Anxiolytic activity of Indian *Hypericum perforatum* Linn: An experimental study

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The putative anxiolytic activity of 50% ethanolic extract of Indian *Hypericum perforatum* (IHp) was investigated in rats using various experimental paradigms of anxiety viz. open field exploratory behaviour (OFB), elevated plus maze (EPM), elevated zero maze (EZM), novelty induced suppressed feeding latency (FL) and social interaction (SI) tests. Pilot studies indicated that single dose administration of IHp had little to no acute behavioural effects, hence the extract of IHp was administered orally at different dose levels once daily for three consecutive days, while lorazepam (LR) (0.5 mg/kg, ip) was administered acutely. IHp extract (100 and 200 mg/kg, po) showed significant anxiolytic effects on all the paradigms of anxiety. The results indicate that IHp and LR induced a significant increase in open field ambulation and slight increase in rearings and activity in centre, whereas grooming and fecal droppings remained unchanged. In EPM, significant augmentation of open arm entries, open arm/closed arm entries ratio and time spent on open arms was noted in IHp treated rats. In EZM test, significant increase in time spent on open arms and entries in open arms were observed, whereas slight increase in head dips and stretched attend postures were also observed. IHp and LR significantly attenuated the novelty induced increase in feeding latency. IHp treated rats also showed significant increase in social interaction in the novel environment. The IHp extracts showed consistent and significant anxiolytic activity in all the tests. The effects induced by 50% ethanolic extract of IHp were less marked than those of lorazepam were.

*Hypericum* species were already known to ancient communities as useful medicinal plants. The use of *Hypericum perforatum* (HP), in particular, as a remedy was described and recommended throughout the Middle Ages. HP Linn is a perennial plant belonging to the Guttiferae family. It is commonly known as St. John's wort. Some taxonomists classify the genus *Hypericum* in a separate family, the Hypericaceae. The genus *Hypericum* encompasses approximately 400 species, of which ten morphologically and chemically distinct species grow in central Europe. HP, is distributed in Europe, Asia, North Africa and North America. Indian HP 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. The name *Hypericum* is a derivation of two Greek words, *hyper* and *eikon* which translate “over” and “icon” as in “over an apparition,” alluding to its use in ancient times for protecting against demonic possession and its reported ability to protect one from “evil spirits.” The species name *perforatum* is based on the perforated appearance of the leaves due to their translucent leaf glands, which can be observed when held up to light.

The primary ancient medical herbalists, including Hippocrates, Pliny, Dioscorides, Theophrastus, and Galen wrote about the medicinal properties of St. John’s wort, noting its use as a vulnerary (wound-healing) and for treatment of neuralgic conditions such as sciatica and hip pain. Mattioli wrote of its use as an emmenagogue, diuretic, and antimalarial. The most common use of *Hypericum* has been for the treatment of depression and various psychological and neuralgic disorders, as an anthelmintic for worms, a vulnerary for minor hemorrhages, for bedwetting in children, and as a diuretic. Besides it has been also used as balm for wounds, burns, ulcers & bites.

Traditionally, the availability of psychotropic drugs has fostered research in the field of psychopharmacology. St. John’s wort has been claim to possess significant antidepressant activity. Although HP has been mentioned in Ayurveda and is known as Bassant, its clinical use does not appear to include nervous disorders. HP appears to have a very high affinity for GABA receptors. However, Indian HP does not appear to have been subjected to in vivo investigations for its behavioural activities. In view of the in vitro studies it is likely that HP may be similar to benzodiazepines which enhance the GABA activity, and may have anxiolytic activity. Therefore, the present

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investigation was undertaken to investigate the anxiolytic activity of Indian HP in rats.

**Materials and Methods**

**Animals**

Adult Charles Foster albino rats (150 ± 10 g), 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 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 h 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 (IHp) was dissolved in 0.2% carboxy methylcellulose (CMC) suspension prior to oral administration. IHp extract was administered orally by using orogastric cannula in the doses of 100 and 200 mg/kg once daily for three consecutive days. Lorazepam (0.5 mg/kg, ip) was used as the standard anxiolytic agent and was administered to one group of rats 30 min. before experiments for comparison. Control rats were treated with the vehicle (0.2% CMC suspension in distilled water). Experiments were conducted on day 3rd, one hour after the last drug administration.

**Behavioural testings**

1. **Open-field test (OFT)**: The open-field apparatus was made of plywood and consisted of squares (61 x 61 cm). The entire apparatus was painted black except for 6 mm thick white lines which divided the floor onto 16 squares. Open-field was lighted by a 40W bulb focussing onto the field from a height of about 100 cm. The entire room, except the open-field, was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min and the following behavioural aspects were noted:
   
   (a) Ambulation: this was measured in terms of the number of squares crossed by the animal;
   
   (b) Rearings: number of times the animal stood on its hind limbs;
   
   (c) Self groomings: number of times the animal groomed facial region, and licked / washed / scratched various parts of its body;
   
   (d) Activity in centre: number of central squares crossed by the animal; and,
   
   (e) Fecal droppings: number of fecal droppings excreted during the period.

