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Original Research Article

EVALUATION OF ANTIDEPRESSANT ACTIVITY OF HYPERICUM HIRCINUM

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Abstract: Objective: To study the effect of ethanol: acetone extract of *Hypericum hircinum* have antidepressant activity on *Wistar albino* rat. **Methods**: Whole plant was collected and extracted using ethanol: acetone by maceration. The crude extracts were screened for the biological activity through the assay behavioral study. Antidepressant activity evaluated by using behavioral methods such as ketamine induced sleep, tail suspension method. **Result**: Hydro alcoholic extract of have significant antidepressant effect on rat. **Conclusion**: The result indicates that whole plant hydroalcoholic extract was an antidepressant agent.

Key words: Antidepressant activity, *Hypericum hircinum*, rat.

Introduction: According to WHO (1978) traditional medicine is defined as the sum total of knowledge or practice whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observations handed down from generation, verbally or in writing. It also comprises the therapeutic practices that have been in existence often for hundreds of years before the development of modern scientific medicine. Anxiety is an emotional state commonly caused by the perception of real or perceived danger that threatens the security of an individual. It allows a person to prepare for or react to environmental changes. Everyone experiences a certain amount of nervousness and apprehension when faced with a stressful situation. This is an adaptive response, and is transient in nature.

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*Hypericum hircinum*is distributed in Africa. It is belongs to the family clusiaceae.

Material and Methods

Collection and authentication of plant materials: Whole plant was collected and its authentication obtained from Cagliari university, Italy (voucher specimen: 232

Herbarium CAG). The plant materials were cleaned, washed with copious amount of distilled water, shade dried, chopped into bits and coarsely powdered in a milled for extraction.

Preparation of crude plant extracts: The plant material was shade dried and reduced to coarse size and subjected for defatting with petroleum ether for four days. Then the petroleum ether was separated out and residue was evaporated to dryness on water bath. The dried material was then macerated with acetone and ethanol (1:1) for 7 days with occasional shaking, followed by distillation and percentage yield was determined.

Phytochemical analysis: Hydro alcoholic extract of plant was subjected to preliminary phytochemical screening.

Experimental animals: *Albino wistar* rat either sex, approximately 125±40g in weight on the day of the testes from the animals house of pinnacle Biomedical Research Institute, Bhopal, were housed 3 or 5 to a cage until the experiments. Room temperature was controlled 23±1℃) and light were 12 hrs cycles. Food and water were available ad libitum.

Acute toxicity study: Acute oral toxicity study was carried out by using Up and Down method, according to OECD 423. Hydroalcoholic extract were administered intraperitoneally (100-2000 mg/kg i.p). Animals were observed continuously for the first 2h for toxic symptoms and up to 24h for mortality [6].

Ethical approval: The protocol was approved by the Institutional Animal Ethical Committee (PBRI/IAEC/2009/PN14) and conducted according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of Experiments on Animals(CPCSEA No.-1283/c09/CPCSEA)

Evaluation of antidepressant activity

- ***Effect of extract on Ketamine-induced sleep:***

Albino wistar rats (male) were divided into following groups by random selection from animal house.

Group 1- vehicle treated
Group 2-HHE (10) treated
Group 3-HHE (20) treated

The effect of plant extracts on ketamine-induced sleeping time was measured as described by Mimura *et.al*. After 30min pre-treatment with the vehicle, HHE (10 and 20 mg/kg i.p), rats were injected with ketamine (100mg/kg, i.p).In the case of the control, rats were pre-treated with solution of injection for water and PEG-400,after 3 0min received only ketamine .The interval between the administration of ketamine until the loss of the righting reflex was recorded as latency of sleep,while the time from the loss to regaining of righting reflex as the duration of sleep(Rabbani et.al .,2004 ; Bastidas *et. al.*, 1998)

- ***Antidepressant activity by using tail suspension test***

Albino wistar rats (male) were divided into following groups by random selection from animal house.

