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Original Article

Evaluation of Antidepressant Activity of Hydroalcoholic Extract of *Commiphora Mukul (Engl.), Burseraceae* in Mice.

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Abstract

Commiphora mukul (CM) is reported for several neurological disorders, but it is not been reported for its antidepressant activity. The aim of the present work was to evaluate the antidepressant activity of a hydroalcoholic extract of Commiphora Mukul (HECM) and its chloroform and ethyl acetate fractions using acute depression models in mice. The effects of acute oral pretreatment of HECM (50, 100, or 200 mg/kg p.o.), chloroform soluble fraction and ethyl acetate soluble fraction of HECM (20mg/kg p.o. and 40 mg/kg p.o.) and fluoxetine (10 mg/kg p.o.) were evaluated using force swim test (FST) and tail suspension test (TST) in mice. Immobility duration in FST and TST was measured one hour after of all the treatments. At the end of TST plasma corticosterone was measured. Both HECM and both the fractions showed dose dependent normalization of immobility duration in both the parameters along with the normalization of plasma corticosterone levels. HECM exerts antidepressant effect in acute models of depression in mice.

Keywords: Commiphora Mukul extract, Guggulsterone, Antidepressant, Serotonin.

1. Introduction

Mental depression is a chronic illness that affects a person's mood, thought, physical health and behavior. The behavioral changes are due to the altered levels of monoamines in brain and plasma corticosterones (Jans et al., 2006). Many antidepressants are currently available in the market but they are associated with many potential side effects (Hueletl-Soto et al., 2011; Khawam et al., 2006) such as dry mouth, constipation, bladder problems, sexual dysfunction (Berton and Nestler., 2006; Frye and Rhodes, 2010; Sequeira et al., 2009) , blurred vision, dizziness, drowsiness, and increased heart rate (Kemp et al., 2003).

Most of antidepressants in general but SSRI in particular are associated with the side effects as sexual dysfunction, nausea, nervousness, insomnia, agitation, decreased sweating with increased body temperature, suicidal behavior (Malkesman et al., 2009). The antidepressants available exhibits many disadvantages such as slow onset of action (Kasper et al., 2006), low response and associated side effects. Therefore, the development of new type antidepressants is an urgent and unmet need, so better remedy is needed to treat the clinical depression. Commiphora mukul, a plant that is native to India, and its active constituents include guggulsterone E, guggusterone Z, guggusterone I-VI, sesanin, camphorene and cambrane-a (Anurekha and Gupta, 2006). Uses of Commiphora mukul includes hyperlipidemia (Urizar and Moore, 2003), rheumatic disorders (Lee et al., 2008), obesity

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(Sharma et al., 2009) and in ischemic heart diseases (Deng, 2007). Guggulsterone (GS) is one of the important constituent of CM. GS is reported to show serotonin releasing property in brain (Srivastava and Kapoor, 1986). So we found it interesting to evaluate the possible antidepressant activity of HECM in acute models of depression.

2. Materials and Methods

2.1 Animals

Swiss albino mice (20-25 g) were purchased from National Toxicology Centre (NTC), Pune. The animals were housed at a temperature of 25±2 ºC and relative humidity of 45-55% under 12:12 light: dark cycle. At the time of housing, animals had free access to the feed pellets (Chakan Oil Mills Ltd., Sangli, Maharashtra, India) and tap water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Maharashtra Institute of Pharmacy, Pune (Approval No: MIP/IAEC/2012-13/M2/Appr/006). All observations were recorded between 8:00 and 15:00 h and each animal was used only once. To avoid subjective bias, the observer was unaware of the given treatment. Each experimental group consisted of 6 animals. Animals were transported from the housing area to the testing area in their own cages and were allowed to adapt the new environment for 3 h prior to the testing.

2.2 *Drugs and Chemicals*

Fluoxetine hydrochloride (fluoxetine), Dexamethasone acetate was purchased from Sigma Aldrich, USA Anesthetic ether was purchased from Research lab, Mumbai, India. The standardized extract was obtained as a gift sample from konark herbals Mumbai. (Batch number- KH/CM/001/12)

2.3 Procedure for fractionation (Asghari et al., 2011; Riaz et al., 2012)

The powder was partitioned with Chloroform and Ethyl Acetate. The solvents were removed from the fractions under reduced pressure and vacuum dried to obtain solid residues. The Fractions were labeled as CH-FR and ETH-FR

2.4 Acute oral toxicity study of extracts and fractions (Malpani et al., 2011)

Acute oral toxicity was performed using Organization of Economic Cooperation and Development guideline number 425. Healthy adult Swiss female mice (20 - 25g) were used. Food, but not water, was withheld for 4 h before the drug was administered orally. The test drug was administered in doses of 2000, 1750, 550 mg/kg p.o. The numbers of mice per dose were three. The mice were observed for 2 h for behavioral, neurological and autonomic profiles and for any lethality or death over the next 48 h.

