

Antidepressant activity of hyperforin conjugates of the St. John's wort, *Hypericum perforatum* Linn.: An experimental study

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Nine extracts of *H. perforatum*, containing hyperforin in conjugated forms, but devoid of free hyperforin and adhyperforin, were subjected to antidepressant screening using the forced swim test (FST). The observed activity was compared with that of SJW extracts containing hyperforin and adhyperforin (in free form). Results indicate that hyperforin conjugates exhibit significant antidepressant activity as evidenced by the reduced immobility period in the FST in rats.

Extracts of *Hypericum perforatum* Linn. (St. John's wort, SJW) have shown significant antidepressant activity in both experimental^{1,2} and clinical studies³. Earlier, hypericin, a naphodiantherene derivative present in SJW, was thought to be the active constituent responsible for the antidepressant activity⁴. However, in later studies the earlier results could not be confirmed⁵. More recently, hyperforin, a prenylated phloroglucinol derivative, present in the plant, has been projected as primarily responsible for the antidepressant activity of SJW⁶⁻⁹. In the present study, SJW collected from Northern parts of India were examined for their hyperforin and adhyperforin contents by HPLC, using authentic markers. Interestingly, in five of the nine extracts examined (SJW 1-5) hyperforin and adhyperforin were vicariously represented by the corresponding conjugates. The remaining four extracts (SJW 6-9) contained hyperforin, adhyperforin and also hyperforin conjugates (as minor entities). In this study, the contribution of hyperforin conjugates, if any, on the antidepressant activity of SJW was studied on forced swim test (FST). FST was selected for this study since its relevance has been demonstrated with a large number of clinically used antidepressants¹⁰.

Animals—Albino rats (CF strain) of either sex, were housed in colony cages maintained at $22 \pm 1^\circ\text{C}$ in a 12 hr dark/light cycle. The rats had free access to water and standard lab chow. SJW extracts were ad-

ministered (50 mg/kg/day) orally for three days in 0.3% CMC suspension. The experiment was performed after 45 min of the final administration of the drugs or the vehicle.

SJW Extracts—In a typical experiment, the dried overground parts of the *H. perforatum* (SJW), collected from Northern India, were extracted continuously with aqueous acetone (Soxhlet, 12h). The solvent was removed from the extract under vacuum. A portion of the dried extract was accurately weighed, dispersed in water and further extracted with chloroform. The chloroform soluble fraction was dried and redissolved in methanol (1 mg/ml) and subjected (20 μl) to HPLC analysis (WATERS ASSOCIATES HPLC assembly equipped with PDA detector; column: reverse phase RP-C18 (Novapak 3.9 \times 150 mm); eluent: acetonitrile - water - phosphoric acid (80:20:01); flow rate: 1.2 ml/min; pressure 1500 psi; scanning at 270 nm) using hyperforin and adhyperforin as markers to eliminate the possibility of their occurrence in the extract. Likewise, the HPLC spectrum of hyperforin conjugates exhibited absence of hyperforin and adhyperforin.

The nature of the hyperforin conjugates was established by comprehensive spectroscopic analyses (UV, ¹HNMR) and crucial chemical transformation of hyperforin¹⁰.

Forced swim test—The rats were placed in a chambers (45 \times 20 cm) containing water up to 25 cm ($25 \pm 2^\circ\text{C}$), so that it could not touch the bottom of the cylinder with its hind limb or tail, or climb over

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Table 1—Percentage abundances of hyperforin, adhyperforin and its conjugates in SJW extracts and their effect on Forced Swim Test (FST) in rats
[Values are mean \pm SE, n=6]

Groups	% Relative Abundance			FST
	Hyperforin	Adhyperforin	Hyperforin conjugates	Immobility period (sec)
Vehicle	-	-	-	115.2 \pm 9.37
SJW 1	-	-	16.31	46.0 \pm 4.25 ^{aa}
SJW 2	-	-	06.32	60.0 \pm 5.51 ^{aa}
SJW 3	-	-	09.64	42.6 \pm 6.14 ^{aa}
SJW 4	-	-	14.31	49.8 \pm 6.14 ^{aa}
SJW 5	-	-	12.42	69.0 \pm 4.45 ^{aa}
SJW 6	4.60	4.15	00.53	52.0 \pm 5.78 ^{aa}
SJW 7	0.33	0.09	02.48	41.8 \pm 5.02 ^{aa}
SJW 8	1.70	0.92	05.21	48.4 \pm 4.93 ^{aa}
SJW 9	0.53	0.31	08.42	39.4 \pm 2.32 ^{aa}
Imipramine	-	-	-	62.3 \pm 3.81 ^{aa}

^{aa} $P < 0.01$ (One way ANOVA followed by Newmann-Keuls test).

Identities of hyperforin, adhyperforin and its conjugates were based on the UV spectral (λ_{\max} 272 nm) and ¹HNMR characteristics. Hyperforin t_R = 10.1; adhyperforin t_R = 11.3; hyperforin conjugates t_R = 2.3 to 6.5.

the edge of the chamber. Two swim sessions were conducted, an initial 15 min pretest, followed by a 5 min test 24 hr later. The period of immobility (animal remains floating in water without struggling and making only those movements necessary to keep its head over water) during the 5 min test period was noted¹¹.

The test paradigm is based on the observation that rats or mice, when forced to swim in a restricted space from which they cannot escape, eventually cease attempts to escape and become immobile, except from the small movements necessary to keep their heads above water. This characteristic behavioural immobility reflects a state of despair in rats and mice. Treatment with antidepressant agents reduce the immobility period¹². SJW extracts containing hyperforin and adhyperforin (SJW 6-9, 50 mg/kg/day) significantly reduced the immobility period in comparison to vehicle treated rats. Interestingly, the SJW extracts (SJW 1-5, 50 mg/kg/day), containing hyperforin conjugates, and devoid of free hyperforin and adhyperforin, also significantly reduced the immobility period in treated rats (Table 1). The antidepressant activity of SJW extracts was compared with that of imipramine (10 mg/kg, ip), a clinically used tricyclic antidepressant.

Both SJW extracts (SJW 1-5), containing only hyperforin conjugates (but free from hyperforin and adhyperforin), and SJW extracts (SJW 6-9), containing hyperforin, adhyperforin and also hyperforin conjugates (as minor entities), showed significant antide-

pressant activity. This was evidenced from the significantly reduced immobility period of the SJW treated rats. Thus, our findings are consonant with the recent reports^{6,9} that hyperforin and adhyperforin contribute to the antidepressant activity of the SJW. Interestingly, the SJW extracts (SJW 1-5), which were completely devoid of free hyperforin and adhyperforin, and contained more polar hyperforin conjugates, exhibited like SJW extract (6-9) containing hyperforin and adhyperforin, significant antidepressant activity in the FST as well (Table 1). The extent of antidepressant activity of hyperforin conjugates and free hyperforins was of same order. In the case of former, the advantage of using the hyperforin conjugates lies in the stability compared to that of hyperforin and adhyperforin¹³.

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