Antidepressant activity of Indian Hypericum perforatum Linn in rodents

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A standardised 50% aqueous ethanolic extract of Indian Hypericum perforatum (IHp) was investigated for its antidepressant activity on various experimental paradigms of depression, viz. behavioural despair (BD), learned helplessness (LH), tail suspension (TS) and reserpine-induced hypothermia (RIH) tests in rats and mice. Pilot studies indicated that single dose administration of Ihp had very little or no acute behavioural effects, hence the Ihp was administered orally at two dose levels (100 and 200 mg/kg, po) once daily for three consecutive days, while imipramine (15 mg/kg, ip), a clinically used antidepressant agent, was administered acutely to rats (CF strain, 150±10g) and mice (Wistar strain, 23±2g) of either sex as the standard drug. Controls animals were treated similarly with equal volume of vehicle (0.3% carboxymethyl cellulose). Indian Hypericum perforatum extract showed significant antidepressant activity on all the paradigms of depression used. Thus Ihp and imipramine treatments significantly reduced the immobility time in BD and TS tests. Significant reduction in escape failures was also observed in LH test. In RIH test Ihp and imipramine inhibited reserpine induced hypothermia in a dose dependent manner. The observed antidepressant activity of Ihp was qualitatively comparable to that induced by imipramine.

Hypericum perforatum, better known as St. John's wort, has been used therapeutically for more than two centuries in Europe. The first association made in the United Kingdom between Hypericum and St. John is reflected in a Gaelic legend dating from the 6th century in which it is said that the missionary, St. Columba, always carried a piece of St. John's wort because of his high regard for St. John1. Hypericum species is a member of family Clusiaceae (alternative name: Guttiferae) in the order of Theales2, but some taxonomists classify the genus Hypericum as a segregated separate family, the Hypericaceae3. Indian Hypericum perforatum is a rhizomatous perennial herb growing up to a height of 3 feet distributed in the western Himalayas at altitudes of 3000-10,500 feet. Hypericum perforatum has been mentioned in Ayurveda and is known as Bassant, though its clinical use does not appear to include nervous disorders4.

In the late 1700s, Jean-Bernard Bossu reported on the use of Hypericum oil for healing wounds5, a use subsequently popularized by modern homeopathic practitioners. The most common use of Hypericum has been for the treatment of depression and various psychological and neurological disorders, as an antihelmintic, a vulnerary for minor hemorrhages, for bedwetting in children and as a diuretic6,9. The use of Hypericum for each of these purposes persists among the modern medical herbalists. Hypericum extracts find extensive use in Europe and Canada for treatment of depression10. Pharmaceutical studies11-13 indicate that the European variety of Hypericum perforatum (Hp) has significant antidepressant activity, somewhat similar to tricyclic antidepressants. These reports were substantiated by clinical studies, which indicated that HP was effective in mild to moderate depression13. Available reports clearly suggest that exploitation of this medicinal plant could not only eventually lead to more rational use of the Hypericum extracts for the treatment of depression, but may also be helpful in the search of antidepressant molecules with potentially novel mechanism(s) or model(s) of action10.

The Indian variety of Hypericum perforatum (IHp) has not been investigated for its neuropsychopharmacological properties, except for a recent report suggesting that 50% ethanolic extract of the plant has significant anxiolytic activity14. The present investigation was undertaken to investigate the antidepressant activity of Indian Hypericum perforatum in rodents. If the Indian variety of HP had similar efficacy as its European counterpart, it could prove to be a...
Materials and Methods

Animals

Adult Charles Foster albino rats (150 ± 10g) and Wistar mice (23 ± 2g) of either sex, were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, and were randomly distributed into different experimental groups. The rats and mice were housed in groups of six in polypropylene cages at an ambient temp. of 25°C±1°C and 45-55 % RH, with a 12: 12 hr light / dark cycle. The animals had free access to standard pellet chow (Brooke Bond- Lipton, India) and tap water given through drinking bottles. Experiments were conducted between 0900 and 1400 hrs.

Drug treatments

50% aqueous ethanolic extract of the whole plant of Indian Hypericum perforatum (IIp) which was standardised to contain 4.5-5.0% hyperforin was dissolved in 0.3% carboxymethyl cellulose (CMC) suspension prior to oral administration. IIp extract was administered orally by using oro-gastric cannula in the doses of 100 and 200 mg/kg once daily for three consecutive days. Imipramine(Sun Pharma, India) (15 mg/kg, ip.) was used as the standard antidepressant agent and was administered 30 min before experiments for comparison. Control rats were treated with vehicle (0.3% CMC suspension in distilled water). Experiments were conducted on day 3rd, one hour after the last drug or vehicle administration.

Behavioural tests

(1) Behavioural despair test in rats—The rat was placed in a chamber (45 × 20 cm) containing 25 cm water (25°C ± 2°C), so that it could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 min pretest, followed by a 5 min test 24 hr later. Drugs were administered after pretest. The period of immobility (animal remains floating in water without struggling and making only those movements necessary to keep its head over water) during 5-min test period was noted.

