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Bioprospecting *Hypericum perforatum* L for Central Activity in Laboratory Animals

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ABSTRACT

Hypericum perforatum L .belonging to the family Clusiaceae is a herbaceous plant, usually growing in shady places among rocks. Although it is not domesticated yet, it is widely distributed in Northern Turkey.[1] The genus *Hypericum* includes numerous species which have been used as medicinal plant for centuries in treatment of truma, burns, rheumatism, neuralgia, gastroenteritis, ulcers, hysteria, bedwetting and depression. *Hypericum perforatum* also known asst. John'swort (SJW) has been extensively studied for anti-depressant activity. The present study has been performed to explore the central effects of *Hypericum perforatum*[2].

INTRODUTION

Medicinal plants are important therapeutic aid for various ailments. The genus *Hypericum* L. comprises some 400 species of trees, shrubs and herbs with opposite gland-dotted leaves. It has a wide distribution, but most species originate from temperate regions and tropical mountains. *Hypericum perforatum* has been extensively studied for anti-depressant activity. Recent research suggests the effectiveness of this herb in treating other ailments, including cancer, inflammation-related disorders, bacterial, viral diseases, as an antioxidant and neuroprotective agent. Interestingly, such properties appear to derive primarily from the constituent hyperforin, rather than from hypericin, which has been investigated for a longer period of time[4]. The present study have been used to explore the central effects of *Hypericum perforatum* and hyperfoliatin are mainly responsible for the activities of *Hypericum perforatum*[5]. In this work, preliminary phytochemical and biological evaluation was performed to evaluate the central activity of *Hypericum perforatum*.

MATERIALS AND METHODS

Plant extract

The plant extract was collected and its authentification was obtained from Cagliari University, Italy (Voucher specimen: 234 Herbarium CAG).

The whole plant was shade dried and subjected for defatting through petroleum ether for four days. The petroleum ether was separated out and residue was evaporated to dryness on water bath. The dried material was macerated with acetone and ethanol (1:1) for 7 days with occasional shaking, followed by distillation.

Animals used

Albino Wistar rats (150-200g) and Albino mice (75-100g) of either sex were used. The approval of institutional animal ethical committee (PBRI/IAEC/2009/PN 17) according to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).CPCSEA no:1283/C09/CPCSEA.

Drugs used

Diazepam, Haloperidol, Ketamine, Apomorphine, PEG-400, Fluoxetine, Lithium sulphate,

Phytochemical evaluation

The preliminary phytochemical investigation of extract was carried out for alkaloids, flavonoids, glycosides, steroids, saponins, tannins, phlobatannins, triterpenes, proteins and carbohydrates by performing specific chemical tests[6].

Biological evaluation

Biological evaluation was carried out by using the effect of extract on gross behavior using Irwin schedule, locomotor activity using open field apparatus, Ketamine induced sleep, elevated plus maze, Haloperidol induced catalepsy, Motor coordination using stumbling board, Lithium induced head twitches, Apomorphine induced stereotyped behavior, anti-depressant activity using tail suspension test[7].