2. **Elevated plus-maze test (EPM)**: The maze had two opposite arms, 50 x 10 cm, crossed with two enclosed arms of the same dimension but having 40 cm high walls. The arms were connected with a central square, 10 x 10 cm, giving the apparatus shape of a plus sign. The maze was kept in a dimly-lit room and elevated 50 cm above the floor. Naive rats were placed individually in centre of the maze, facing an enclosed arm. Thereafter, number of entries and time spent on the open and enclosed arms were recorded during the next 5 min. An arm entry was defined when all four paws of the rat were in the arm. Observations were made by a neutral 'blind' observer.

3. **Elevated zero-maze test (EZM)**: The maze comprised of a black perspex annular platform (105 cm in diam, 10 cm width) elevated to 65 cm above the ground level, divided equally into four quadrants. The two opposite quadrants were enclosed by a black perspex wall (27 cm high) on both the inner and outer edges of the platform, while the remaining two opposite quadrants were surrounded by perspex "lip" (1 cm high) which served as a tactile guide to animals on these open areas. The apparatus was illuminated by dim white light arranged in such a manner as to provide similar lux levels in open and enclosed quadrants. Rats were placed on one of the enclosed quadrants for a 5 min test period. The maze was cleaned with 5% ethanol/water solution and dried thoroughly between test sessions. During the 5 min test period, time spent on open arms, number of 'head dips' over the edges of platform, and number of 'stretched attend postures' from closed to open quadrants were recorded. Animals were scored as being in the open area when all four paws were in the open quadrants and in the enclosed area only when all four paws had passed the open-closed divide.

4. **Social interaction test (SI)**: The rats were first housed individually for 5 days before testing. The apparatus used for the test was a wooden box (60 x 60 x 35 cm) with a solid floor and was placed in a dimly lit room. On day 6, the rats were placed individually in the box and given two 7.5 min familiarization sessions at 2 hr interval. On day 7, rats were paired on weight and sex basis and placed in the box for 7.5 min. During this time total time spent by the rat pair in "social interaction", including sniffing, following, grooming, kicking, boxing, biting and crawling under
or over the partner, was recorded by a neutral 'blind' observer.

5. Novelty induced suppressed feeding latency (FL) test: The test apparatus was a wooden box (60 x 60 x 35 cm) with a solid floor placed in a dimly lit room. The floor of the wooden box was covered with 2 cm layer of wooden chips, and laboratory chow pellet was placed on the floor. A similar arrangement was made in the home cages of the rats. Food was removed from the home cage 48 hr prior to testing, but water was provided ad libitum. Naive rats were placed individually in the test chamber and the latency to begin eating (defined as chewing of the pellet and not merely sniffing or playing with it), was recorded. If the rat had not eaten within 300 sec, the test was terminated and a latency score 300 sec was assigned. Observations were made by a neutral 'blind' observer.

Statistical analyses: 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 (two-tailed) for only those responses which yielded significant treatment effects in the ANOVA test.

Results

Open-field exploratory behaviour: Rats treated with both the doses of IHp extract showed dose dependent significant increase in open field ambulation, rearings, self grooming and activity in centre in comparison to vehicle treated rats, evincing significant anxiolytic activity of IHp. However the open-field fecal droppings remain unchanged. Lorazepam (LR) also induced significant anxiolytic activity and the effects were found to be more than that of IHp extract (Table 1).

Elevated plus maze behaviour: IHp treated rats exhibited dose dependent significant increase in time spent in open arms, entries made in open arms and significant decrease in time spent in enclosed arms and entries in enclosed arms in comparison to control rats. The result obtained by open/closed time and entries ratios also indicated significant anxiolysis in rats by IHp extract. LR caused more anxiolysis in comparison to IHp extract (Table 2).

Elevated zero maze behaviour: The rats treated with IHp extract showed anxiolysis in terms of significant increase in time spent in open arms, entries in open arms and number of head dips on elevated zero maze. However the response stretched attend postures remain unchanged. LR also caused significant anxiolytic activity and the effects were comparable to that of IHp extract (Table 3).

| Table 1 — Effect of 50% ethanolic extract of Indian Hypericum perforatum (IHp) and lorazepam (LR) on open field exploratory behaviour in rats 

