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PHARMACOLOGICAL EVALUATION OF ANXIOLYTIC ACTIVITY OF *CANNABIS SATIVA* EXTRACT IN ALBINO MICE

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ABSTRACT

The present work was performed to evaluate the anxiolytic activity in mice of ethanolic extract of *cannabis sativa*. The anxiolytic activity was evaluated by marble burying apparatus. Anxiolytic activity of *cannabis sativa* extract was compared with diazepam. Physically induced depression parameters showed various behavioural abnormalities in experimental animals. Mice were divided into 5 groups of 6 animals in each group. In marble burying apparatus Group I- Normal saline (as per body weight), Group II- Diazepam (2mg/kg), Group III-*Cannabis sativa* (10 mg/kg), Group IV-*Cannabis sativa* (20 mg/kg), Group V-*Cannabis sativa* (40 mg/kg). Analyzed the animals for anxiolytic effect of *Cannabis sativa* extracy by using marble burying apparatus. The result showed that *Cannabis sativa* extract at gradually increasing concentration 10 mg/kg, 20 mg/kg and 40 mg/kg significantly decreases marble burried in marble burying apparatus. Thus, *Cannabis sativa* extract may be included in effective treatment strategy of anxiolytic disorder.

KEYWORD: Anxiolytic activity, *Cannabis sativa*, Marble burying apparatus, Diazepam.

List of abbreviations

OCD	Occlusive Compulsive Disorder
Fig.	Figure
I.P.	Intra Peritoneal
P.O.	Orally
SEM	Standard Error Mean
GAD	Generalized Anxiety Disorder
PTSD	Post-traumatic Stress Disorder

INTRODUCTION

It has observed that most of the people are suffering from mental stresses and Anxiety, apart from anxiety number of symptomatic and asymptomatic disorders are the major challenge on the mental health now a days. On average up to 33.7% of the population are affected by an anxiety disorder during their lifetime (Bandelow & Michaelis, 2015). Anxiety is usually defined as an emotional state, marked by ill-adaptive and excessive to emotional sensitivity to potential hazardous situations. The anxiety pathology goes to emotional disturbances with an eternal effect towards the possibility of future, vaguely defined negative events (Nutt *et al.*, 2008). In the fourth edition of the Medical and Statically Manual of Disorders (DSM-IV) (Sugiura & Waku, 2000), according to the current definition of anxiety disorders, the major medical entities in this group are.

A) Generalized anxiety disorder (GAD), with general irritability, anxiety attacks, persistent fear/ anxiety symptoms and secondary phobic avoidance.
B) Stress disorder, characterized by brief (2-10 min) anxiety or stress spells followed by somatic and cognitive symptoms.
C) Social anxiety disorder (or social phobia), defined as excessive social agitation and the avoidance of social situations.
D) Obsessive compulsive disorder (OCD), characterised by recurring and intrusive anxiogenic thought (obsession) and stereotyped behaviour (compulsion) aimed at reducing the distress caused by obsession.
E) Post-traumatic stress disorder (PTSD), which results in a long-lasting anxiety reaction, re-experience/flashback syndrome, avoidance and emotional numbness.

Obsessive compulsive disorder (OCD) causes extreme changes in thought processes and behaviour. The disorder's main signs are characterized by persistent and recurring thoughts that cause distress (obsession) and compulsion that help to intensify the distress (Diniz *et al.*, 2012). Obsessions and compulsions are usually ego-dystonic, meaning the person is conscious of their symptoms but cannot regulate them (Rasmussen & Eisen, 1992). Most

people experience obsessive-like thought and/or compulsive behaviour but their frequency and intensity is what separates OCD obsession and compulsion. OCD is characterized by high rates of unreasonable and/or null standard responces (Leckman *et al.*, 2010). Polpular therapies, frequency opting to extend or change method in pharmacological and non-pharmacological ways. Patients with OCD have shown defects in corticostriatal-thalamocortical circuits including the orbito frontal cortex (OFC), anterior cingulatede gyrus, insula, striatum and thalamus (Monteiro & Feng, 2016). Such experiments are consistent with the survival of various dimensions of OCD symptoms, as different patterns of brain activity indicate contamination / washing, trusting and hoarding subgroups (Chaudhary *et al.*, 2015). The drug commonly used to treat depression is agomelatine, which is non-selective agonist of melatonin (Bhutada *et al.*, 2013). It has excellence binding potential for MT1/MT2 melatonin receptor and also functions as an antagonist of the 5-HT2C serotonin receptor (Loiseau *et al.*, 2006). Aglomelatine does not interact specifically with the serotonin, norepinephrine or dopamine intake (Zupancic & Guilleminault, 2006). Nevertheless, by inhibiting 5HT-2C receptors, it increases norepinephrine and dopamine secondarily within the brain's frontal cortex (Joel, 2006). This compound's may be antidepressant activity due to effect mentioned above. By analyzing the above data, it appears that this uncontrolled behaviour could be altered by the OCD melatonergic system and therefore this study aims to investigate the role of melatonergic system in modulating compulsive (uncontrolled) behaviour in rodants (Bagdy *et al.*, 2001). Both conditions are accompanied by divergent neurobiological changes along with the restoratiuon of their various clinical features and and phenomenological manifestation, and responds to slightly different pharmacotherapeutics stratigies (Joel, 2006). Animal's models and research paradigms for anxiety like behaviour have made a significant contribution to our understanding of the neural bases of anxiety disorders and to the development innovative therapies.

