Pakistan Journal of Pharmaceutical Sciences
Vol. 17, No.2, July 2004, pp.13-19

ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACTS OF ST. JOHN'S WORT *(HYPERICUM PERFORATUM)* PREPARATION

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ABSTRACT

The analgesic effect of St. John's Wort plant preparation was evaluated using chemically induced pain models in adult albino mice. The intraperitoneal (i.p.) administration of 30-100 mg/kg of the preparation produced significant analgesic effect (75%) in acetic acid induced writhing and formalin licking tests. Based on its inhibitory effects on acetic acid induced writhes and both phases of the formalin test, the plant preparation seems to have central and peripheral analgesic properties. Its effect was found twice more potent than ibuprofen (100 mg/kg), a standard reference drug, in the acetic acid induced writhing test.

INTRODUCTION

Hypericum perforatum also known as St. John's Wort, Klamath weed and goat weed, is perennial which, grows in sunny areas and well drained sandy soil, commonly seen growing by the road side and along railroad beds (Alan and Miller, 1998). The plant is native to Europe and Asia. St. John's Wort has been known for centuries for its putative medicinal properties including antidepressant, anxiolytic antibiotic, antiviral and wound healing effects (Miller, 1998). The traditional indications of the plant include; rheumatism, hemorrhoids, neuralgia, snake bite sprains analgesia and depression (Snow, 1996 and Upton, 1997). Other species of Hypericum such as *Hypericum patulam* and *H. hookerianum* have also been used in folk medicine for spasmolytic, stimulant, hypotensive and antibacterial activities (Chopra and Nair, 1956). *H. perforatum* has recently got much popularity as antidepressant and is extensively used in U.S.A., Brazil, China and Germany for the treatment of depression (Linde *et al*., 1996; Volz, 1997 and Daudt *et al*., 2000). It has also been used as analgesic and anti-inflammatory agent in traditional practices. The phytochemical investigation of the plant revealed that it contains hypericin, pseudohypericin (Naphtodiantrones), hyperoside, rutin, quercitin (Flavonoids) and hyperforin. The weak analgesic effect of the crude ethanolic extracts from *Hypericum perforatum* in mouse writhing test has also been reported (Jakovljevic *et al*., 2000). Considering this property we evaluated recently marketed preparation of methanolic extracts of St. John's Wort (Deprisin®) in Pakistan, for peripheral and central analgesic activities in chemical (acetic acid and formalin) induced pain models.

EXPERIMENTAL

Herbal extracts and chemicals

The chemicals used in this study include; formalin 36% (Fluka chemie, Busch, Switzerland),

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acetic acid (Sigma Chemicals Company, St. Louis, USA) and ibuprofen (Brufen tab. Knoll Pharma, Karachi). The plant extracts of St. John's Wort (Deprisin), provided by Medics Laboratories, Karachi, Pakistan.

Animals

NMRI mice (22-26 gm) of either sex were obtained from animal house facility of H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan. Animals were housed 10 per cage under standard environmental condition with 12h light: dark cycle with free access to food and water.

Acute toxicity test

Male mice (25-30 gm) were injected i.p. with vehicle (10% DMSO) or different doses (100-1000 mg/kg of plant preparation. Animals were observed for 1-3 hours for any behavioral changes and neurotoxic effects of the plant preparation. Any mortality occurred within one week of treatment was noted and LD_{50} value (Dose causing 50% mortality in experimental animals) was determined.

Acetic acid induced writhing in mice

The writhing test was performed as described by Koster *et al*. (1959). Mice (25-30 gm) of either sex were administered intraperitoneally, using different doses of St. John's Wort preparation (30-100 mg/kg) or standard analgesic drug ibuprofen (100 mg/kg) 30 minutes prior to administration of acetic acid (0.9%). Animals were observed individually and the number of writhes (abdominal constriction) was counted for 20 minutes commencing 5 min. after injection of acetic acid. The number of writhes of treated group was compared to control group and represented as percent inhibition.