2.5 *Treatment schedule in animals*

Different set of animals (n=6) were used for the experiments. Group I Mice were treated as stress control, the Mice in Group II were administered with fluoxetine (10 mg/kg), and Group III, IV and V Mice were treated with HECM (50, 100, 200 mg/kg, p.o.). In other set of experiments the mice were treated with CHFR or ETHFR in a dose of 20 and 40 mg/kg p.o. The dose of fractions was decided depending upon results of extracts.

2.6 *Force swim test* (Gu et al., 2011)

The FST used was the same as described in detail elsewhere (Porsolt et al., 1977), with some of the modification. Briefly, Mice were individually placed in the glass cylinder (20 cm in height, 14 cm in diameter) filled with 10-cm high water (25 ± 2 ◦C). All animals were forced to swim for the period of 6 minutes, and the duration of immobility was recorded for later 4 minutes. The immobility period was regarded as the time spent by the rat floating in the water without struggling and making only those movements necessary to keep its head above the water.

2.7 *Tail suspension test* (Gu et al., 2011)

Mice and mice were individually suspended by tail with the help of clamp (1 cm from the tip of the end) in a box (25 cm ×25 cm ×30 cm) with the head 5 cm from the bottom. Testing was carried out in a room with minimal background noise. Animals were suspended for a total of 6 minutes, and the duration of immobility was recorded during the later 4 minutes interval of the test. Animals were considered immobile only when they hung passively and completely motionless.

2.8 *Estimation of plasma corticosterone* (Sheikh et al., 2007)

Immediately after the TST, blood was collected from retro orbital plexus in EDTA coated tubes kept in ice and centrifuged at 1000 g for 20 m at 4°C. Plasma was separated. HPLC/PDA system (Waters, USA) was used for the quantification of plasma corticosterone level according to (Woodward and Emery (1987), using dexamethasone acetate as an internal standard. Briefly, 500 µL of plasma containing a known quantity of dexamethasone was extracted with 5mL of dichloromethane (DCM). The extract was evaporated to dryness and was dissolved in 100 µL of mobile phase. Twenty microliter of the extract was injected into the HPLC system for the purpose of quantification. Mobile phase was made up of methanol: water (70:30). At the flow rate of 1.2 mL/min and corticosterone was detected at 250 nm using a PDA detector (Model 2487, Waters, USA).

3. Results and Discussion

3.1 Percent yield obtained

Percent yield of Chloroform and ethyl acetate fractions with respect to weight of the starting material was found to be 2.2 % and 1.8 % respectively.

3.2 Photochemical Analysis

The HECM showed all the tests positive for steroids, flavonoids, tannins, alkaloids, glycosides. Whereas the chloroform fraction showed the presence of steroids, alkaloids and ethyl acetate fraction showed the presence of steroids, flavonoids, tannins, alkaloids, glycosides.

Identification of Guggulsterone by HPLC in HECM

The extract was analyzed for the content of active ingredient, guggulsterone by HPLC. A sample solution of 10 mg of HECM was dissolved in 10 ml demineralized water. For identification of E and Z isomers in herbal extracts, the HPLC technique with photo diode array detector and gradient solvent was applied. The guggulsterone peaks were identified at 242 nm on symmetry C18 steel reversed-phase column (SC-04: 125 × 4.0 mm, 5.0 µm particle size) (Figure-4, 5). The mobile phase used was acetonitrile /water (46 / 54 v/v), the flow rate of 1.0 mL /min was applied. (Asghari et al., 2011).

3.3 Acute oral toxicity study

The drug was found to be safe at 2000 mg/kg p.o.