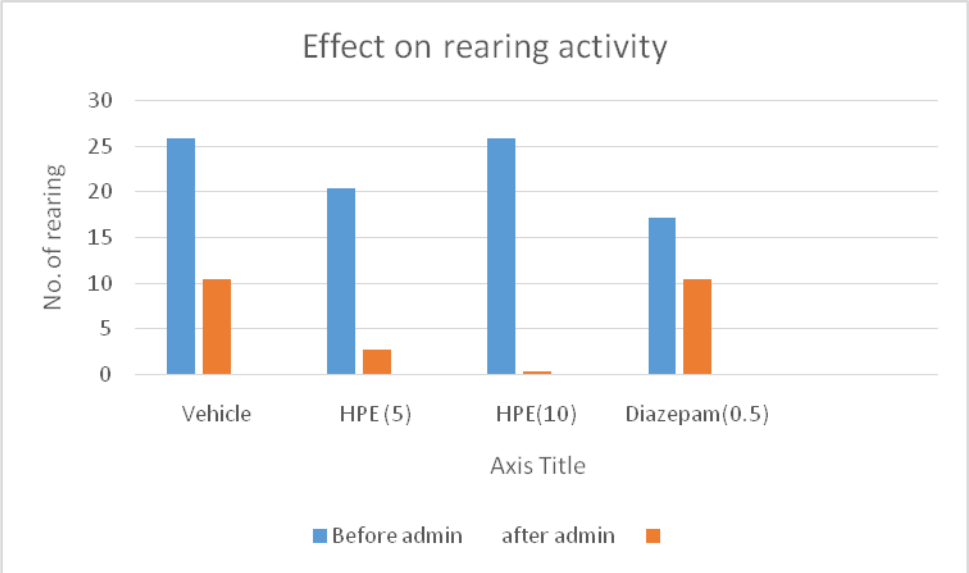
RESULTS AND DISCUSSION

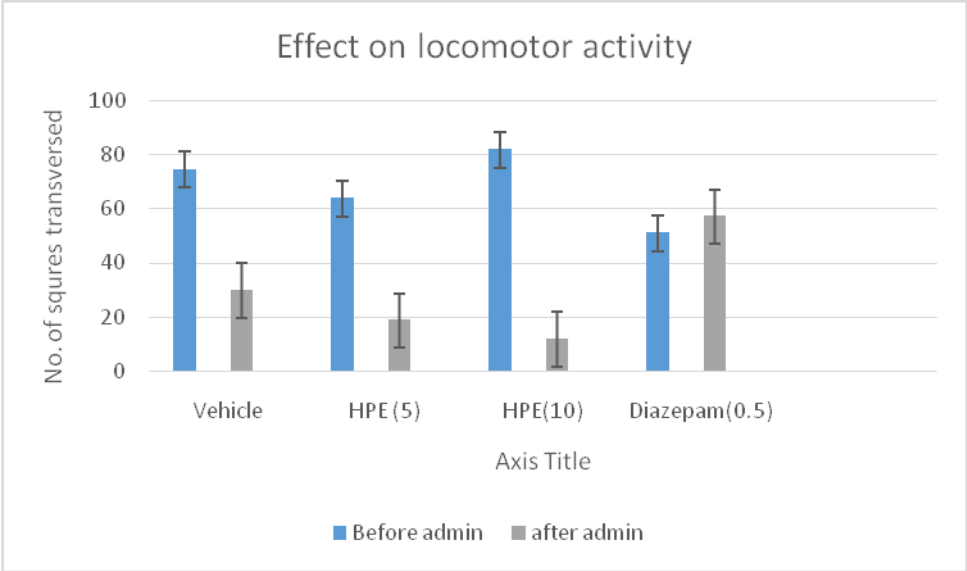
Phytochemical evaluation

Phytochemical constituents	Results
Glycosides • Keller killiani Test • Modified Borntrager Test • Coumarin Glycosides	 + + -
Flavonoids	+
Alkaloids • Hager's test • Wagner's test • Mayer's test	 + – –
Steroids • Salkowski test	 +
Saponins • Frothing test	 –
Tannins	-
Phlobatannins	-
Triterpenes	-
Proteins • Biuret test	 –
Carbohydrates • Molish's test • Iodine test	 + -