Pharmacological treatment for anxiety disorder therapy

Various pharmacological treatments for anxiety disorders are widely used now a days which are more tolerable, available over the past half century. At the same time, research is going on to understand the neurobiological and physiological mechanisms involved in chronic anxiety and stress responses, suggesting new approaches to the treatment of anxiety disorders (Pollack *et al.*, 2008). Evidence based data from patients with depression, and some uncontrolled data with anxiety, suggest that about 20% of patients may need 10–12 weeks or longer before responding. However, between one-third and one-half of patients on a modern

antidepressant do not achieve sustained remission from anxiety (Tedeschini *et al.*, 2010). List of various classes of compound are given below to treat different type of anxiety.

1. Generalized anxiety

a. Benzodiazepines

b. Buspirone

c. Selective inhibitor for reuptake of serotonin

2. Panic hit

a. Benzodiazepines for high potency

b. Antidepressants tricyclic

c. Selective reuptake serotonin antagonists

d. Inhibitors of monoamine oxidase

3. Post-traumatic stress

a. Selective inhibitors to reuptake serotonin

b. Low dose antipsychotic drugs

4. Compulsive obsessive disorder

a. Antidepressants tricyclic

b. Selective inhibiotors of serotonin reuptake

Paradigms for testing rodent like anxiety behaviour

Various test paradigms have been developed to assess behavioral parameters indicating anxiety in rodents. Tests for anxiety in rodents are suggested to identifying the profile of anxiety-related behavior in these animals.

1. Unconditional worries

a. Socio-anxiety

i) Ultrasonic vocalization induced in the maternal separation (for pups)

ii) Social interplay

b. Approach-based assessments/conflict avoidance

i). Open check area

ii). Abandoment protectrion

iii). Uplified plus

iv). Labyrinth high up T-maze

v). Zero maze

vi). Case light/dark

vii). Check on emergence

c Tests focused on defensive actions with antipredators

i). Mouse battery test defence

ii). The urinary sensitivity test for predators

iii). Exposure check with predator

d Other trials

i). Supression of feeds induced by novelity

ii). Marble burying

iii). To bury the defensive

2. Conditioned fears

a. Tests on conditional angst

b. Check operating conflict

i). Geiller-seifter test (Conditional eating supression)

ii). Vogel test (Conditioned drinking supression)

In recent decades, converging epidemiological, clinical, and preclinical finding have illustrated a central consequence of cannabis and its endogenous function in anxiety control.

Overview of plant *Cannabis sativa*

The herb genus marijuana has become synonymous with recredential medication marijuana in many areas. While cannabis plants are grown and used for food, fiber, fuel, medicine and shelter in various parts world, mainly cultivated, particularly in the United states is called cannabinoids for the major and essential chemical constituents (Sarma *et al.*, 2020). These cannabinoids are well known to be effective in treating a number of conditions of human disease (Kogan & Mechoulam, 2007), but were also misused due to their addictive, toxic and non-therapeutically beneficial properties (Huestis, 2007). Cannabis plants are thus seen as both a botanical blessing and a threat to society. As a plant with a long history of cultivation and use, cannabis has been spread to a range of ecosystem in Central Asia, the Northwest Himalayas and, most likely, China (Potter, 2014). Tropical and temperate areas of the world, by communities' drawn and addicted to poisonous resin and to the practical use of fibres and extractable fruit oil (Ames, 1958). This dichotomy of uses for cannabis as a medical and recreational substance and as a source of fiber and oil has continually stimulated public and scientific interest and curiosity about the plant's nature, leading to earlier studies of the plant's botany and other aspects. Cannabis, along with the genus Humulus (hops) and the genus

Celtis (hack-berry and sugarberry) is a member of the Cannabaceae family (McPartland, 2018).