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>Ambulation (N)</th>
<th>Rearings (N)</th>
<th>Self groomings (N)</th>
<th>Activity in centre (N)</th>
<th>Fecal droppings (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12</td>
<td>46.08 ± 5.40</td>
<td>9.66 ± 2.01</td>
<td>7.58 ± 1.67</td>
<td>1.25 ± 1.21</td>
<td>3.58 ± 1.37</td>
</tr>
<tr>
<td>IHp  (100mg/kg)</td>
<td>6</td>
<td>74.00 ± 14.87</td>
<td>10.00 ± 2.60</td>
<td>8.16 ± 1.16</td>
<td>2.66 ± 1.50</td>
<td>2.83 ± 0.75</td>
</tr>
<tr>
<td>IHp  (200mg/kg)</td>
<td>6</td>
<td>83.83 ± 6.11</td>
<td>11.33 ± 2.80</td>
<td>7.66 ± 1.50</td>
<td>4.66 ± 2.16</td>
<td>3.16 ± 0.75</td>
</tr>
<tr>
<td>LR  (0.5mg/kg)</td>
<td>6</td>
<td>85.66 ± 3.55</td>
<td>16.66 ± 2.50</td>
<td>12.16 ± 1.83</td>
<td>6.00 ± 1.41</td>
<td>2.33 ± 0.81</td>
</tr>
</tbody>
</table>

Superscripts a,b,c indicate statistical significance respectively in comparison to vehicle, IHp (100mg/kg) and lorazepam treatments. a, b and c denote P<0.05 and ≤0.01 respectively.

| Table 2 — Effect of 50% ethanolic extract of Indian Hypericum perforatum on the elevated plus maze behaviour in rats. Abbreviations are same as in Table 1 

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>Time spent on (sec)</th>
<th>Entries on open arms</th>
<th>Ratio of open/enclosed arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12</td>
<td>214.94 ± 5.43</td>
<td>7.83 ± 2.79</td>
<td>0.37 ± 0.11</td>
</tr>
<tr>
<td>IHp  (100mg/kg)</td>
<td>6</td>
<td>165.89 ± 5.43</td>
<td>64.96 ± 7.37</td>
<td>0.40 ± 0.12</td>
</tr>
<tr>
<td>IHp  (200mg/kg)</td>
<td>6</td>
<td>158.46 ± 5.43</td>
<td>69.74 ± 2.18</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>LR  (0.5mg/kg)</td>
<td>6</td>
<td>164.14 ± 4.65</td>
<td>70.16 ± 3.57</td>
<td>0.78 ± 0.09</td>
</tr>
</tbody>
</table>

Superscripts a,b,c indicate statistical significance respectively in comparison to vehicle, IHp (100mg/kg) and lorazepam treatments. a,b and c denote P<0.05 and ≤0.01 respectively.
Table 3—Effect of 50% ethanolic extract of Indian Hypericum perforatum on the elevated zero maze behaviour in rats. Abbreviations are same as in Table 1.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>Time spent on open arms (in sec.)</th>
<th>Head dips (N)</th>
<th>Stretched attend postures (N)</th>
<th>Entries in open arms (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12</td>
<td>51.28 ± 3.55</td>
<td>8.75 ± 1.71</td>
<td>3.83 ± 1.02</td>
<td>4.75 ± 1.35</td>
</tr>
<tr>
<td>IHp (100mg/kg)</td>
<td>6</td>
<td>70.14 ± 3.62^a</td>
<td>12.00 ± 3.03^a</td>
<td>3.00 ± 0.89</td>
<td>8.00 ± 1.67^acc</td>
</tr>
<tr>
<td>IHp (200mg/kg)</td>
<td>6</td>
<td>72.94 ± 2.59^aa</td>
<td>12.66 ± 4.71^a</td>
<td>2.50 ± 1.37^c</td>
<td>9.66 ± 1.36^abcc</td>
</tr>
<tr>
<td>LR</td>
<td>6</td>
<td>73.14 ± 1.89^aa</td>
<td>14.16 ± 1.60^aa</td>
<td>3.66 ± 0.81</td>
<td>11.33 ± 1.21^aa</td>
</tr>
</tbody>
</table>

Superscripts ^abc indicate statistical significance respectively in comparison to vehicle, IHp (100mg/kg), and lorazepam treatments. ^ab and ^acc denote P<0.05 and <0.01 respectively.

Social interaction: The rats treated with IHp extract spent significantly more time in social interaction in comparison to control rats and effects of IHp extract was found to be dose dependent. LR also caused significant increase in social interaction in rats and its effects was comparable to that of higher dose (200mg/kg) of IHp (Table 4).

Novelty induced suppressed feeding latency: IHp caused dose dependent significant attenuation of novelty induced feeding latency in rats in comparison to vehicle treatment. LR also induced similar effects, however, its effect was observed to be more than that of IHp extract (Table 5).