3.4 Effects of HECM on the immobility time in the Force swim test (FST)

Immobility duration in saline treated animals was found to be 76.17 ± 1.81 seconds as seen in figure number-1. Treatment with a standard drug fluoxetine significantly ($P < 0.001$) reduced immobility duration by 49.89% as compared to saline treated animals. Treatment with the test drug HECM at (100 mg/kg p.o.) and (200 mg/kg p.o.) significantly ($P < 0.001$) reduced the immobility duration to 16.42% and 34.59% as compared to saline treated group. Treatment with both chloroform and ethyl acetate at both the doses (20 mg/kg p.o) and (40 mg/kg p.o.) significantly ($P < 0.001$) reduced immobility duration to 15.98%, 15.33%, 18.83% and 28.89% as compared to saline treated group.

3.5 Effects of HECM on the immobility time in the Tail Suspension Test (TST)

Immobility tome in saline treated group was found to be 127.0 ± 4.45 seconds as seen inm figure no-2. Treatment with fluoxetine (10mg/kg p.o.) significantly ($P < 0.001$) reduced the immobility duration to 57.23% as compared to saline treated group. Treatment with HECM (100, 200mg/kg p.o.) significantly ($P < 0.001$) reduced immobility duration to 23.37%, 31.76% as compared to saline treated group. Treatment with the both chloroform and ethyl acetate fractions (20, 40mg/kg p.o.) significantly ($P < 0.001$, $P < 0.01$) attenuated the duration of immobility to 51.44%, 47.50%, 46.98% and 47.89% as compared to saline treated group.

3.6 Effect on Plasma corticosterone levels

The corticosterone in normal saline treated animals (control) was found to be 114.8 ± 4.13 ng/ml of blood plasma as seen in Figure no-3. Acute stress significantly ($p < 0.001$) increased the level of corticosterone in plasma as compared to saline treated group. The percentage rise was 194.4% Treatment with HECM at all dose levels significantly ($p < 0.001$) restored the plasma corticosterone

concentration to normal as compared to stressed animals. The HECM (50, 100 and 200 mg/kg) showed percentage reduction by 24%, 37% and 61% respectively. On the other hand fluoxetine reduced the corticosterone only by 0.39%.

The belief that "natural is better", complementary and alternative medicines are widely becoming popular, even among those taking prescription drugs (Hunt et al., 2010). Some herbal supplements has been reported to have appreciable effects on memory, mood, insomnia and appetite. As a result, some psychiatric conditions particularly depression were thought to be among the most common conditions which has been treated with complementary and alternative therapies in some surveys (Kessler et al., 2001; van der Watt et al., 2008). Commiphora mukul, an herb medicine widely used in Eastern-Asia countries, in some remedies like hyperlipidemia (Lata S. et al., 1991). There is no study reported for its effect on depressive type of disorders. So, we found it interesting to study the effect of the extract as well as chloroform and ethyl acetate fractions in acute models of depression. The present study is the first case to demonstrate that acute administration of HECM (50, 100 and 200 mg/kg, p.o.) had a specific antidepressant-like effect in FST and TST in mice. Moreover, it was noteworthy that the anti-immobility effect which was produced by HECM was comparable to that produced by the classical serotonin booster antidepressant fluoxetine.

Since serotonin is the main neurotransmitter involved in the depression, it has been proposed that an increase in 5HT neurotransmission might counteract the depressive illness (Owens and Nemeroff, 1994). Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants. In the present study HECM at 200 and 400 mg/kg p.o. exhibited significant antidepressant activity which might be due to increased brain serotonin level as reported earlier.

The results obtained by HECM might be attributed to the presence of some major biologically active phytoconstituents i.e. steroids such as guggulsterone, flavonoids such as naringenin and quercetin, terpenoids as cembranoids, α- pinene, 1, 8-cineole, α-terpinol, mansumbinoic acid, mansumbinone. The flavonoids have been reported for its antianxiety, antidepressant, antiparkinsonism and antiepileptic activity (Jager et al., 2012).

Terpenoids are also playing a vital role in treating some behavioral disorders. Terpenoids as cembranoids are known for their neuroprotective activity (Ferchmin et al., 2009). Whereas α- pinene and 1,8 - Cineole have been reported for their antistress effect and effect on cognitive performance (Hitomi Akutsu et al., 2002, Mark Moss et al., 2012). GS is reported to show serotonin releasing property in brain (Srivastava and Kapoor, 1986).

