Anti-stress activity of Indian Hypericum perforatum L.

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Received 8 September 2000; revised 27 December 2000

Hypericum perforatum L (HP) has been used since ancient times in European folklore medicine for its many medicinal properties. Modern usage is still quite diverse and includes wound healing, kidney and lung ailments, insomnia and depression1. This widely grown plant has been in use by tribals of this country for ailments such as fever, joint pain, general weakness, sleep disorders and delayed wound healing. Standardised extracts of HP are widely used in the treatment of psychogeriatric disorders and especially for mild forms of depression in Europe2. Several clinical studies have confirmed the antidepressant activity of the extracts derived from HP3-5. The extract of Indian Hypericum perforatum (IHp) has also shown significant neuropsychopharmacological actions5-9, but has not been commercially in this country. Since this plant is of great market value it becomes important to investigate its putative neuropsychopharmacological properties. In this study, the anti-stress adaptogenic activity of IHp was investigated in view of its reported anxiolytic, antidepressant and nootropic activity5-7.

Materials and Methods

Drug treatments—The plant (IHp) was collected during August from Company Garden, Saharanpur, India. A specimen of the plant is preserved with Indian Herbs, Saharanpur. 50% ethanolic extract (yield 26.75% w/w, standardised for 4.5-5% hyperforin, HPLC) of the dried leaves, flowers and stem of the plant was orally administered as 0.3% carboxymethyl cellulose (CMC) suspension, in the doses of 100 and 200 mg/kg, po and PG (100 mg/kg, po). The results indicate that IHp has significant anti-stress activity, qualitatively comparable to PG, against a variety of behavioural and physiological perturbations induced by chronic stress, which has been proposed to be a better indicator of clinical stress than acute stress, and may indicate adaptogenic activity.

Hypericum perforatum L (HP) has been used since ancient times in European folklore medicine for its many medicinal properties. Modern usage is still quite diverse and includes wound healing, kidney and lung ailments, insomnia and depression1. This widely grown plant has been in use by tribals of this country for ailments such as fever, joint pain, general weakness, sleep disorders and delayed wound healing. Standardised extracts of HP are widely used in the treatment of psychogeriatric disorders and especially for mild forms of depression in Europe2. Several clinical studies have confirmed the antidepressant activity of the extracts derived from HP3-5. The extract of Indian Hypericum perforatum (IHp) has also shown significant neuropsychopharmacological actions5-9, but has not been commercially in this country. Since this plant is of great market value it becomes important to investigate its putative neuropsychopharmacological properties. In this study, the anti-stress adaptogenic activity of IHp was investigated in view of its reported anxiolytic, antidepressant and nootropic activity5-7.

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Animals—Adult inbred Charles Foster albino rats (150±10g), 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 animals 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. Animals were provided with commercial food pellet (Booke Bond-Lipton, India) and water ad libitum. Experiments were conducted between 0900 and 1400 hrs. *Principles of laboratory animal care* (NIH publication number no. 85-23, revised 1985 guidelines were followed.

Induction of chronic stress—The method of Armando et al.10 was used. The rats were randoml
assigned to the unssressed control, stress and drug treated stress groups. Those assigned to the vehicle or drug treated groups were subjected daily (including Sundays) to 1 hr of footshock through a grid floor in a standard conditioning chamber with the escape route closed. The duration of each shock (2 mA) and the intervals between the shocks were randomly programmed between 3 and 5 sec and 10 and 110 sec, respectively in order to make them unpredictable. Animals were sacrificed on day 14, 1 hr after the last shock procedure on completion of the test procedure involved.

Techniques used for assessment of stress intensity—The following parameters were used to assess the intensity of stress-induced effects:

(a) Gastric ulceration: The stomach was removed and split open along the greater curvature. The numbers of discrete ulcers were noted by the help of a magnifying glass. The severity of the ulcers was scored after histological confirmation as, 0=no ulcers, 1=changes limited to superficial layers of the mucosa with no congestion, 2=half the mucosal thickness showing necrotic changes and congestion, 3=more than two-third of mucosal thickness showing necrotic changes and congestion, and 4=complete destruction of the mucosa with marked haemorrhage. Thereafter, the period ulcer severity score was calculated after adding up individual scores.

(b) Adrenal cortex and spleen weights: The adrenal gland and spleen were removed and weighed.

