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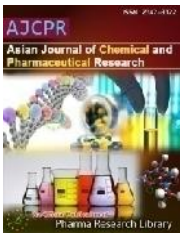

Research Article **Open Access**

Evaluation of antidepressant activity of hypericum perforatum using experimental models of depression in rats

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A B S T R A C T

Depression constitutes the second-most common chronic condition in clinical practice, exceeded only by hypertension. Despite recent progress achieved in the development of clinically relevant antidepressant drugs in recent years, the currently available antidepressant therapy is not at totally effective and it is associated with many undesirable collateral effects 32-35. In addition, only 60% of patients are responsive to the treatment with the available antidepressants. For this reason, the search for new drugs for the control of the symptoms associated with depressive disorders is still desirable. In the present study, 7 days pretreatment with CR at the doses of 100, 200 and 400 mg/kg showed antidepressant activity in the forced swim test and tail suspension tests. The FST is the tool most widely used for assessing antidepressant activity preclinically. The widespread use of this model is largely a result of its ease of use, reliability across laboratories, and ability to detect a broad spectrum of antidepressant agents49. Most clinically active antidepressants are effective in the FST, while neuroleptics and anxiolytics produce different effects.34 In the forced swim test, HP significantly reduced immobility period suggesting anti-depressant activity and the activity was comparable to the reference drug IMP. Immobility is a state of lowered mood or hopelessness, which the rats experience when they are allowed to swim in a restricted space from which they cannot escape.

Keywords: Hypericum Perforatum (Hp), Antidepressant Activity

A R T I C L E I N F O

CONTENTS

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1. Introduction

Depression is the most common of the affective disorders (disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression

accompanied by hallucinations and delusions. Worldwide, depression is a major cause of disability and premature death. The symptoms of depression include emotional and biological components:

Emotional symptoms:

- Misery, apathy and pessimism
- Low self-esteem: feelings of guilt, inadequacy and ugliness
- Indecisiveness, loss of motivation.

Biological symptoms:

- Retardation of thought and action
- Loss of libido
- Sleep disturbance and loss of appetite.

There are two distinct types of depressive syndrome, namely unipolar depression, in which the mood swings are always in the same direction, and bipolar affective disorder, in which depression alternates with mania. Mania is in most respects exactly the opposite, with excessive exuberance, enthusiasm and self-confidence, accompanied by impulsive actions, these signs often being combined with irritability, impatience and aggression, and sometimes with grandiose delusions of the Napoleonic kind. As with depression, the mood and actions are inappropriate to the circumstances.Unipolar depression is commonly (about 75% of cases) non-familial, clearly associated with stressful life-events and accompanied by symptoms of anxiety and agitation; this type is sometimes termed reactive depression. Other patients (about 25%, sometimes termed endogenous depression) show a familial pattern, unrelated to external stresses, and with a somewhat different symptomatology. This distinction is made clinically, but there is little evidence that antidepressant drugs show significant selectivity between these conditions. Bipolar depression, which usually appears in early adult life, is less common and results in oscillating depression and mania over a period of a few weeks. There is a strong hereditary tendency, but no specific gene or genes have been identified either by genetic linkage studies of affected families, or by comparison of affected and non-affected individuals.

Theories of Depression

The Monoamine Theory:

The monoamine biochemical theory of depression is the monoamine hypothesis, proposed by Schildkraut in 1965, which states that depression caused by a functional deficit of monoamine transmitters at certain sites in the brain, while mania results from functional excess. (Baker and Dewhurst, 1985; Maes and Meltzer, 1995; Manji et al., 2001). The monoamine hypothesis grew originally out of associations between the clinical effects of various drugs that cause or alleviate symptoms of depression and their neurochemical effects on monoaminergic transmission in the brain.

Pharmacological Evidence

The pharmacological evidence does not enable a clear distinction to be drawn between the noradrenaline and 5-HT theories of depression. Clinically, it seems that inhibitors of noradrenaline reuptake and of 5-HT reuptake are equally effective as antidepressants though individual patients may respond better to one or the other.