Formalin test

Mice were injected 20µl (.02 ml) of 1% formalin (in 0.9% saline) into sub-plantar space of the hind paw and observations were made as described by Hunksaar and Hole (1987). The duration of paw licking was determined between 0-5 min. (first phase) and 15-30 (second phase) after formalin injection. Animals were treated intraperitoneally either with ibuprofen (100 mg/kg) or different dose of St. John's Wort preparation (30-100 mg/kg) 30 minutes prior to formalin injection. The Control animals received only the vehicle used to dilute the substances (saline or 10% DMSO in saline, 10 ml/kg). The paw licking time of treated animals was compared to control group and represented as percent inhibition.

Statistical Analysis

The results of the experiment are represented as mean ± standard error of mean. The difference between control and test group was estimated by means of Student's t-test. The results were considered significant when $P<0.05$.

RESULTS

Acute toxicity

The intraperitoneal (i.p) administration of St. John's Wort preparation produced 30% mortality at 400 mg/kg which increased to about 60% mortality at the dose of 500 mg/kg. The mortality of the animals increased to 83% and 100% at doses of 800 mg/kg and 1 gm/kg respectively (Table-1).

Table-1
Acute toxicity of St. Jhon's Wort preparation

Treatment	Dose (mg/kg) i.p.	n	Percent mortality
St. John's Wort	100	6	0
	200	6	0
	400	6	30
	500	12	58
	800	6	83
	1000	12	100
DMSO (10%, 10 ml/kg)	-	20	0

Mice were administered (i.p) different doses of plants extracts. Animals were observed initially for 1-3 hours for acute symptoms of toxicity. Any mortality observed during one week later was registered.

Effect of acetic acid induced writhing

St. John's Wort significantly ($p<0.001$) reduced the acetic acid induced number of writhes (Control= 73 ± 3) in mice. The analgesic effect of the extract was maximum (88%) at dose of 100 mg/kg. At the same dose ibuprofen, a standard reference drug produced half of the response as exhibited by St. John's Wort preparation (Table-2). Thus indicating the superior potency and efficacy of the plant preparation, over standard analgesic compound.

Table-2
Effect of St. John's Wort on acetic acid induced writhing response in mice

Treatments	Dose (mg/kg) i.p.	No. of Writhes	% Inhibition
St. John's Wort	30	22 ± 3.8	70***
	70	20 ± 3	73***
	100	9 ± 2.2	88***
Ibuprofen	100	41.8 ± 6	43**
Control (10 ml/kg)	-	73 ± 3	0

Mice received different treatments (i.p.) 30 min. before the administration of 0.9% acetic acid (10 ml/kg). Data show the number of writhes (mean ± S.E.M) produced between 5-25 min. after admin. of acetic acid and the percent inhibition for each group (n=4-20).
Asterisks indicate significant difference at **P<0.01 and ***P<0.001.

Effect on formalin test

The extracts of St. John's Wort exerted significant dose dependent inhibitory effect on both phases of the formalin test (Figs. 1a and b). The maximum effect of extracts occurred at dose of 100 mg/kg, in this case the animal's total time spent in paw licking reduced from 49.7±3 to 12.3±2.6 (75% reduction) in first phase and from 82.5±5.5 to 14±4.4 (83% reduction) in second phase of the test. Whereas at the same dose ibuprofen, a standard analgesic compound, exhibited

greater effect on the second phase of the nociceptive responses in mice, producing about 18% and 72% reduction in paw licking responses of the animal in the first and second phase respectively (Table-3).

Table-3
Effect of St. John's Wort (Hypericum) on the formalin induced nociception in mice

Group	Dose (mg/kg)	Licking time (Sec)		% Inhibition	
		1st phase	2nd phase	1st phase	2nd phase
Hypericum	30	29.4 ± 5	28 ± 4	40*	66*
	50	23 ± 7	16.8 ± 4.4	54*	80**
	100	12.3 ± 2.6	14 ± 4.4	75**	83**
Ibuprofen	100	40.8 ± 5	23.2 ± 3.3	18	72**
Control	10ml/kg	49.7 ± 5	82.5 ± 5.5	-	-

Mice received (i.p.) different treatments 30 min before the subplantar injection of 20µ1 formalin. Data represent mean ± s.e.m. (n=6-12) of the licking time (Sec) in the first phase (0-5 min.) and second phase (15-30 min.). Asterisks indicate significant difference at *P<0.05 and **P<0.01.