The specific receptor studies are further needed to demonstrate the possible mechanism of action. Chronic social stress is one of the most important factors responsible in the triggering and/or worsening of anxiety and depressive disorders in humans. It has been hypothesized that life stress alters the dynamic regulation of the autonomic, neuroendocrine, and immune systems (Checkley, 1996; Pike et al., 1997). Moreover plasma concentration of corticosterone has been shown to be increased in depressed individuals (Hauger et al., 2009). The importance of coordinated functional interactions between the HPA axis and sympathetic – adrenal – medullary system in promoting adaptive responses to stress from anxiety, or fear has been emphasized from past studies in animal models (Vermetten and Bremner, 2002a, b). In present study, we found significant decrease in plasma corticosterone level in HECM treated group dose dependently. Further in order to understand the phytoactive constituents responsible for antidepressant activity, we fractionated HECM and explored the antidepressant activity of fractions. The fractions were administered at a dose of 20 and 40 mg/kg p.o. CH-FR at 20 as well as 40 mg/kg p.o. showed significant decrease in immobility duration as compared to stress control group. The higher dose exhibited better antidepressant activity.

From the chromatogram (Fig 5.), HECM has shown to contain E and Z Guggulsterones as major steroidal content. Guggul resin and it's fractions has already been reported to enhance the biosynthesis of catecholamine in normal and hyperlipaemic animals (Srivastava et al.,

1984) and E isomers of guggulsterone were reported to enhance the level of biogenic monoamines and dopamine β-hydroxylase (DBH) activity (Srivastava et al., 1986). The antidepressant activity obtained in our study might be attributed to increased level of 5-HT in brain as reported earlier.

Thus our experiment for the first time reports antidepressant activity of Commiphora mukul extract. Further studies are designed where the phytoconstituents will be isolated and studied for its antidepressant activity.

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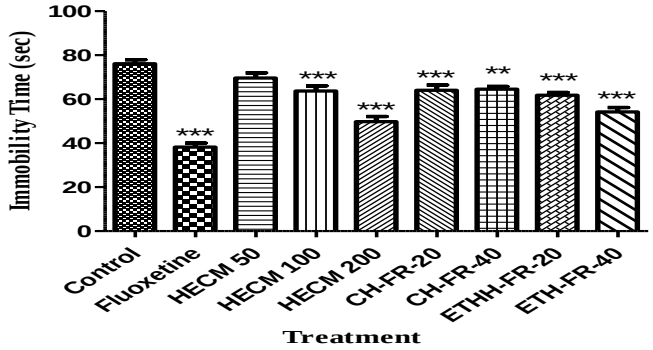


Fig. 1. Effect of HECM and fractions on Force swim test.

(The data was analyzed by one way ANOVA followed by Dunnet's test (n=6). Where ***P < 0.001, **P < 0.01 as compared to control group.)

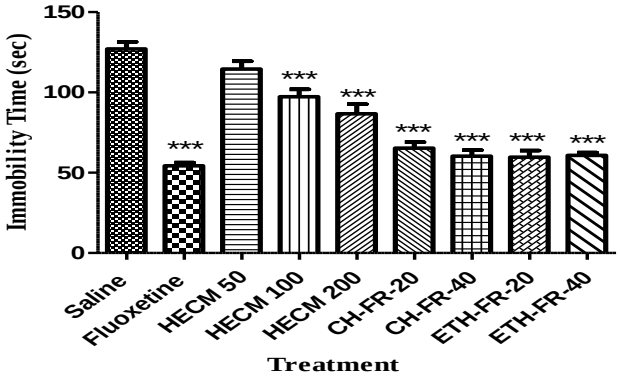


Fig. 2. Effect of HECM and fractions on Tail suspension test.

(The data was analyzed by one way ANOVA followed by Dunnet's test (n=6). Where ***P < 0.001, **P < 0.01 as compared to control group.)