BIOLOGICAL EVALUATION

Effect of extract on Locomotion using open field apparatus

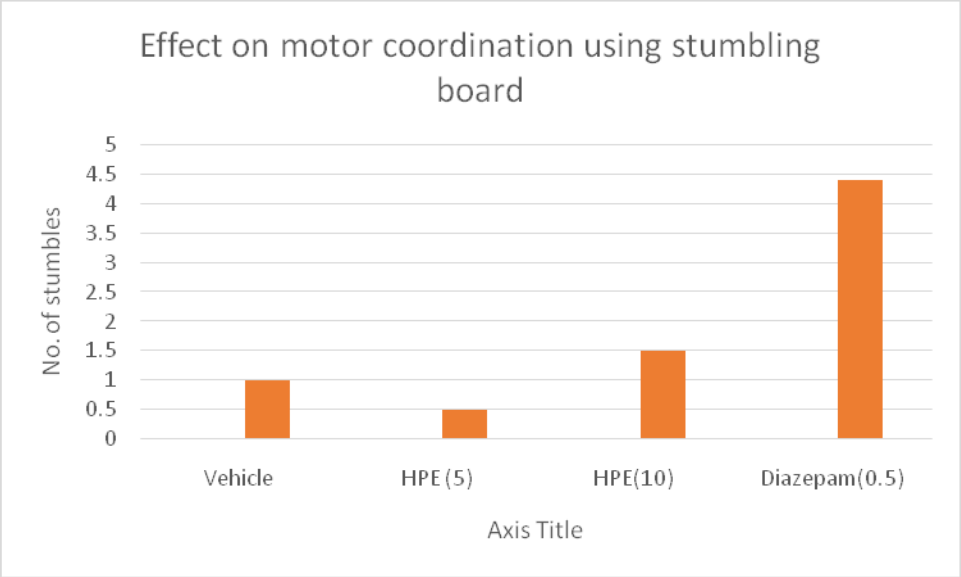




The locomotion and rearing were measured for 5 minutes before and after the administration of extract.

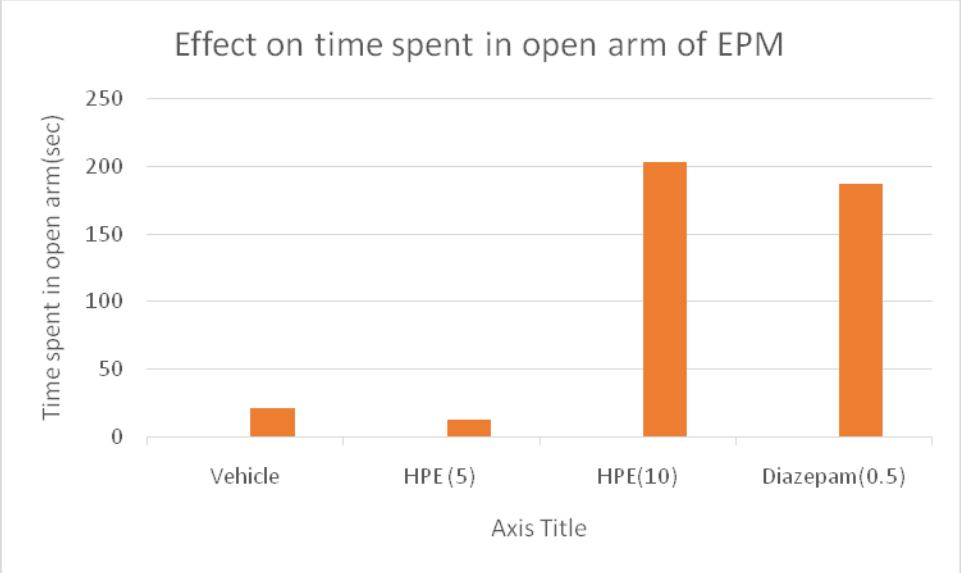
The rearing and locomotion were significantly reduced by both the doses.