AIM & OBJECTIVES OF THIS STUDY

The purpose of this research is "Pharmacological assessment of the anxiolytic effect of *cannabis sativa* in mice". Anxiety is a feeling of fear or stress, and is generally transient and manageable. The goal of fear may be true of that can be just an imagination, often general causes of anxiety are examination tension, arrangement of money for family survival, the incident that had already occurred, fear of standing in front of crowd, and speaking among them. A normal part of life is worry, doubt and fear. *Cannabis sativa* plant has shown effective results in the treatment of anxiety, spastic disorder, pain, antiemetic, epilepsy, glaucoma, bronchial asthma and mood disorder, based on literature survey. The main objectives of current study are.

- Collection and authentication of *Cannabis sativa*.
- Extraction of ethanolic extract of *Cannabis sativa*.
- To evaluate the effects of anti-anxiety in mice.
- Validate the burying apparatus of marble.

MATERIAL AND METHODOLOGY

Plant collection and authentication

Cannabis sativa leaves were collected from P.G. Hostel for research during the month of January, Distt-Bareilly, and Uttar Pradesh. Dr. Alok srivastava described the plant taxonomical, and authenticated it Head, Derpartment of plant science, M.J.P. Rohilkhand University, Bareilly, 243006, Uttar Pradesh.

Preparation of ethanolic extract of *Cannabis sativa*

With the aid of a grinder, dried shade leaves of *Cannabis sativa* were powdered. Extraction was archived by packaging the coarsely powdered (80gm) substance in a soxhlet assembly (with a round bottom flask containing ethanol and a reflux condenser at the top). Round bottom flask was held at temperature up to 55^0C on heating mantle. It does not surpass as MERK INDEX says ethanol BP is 64^0C at 760 mm Hg. After this solvent had been extracted by evaporation, a semi solid extract was then collected.

Animals

All experiments were performed using healthy albino mice of either sex (20-40 gm) collected from the Department of Pharmacy's animal house , M.J.P. Rohilkhand University, Bareilly, 243006, Uttar Pradesh and animals held at ambient temperature of 25±2 ^{0}C and 55-65 relative humidity with 12 hours light and dark period. The animals had free access to stand tap water and pellet chow. Animals were kept in groups for at least a week before they were used for experimentation.

Drugs and Chemicals

Diazepam as normal medication, distilled water, Ethanol is taken from M.J.P. Rohilkhand University, Department of Pharmacy.

Glassware and Apparatus

Measuring cylinder, funnel, beaker, glass rod, soxhlet apparatus, round bottom flask, condenser, heating mantle and balance weighing.

Experimental Protocol

Treatment

The present study employed five groups and each group consisting of six rats of both sexes as mention below. All the solutions were freshly prepared and administered in animals by intra-peritoneal and oral route (Imam *et al.*, 2016).

List of handled groups

To evaluate the anxiolytic activity of *Cannabis sativa* in albino mice all the selected animals are divided in six groups and the list of groups are given in **Table 1** and the Marble burying apparatus is shown in **Fig. 1**.

Fig. 1. Marble burying apparatus.

Table 1.- List of Animal group for anxiolytic activity evaluation.

Groups	Treatment
Group I	Normal saline (as per body weight)
Groups II	Standard drug Diazepam (2mg/kg, i. p.)
Groups III	10mg/kg Body weight Drug Extract
Groups IV	20mg/kg Body weight Drug Extract
Groups V	40mg/kg Body weight Drug Extract

Group I Animal- All Group I mice were treated with normal saline (as per body weight) up to 30 minute for five days before the experiment begins. **Group II animals-** All the mice were treated with standard drug diazepam 2mg/kg (Standard), intraperitonial, before the experiment begins for 30min. **Group III animal-** All the mice were treated with *Cannabis sativa* ethanolic extract at dose of 10mg/kg (Test I), orally up to 30 min for five days before the experiment begins. **Group IV animal-**All the mice were treated with *Cannabis sativa* ethanolic extract at dose of 20mg/kg (Test II), orally up to 30min for five days before the experiment begins. **Group V animals-** All the mice of this group were treated with *Cannabis sativa* ethanolic extract at dose 40mg/kg (Test III), orally up to 30min for five days, before the experiment begins.