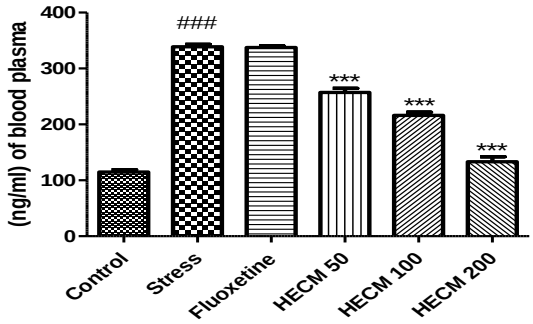
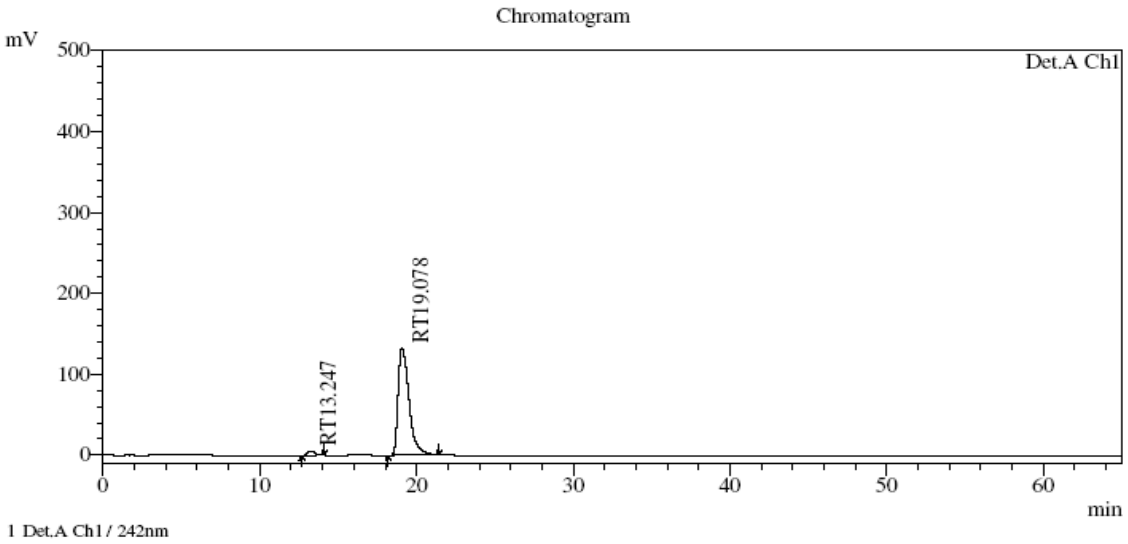


Fig. 3. Effect on plasma corticosterone.

(The data was analyzed by one way ANOVA followed by Dunnet's test (n=6). Where ***P < 0.001 as compared to control group.)

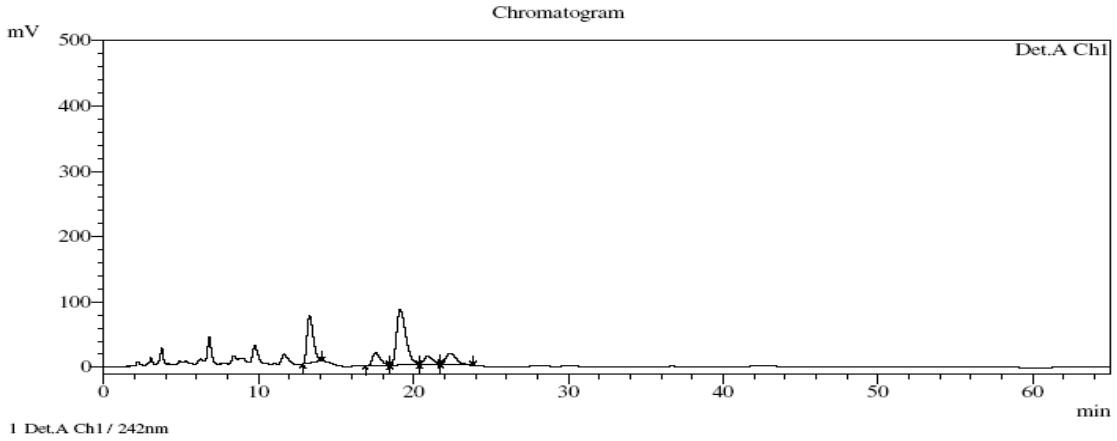


PeakTable

Detector A Ch1 242nm

Peak#	Ret. Time	Name	Area	Area %
1	13.247	RT13.247	165041	2.487
2	19.078	RT19.078	6471502	97.513
Total			6636544	100.000

Fig. 4. Reference standard chromatogram.



PeakTable

Detector A Ch1 242nm

Peak#	Ret. Time	Name	Area	Area %
1	13.292	RT13.292	2013827	26.548
2	17.557	RT17.557	711768	9.383
3	19.148	RT19.148	3554567	46.860
4	20.929	RT20.929	514061	6.777
5	22.356	RT22.356	791303	10.432
Total			7585527	100.000

Fig. 5. Chromatogram – *Commiphora mukul* extract.

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