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Antagonism of Antinociceptive Effect of Hydro-Ethanolic Extract of *Hypericum perforatum* Linn. By a non Selective Opioid Receptor Antagonist, Naloxone

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Abstract: Hydro-ethanolic crude extract of *Hypericum perforatum* Linn. family hypericaceae (St. John's Wort) aerial parts (Hp. Cr) was studied for its possible antinociceptive effect against acetic acid-induced abdominal constriction assay in mice. Hp. Cr (10-20 mg kg^{-1}), opium (10-30 mg kg^{-1}), morphine (0.75-3.0 mg kg^{-1}) and aspirin (50-100 mg kg^{-1}) showed dose-dependent antinociceptive effect. In animals treated with naloxone (0.5 mg kg^{-1}), the antinociceptive effect of Hp. Cr was significantly reduced similar to that of opium, while effect of aspirin remained unchanged. These results suggest that the antinociceptive effect of *Hypericum perforatum* may be mediated through activation of opioid receptors.

Key words: Antinociceptive effect, *Hypericum perforatum*, mice, opioid receptors

INTRODUCTION

Hypericum perforatum Linn., commonly known as St. John's Wort has been used as a medicinal herb for over 2000 years. Over the past two decades, its application as a plant extract for treating depression and other central nervous system disorders has led to rigorous scientific investigations (Di Carlo *et al*., 2001; Kasper, 2001). In addition, its oil is used in colitis and gastric ulcers (Weiss, 1988) and asthma (Linde *et al*., 2001; Gilani *et al*., 2005).

Phytochemical studies revealed the presence of multiple chemicals, such as flavonoids, hypericins, hyperforin, hyperin, quercitrin, rutin, saponins and tannins (Duke, 1992). The plant has been known to possess antibacterial (Negrash and Pochinok, 1972), antiviral (Meruelo *et al*., 1988), sedative (Girzu *et al*., 1997), analgesic and anti-inflammatory (Kumar *et al*., 2001) properties. Although the plant has been known to possess analgesic effect, possible mechanism of action remained unknown. In this investigation, we report the opioid receptor mediated antinociceptive effect of *Hypericum perforatum*.

MATERIALS AND METHODS

Plant material: The aerial parts (stems+leaves+flowers) of the plant were collected from the Galyat areas of district Abbottabad, Pakistan in May 2002. The plant identification was carried out by a taxonomist/ pharmacognosist, Dr. M. Ibrar, Associate Professor at the Department of Pharmacy, University of Peshawar. A voucher specimen (PUP 012529) has been submitted to the herbarium of Botany Department of the same University. A sample spacemen was also retained for our own record.

Preparation of crude extract: The plant materials were cleaned, shade dried and coarsely ground. The powdered material was soaked in 70% aqueous-ethanol for three days with occasional shaking. It was first filtered through a muslin cloth and then through a filter paper. This process was repeated twice more and the combined filtrate was evaporated on rotary evaporator under reduced pressure to a thick, semi-solid mass of dark brown color i.e., the crude extract (Hp.Cr), yielding approximately 19.6%. Hp.Cr was solubilized in normal saline (0.9% sodium chloride) for use *in vivo* experiments.

Chemicals: Opioids were obtained from Anti Narcotic Force, Peshawar Division through proper channel, while aspirin, naloxone hydrochloride and acetic acid were purchased from Oval Pharmaceutical Lahore and Sigma Chemicals Co., St Louis, MO, USA., respectively. All chemicals used were of the analytical grade available. All drugs were dissolved in normal saline on the day of experiment.

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Animals: Balb-C mice (20-25 g) bred in our own animal house and either sex were used in this study. Animals were housed at the Animal House of Pharmacy Department of the Peshawar University. Mice were kept in metal cages with sawdust bedding. Prior to experiment, food and water was withdrawn and the animals were transferred to a separate box and tested individually in a two compartment wooden box, each measuring 18×14×10 inches (length×width×height), so that two animals could be observed separately at the same time. Experiments were conducted during the light phase between 10.00 and 16.00 h strictly in accordance with the procedures laid down under the United Kingdom Animal Scientific Procedure Act (1986).

Preliminary phytochemical analysis: The crude extract was screened for the presence of anthraquinones, coumarins, flavonoids, saponins, sterols, tannins and terpenes by using the method of Wagner and Bladt (1994).

Antinociceptive test: Different groups of six mice each were randomly assigned to one of the treatment groups with appropriate controls. Each of the treatment groups received either *Hypericum perforatum* extract or opium or morphine or aspirin 45 min before 1% acetic acid challenge (10 mL kg^{-1}), while the control animals received same volume of normal saline. Following acetic acid administration, animals were placed in individual cages and the numbers of abdominal constrictions were counted for 20 min. The abdominal constriction was defined as a posture with the abdomen flattened, the back depressed and the hind limbs extended (Millan *et al*., 1994). Naloxone (0.5 mg kg^{-1}) was given to the animals 5 min before the acetic acid challenge. All the treatments were administered intra-peritoneally except naloxone, which was injected subcutaneously.

