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Anti-depressant Activity of *Commiphora mukul*

*** Kavita kumari** Department of Pharmacology, Vidhyasthali institute of technology, science and management, Jaipur. * Corresponding Author

Sneha Bhataia Senior demonstrator, Department of Pharmacology, R.N.T. Medical college, Udaipur

ABSTRACT

The antidepressant activity of Commiphora mukul were studied at doses of 200 mg and 400 mg/kg against forced swimming test and tail suspension test in albino wistar rats. Fluoxetine (10mg/kg, orally) was selected as reference standard and it showed significant antidepressant activity in albino wistar rats. A dose dependent reduction in immobility time was observed in FST and TST. Whereas the extract significantly increases the exploratory behaviour in rats. The finding from the present investigation indicates that extract has significant anti-depressant activity as shown by its effects on different experimentally induced different models.

KEYWORDS : *Commiphora mukul*, Antidepressant, Forced swimming test, Tail suspension

INTRODUCTION

According to the World Health report, approximately 450 million people suffer from mental or behavioral disorders, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3 % of the global burden of disease and will rise to 15 % by 2020. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide has progressed constantly demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. Anxiety and depression are extremely dramatic and debilitating multifacetic disorders and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurological aspects of depression and anxiety. [1&2] Depression is an important health care problem in the world that is characterized by several signs such as intense sadness, despair and recurrent thoughts of death or suicide.

Prevalence of this disorder is about 13-20% of population.[4] Approximately two third of depressed patients has suicide thoughts and 10-15% of whom attempt suicide before the age of 40.[5] Although several synthetic drugs are available for treatment of depression, side effects such as dry mouth, hypotension, fatigue, sexual dysfunction and drowsiness limit the use of these treatments.[6] In addition, the success rate of medication is low and at least 40% of the patients do not respond to the antidepressant drugs.[7] Therefore, researches for new antidepressant drugs with fewer side effects are needed. Nowadays, medicinal plants are largely investigated for treatment of depression.[8] Several plants such as *Crocus satvous, Echium vulgar, Rosmarinus officinalis, Hypericum reflexum* and *Ginkgo biloba.* It may have beneficial effects on stress, tension and depression. Animal had free access to food and water. Experiments were carried out between 9:00 and 17:00. The animals were placed in the experimental room 24 h before the test for acclimatization.[9]

MATERIALS AND METHODS:

Collection and authentication of plant materials

The plant materials leaves and stems of *Commiphora mukul* belonging to the family *Burseraceae* were collected in the month of May 2014 from the local areas of Jaipur district, Rajasthan, India. The plant material was identified and authenticated by Rajasthan University.

Authentication No. – RUBL211428

Preparation of ethanolic extract of *Commiphora mukul*

Plant material was collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant material was then shade dried and pulverized in a hand mill followed sieving (sieve no. 40) to obtain coarse powder.

About 180 gm of dry powder was extracted with ethanol (40-60^{0}C) for 48 hr in soxhlet extractor. The ethanolic extract was filtered with Whatman filter paper, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The final obtained extract was weighed to obtain **27.4 g** and percentage yield was calculated and stored in a cool place.

Phytochemical analysis

The ethanolic extracts of Commiphora mukul were subjected to preliminary phytochemical screening.

Ethical approval and preparation of experimental animals:

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), Institute of biomedical & industrial research, Jaipur, India. (1517/PO/Q/11/CPCSEA) and all the experiments was conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

About 24 albino wistar rats of either sex weighing between 150-200 gms, procured from disease free animal house of Jaipur were used for the present study. Animals had free access to food and water and maintained under standard laboratory conditions (Temp. 24± 2^{0}C and relative humidity 30-70%) with a natural light and dark cycle ratio of 12:12 . The animals were acclimatized for at least five days before behavioural experiments. Experiments were carried out between 9.00 and 15.00 hrs. Experimental protocol was approved by the institutional animals' ethics committee before the start of the study.

Acute toxicity studies

The acute oral toxicity study was carried out according to OECD 423 guidelines which are based on a stepwise procedure with the use of a minimum number of animals (wistar rats of 150-200 g) per step. Absence or presence of compound related mortality and behavioural changes of the animal's dose at one step were determined the next step. Mortality in each group within 24 h was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The median lethal dose (LD50) was calculated using the second phase.[32(10)]

Selection of animals specie:

Healthy young albino wistar rats of either sex weighing 150-200 gm were used for acute toxicity study. Prior to dosing animals was fasted approximately 18 hr during the fasting period the rats were examined for health and weight (initial). Six (3 male and 3 female) healthy rats were selected for test.

Dose calculation: Doses were calculated based on the initial body weight.

Body weight: Individual body weight of the animals were recorded prior to test substance administration and again on day 7 and 14.

Cage Side Observations: The animals were observed for mortality, signs of gross toxicity and behavioural changes at 1 and 3 hrs. post-dosing and at least once daily there after 14 days. Observations included gross evaluation of skin and fur, eyes, respiration, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convultions, salivation, diarrhea and comma.