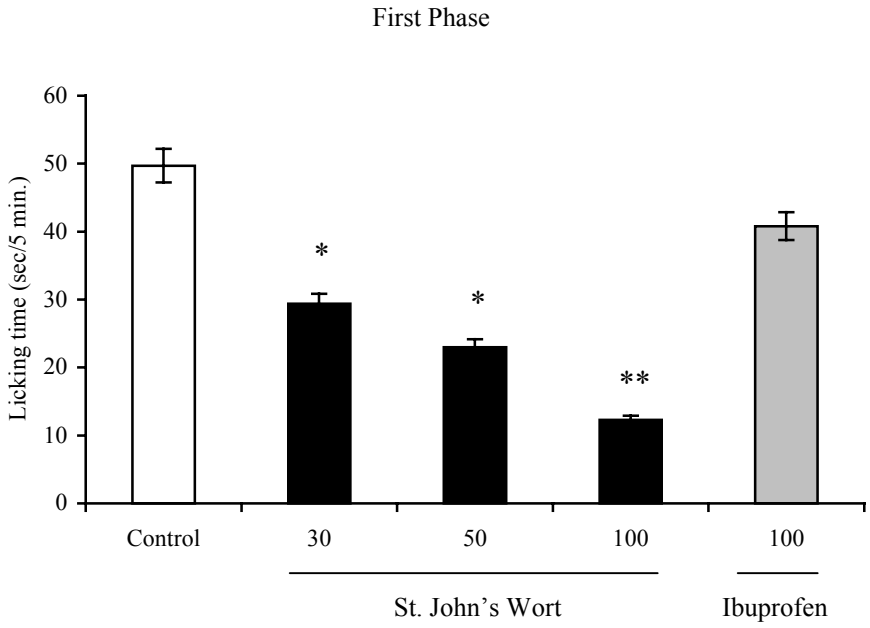

Fig. 1a: The antinococeptive effects of different doses of St. John's Wort extracts and ibuprofen on the first phase of formalin-induced pain response in mice. Mice received treatments 30 minutes prior to the administration of formalin (1%). Values represent mean ± standard error of mean of licking time in seconds. Asterisks indicate significant difference at *P<0.05 and **P<0.01.

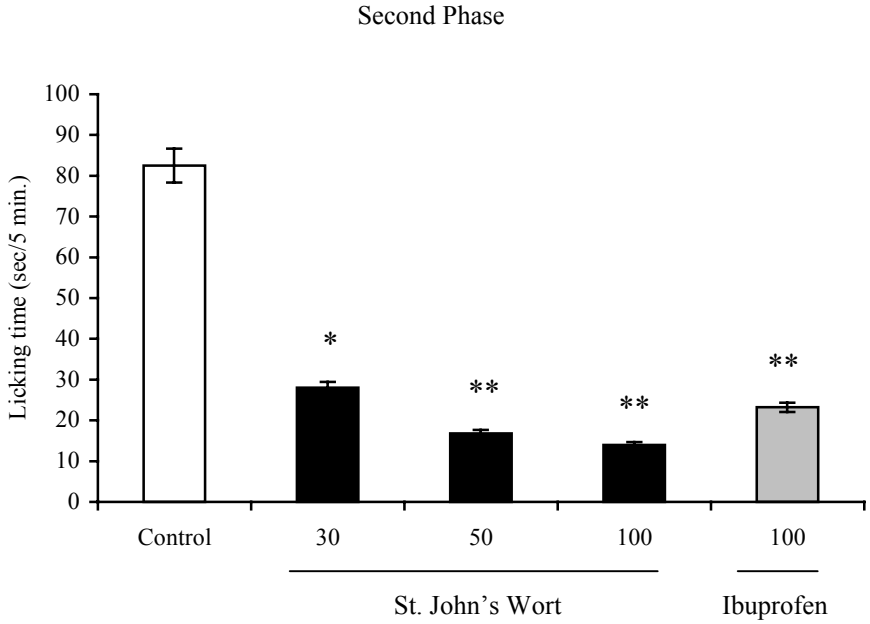

Fig. 1b: The antinococeptive effects of different doses of St. John's Wort extracts and ibuprofen on the second phase of formalin induced pain response in mice. Mice received treatments 30 minutes prior to the administration of formalin (1%). Values represent mean ± standard error of mean of licking time in seconds. Asterisks indicate significant difference at *P<0.05 and **P<0.01.