The data was presented as percent abdominal protection, calculated by the following formula:

$$\% \text{ Protection} = \frac{\text{Mean control group - Mean treated group}}{\text{Mean control group}} \times 100$$

RESULTS

Preliminary phytochemical analysis: Hp. Cr was found to contain flavonoids, saponins and tannins while the test for the presence of anthraquinones, coumarins, sterols and terpenes were negative.

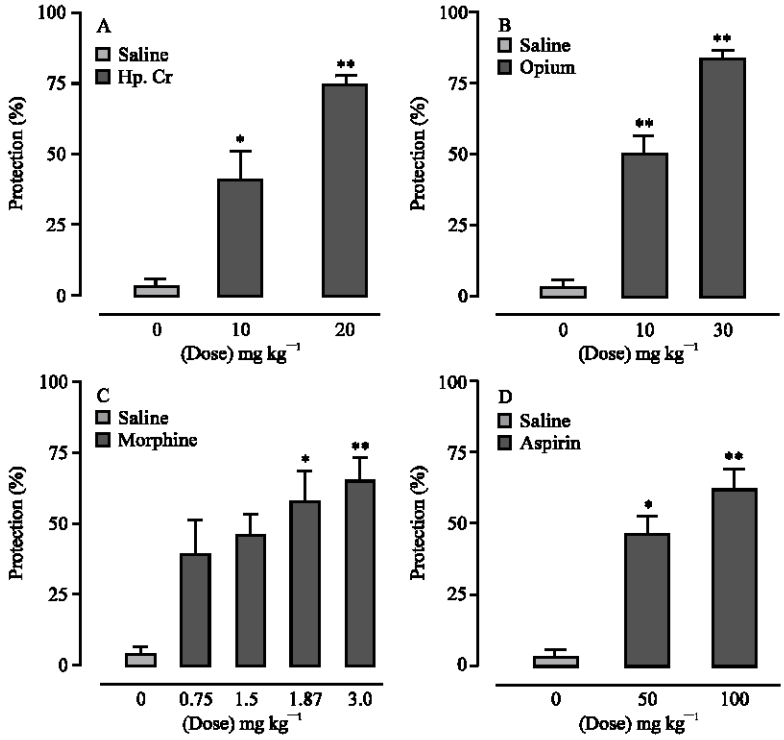

Fig. 1: Antinociceptive response of the (A) crude extract of *Hypericum perforatum* (Hp. Cr), (B) opium (C) morphine and (D) aspirin in the abdominal constriction assay. Each column represents the mean±SEM of 6 mice. *p<0.05, **p<0.01, ANOVA with Dunnett post hoc test represent significant difference between saline and drug treated groups

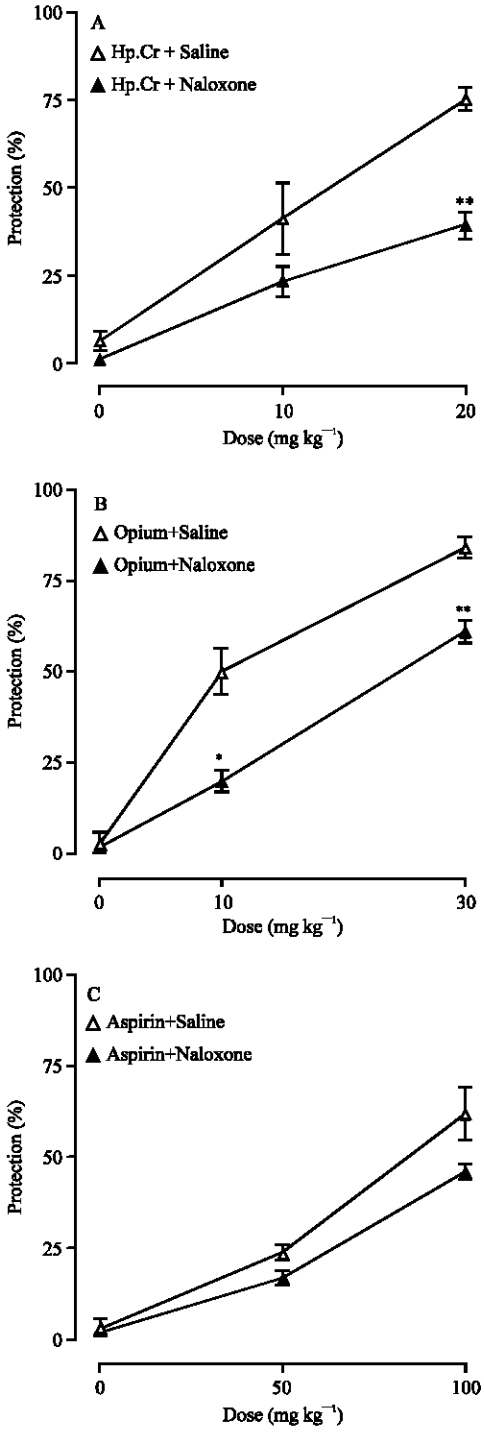

Fig. 2: Antinociceptive effect of various doses of (A) crude extract of *Hypericum perforatum* (Hp. Cr), (B) opium and (C) aspirin in the absence and presence of naloxone in the mice abdominal constriction assay. Each point represents the mean±SEM response of 6 mice. *p<0.05, **p<0.01, Student's t-test