2. Materials and Methods

Drugs and Chemicals

Thiobarbituric acid and DTNB reagent (HiMedia Laboratories Ltd., Mumbai), Trichloroacetic acid (Qualigens Fine Chemicals, Mumbai), Riboflavin (Astra IDL, Bangalore), Sodium dihydrogen phosphate and Disodium hydrogen phosphate (S.D. Fine Chemicals, Mumbai), Lorazepam (Ranbaxy, India), 1,1,3,3,-Tetraethoxy propane, O-Dianisidine, Imipramine hydrochloride, 5-Hydroxy Tryptophan (5-HTP), Clonidine and L-DOPA (Sigma, St. Louis, USA) were used in the study. The other chemicals and solvents used were of analytical grade and purchased from commercial suppliers. Imipramine (IMP), 5-HTP, clonidine, L-DOPA, Lorazepam was administered intraperitoneally by dissolving in normal saline.

Animals

Healthy Adult Male mice of 5 weeks old with Average weight in the range of 40-60gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies [31]

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organisation for Economic Co-operation and Development) guidelines no 423. Female Albino wistar mice (30-40 g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/5, 1/10 and 1/20 doses of 2000 mg/kg i.e. 100 mg/kg and 200 mg/kg 400mg/kg were chosen for subsequent experimentation.

***In-Vivo* Models of Depression Employed in the Study**

1. Forced swimming test (FST)
2. Tail suspension test (TST)
3. 5-HTP induced head twitches in mice
4. Clonidine-induced aggression in mice
5. L-DOPA-induced hyper activity and aggressive behavior in mice (LHA)

Statistical Analysis

Results were expressed as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found statistically significant ($P < 0.05$)

3. Results and discussion

Results: %Yield value of Ethanolic Extract from Aerial Parts of *C. Racemosa* was found to be **56.3%**

Preliminary Phytochemical Screening

Investigation revealed the presence of steroid, Alkaloid, Tannins, phenols & Flavonoid in Ethanolic Extract of *Hypericum perforatum (*CR)

Acute toxicity studies

As per (OECD) draft guidelines 423 Female albino mice were administered *C. Racemosa* and doses was be selected in the sequence (1.75-5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as $1/10^{th}$ and $1/20^{th}$ i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose of antidiabetic investigations.

1. Forced Swim Test (FST)

The results (Table. 1) showed that both *CR* (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the *CR* (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig. 1).

Table 1: Effect of *CR* and imipramine (IMP) on forced swim test (FST) in rats

Group no.	Treatment (dose in mg/kg)	Immobility period (sec) Mean ± SEM
I	Control (0.3% CMC) + FST	149.2±1.9
II	*C.Racemosa* (100 mg/kg, p.o.) + FST	120.8±3.7
III	*C.Racemosa* (200 mg/kg, p.o.) + FST	88.6±1.6*
IV	*C.Racemosa* (400 mg/kg, p.o.) + FST	66.6±1.4*
V	Imipramine (15 mg/kg, i.p.) + FST	77.6±1.8*

Each column represents mean ±S.E.M of immobility period (sec), n = 6. *= $p<0.001$ compared to control

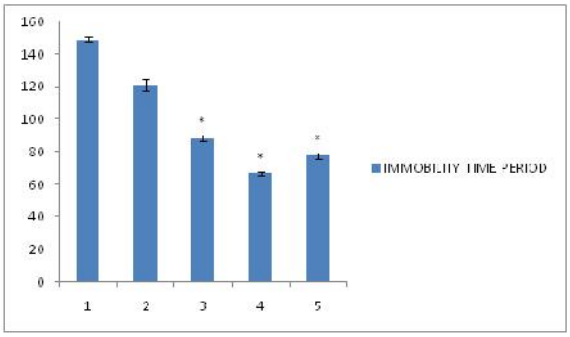

Figure 2: Effect of *CR* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on forced swim test (FST) in rats. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. *= $p<0.001$ compared to control

2) Tail Suspension Test (TST)

The results (Table. 2) showed that both *CR* (100,200,400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the *CR* (100, 200 and 400 mg/kg) and IMP treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig. 2).