Dose selection: Dose was selected on the basis of maximum tolerable dose, as there was no lethality observed up to 2000 mg/kg. Thus dose took as 1/10 and 1/5 of 2000mg/kg (i.e.200mg/kg and 400 mg/kg) for further investigation.

Drugs use
Fluxetine hydrochloride was used in this study as standard. All drugs were dissolved in distilled water and administered either intra peritoneally (i.p.) or orally (p.o.) used. Distilled water was used as the vehicle.

Pretreatment with drugs:
Commiphora mukul stem and leaves ethanolic extract was dosed at 200 and 400 mg/kg for 14 days, by oral route to the rat, FST and TST were carried out at 14th day, 1 hr after dosing the animals. Fluoxetine (10 mg/kg *P.O.*) was used as reference positive standard and was dosed only at day 14. For interaction of *Commiphora mukul ethanolic extract* with conventional antidepressant drugs, *Commiphora mukul ethanolic extract* was dosed at sub-effective dose for 14 day and conventional antidepressant were also administered at sub-effective doses only at 14th day and 1 hr after dosing, animals were subjected for forced swim test and TST.

STUDY DESIGN
The animals were selected randomly for each experiment and divided into 4 equal groups. Drugs (Fluoxetine) administered orally (P.O.) for 7 & 14 successive days. Sixty minutes after last dose, immobility period was recorded in two different animal models of depression. Overnight fasted animals were selected randomly on the day of experiment for administration of vehicle, standard drug and study drug. The animals were acclimatized one hour before for behavioural tests. Thirty minutes and 1 hour time interval between drug administration and a behavioural test were maintained in case of i.v. and oral administrations respectively.

The animals were divided into four groups of six animals in each as follows:

Group I (n=6) – Control, received distilled water,

Group II (n=6) – (Standard) Fluoxetine (TST and FST) 10 mg/kg,

Group III (n=6) – Ethanolic extract of *Commiphora Mukul* 200 mg/kg,

Group IV(n=6) – Ethanolic extract of *Commiphora Mukul* 400 mg/kg,

The antidepressant activity was carried out using two different models. Further the effect of drugs was evaluated in both test.[2]

Assessment of Antidepressant activity:
Forced swimming test (FST):
Rats of either sex were individually forced to swim in an cylindrical container (diameter 10cm, height 25cm), containing 19cm of water at 25±1^{0}C. All the rats of either sex were divided in four different groups. The first group assigned as control receiving only vehicle.

Assessment of Antidepressant activity:
Forced swimming test (FST):
Rats of either sex were individually forced to swim in an open cylindrical container (diameter 10cm, height 25cm), containing 19cm of water at 25±1°c. All the rats of either sex were divided in four different groups. The first group assigned as control receiving only vehicle. The other third and fourth groups received acute dose of ethanolic extract of CM (200, 400mg/kg). The second group received standard drug Fluoxetine (10mg/kg). The total duration of immobility was recorded during the last 6min of the 10min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect.[28-31]

Tail suspension test (TST) :
Tail suspension test is behaviour despair model of depression, employed in rodents to predict antidepressant potential by decreasing immobility period produced by several different classes of antidepressant drugs. It has been reported that tail suspension test is less stressful and has higher pharmacological sensitivity than forced swim test, the other commonly employed model to study antidepressant activity. Treatment was given 60min prior to study as described by study design. Rats were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min period. The animal was considered immobile when it did not show any movement of the body except for those required for respiration and hanged passively[33]. Mice were considered immobile when they were completely remain motionl

OBSERVATION AND RESULTS
Commiphora mukul was extracted by ethanolic solvent extraction process.

The ethanolic extract of *Commiphora mukul* were subjected to preliminary phytochemical screening.

Table.1: Phytochemical screening of chemicals in ethanolic extracts of *Commiphora mukul:*

Sr. no.	Extract test for	Ethanolic extract
1.	ALKALOIDS	Absent
2.	STEROLS	Present
3.	TRITERPINOIDS (Terpenes)	Present
4.	TANNIN	Absent
5.	FLAVANOIDS	Present
6.	VOLATILE OIL	Absent
7.	RESINS	Present
8.	GUM	Present
9.	Acid	Present
10.	Alcohal	Present
11.	Carbohydrate	Absent
12.	Glycosides	Absent
13.	Fatty acids	Present

A dose response antidepressant screening was performed using 200 and 400mg/kg of ethanolic extract of *Commiphora mukul* leaves and stems against depression by models. Depression were scored and analysed as described earlier. The study indicates a dose dependent anti depressant activity of ethanolic extract *Commiphora mukul* leaves and stems.