Marble Burying Apparatus

Using regular acrylic cage (22x32x13.5) containing a 5cm layer of saw dust and 24 glass marble (1.5 cm in diameter) distributed evenly on saw dust in the cages. Once daily p.o. the *Cannabis sativa* (10, 20 and 40 mg/kg) and vehicle are administered. The last dose will be given on the 5^{th} day for a 5 days, 60 min. before the experiment. The regular Diazepam drug will be given at 2mg/kg i.p. 60 min. before the experiment and the animal were held in the cages for 30 min., and the amount of marbles buried in the saw dust was at least two third (Avijit *et al.*, 2010).

Method of testing

Mice were individually placed in the illuminated part of the cage facing in the opposite direction of the burying cage opening and the following parameters were recorded during the 30 minute test session, the total number marbles buried in the cage.

Parameter observed

During the 30 minute test session, one parameter was observed in the marble apparatus for the evaluation of anti-anxiety activity i.e. number of marble burry.

Statistical analysis

All results were expressed as mean ±S.E.M. and the intergroup variation were measured by t-test. Accordingly the use of graph pad prism software was considered statistically significant.

RESULTS

Following groups are evaluated by using marble burying apparatus for occlusive compulsive disorder. The burying of marble is counted during the time session of each 30 min. Each value of burying is the mean value obtained from marble burying apparatus and expressed as mean ±SEM. According to marble burying apparatus graph were plotted and their activities were evaluated.

Effect of drug on physically induced anxiolytic using marble burying apparatus

Each value reflects mean ±SEM (n=6), compared with marble burying control during the 30 minute time session. The effect of *Cannabis sativa* and standard drug on marble buried is given in **Table 2** and in **Fig.2.**

Table 2: Effect of drug on marble buried (Normal saline)

Group	No. of marble buried
Control (Normal saline)	17.666±1.021
Test I (*Cannabis sativa* 10mg)	16.166±1.077
Test II (*Cannabis sativa* 20mg)	15.0±0.894
Test III (*Cannabis sativa* 40mg)	10.166±0.600
Standard (Diazepam)	4.666±1.073

The test group consists of six animals in each group treated with dose 10, 20 and 40 mg/kg *cannabis sativa* extract and the normal group treated with 2mg/kg Diazepam for five days, respectively. They were examined in marble burying apparatus after care to determine their anti-anxiety effects during the 30 minute time session.

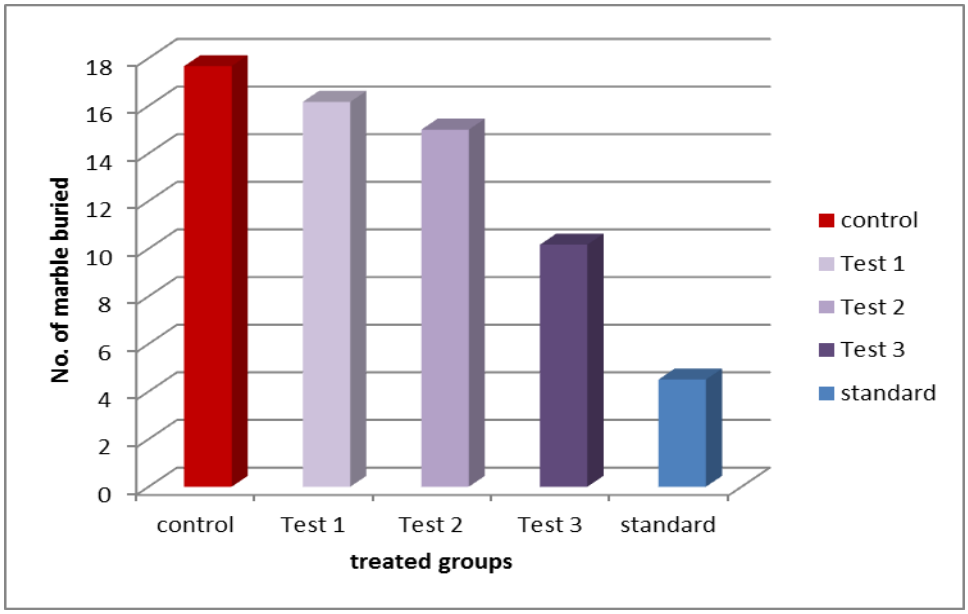

Fig. 2. Graphical representation of effect of drug on marble buried.