(2) Learned helplessness test—This model is based on the assumption that exposure to uncontrollable stress associated with repeated experiences of failure to escape from the stress produces a helpless situation, which results in performance deficits in subsequent learning tasks. A typical experiment involves two parts:

(a) Inescapable shock treatment—Rats were subjected to footshocks in a two compartment jumping box with the escape route to the adjoining unelectrified ‘safe’ chamber closed. A constant current shocker was used to deliver 60 scrambled shocks (15 sec duration, 0.8 mA every min) through the steel mesh grid floor. Control animals were placed in the chamber for 1 hr without experiencing shocks. This exercise was repeated 48 hr later on day 3.

(b) Conditioned avoidance training—On day 3, after the second inescapable shock treatment, the rats were subjected to avoidance training where a rat was placed in the electrified chamber and allowed to acclimate for 5 min before being subjected to 30 avoidance trials, with an inter-trial interval of 30 sec. During the first 3 sec of each trial, a light signal (conditioned stimulus, CS) was presented, followed by footshock (0.8 mA for 3-sec duration, unconditioned stimulus, UCS). The avoidance response is characterized by escape to the adjoining unelectrified chamber during CS, and was designated as ‘escape response’. Failure to exhibit escape response during CS was assessed as ‘escape failure’, which is said to represent depressive behaviour. Antidepressants reduce or even eliminate escape failures. This model has excellent predictive validity and is extensively used to screen antidepressants.

(3) Tail suspension test in mice—This test is a variant of the behavioural despair test in which immobility is induced by suspending a mouse by its tail. In a typical experiment a mouse is hung on a wire in an upside down posture so that its nostril thus touches the water surface in a container. After initial vigorous movements, the mouse assumes an immobile posture and the period of immobility during a 5-min observation period were noted. This test is reliable and rapid screening method for antidepressants, including those involving the serotonergic system.

(4) Reserpine induced hypothermia—On the day before testing, rats were dosed with 2 mg/kg reserpine (Sigma, USA) subcutaneously. Rats had free access to food and water. Eighteen hours after reserpine administration, the animals were placed into individual cages. The initial rectal temperature was determined by insertion of an electric thermometer (telethermometer) to a constant depth of 5cm. Fol-
lowing administration of the test compound, the rectal temperature was measured again at 60 minutes interval for 7 hours.

Statistical analysis—The data are expressed as means ±SDs for each treatment group. The data obtained from each response measures were subjected to Kruskal-Wallis one way analysis of variance (ANOVA) and inter group comparisons were made by Mann-Whitney-U-test for only those responses which yielded significant treatment effects in the

ANOVA test$^{20}$.

Results

Behaviour despair test—In the initial experiments acute administration of even high doses of the ethanolic extract of IHp did not reveal any antidepressant like effects in this test. Repeated oral administration of IHp extract for three consecutive days did, however, dose dependently reduced the immobility time in rats. Imipramine also showed similar activity and
the effects were comparable to that of higher dose of IHp extract. The observed effects of the extract are summarised in Fig. 1.

_**Learned helplessness test**_—Control rats with prior experiences of inescapable shocks exhibited marked increase in escape failures as compared to those with no such prior experiences. The escape failures significantly and dose dependently decreased in rats treated with both the doses of IHp. As in behaviour despair test, oral dose of 200 mg/kg, of IHp was almost equieffective to 15 mg/kg, ip dose of imipramine. The dose-effect responses are shown in Fig. 2.

_Tail suspension test with mice—Hypericum extract_ caused a significant and dose dependent decrease of immobility time in tail suspension test. This effect is regarded as indicative for antidepressant activity. Imipramine also showed significant antidepressant activity and the effects were comparable to that of higher dose of IHp (Fig. 3).

_Reserpine induced hypothermia_—Data obtained demonstrate that IHp extract completely antagonised reserpine induced hypothermia. Imipramine also showed complete antagonism of reserpine induced hypothermia and its effects are comparable to that of IHp (Fig. 4).

**Discussion**

The results of the present study clearly demonstrate significant antidepressant activity of an extract of IHp as assessed by the behavioural despair, learned helplessness, tail suspension and reserpine induced hypothermia tests in rats and mice.

Amongst a wide variety of proposed and critically assessed _in vivo_ models of depression, the two most commonly used paradigms are learned helplessness and behaviour despair forced swim tests. A few reports have also demonstrated the efficacies of standardised _Hypericum_ extracts in these animal models. In learned helplessness test, rodents are exposed to inescapable and unavoidable electric shocks in one situation, later fail to escape shock in a different situation when escape is possible. This phenomenon was evaluated as a potential animal model of depression. A drug is considered to be effective, if the learned helplessness is reduced and the number of failures to escape is decreased. Behavioural despair was proposed as a model to test antidepressant activity by Porsolt _et al._. It was suggested that mice or rats forced to swim in a restricted space from which they can not escape, exhibit a characteristic immobility. This behaviour reflects a state of despair, which can be reduced by several agents, which are therapeutically effective in human depression. Apart from these two paradigms, the observed results in tail suspension and reserpine induced hypothermia tests, provide additional measures for assessing antidepressant activity. In tail suspension test, the immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesised to reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempt to escape when suspended by tail. Reserpine induced hypothermia test has been proven as a simple and reliable method to detect antidepressant activity. However, the reversal of hypothermia is not specific for antidepressants. Amphetamines and some antipsychotic agents (chlorpromazine) can also antagonize the fall in body temperature. The different time course of antidepressants (slow onset of action, long lasting effect) and amphetamines-like drugs (quick onset of action, short lasting effect) allows differentiation between two group of drugs.