Group 1- vehicle treated
Group 2-HHE (10) treated
Group 3-HHE (20) treated
Group 4- Fluoxetine(20) treated

The total duration of immobility induced by tail suspension was measured according to method described by *steru et al* (1985) as a facile means of evaluation potential antidepressants. Rats were administered vehicle, HHE (10 and 20 mg/kg i.p) and fluoxetine (20mg/kg i.p)before 30 minute of the test .Rats were suspended for 6 min on the edge of a table 50cm above the floor by the adhesive tape placed approximately 1cm from the tip of the tail. Latency to immobility was recorded after suspended the rat and first 2minutes discarded and duration of immobility was recorded for last 4 minutes. Rats was considered to be immobile when it did not any movement of body hanged passively.

Statistical Analysis: Data expressed as means ± S.E.M. All result obtained from different tests were compared against the control group by using analysis of variance (ANOVA) and followed by Dunnett's test.

Results: Phytochemical analysis

Table 1: The results of the chemical tests performed

TESTS	RESULT
Carbohydrate	+
Proteins	-
Amino acids	_
Glycoside	+
Steroids	+
Flavonoid	+
Tannins	-
Alkaloids	+
Saponins	+
Phlobatannins	_
Triterpenes	_

+ indicate presence, - indicate absence

Determination of LD50 using OECD guidelines

Animals were observed for mortality for 24 hr. no mortality was observed and the preparation was found to be safe up to a dose of 2000mg/kg.

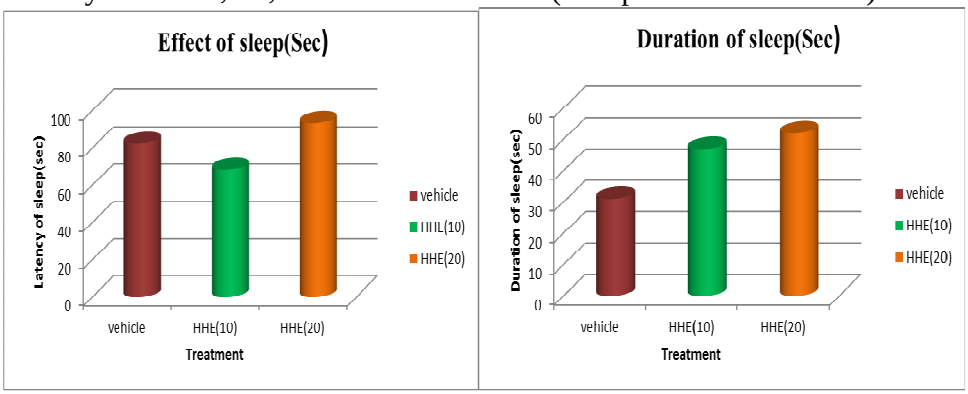
Antidepressant activity

The vehicle treated rats the righting reflex was lost after 82.25±8.3sec of ketamine injection. Injection of plant extracts (30min prior to ketamine) at doses of 10 and 20 mg/kg did not change the latency to sleep significantly (68.25±15.5 and 93.25 ±32.4 respectively).In rats, treated by extracts (10mg/kg and 20mg/kg) increased the duration of sleep (47± 9.7 min 52±5.8min respectively) against the vehicle treated groups (31±4.08min). Data are shown in table 3 and graphical representation in Fig .1

Table 2.Effect of extract of *H.hircinum* extract on ketamine induced sleep in rat

Treatment doses (mg/kg i.p)	Latency of sleep(sec)	Duration of sleep(min)
Vehicle	82.25±8.3	31.0±4.08
HHE(10)	68.25±15.5	47.0±9.7*
HHE(20)	93.25±32.4	52.0±5.8*

N=4, Using one way ANOVA, F2,9=10.P=0.005.P<0.05(Compared to the vehicle)