Cannabis sativa (10, 20 and 40 mg/kg,i.p.) when compared to Distilled water (as per body weight), and Diazepam (2mg/kg, i.p.) administered group. The result was found to be significant decreases (P<0.0001) marble burying in *Cannabis sativa.*

DISCUSSION

The concept of marble burying behaviour has been proposed as a useful model for testing the anxiolytic (compulsive anti-obsessive disorder). Diazepam treated community consists of six animals that are treated with Diazepam (2 mg/kg, i.p.) 30 minute before the experiment is started. Diazepam is composed of the drug class Benzodiazepine; it is one of the most frequently prescribed drugs for anxiety treatments. The amount of marble buried in marble burying apparatus is found to diminish substantially, the number of marble buried, compared to study Test I (10 mg/kg), Test II (20 mg/kg) and Test III (40 mg/kg) ethanolic extract of *Cannabis sativa* and control group administered, diazepam shows better marble burying apparatus. As a result, diazepam reveals that mice have a strong anxiolytic function (Chaudhary *et al.*, 2016).

Test I (10 mg/kg), Test II (20 mg/kg) and Test III (40 mg/kg) of the community treated with *Cannabis sativa* consists of six animals that are treated daily. In Test I (16.166±1.077), the number of marble burying is higher than in Test II (15.0±0.894) and Test III (10.166±0.600). That means the strength of extract when increases then the number of marble buried were decreases.

Test I: (*Cannabis sativa*, 10 mg/kg ethanolic extract) treated group consists of six animals which are treated with extract I, orally for five days. The test revealed that when compared to the control group (distilled water), there is a significant difference in amount of marble burying. So extract (*Cannabis sativa* ethanolic extract 10 mg/kg) does not exhibit anti-anxiety activity in the mice. **Test II:** (*Cannabis sativa*, 20 mg/kg ethanolic extract) treated group consists of six animals which are treated with Test II, orally for five days. Here the buried marble number decreases as a contrast to control group (normal saline) in during 30 minute of time period. The whole parameter shows that a 20 mg/kg dose of *Cannabis sativa* ethanol extract has an anxiolytic effect. Even Test II in mice shows anxiolytic activity. **Test III**: (*Cannabis sativa*, 40 mg/kg ethanolic extract) treated group consists of six animals which are treated with Test III, orally for five days. The amount of marble buried there is much smaller than that of control group (normal saline), ethanolic extract of *Cannabis sativa* dose 10 mg/kg and 20 mg/kg cannabis sativa in marble buried in marble burying chamber. The results showed that dose 40 mg/kg has more anti-anxiolytic effect compared to 10 mg/kg and 20 mg/kg, but as Diazepam (2 mg/kg) it is not effective. It can assess ethanolic dose of *Cannabis sativa* 40 mg/kg on behalf of this study to have anti-anxiety effect in mice. Such development could be due to increased serotonin and neurotransmitter nor-epinephrine levels in mice's brain. This study is supported by earlier anxiety related studies of *Cannabis sativa* (Garabadu & Krishnamurthy, 2014).

SUMMARY AND CONCLUSION

Anxiolytic is something of an emotional response. It is an overwhelming feeling most people feel when the challenge faces them. Mild anxiety needs no medication, but anxiety is intense; it causes distress, lasts longer, and interfaces with the daily activities, which is medical concern. Anxiety is the mental disorder most common in human. The aim of the present study is to investigate the effect of *Cannabis sativa* in mice using a marble burying apparatus. This apparatus is quick, less time consuming, it may or may not test the anti-anxiolytic behaviour of the agent during the time session of 30 minute following parameter being reported on behalf of these parameters. It reflects anxiolytic behaviour as the amount of marble buried by the animals in marble burying apparatus decreases so; such criteria were therefore used to evaluate anxiolytic function of different doses of *Cannabis sativa*. Based on the results obtained in this study, the following salient finding can be summarized.

- Diazepam (2 mg/kg) has been shown to be a powerful anxiety drug in mice. Diazepam has given us the best results relative to all other groups and there is no significant difference compared to group monitoring.
- There is a significance difference between *Cannabis sativa* (10 mg/kg) and control group. And, in mice this has lower anxiolytic activity.
- There is a significant difference between *Cannabis sativa* (20 mg/kg) and control group. So, this operation in mice is anxiolytic. Compared to Diazepam (2 mg/kg), this dosage indicates minute anxiolytic action.
- Cannabis sativa (40 mg/kg) has more anxiolytic activity than other *Cannabis sativa* levels. The results also showed that compared to the control group, there is significant, but it is not as potent as diazepam. So this dose can be considered effective in mice as a *Cannabis sativa*.

From the above data it can be inferred that the ethanolic extract of *Cannabis sativa* at 10 mg/kg dose does not exhibit anxiolytic activity, does 20 mg/kg proves to be the effective does then all other doses of ethanol extract of *Cannabis sativa* bit is not as potent as regular diazepam (2 mg/kg) in marble burying apparatus. The ethanolic extract of *Cannabis sativa* at 40 mg/kg shows a higher dose of anxiolytic activity.

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