Our present behavioural observations confirm that 50% aqueous ethanolic extract of _Hypericum perforatum_ (IHp) possesses significant antidepressant activity.

Recently, _Hypericum_ extracts containing hyperforin have been reported to exhibit anxiolytic in rats on various paradigms of anxiety. A standardised extract of _Hypericum perforatum_ (Hp) has been reported to possess psychotropic activities like antidepressants in a water wheel and isolation induced aggression tests in mice. Other researchers have also reported similar antidepressant activity in Hp extract using tail suspension and forced swim tests. If Hp shares similar mechanism with currently used antidepressants, this is not apparent so far. The available reports indicate that Hp appears to affect multiple neurotransmitters without fitting easily into known antidepressant categories. Although _Hypericum_ demonstrates monoamine oxidase (MAO) inhibition _in vitro_, this effect has not been demonstrated _in vivo_, nor have there been any reported cases of MAO inhibitor-associated hypertensive crisis in humans using Hp. While previous studies showed that hypericin inhibits MAO in concentrations of 50 μg/mL, others have failed to confirm this effect. Bladt and Wagner reported that _Hypericum_ fractions with the greatest MAO inhibition contain the highest concentration of
flavonoids. Computer modeling of Hypericum constituents also suggests flavonoids to be the most likely MAO inhibitor fraction, due to structural similarity to taloxone and brofaromine, two known inhibitors of MAO \(^A\). In another study, the xanthone fraction was found to be a particular strong inhibitor of MAO \(A\) in vitro \(^{41}\).

Earlier studies suggested that hypericin, a MAO inhibitor may be active constituent of Hypericum perforatum. However, many recent studies indicate that the major active constituent of Hypericum perforatum may be the acylphloroglucinol, hyperforin. Extracts rich in hyperforin and devoid of hypericin have shown significant antidepressant activity in animal models of clinical depression. However, although hyperforin may be the major antidepressant component of Hypericum perforatum extract, there may either unidentified antidepressant component \(^{42}\). Hyperforin containing Hp extracts have been shown to inhibit synaptosomal uptake of serotonin, norepinephrine and dopamine with about similar affinities and to lead to a significant down-regulation of cortical \(\beta\)-adrenoceptors and 5-HT\(_2\)-receptors after subchronic treatment of rats. This reuptake inhibiting properties are not shown by hypericin \(^{43}\). Preliminary investigations of IHp indicate that it contains significant amount of hyperforin and is devoid of hypericin (S. Ghosal, unpublished data). A comparative chemical evaluation of the European and Indian varieties of Hypericum perforatum is in progress.

A recent meta-analysis of the existing double blind studies demonstrates that hydroalcoholic extracts of Hypericum perforatum are more effective than placebo and equieffective to some standard antidepressant drugs like amitriptyline, imipramine and maprotiline in the treatment of mild to moderately severe depressive disorders \(^{7,44,45}\). Furthermore, it has also been shown that treatments with such extracts seem to be devoid of major side effects typical for tricyclic antidepressant (TCA) or for the specific serotonin reuptake inhibitors (SSRI) and are less expensive \(^{7,45-48}\). Depressive disorders are now regarded as a major health problem \(^{49,50}\). Despite considerable progress made during the last 5 decades, successful treatment of clinical depression with currently available therapeutic agents can be achieved only in 65-75\% of patients, of which only 40-50\% achieve complete recovery \(^{51,52}\). Such a situation necessitates the development of more effective antidepressants \(^{52,53}\). The first generation of antidepressants, the TCA, discovered only after fortuitous clinical findings with imipramine \(^{53}\), are still widely used because of their familiarity and low cost. The introduction of second-generation antidepressants may have reduced the risks of adverse effects of the first generation tricyclic antidepressants, but made little impact on improving the effectiveness of treatment \(^{53,54,55}\). The search for new molecules as target for antidepressant drug discovery, therefore, remains a continuing challenge for modern psychiatry. It has been pointed out that, like in various other therapeutic areas \(^{56,57}\), investigations of traditional herbal products may provide a good chance for novel treatments for affective and other CNS disorders \(^{58,59}\).

It has been pointed out that although the majority of world’s health care services use herbal medicines \(^{50}\), the wide acceptance and rational uses of such botanical medicines is possible only when the active constituents and their modes of action are known. The case of Indian Hypericum extract does not also seem to be much different. Our experimental findings, thus, not only confirm the previous studies but also provide rationale for use of IHp as an antidepressant. Chemical investigations on IHp are now in progress.

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References