Effect of extract on motor coordination using stumbling board



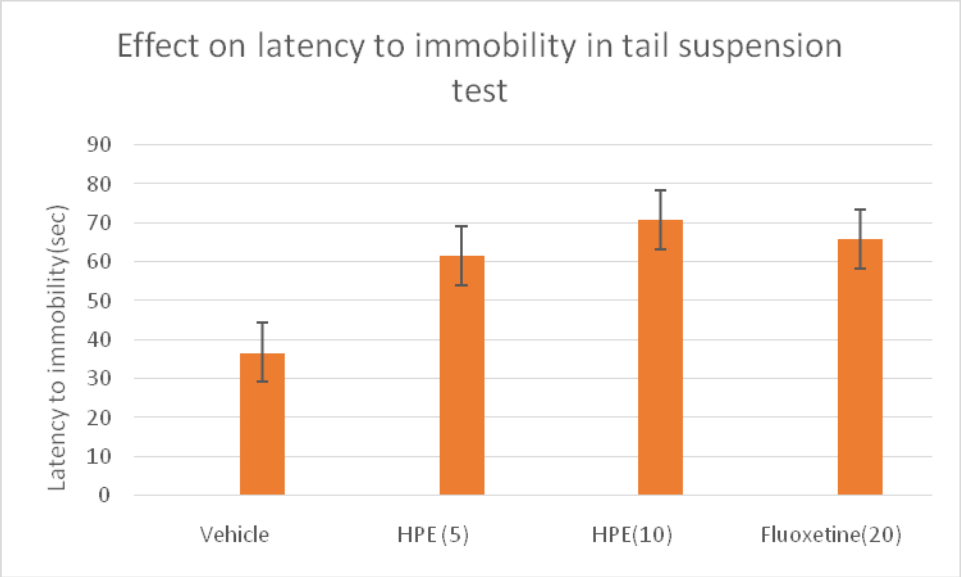
There are no significant changes in motor coordination by both doses of extract compared to vehicle and standard.

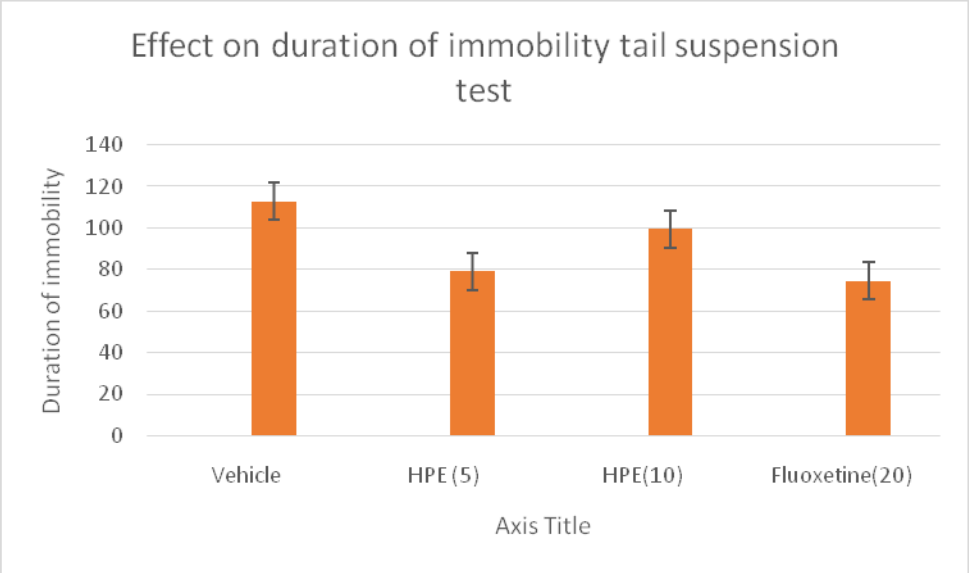
Effect of extract on duration of time spent on open arm of elevated plus maze



Diazepam significantly increased the time spent by rats in open arm. HPE 10mg/kg increased the time spent on open arms compared to vehicle. But HPE 5mg/kg did not show significant results. Increase in the time spent in open arm suggest that the extract has anxiolytic activity.