Methods used to assess stress-induced perturbations

(a) Stress-induced ‘behavioural depression: The following methods were used to assess behavioural depression

(i) Stress-induced ‘behavioural despair’ test: Rats were forced to swim individually in a polypropylene vessel (45x40x30 cm) with a water level of 20 cm, which ensured that the rat’s feet did not touch the floor of the vessel and that it could not climb out of it. The rat was allowed to swim for 10 min. Thereafter, during the next 5 min, the total period of immobility, characterized by complete cessation of swimming with the head floating above water level, was noted. This immobility period, after initial frenzied attempts to escape, is postulated to represent ‘behavioural despair’ as an experimental model of endogenous depression.

(ii) Learned helplessness test: On day 12 of the investigation, rats were subjected to footshock (60 scrambled shocks, 15 sec duration, 0.8 mA, every min) in a two compartment jumping box (Techno) with the escape door to the adjoining unelectrified compartment closed. The exercise continued for 1 hr. On day 14, 48 hr later, the rats were subjected to avoidance training, using the same apparatus but keeping the escape route to the unelectrified chamber open. During this avoidance training the rats were placed in the electrified chamber and allowed to acclimatize for 5 min before being subjected to 30 avoidance trials, with an inter-trial interval of 30 sec. During the first 3 sec of the trial, a buzzer stimulus (conditioned stimulus, CS) was present followed by electroshock (unconditioned stimulus, UCS) (0.8 mA) delivered via the grid floor for the next 3 sec. The avoidance response was characterized by escape to the adjoining ‘safe’ chamber during CS. Failure to escape during UCS within 15 sec assessed as ‘escape’ failure which is postulated to indicate despair or depression.

(iii) Stress-induced inhibition of male sexual behaviour: A male rat was placed in a cage in a dimly room for 10 min with 2 oestrinised (sequentially treated with oestradiol valerate 5 μg/rat, followed 48 hr later by hydroxyprogesterone 1.5 mg/rat, sc) female rats. The total numbers of mounts were counted.

Stress-induced cognitive dysfunction: The following parameters were used to assess the effect of stress on retention of a learned task as memory:

(i) Active avoidance test: Rats were trained for an active avoidance task before subjecting them to stress. During training, the rat was placed in the right electrified compartment of a shuttle box (Techno) and allowed to acclimatize for 5 min. Thereafter, the animal was subjected to 15 sec of a buzzer stimulus (CS) which was followed by electric shock (1 mA, 50 Hz) given through the grid floor (UCS). The rats were given at least 10 trials, with an inter-trial interval of 60 min, until they reached the criterion of 100% avoidance response of jumping to the unelectrified left chamber of the shuttle box during CS. The test was repeated on day 14 in order to assess the retention of the active avoidance learning.

(ii) Passive avoidance test: The test apparatus was a rectangular box (45x30x40) with an electrified grid floor. An 8 cm high platform (17x12 cm) was fixed to the centre of the floor. A rat was placed on the platform and allowed to step down. 24 hr later, on day 1 of the experiment, the rat was again placed on the platform and on stepping down, received footshock (0.75 mA, 2 sec) through the grid floor. The rat was
given 3 more trials until the latency of step down had stabilised. The test was repeated on day 14 and retention of learning as memory, for each rat was recorded.\(^7\)

Statistical analysis: The values are expressed as mean \pm SD. Statistical significance of the differences between control and treated groups was calculated using Kruskal Wallis one way analysis of variance (ANOVA) followed by Mann-Whitney U-test (two tailed). \(P<0.01\) was considered to be significant.

Results

(1) Gastric ulceration: Chronic stress markedly increased the incidence, number and severity of gastric ulcers. IHp (100 and 200 mg/kg, po) and PG (100 mg/kg, po) significantly reduced these stress-induced gastric indices (Table 1).

(2) Adrenal cortex and spleen weights: Chronic stress significantly increased adrenal gland weight and reduced that of spleen. These stress-induced changes were attenuated by IHp (100 and 200 mg/kg, po) and PG (100 mg/kg, po) (Table 2).

(3) Stress-induced ‘behavioural despair’ and ‘learned helplessness’ test: Chronic stress increased the duration of immobility test, while increasing escape failures with concomitant decrease in avoidance response in the learned helplessness test, features indicative of depression. IHp (100 and 200 mg/kg, po) and PG (100 mg/kg, po) tended to reverse the stress-induced behavioural changes (Tables 3 and 4).

(4) Stress–induced inhibition of male sexual behaviour: Chronic stress significantly decreased the sexual behaviour of male rats, as indicated by decrease in the number of mountings. This stress effect was reversed by IHp (100 and 200 mg/kg, po) and PG (100 mg/kg, po) (Table 3).

(5) Active and passive avoidance tests: Chronic stress produced significant decrease in the retention of acquired active and passive learning. These stress induced memory deficits were reduced by IHp (100 and 200 mg/kg, po) and PG (100 mg/kg, po) (Tables 3 and 5).

