of *l*-THP at a dose of 3, 10 and 20mg/kg is found to be significantly (***p<0.001) decreases the rate of Apomorphine self-administration which is indicated by decreases number and time spent by treated animals in the light compartment; moderately significant (**p<0.01) at a dose of 10mg/kg and 20mg/kg and less significant (*p<0.05) at 3mg/kg in decreasing the number of response (lever press). This might contribute to the pharmacotherapeutic activity of *l*-THP as an antiaddictive agent in treatment of Apomorphine addiction. The mechanism involved might be due to decrease in the extracellular level of DA in NAc and Striatum. First of all, *l*-THP is known to have antagonist property on DA receptors, particularly D2 and D3 receptor and would play an important role in reducing addiction and craving. D3 receptors are located post-synaptically and its subset are located presynaptically, its neuroanatomical location is restricted to express in distinct area of limbic system such as NAc, this NAc is involved in the various neurological and psychiatric disorders such as drug abuse, Parkinson's disease, etc.

In reflection with anticraving study, administration of (3, 10 & 20mg/kg) of *l*-THP was observed and found that it was highly significant (***p<0.001) in decreasing the runtimes of treated animals toward goal box associated to addiction, decreased in number of entries and time spent of treated animals in the open arms which is previously paired to addiction induced. These indicate that the drug may have anticraving property which may be contributed by the normalizes or stabilisation of the dysregulated Dopaminergic/ opioidergic, GABAergic/ glutamatergic and Serotonergic system in associated with reward, relief and obsessive pathway.

In antirelapsing study, *l*-THP was found to be non-significant (^{ns}p>0.05) as the number of entries and time spent by animals in the light compartment was found to be increased upon re-exposure after 2 weeks of treatment free period. Hence, can be conclude that the drug has very less significant antirelapsing

property. This may be due to maladapted of brain DA neurons towards Apomorphine upon long term exposure which usually could be readapted by existing drug but fails to produce by this test drug on termination of treatment.

The levels of dopamine release in positive control and treatment group was estimated and found that there is an increased in DA level in positive control group and gradually decreased in DA level (upto the range almost equal to sham control group) in the treatment group. This contribute to the effectiveness of *l*-THP in controlling and maintaining of DA release and level- a neurotransmitter that upon imbalance can leads to changes in normal function of the body, by either blockade of DA autoreceptors or by activation of the firing rate of DA neurons.

In histopathological study, the section of positive control group was found to have changes in the brain cerebral cortex with distortion of normal architecture and degeneration of neurons with perineuronal vacuolation and pyramidal cell degeneration. Whereas, in treatment groups normal architecture of neurons and pyramidal cells is seen, similar to those of the Sham control group. This contribute that *l*-THP may have the tendency to improve, stabilize and normalize the affected brain.

CONCLUSIONS

The present study supports the preclinical finding of *l*-THP as a therapeutic agent for the treatment of addiction. Treatment of *l*-THP at the dose of 3, 10 and 20mg/kg on Apomorphine-induced addictive rats for its antiaddictive and anticraving response were found to be effective. The study reveals that *l*-THP can attenuate Apomorphine discriminative stimulus property even at a very less dose of 3mg/kg which confirms its efficacy in addiction and craving treatment. But, in anti-relapsing study *l*-THP was found to be less significant. Based on the above outcome it can be concluded that the *l*-THP can be used in the management of Apomorphine and other narcotic drug addiction.

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