Table 2: Effect of *CR* and Imipramine (IMP) on tail suspension test (TST) in mice.

Group no.	Treatment (dose in mg/kg)	Immobility period (sec)
I	Control (0.3% CMC) + TST	148.5±4.3
II	*C.Racemosa* (100 mg/kg, p.o.) + TST	113.5±4.3[a]
III	*C.Racemosa* (200 mg/kg, p.o.) + TST	92.5±2.63[a]
IV	*C.Racemosa* (400 mg/kg, p.o.) + TST	81.0±3.01 [a]
V	Imipramine (15 mg/kg, i.p.) + TST	72.5±2.75 [a]

Each column represents mean±S.E.M. of immobility period (sec), n=6. a = $p<0.001$ compared to control

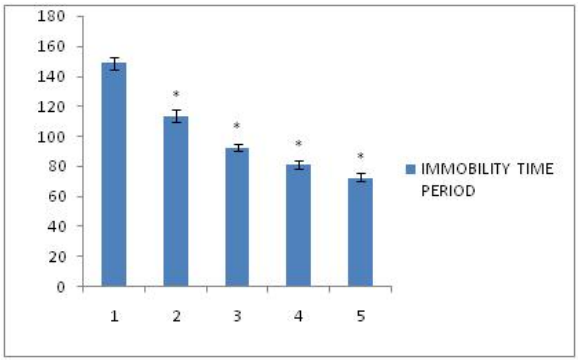

Figure 3: Effect of *CR* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on tail suspension test (TST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = $p<0.001$ compared to control

3) 5-HTP induced head twitches in mice

Table.3. illustrates the effect of *CR* and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of CR (100, 200 and 400 mg/kg, $p<0.01$, $p<0.001$) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase ($p<0.001$) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *CR* was significantly higher than IMP ($p<0.001$) (Fig. 3).

Table 3: Effect of *CR* on 5-HTP-induced head twitches in mice.

Group no.	Treatment (dose in mg/kg)	Head twitches Mean ± SEM
I	Control (0.3% CMC)	13.67±0.8
II	*C.Racemosa* (100 mg/kg, p.o.)	19.83±1.0[a]
III	*C.Racemosa* (200 mg/kg, p.o.)	24±1.36[b]
IV	*C.Racemosa* (400 mg/kg, p.o.)	31.5±1.3 [b]
V	Imipramine (15 mg/kg, i.p.)	21.5±1.2 [b]

Each column represents mean ± S.E.M. of number of head twitches, n=6. a = p<0.01, b = p<0.001 compared to control

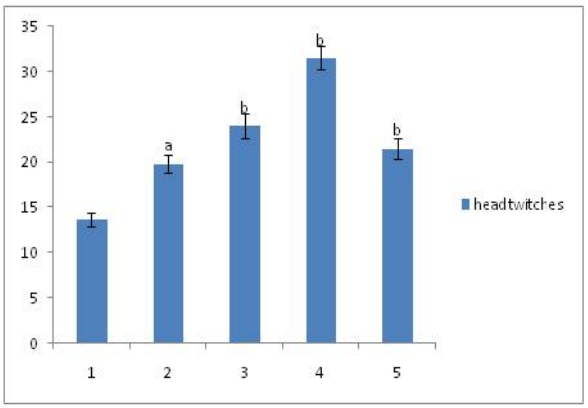

Figure 4: Effect of *CR* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on 5-HTP-induced head twitches in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001, compared to control

4) L-DOPA induced hyperactivity and aggressive behavior in mice: The effect of *CR* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 4. Post-hoc analysis revealed that three doses of *CR* (100,200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group (Fig. 4).