Antinociceptive activity: Hp. Cr dose-dependently (10-20 mg kg^{-1}) exhibited analgesic effect against the acetic acid-induced abdominal constrictions. Statistical analysis showed a significant difference at doses of 10 mg kg^{-1} ($p<0.05$) and 20 mg kg^{-1} ($p<0.01$) as compared to saline treatment (Fig. 1A).

Opium (10-30 mg kg^{-1}) decreased the acetic acid-induced abdominal constrictions. The *antinociceptive* effect observed at each dose was significantly higher than the saline treated group ($p<0.01$) as shown in Fig. 1B.

Morphine dose-dependently (0.75-3.0 mg kg^{-1}) decreased acetic acid-induced abdominal constrictions. Statistical analysis showed significant *antinociceptive* activity at doses of 1.87 mg kg^{-1} ($p<0.05$) and 3 mg kg^{-1} ($p<0.01$) in comparison to saline treatment (Fig. 1C).

Aspirin at the doses of 50-100 mg kg^{-1} significantly decreased acetic acid-induced abdominal constrictions ($p<0.01$) in comparison to saline treated group (Fig. 1D).

The *antinociceptive* dose-response relationship of Hp. Cr+saline and Hp. Cr+naloxone demonstrated that naloxone significantly antagonized the *antinociceptive* effect of the 20 mg kg^{-1} dose of the plant extract ($p<0.01$) as shown in Fig. 2A.

Naloxone antagonized the *antinociceptive* effect of opium. Student t-test showed significant antagonism of 10 mg kg^{-1} ($p<0.05$) and 30 mg kg^{-1} ($p<0.01$) doses of the opium (Fig. 2B).

Although, naloxone caused a slight shift in the aspirin-induced analgesia dose-response curve to the left, but the difference was not statistically significant (Fig. 2C).

DISCUSSION

The findings of preliminary phytochemical analysis of the crude extracts of aerial parts of *Hypericum perforatum* are in accordance with the reported phytochemical constituents, such as flavonoids, saponins and tannins (Duke, 1992). The results of *in vivo* studies revealed that the plant extract produced dose-dependent antinociceptive effect inhibited by naloxone, a non-selective opioid receptor antagonist (Gray *et al*., 1998) similar to that of opium, an opioid receptor agonist. Due to short supply, the interaction of morphine with naloxone could not be observed. As expected, the antinociceptive effect produced by non-narcotic antinociceptive, aspirin remained unaltered any significantly by treatment of animals with naloxone. These observations hint that the antinociceptive effect induced by the *Hypericum perforatum* is likely to be mediated through opioid receptors activation. The plant

preparations are known to interact with the opioid receptors and inhibit the binding of naloxone to mu (μ) and kappa (κ) receptors (Simmen *et al*., 1998), which not necessarily mean antonociceptive effect as the receptor binding assay does not reflect whether or not receptors are activated. This is the first functional study showing its antinociceptive effect, mediated possibly through the activation of opioid receptors. The antinociceptive effect might be due to the presence of flavonoids, as they are reported to produce such effect (Vasilchenko, 1986).

Pain is regulated through two descending pathways from the brain. A pathway stretching from the locus coeruleus of the medulla to the dorsal horn (spinal cord) acts as an antinociceptive by releasing noradrenaliene, which inhibits the release of Substance P. The second pathway, which involves midbrain and medulla, releases 5-hydroxytryptamine (5-HT) in the dorsal horn. The later pathway is vital to the understanding of the actions of endogenous opioids, because the areas along the pathway are rich in opioid peptides and opioid receptors and 5-HT induces the release of opioid peptides (Sherwood, 1993).

There are several reports in the literature which suggested that *Hypericum perforatum* extract blocks synaptic reuptake of 5-HT, noradrenaline, dopamine, GABA and L-glutamate (Rommelspacher *et al*., 2001; Simmen *et al*., 1998; 2001), hence the role of the neurotansmitters (5-HT and/or noradrenaline) can not be totally ignored in the mediation of antinociceptive response induced by the plant extract. *Hypericum perforatum* exhibits calcium channel blocking activity (Gilani *et al*., 2005), which might also be contributing in its antinociceptive action up to some extent as calcium channel blockers are well known for the pain relieving effect (Krishtal *et al*., 2001).

In conclusion, the hydro-ethanolic crude extract of *Hypericum perforatum* possess naloxone-sensitive antinociceptive effect, mediated through opioid receptors, though additional mechanism cannot be ignored.

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