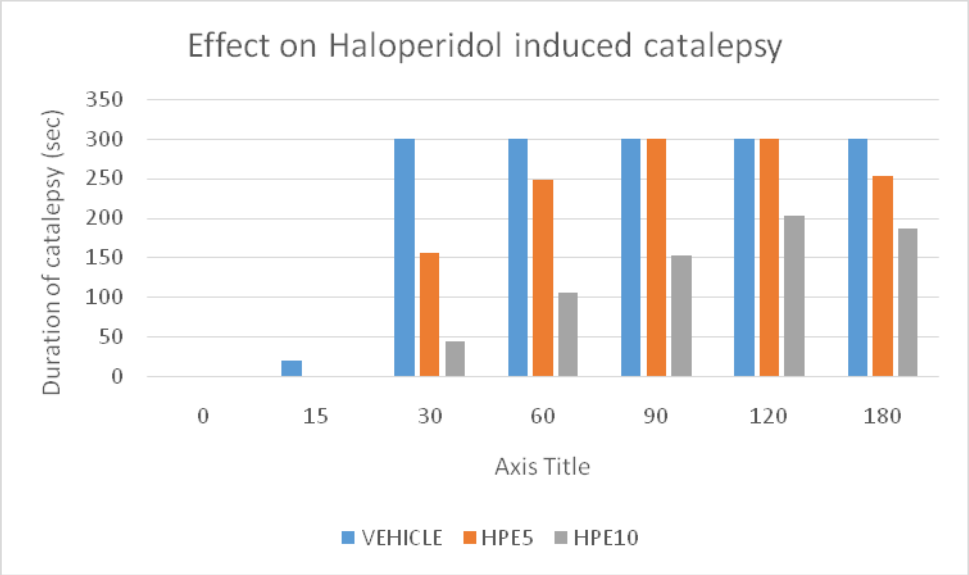
Effect of extract on duration of immobility using tail suspension test.





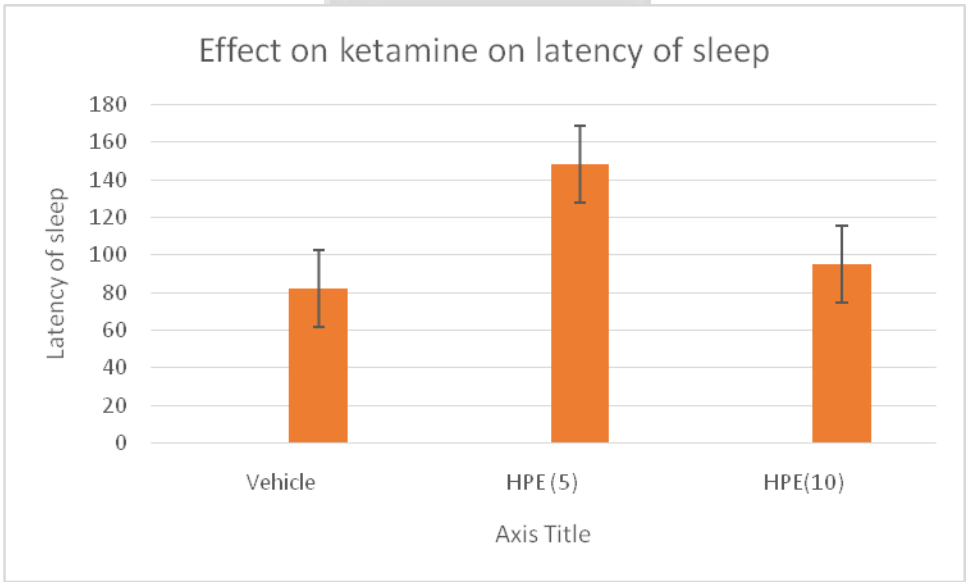
The duration of immobility of animals decreased at 5mg/kg compared to vehicle. Both the doses of extracts and fluoxetine altered latency to immobility significantly. Fluoxetine decreased the duration of immobility.