Table 4: Effect of *CR* and Lorazepam on L-DOPA-induced hyperactivity and aggressive behavior in mice.

Group no.	Treatment (dose in mg/kg)	Behavioral score
I	Control (0.3% CMC)	1
II	*C.Racemosa* (100 mg/kg, p.o.)	2.2 ± 0.1[a]
III	*C.Racemosa* (200 mg/kg, p.o.)	2.3 ± 0.1 [a]
IV	*C.Racemosa* (400 mg/kg, p.o.)	2.7 ± 0.1 [a]
V	Lorazepam (2.5 mg/kg, i.p.)	2.2 ± 0.1 [a]

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control

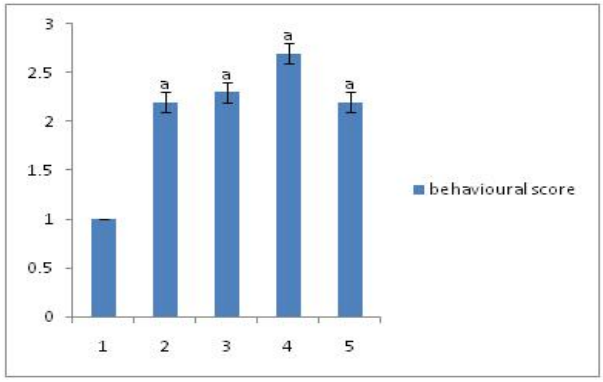

Figure 5: Effect of *CR* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on L-DOPA-induced hyperactivity and aggressive behavior in mice. Each column represents mean ± S.E.M. of number of head twitches, n=6. a= p<0.001, compared to control

5) Clonidine induced aggression in mice

Table. 5. Indicates the effect of *CR* (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *CR* (p<0.001) significantly increased the latency to first attack and decrease the no. of bouts compared to control (Fig. 5).

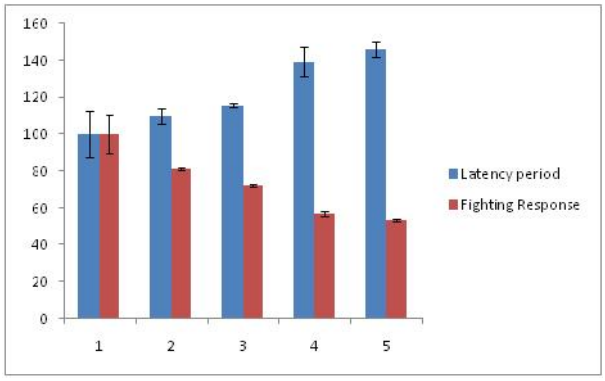

Figure 6: Effect of *CR* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on clonidine induced aggression in mice. Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

Table 5: %response for clonidine induced aggression

Group no.	Treatment (dose in mg/kg)	% Response(MEAN ± SEM)	
		Latency to 1st attack	Fighting response
I	Control (0.3% CMC)	100 ± 12.7	100 ± 1.1
II	*C.Racemosa* (100 mg/kg, p.o.)	109.8 ± 4.4 [a]	81.395 ± 0.6 [a]
III	*C.Racemosa* (200 mg/kg, p.o.)	115.5 ± 1.1 [b]	72.09± 0.6 [b]
IV	*C.Racemosa* (400 mg/kg, p.o.)	139.2 ± 8.2 [b]	56.976 ± 1.2 [b]
V	Lorazepam (2.5 mg/kg, i.p.)	146.0 ± 3.9 [b]	53.48± 0.6 [b]

Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control

4. Conclusion

The results from the present study confirm the antidepressant activity of *Hypericum perforatum* , since it reduced the immobility in both FST and TST.In the present study, *CR* significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively.

Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with *Hypericum perforatum*, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in rat brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of *CR* might be a part of the mechanism for its antidepressant activity. Results from behavioral experiments indicate that the antidepressant activity of *Hypericum perforatum* , might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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