DISCUSSION

Acetic acid induced abdominal constriction in mice is widely used method for evaluation of peripheral analgesic effect (Gene *et al*., 1998). Various peripherally acting analgesic drugs such as ibuprofen, aspirin and indomethacin have been reported to inhibit acid induced writhing (Gene *et al*., 1998 and Okpo *et al*., 2001) in mice. St. John's Wort extracts (30-100 mg/kg) reduced the number of mouse abdominal contractions to a significant extent (P<0.001) and maximal inhibition 88% observed at 100 mg/kg of extracts. Ibuprofen, a standard reference compound also inhibited the writhing response in mice. It is established that nonsteriodal anti-inflammatory drugs (NSAIDs) such as ibuprofen inhibits the synthesis of prostaglandin; which increases the sensitivity of nociceptor and perception of pain (Curtis and Curtis, 1990). Therefore, it seems that the analgesic effect of St. John's Wort preparation might have occurred via prostaglandin synthesis inhibition.

The formalin model is very useful for elucidating mechanism of pain and analgesia (Tjolsen *et al*., 1992) and it was used to verify the central or peripheral mechanism of the analgesic effect of St. John's Wort preparation. It is reported that formalin induces persistent pain in mice paw involving two distinct phases, a neurogenic pain, which corresponds to the first phase and this, is followed by an inflammatory pain (second phase) this is accompanied by the release of inflammatory mediators (Hunskar *et al*., 1985 and Murray *et al*., 1988). Drugs that mainly act centrally such as narcotics (Morphine) inhibit both phases of formalin-induced pain while

peripherally acting drugs such as ibuprofen and aspirin inhibit only the second phase (Santos *et al*., 1998). Since St. John's Wort preparation exerted significant dose related inhibition of both the neurogenic (first phase) and inflammatory (second phase) of formalin induced licking test. This indicates that central and peripheral mechanism contribute in the overall antinociceptive effect of St. John's Wort preparation. Ibuprofen, a standard analgesic compound also produced significant reduction in pain response of the animal but the effect was predominant on the second phase thus further strengthening the peripheral analgesic action of the plant preparation in addition to central mechanism of analgesia. These findings were consistent with the early reports that NSAIDs including ibuprofen and aspirin are efficacious solely in the second phase of the formalin test.

Two different analgesic testing methods were employed in the current investigation with the objective to identifying possible peripheral and central effects of the St. John's Wort extracts. Using, both acetic acid induced writhing and formalin induced paw licking response in mice, it was observed that the plant extracts possessed analgesic effects against both models. The observations also indicated that the extracts have both peripheral (writh reduction) and central (licking suppression in the first phase of formalin) effects. From the present work alone, the exact mechanism(s) of the analgesic effects of the St. John's Wort in not readily apparent. It can however, be speculated that it may be linked to processes involved in the prevention of sensitization of nociceptors, down regulation of the sensitized nociceptors and/or blockade of the nociceptors at peripheral and/or central levels (Ferreira, 1990). Thus the present studies warrant further studies for the confirmation of mechanism involved in analgesic effect of the St. John's Wort extracts.

CONCLUSION

In summary, the results from the present study demonstrate that St. John's Wort preparation possesses profound analgesic activity. The herbal preparation exhibited inhibitory effect on acid induced abdominal contraction and licking responses in both phases (neurogenic and inflammatory) of formalin induced pain. Apparently analgesic effect of St. John's Wort preparation is probably mediated via inhibition of the prostaglandin synthesis whereas central inhibitory mechanisms cannot be ruled out. These results also validate the traditional use of the plant as analgesic and other conditions associated with pain such as trauma, burns, rheumatism and neuralgia.

Further studies are needed for the confirmation of its anti-inflammatory activity and the mechanism of analgesia.

ACKNOWLEDGEMENT

The authors are grateful to Medics Laboratories, Karachi, Pakistan for providing the authenticated samples of St. John's Extracts.

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