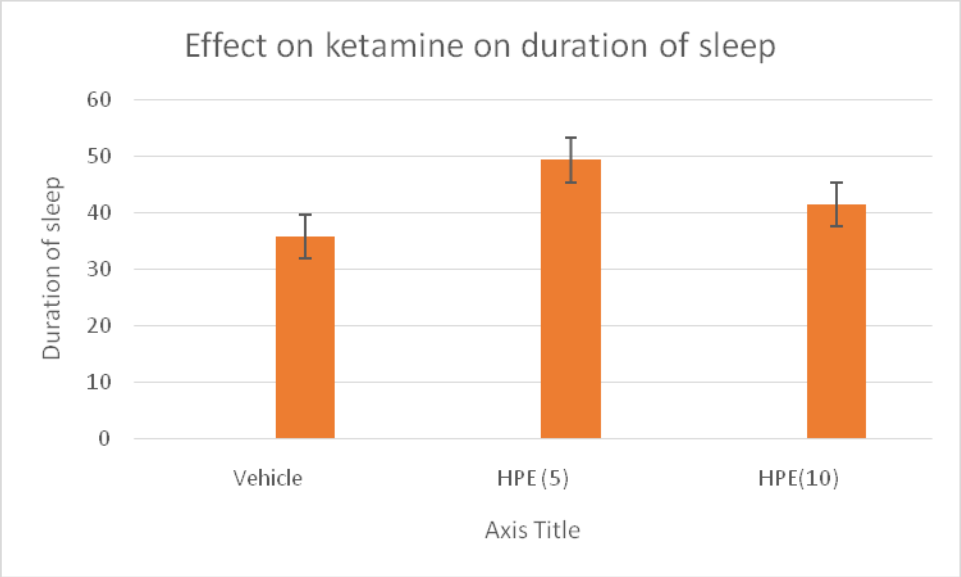
Effect of extract on haloperidol induced catalepsy



Extract inhibited haloperidol induced catalepsy at dose 10mg/kg significantly indicating that the extract either improves dopaminergic transmission or stimulated dopamine D_2 receptors. But low dose of 5mg/kg did not show any significant result.

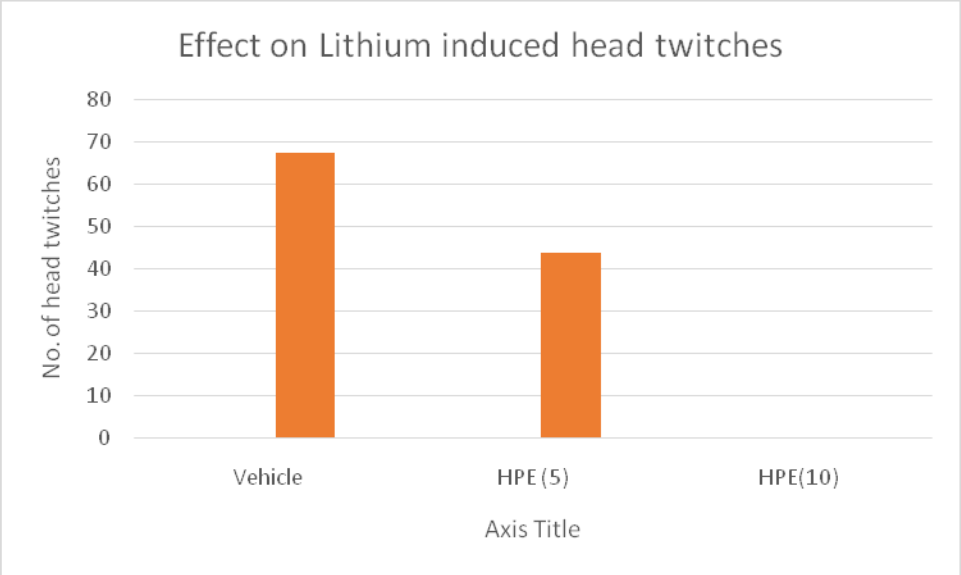
Effect of extract on ketamine induced sleep