Discussion

Most of the animal models of anxiety now in use were developed for benzodiazepines (BDZ) and, since these compounds also exhibit significant muscle relaxant and anticonvulsant effects, evaluation of anxiolytic activity, even with non-BDZ compounds, invariably now includes test for these neuropharmacological actions. The sedative, amnesic and ataxic effects of BDZ and non-BDZ anxiolytics are definite drawbacks when these drugs are used for the treatment of anxiety. However, since the question of reliability and validity is foremost in establishing animal tests, recourse has to be taken to compare the pharmacological profile of activity of a putative anxiolytic agent with that elicited by a BDZ. As such, despite the additional effects that lorazepam is known to have, it was used to validate the anxiolytic activity of Indian Hypericum perforatum (IHp).

In the open field test, when animals are taken from their home cage and placed in a novel environment, they express their anxiety and fear by decrease in ambulation and exploration, freezing, rearing and grooming behaviours, and increase in defecation due to heightened autonomic activity. These behavioural changes are attenuated by classical anxiolytics and
augmented by anxiogenic agents\textsuperscript{19}. Likewise, the elevated plus maze and elevated zero maze tests are based on the principle that exposure to an elevated and open maze arm leads an approach—conflict that is considerably stronger than that of evoked by exposure to an enclosed arm of the maze. Thus, open/enclosed arms entries and time ratios provide a measure of fear-induced inhibition of exploratory activity. These responses are increased by anxiolytics and reduced by anxiogenic agents\textsuperscript{20}. Furthermore, anxiolytics increase the social interaction and decrease the feeding latency respectively in the social interaction and novelty induced suppressed feeding latency tests. However the anxiolytic activity of IHp was found to be less marked than that of the common BDZ anxiolytic agent lorazepam.

Recently, Hypericum extracts containing hyperforin has been reported to exhibit anxiolysis in rats on elevated plus maze test\textsuperscript{21}. A standardised extract of Hypericum perforatum has been reported to possess psychotropic activities like antidepressant in a water wheel and isolation induced aggression tests in mice\textsuperscript{22}. Other researchers have also reported similar antidepressant activity in HP extract using tail suspension test and forced swim test in animals\textsuperscript{23,24}. Hypericum has also been reported to be a sedative\textsuperscript{25} and useful in the treatment of chronic tension headaches\textsuperscript{26}. In addition to the antidepressant effect, Hypericum has been used for a wide variety of neurological conditions, including anxiety, trigeminal neuralgia, neurosis, migraine headaches, fibrositis, dyspepsia and sciatica\textsuperscript{17,26}. We now report the presence of significant anxiolytic property in the 50% ethanolic extract of IHp.

If HP shares a 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 HP demonstrates monoamine oxidase inhibition in vitro, this effect has not been demonstrated in vivo, nor have there been any reported cases of monoamine oxidase inhibitor-associated hypertensive crisis in humans using HP\textsuperscript{31}. Other proposed mechanisms involve effects of monoamine uptake. In vitro HP inhibits not only uptake of serotonin but also that of norepinephrine and dopamine\textsuperscript{32}. However, the concentrations required to attain these effects are quite high, and the chances of attaining blood concentrations necessary for these effects are remote. The most potent effect thus so far reported is for the GABA receptors, with effects shown at IC\textsubscript{50} approximately 75 ng/ml for GABA\textsubscript{A} and mg/ml for GABA\textsubscript{B}. The crude extract of Hypericum had significant receptor affinity for adenosine, GABA\textsubscript{A}, GABA\textsubscript{B}, serotonin, benzodiazepine, inositol triphosphate (IP\textsubscript{3}), and monoamine oxidase (MAO\textsubscript{A,B})\textsuperscript{33}. These data are consistent with recent pharmacologic evidence suggesting that several constituents of this plant may be important for the reported psychotherapeutic activities. It is conceivable that the very high affinity of Hypericum extract for GABA receptors may be important for its anxiolytic activity. The significance of this GABA binding is unknown, but there is considerable literature on the role of GABA in affective disorders. GABA\textsubscript{A} stimulation has been found to enhance receptor down regulation during imipramine treatment\textsuperscript{34}. Fenogabine, a GABAergic agent, has also been reported to be an effective antidepressant\textsuperscript{35}. GABA plasma levels has been reported to be low in both bipolar and unipolar depression\textsuperscript{5,37} and benzodiazepines, which enhance GABA\textsubscript{A} activity, may be effective antidepressants as well as anxiolytics\textsuperscript{38}. GABA has also been found to be one of the major constituents of Hypericum perforatum\textsuperscript{25} and crude extract of Hypericum has been observed to have significant affinity for BDZ receptors in in vitro studies\textsuperscript{39}. Therefore, the observed anxiolytic effect of IHp in the present study, may be attributed to its high affinity for GABA and BDZ receptors. However, further studies should be planned to characterize the Hypericum effects. Studies with GABA agonists and antagonists with Hypericum should also be carried out to specify its intrinsic activity.

References
3. Fernie W T, Herbal simples, (John Wright & Co, Bristol) 1897.