Discussion

Stress research in laboratory animals has assumed an important role in understanding the biological and behavioural consequences of external or internal stressors, which threaten to perturb homeostasis, and may induce a number of clinical diseases when the body fails to counter the stress situation.\(^8\) A variety of stressful situations have been employed and the lack of consistency of the stress protocols is astounding.\(^8\) Likewise, there is wide variation in the physiological consequences of the stressors utilized in animal research.\(^9\) However, it is now widely accepted that chronic inescapable intermittent stress, particularly of an unpredictable pattern, is more likely to induce neural, endocrine and biochemical perturbations than either acute or chronic stress of a

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**Table 1—Effects of IHp and Panax ginseng compared to vehicle on chronic stress-induced gastric ulcerations in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer incidence (%)</th>
<th>Number of ulcers</th>
<th>Severity of ulcers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle+Stress(VS)(10)</td>
<td>—</td>
<td>100</td>
<td>8.7±1.7</td>
<td>16.8±3.3</td>
</tr>
<tr>
<td>IHp+VS (6)</td>
<td>100</td>
<td>67*</td>
<td>4.5±2.3</td>
<td>8.7±4.5*</td>
</tr>
<tr>
<td>IHp+VS(6)</td>
<td>200</td>
<td>33*</td>
<td>2.3±1.7*</td>
<td>4.8±3.5*</td>
</tr>
<tr>
<td><em>Panax ginseng</em>+VS(6)</td>
<td>100</td>
<td>67*</td>
<td>2.3±1.0*</td>
<td>4.8±2.0*</td>
</tr>
</tbody>
</table>

Values in parentheses indicate number of animals; * indicates difference with VS (ANOVA followed by Mann-Whitney U-test).

**Table 2—Effects of IHp and Panax ginseng compared to vehicle on chronic stress-induced changes in adrenal gland and spleen weights in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Adrenal gland wt (mg/100g)</th>
<th>Spleen wt (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle(10)</td>
<td>—</td>
<td>24.15±2.10</td>
<td>195.70±11.00</td>
</tr>
<tr>
<td>Vehicle+Stress(VS)(10)</td>
<td>—</td>
<td>41.10±3.75*</td>
<td>128.65±8.54*</td>
</tr>
<tr>
<td>IHp+VS (6)</td>
<td>100</td>
<td>30.74±2.90*</td>
<td>157.75±7.05*</td>
</tr>
<tr>
<td>IHp+VS(6)</td>
<td>200</td>
<td>27.05±2.54*</td>
<td>178.48±6.10*</td>
</tr>
<tr>
<td><em>Panax ginseng</em>+VS(6)</td>
<td>100</td>
<td>28.45±2.70*</td>
<td>170.10±9.15*</td>
</tr>
</tbody>
</table>

Values in parentheses indicate number of animals; * indicates difference with vehicle treated group; * indicates difference with VS (ANOVA followed by Mann-Whitney U-test).
predictable nature\textsuperscript{15}. The validity of the method used in the present study is demonstrated by the biological effects induced by it, which include gastric ulcerations, increase in adrenal gland weight and decrease in the weight of the spleen. All these parameters have been conclusively shown to be stress-induced effects\textsuperscript{20}.

The prevention and management of stress disorders remains a major clinical problem. Benzodiazepines (BDZs) appear to be effective against acute stress but fail to prevent the consequences of chronic stress\textsuperscript{18}. In addition, the problems of tolerance and physical dependence exhibited by BDZs, on prolonged use, limit their utility\textsuperscript{18}. An answer to this vexing problem was first provided when Brekhman and Darymov\textsuperscript{21} reported that some plant-derived agents could induce a state of non-specific increase of resistance to affect internal homeostasis. These agents, named adaptogens, appeared to be effective only when the physiological perturbations were discernible following prolonged illness, old age and exposure to chronic stress\textsuperscript{21}. Adaptogens like Panax ginseng (PG) were shown to be effective in attenuating stress induced adverse effects in astronauts, soldiers and athletes in the USSR\textsuperscript{21}. PG, the first clinically used adaptogen, has been extensively investigated experimentally and clinically for its stress-attenuating activity\textsuperscript{22}.

Both IHp and PG prevented chronic stress-induced gastritis, in the term of the incidence and severity of the ulcers. Involution of the spleen and increase in adrenal gland weight, are also consequences of chronic stress\textsuperscript{18}, both responses being reversed by IHp and PG.