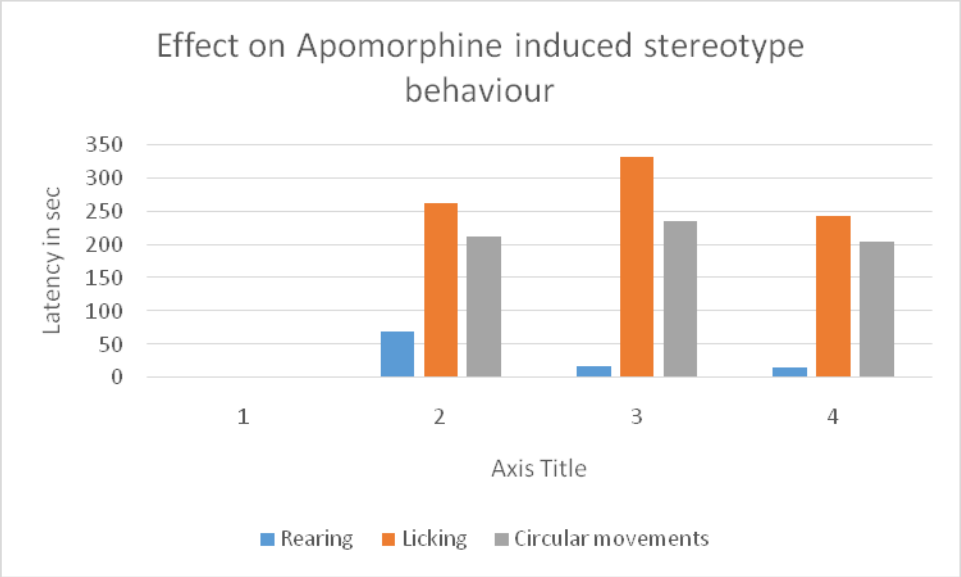
Injection of plant extracts of 5 and 10mg/kg did not significantly change the latency and duration of sleep compared to vehicle. Since anxiolytics should not have sedative effect, the extract can act as an anxiolytics.

Effect of extract on lithium induced head twitches



The vehicle treated rats exhibited 67.25 head twitches in one hour. A significant decrease in number of head twitches was observed with 10mg/kg of extract. The lower dose fails to modify the number of head twitches.

Effect of extract on apomorphine induced stereotype behavior



The administration of 5mg/kg and 10mg/kg extracts did not alter the licking and circular movements compared to vehicle. Number of rearing at doses of 5mg/kg and 10mg/kg significantly decreased compared to vehicle.

CONCLUSION

The *Hypericum perforatum* extract was safe as the LD 50 was greater than 2000mg/kg. The extract was reduced significant effect on haloperidol induced catalepsy, indicating that the extract either improves dopaminergic transmission or stimulated dopamine D_2 receptors. The behavioral action of dopamine D_3 receptor is believed to act as a brake on D_1, D_2 mediated behaviors. The extract reduced locomotor activity and rearing. The extract significantly increased the time spent in the open arm[8]. Results showing the anxiolytic effect at higher dose of extract. Lack of any significant effect on ketamine induced sleep indicates that the extract is safe and does not potentiate depressant effect of ketamine. The inhibition of lithium induced head twitches by the extract suggests that the extract inhibits serotonin release or blocks 5-HT_2 receptors. The extract in lower dose exhibited antidepressant activity as indicated by decrease in the duration of immobility. This observation is in congruence with the observation that antidepressants inhibit haloperidol induced catalepsy. Thus, the extract has well sustained antidepressant activity. And this activity is mediated via dopaminergic mechanism. Therefore,

we can conclude that the extract of *Hypericum perforatum* is worth investigating for assessment of anxiolytic and antidepressant activity and other diseases where dopaminergic stimulation is necessary[9].

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