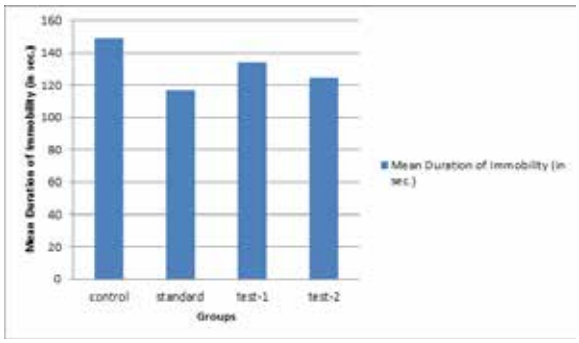
Table No.2: Results of the effects of the ethanolic and aqueous extract of *Commiphora mukul* leaves and stems against antidepressant by forced Swim Test in Rats .
Observation:

GROUP	TREATMENT	DOSE (mg/kg)p.o	Mean Duration of immobility (sec.)
1	Control	1ml/kg	149 ± 2.469
2	Fluoxetine	10mg/kg i.p	117 ± 2.875**
3	Ethanolic extract of *Commiphora mukul*	200mg/kg	134 ± 3.276*
4	Ethanolic extract of *Commiphora mukul*	400mg/kg	125 ± 3.055**

One way ANOVA followed by Dunnet's test Values are mean ± S.E.M

(n = 6), in each group *p < 0.05, **owed p < 0.01 when compared to control.

Fig. 1. Graphical representation of Forced swimming test.



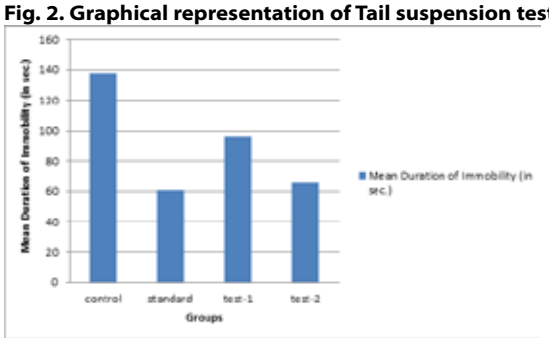
The effect of ethanolic extract of Commiphora mukul 1 and 2 are tabulated as shown in table .Duration of immobility a measure of antidepressant activity was recorded in the last 6 minutes of 10 minutes test session. Statistically significant (P<0.05and P<0.001) reduction in duration of immobility was observed in ethanolic extract *Commiphora mukul*-1 and -2 treated animals respectively. The effect of 400mg/kg was nearly equal to Fluoxetine treated animals (P<0.001), hence the effect of 400 mg/kg can be considered antidepressant dose of test formulation. Acute effect of ethanolic extract of *Commiphora mukul*-1 and 2 test formulation.

Table.3: Results of the effects of the ethanolic and aqueous extract of *Commiphora mukul* leaves and stems on Tail Suspension Test in rats at different time intervals.
Observation:

GROUP	TREATMENT	DOSE (mg/kg)p.o	Mean Duration of immobility (in sec)
1	Control	1ml/kg	137.7 ± 14.96
2	Fluoxetine	10mg/kg	60.33 ± 4.310***
3	Ethanolic extract of *Commiphora mukul*	200mg/kg	96.67 ± 10.56*
4	Ethanolic extract of *Commiphora mukul*	400mg/kg	65.67 ± 12.42***

Values represents the mean ± SEM,*P< 0.05, ***P<0.001, when compared to vehicle treated animals.

Fig. 2. Graphical representation of Tail suspension test.



Statistical analysis
The results will be express as mean ± S.E.M. The differences will be compare using one way analysis of vasriance (ANOVA) and subsequently follow by Bonferroni's test

DISCUSSION
The prevention and management of stress disorders remains a major clinical problem. Hence it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. In this work, it was demonstrated that the administration of different doses of the ethanolic extract of *Commiphora mukul* in rats was able to induce antidepressant effects. In forced swimming test, the extract can decrease the immobility time in rats with mild sedative effect. It was found that *Commiphora mukul* can produce antidepressant like activity at a dose of 200mg and 400mg/kg body weight in a dose dependent manner. The decrease in the immobility time is accompanied with the increase in swimming time. Previous demonstrated that many neurotransmitters were involved in the pathophysiology of depression. Numerous studies have demonstrated that antidepressant drugs such as Fluoxetine, Imipramine stimulated the action of serotonin and act by inhibiting the reuptake of biogenic amines in CNS. These drugs were widely used as antidepressant drugs and agreed with studies in animal models, such as forced swimming test. An antidepressant drugs reduce the exploratory behaviour depending upon the concentration. At present, the study revealed that the ethanolic extract of Commiphora mukul significantly reduces the number of head dip pings and numbers of line crossings were the indicator of exploratory behaviour. The findings from the present investigation indicate that EECM (Ethanolic extract of commiphora mukul) possesses significant antidepressant activity as shown by its mitigating effects on different experimentally induced stress models in rats and mice The widespread use of FST is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rodents following initial escape oriented movements develop an immobile posture when placed inside an inescapable cylinder filled with water. The immobility is thought to reflect either a failure of persistence in escape directed behavior (i.e., despair behavior) or the development of a passive behavior, meaning the loss of the animal's ability to cope with stressful stimuli. Markedly showed a significant decrease in the time spent immobile by rodents. By performing tail suspension test, the reduced immobility time directed the antidepressant effect.[2]

Conclusion
Ethanolic extract of commiphora mukul has significant anti-depressant activity as shown by its effects on different experimentally induced different models.

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