There is considerable experimental and clinical evidence to suggest that chronic stress induces endogenous depression\textsuperscript{23}. A number of animal models of depression are based on the use of uncontrollable stress and the biochemical correlates of such tests are consonant with those seen in chronic stress, including monoamine deficiency and increased activity of the corticotrophin-releasing factor\textsuperscript{23}. Both IHp and PG were able to reverse chronic stress-induced indices validated as animal models of depression. Chronic stress is known to affect other endocrine responses as well, which can induce sexual debility in males\textsuperscript{24} and perturb glucose metabolism\textsuperscript{25}. Maturity-onset diabetes mellitus may represent a state of stress-induced disturbance in glucose homeostasis\textsuperscript{25}. IHp and PG reversed chronic stress-induced inhibition of male sexual behaviour.

Stress is known to interfere with cognitive functions, tending to retard the memory engram rather than the acquisition of learning\textsuperscript{26}. The mechanisms involved in the memory-attenuating effect of stress remains conjectural but a similar neurochemical basis operating in the induction of stress-induced depression, may be responsible\textsuperscript{26}. IHp and PG

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Treatment & Dose (mg/kg) & Escape failures (N) & Avoidance response (N) \\
\hline
Vehicle (10) & - & 14.75±1.80 & 3.70±0.71 \\
Vehicle+Stress(VS)(10) & - & 26.08±1.95\textsuperscript{a} & 0.83±0.18\textsuperscript{a} \\
IHp+VS (6) & 100 & 18.35±1.55\textsuperscript{b} & 1.45±0.55\textsuperscript{b} \\
IHp+VS(6) & 200 & 15.70±1.24\textsuperscript{b} & 2.70±0.64\textsuperscript{b} \\
Panax ginseng+VS(6) & 100 & 15.95±1.20\textsuperscript{b} & 2.65±0.58\textsuperscript{b} \\
\hline
\end{tabular}
\caption{Effects of IHp and Panax ginseng compared to vehicle chronic stress-induced changes in learned helplessness test in rats.}
\end{table}

Values in parentheses indicate number of animals; \textsuperscript{a} indicates difference with vehicle treated group; \textsuperscript{b} indicates difference with VS (ANOVA followed by Mann-Whitney U-test).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Treatment & Dose (mg/kg) & Number of mountings (N) & Active avoidance response on day 14 (%) \\
\hline
Vehicle (10) & - & 117.05±10.15 & 6.15±0.80 \\
Vehicle+Stress(VS)(10) & - & 260.92±7.35\textsuperscript{a} & 1.70±0.74\textsuperscript{a} \\
IHp+VS (6) & 100 & 181.47±8.63\textsuperscript{b} & 3.25±0.78\textsuperscript{b} \\
IHp+VS(6) & 200 & 139.28±6.10\textsuperscript{b} & 4.00±0.95\textsuperscript{b} \\
Panax ginseng+VS(6) & 100 & 142.45±6.58\textsuperscript{b} & 4.15±0.86\textsuperscript{b} \\
\hline
\end{tabular}
\caption{Effects of IHp and Panax ginseng compared to vehicle chronic stress-induced increase in swim stress immobility, suppression of sexual behaviour and memory deficit in active avoidance response in rats.}
\end{table}

Values in parentheses indicates number of animals; \textsuperscript{a} indicates difference with vehicle treated group; \textsuperscript{b} indicates difference with VS (ANOVA followed by Mann-Whitney U-test).
attenuated the stress-induced deficit of retention of learned tasks, both in the active and passive avoidance parameters, thus facilitating memory and its recall.

The findings indicate that, like the standard adaptogen PG, IHp can attenuate chronic stress-induced biochemical, behavioural and physiological perturbations in rats. PG has earlier been reported to reverse chronic stress induced effects in humans. Japanese traditional medicinal plant formulations, like Goya-jinko-gan, Kysushin and Reiousan, essentially based on Ginkgo biloba, have been reported to reduce the adverse effects of chronic hanging stress on sexual and learning behaviours in mice.

Increased generation of oxidative free radicals (OFR), or impaired antioxidant defence mechanisms, have been implicated in chronic stress induced perturbed homeostasis including immunosuppression, inflammation, diabetes mellitus, peptic ulceration and other stress-related diseases. IHp have been shown to exert significant antioxidant activity induced by augmented activity of OFR scavenging enzymes, superoxide dismutase, catalese and glutathione peroxide. Thus, the observed adaptogenic antistress effect of IHp may be at least partly due to its antioxidant activity.

The present investigation indicates that IHp has significant adaptogenic activity as shown by its mitigating effects on several chronic stress induced physiological and behaviour perturbations, comparable to that induced by the well accepted adaptogenic agent, Panax ginseng.

Acknowledgement

Vikas Kumar is grateful to ICMR, New Delhi for award of Senior Research Fellowship.

References