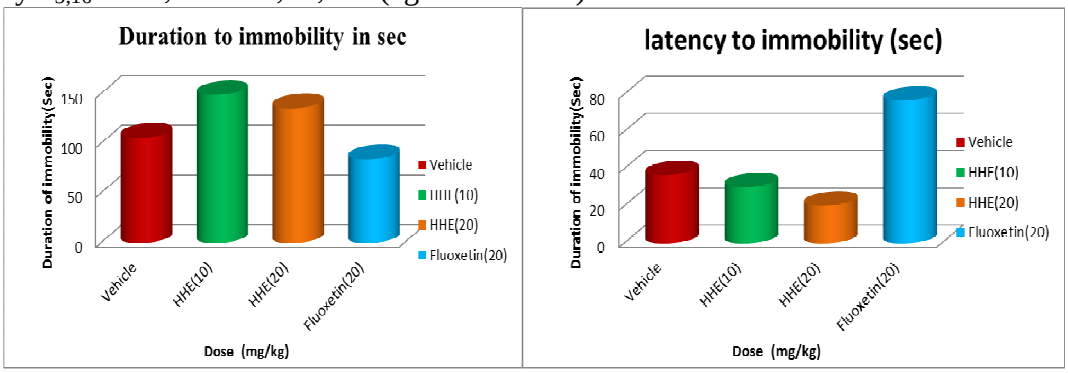


Antidepressant activity using tail suspension test: The result is obtained after administration of HHE(10 and 20mg/kg.i.p) showed that the duration of immobility of rats increased compared to vehicle, namely, the animals were not active in both employed does compared to vehicle ,which means that the depressant effect was stronger .Depressant effect of extract was stronger. Depressant effect of extract was stronger as 10mg/kg i.p (statistically significantly) but on 20mg/kg i.p was not statistically significant. Both the extracts were not altered latency to immobility significantly. On the other hand, fluoxetine increased the latency to immobility and decreased the duration of immobility. One rat was died after 15 min of the test on20mg/kg i.p extract dose.

Table 9. Effect of *H.hircinum* extract on latency and duration of immobility of rats

Treatments Dose (mg/kg i.p)	**Latency to immobility in sec**	**Duration of immobility in (sec)**
Vehicle	36.75±9.0	106.5±12.35
HHE(10)	30.25±7.8	149.25±9.2*
HHE(20)	20.25±5.7	136.0±13.2
Fluoxetine(20)	76.75±3.2	84.75±7.8

N=4, for latency to immobility $F_{3,6}$ =13.32,P=0.0001,P<0.05(against vehicle),For duration of immobility $F_{3,16}$=7.15,P=0.003,*P,0.05(against vehicle)



Discussion: The *Hypericum hircinum* extracts where subjected to phytochemical screening. The result indicates that hydro alcoholic extract of plant shows the presence of carbohydrates, saponins, steroids, flavonoid and alkaloid.

The present work demonstrated that the hydroalcoholic extract of *Hypericum hircinum* had antidepressant activity in rat by Effect of extract on ketamine –induced sleep method and tail suspension method. Our pre-clinical experiment demonstrates the depressant effect of *H.hircinum* extract using this animal model of depression. In the present study, however, we used doses (10 and 20 mg/kg.i.p)of extract. The highest dose in our study was 20mg/kg i.p doses of extract reported in the literature usually range from 2.5 -200mg/kg. The result obtained after administration of the extract showed that immobility time of the animal increased at 10mg/kg i.p. strongly compared to 20mg/kg i.p. which means the depressant effect was stronger .For both doses administered there were difference compared to the vehicle , that is, they led to increment immobility time, the immobility displayed in rodents subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorder in humans.

Conclusion: Phytochemical screening indicated presence of various phytoconstituent detected is known to have beneficial use in industries and medical science. Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavonoids, saponins, alkaloids, steroids, glycosides possess activity against many CNS disorders.

Duration of sleep induced by ketamine rats promoted by both the doses of extracts,20mg/kg dose produced more effect than lower dose and suggesting sedative effect of preparation. The result obtained after administration of the extract showed that the immobility time of animals increased at 10mg/kg strongly compared to 20mg/kg which means that the depressant effect was stronger. The immobility displayed in rodents subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Results of tail suspension test suggested CNS depressant activity.

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