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Research Article

EVALUATION OF ANALGESIC ACTIVITIES OF METHANOLIC EXTRACT OF MEDICINAL PLANT *JUNIPERUS COMMUNIS* LINN

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ABSTRACT

The present study evaluates the analgesic activity of methanolic extract of *Juniperus communis* (Cupressaceae) in adult Albino mice. The extract at a dose of 100mg/kg and 200mg/kg were investigated for its analgesic activity with Acetylsalicylic acid (100mg/kg) as standard. Different *in vivo* tests like Acetic acid induced writhing, formalin test and tail flick tests were performed to establish this activity. The effect of the extract and Pethidine(10mg/kg) i.p on tail flick test was inhibited by Naloxone(2mg/kg) i.p. The extract showed a dose dependent and significant ($P<0.01$) inhibition of writhing response. In case of formalin test, the extract showed prominent dose dependent inhibition in the late phase comparable to Aspirin ($P<0.01$). The tail flick test revealed the central activity of the extract mainly after 30mins of administration. This central analgesic activity was confirmed by the blocking effect of naloxone. This has been observed that the plant showed significant activity as antinociceptive agent and was proved to act both peripherally and centrally. So this study provides the rationale behind the use of this plant as a potent and safe analgesic in the future.

Keywords: *Juniperus communis*, Analgesic, Writhing Test, Formalin Test, Tail Flick test, Pain.

INTRODUCTION

Plants with medicinal values have immense use as folk medicine, providing cure to a wide range of diseases. Our investigational plant *Juniperus communis* var. Saxatilis belongs to family Cupressaceae which is an evergreen dense shrub, having sharply pointed, scented leaves, with a bluish white surface. This is widely distributed across the northern hemisphere and in Himalayas from Kumaon at an altitude of 1600-4600m[1]. Besides being a good diuretic agent, this species is also used as carminative, in rheumatoid arthritis and as antifungal agent. The dried berries are known for their good flavoring property and for stimulating appetite[2]. Research work on the anti-inflammatory, anti-pyretic[3] and antimicrobial[4] activities has also been successfully conducted to evaluate its effectiveness. The anti-inflammatory effect followed by the study of antiulcer activity revealed that this plant being a potent analgesic acts as ulcer protective[5] agent.

Pain is an unpleasant feeling often associated with tissue damage. Tissue injury is the immediate cause of pain as it releases different chemical mediators like prostaglandins, bradykinins, substance P which act on the nociceptors causing this sensation. The nociceptive stimulus is transmitted to the CNS by small myelinated Aδ- fibres or by unmyelinated thin C-fibres[6]. It is often classified as chronic and acute. Acute pain may be characterized by its quick onset and short duration, lasting for hours. On the other hand, chronic pain is often associated with persistent pain over a large period of time[7, 8].

The purpose of this study was to evaluate the analgesic activity of the extracts obtained from *J. communis* leaves, using different acute and chronic pain models in mice.

MATERIALS AND METHODS

Plant material and extraction

Fresh leaves of the plant *Juniperus communis* was obtained form Sikkim. The herbarium sample was identified and authenticated by The Botanical Survey of India, Botanical Gardens, Howrah, West Bengal.

The leaves thus obtained were dried under shade and then powdered. The powdered material (250gm) was defatted using petroleum ether 40-60 by maceration which was further extracted using Methanol (MeOH) for 8-9hrs using Soxhlet Apparatus. The solvents were evaporated using Rotary Vacuum Evaporator (Hahn Vapor HS-2005V, Hahnshin Scientific Co, Korea) and then it was freeze dried using Freeze Drier (IIC Industrial Corp., Kolkata, India).

Chemicals and Drugs

The following chemicals and drugs were used: Methanol, Petroleum ether 40-60, Acetic acid (Merck Ltd, Mumbai, India), Formalin (Rankem Fine Chem Limited, New Delhi, India), Aspirin (Colsprin-100, Reckitt-Benckiser (India) Ltd, Mysore, India), Pethidine (Pethitroy-100, Troikaa Pharmaceutical, Gujrat, India), Naloxone HCl (Nalox, Samarth Life Science Pvt. Ltd, Mumbai, India).

Animals

Swiss Albino mice (20-25gm) obtained from Indian Institute of Chemical Biology (IICB), Kolkata were distributed into groups and housed in standard polypropylene cages under a 12hr light dark cycle at 24±1 ^{0}C with free access to standard dry pellet diet (Hindusthan Unilever Ltd, Kolkata, India) and water *ad libitum.* They were kept for 2 hrs prior to the experiment to get acclimatized with the laboratory environment. The animals were used only once and were sacrificed by cervical dislocation after performing each test. The procedures were maintained in accordance to the Departmental Animal Ethics Committee recommendations (Approval No: 147/1999/CPCSEA).

Acute toxicity studies

Swiss Albino Mice (20-25gm) of either sex (n=10) were used for the toxicity study done in accordance to the guidelines given by OECD[9, 10]. This study showed no mortality up to a dose of 5gm/kg p.o. for 24hrs period. Based on this result, the effective dose was fixed at 100mg/kg, 200mg/kg body weight for performing the analgesic study. No adverse effects of the extract were noted during this study.

Preliminary Phytochemical Screening

The Methanolic extract of *Juniperus communis* (MEJC) was screened by different chemical test for the identifying the basic chemical constituents present in the extract. The standard chemical tests for alkaloids, tannins, flavonoids, terpenoids and steroids were performed to get a preliminary idea of the chemical constituents[11, 12].

Analgesic Activity

The animals were divided in four groups having six in each group and the following invivo tests were performed.

Writhing Test

The method described earlier was used to evaluate the antinociceptive effect (Koster R, 1959)[13]. The animals were orally administered with MEJC (100mg/kg, 200mg/kg) and 30mins after

treatment, the animals were injected with 0.6% Acetic acid solution i.p. to induce the characteristic abdominal constriction and stretching called writhing. Normal saline (0.1ml/10g) and Aspirin (100mg/kg) was given to control and reference group respectively[14, 15,16]. The mice were observed and the number of writhes was noted for 0-20mins. The percentage inhibition was calculated by comparing with control group by the standard formula[17]:

Percentage inhibition = [(Control mean-Treated mean)/Control mean] x 100

Formalin Test

The method used was similar to that described previously (Hunskaar S, Hole K, 1987)[18]. The control group received normal saline (0.1ml/10g) and standard group Aspirin (100mg/kg). MEJC (100mg/kg & 200mg/kg) was orally administered and after 30 minutes of treatment, 20µl of 1% Formalin solution was injected subcutaneously in the right hind paw of the mice. The time spent in licking and biting of the affected paw was noted. The total paw licking response was measured as early phase (0-5mins) and late phase (15-20mins) after formalin injection[19, 20]. The percentage of pain inhibition was expressed by the given formula:

Percentage inhibition = [(Control mean-Treated mean)/Control mean] x 100

Tail flick test

The animals were tested for tail flick by Analgesiometer (Techno Electronics, Lucknow, India) as it was described earlier (Miranda et al, 2003)[21] . The basal time was noted at first for each animal. Current through the naked nichrome wire was set at 5 Amp over which 1-2cms from the tip of the tail was exposed to check out the response. The cut off time was set at 10 sec to prevent any tissue damage. The time (in second) required for the animal to withdraw (flick) its tail from the heat source was measured[22, 17]. The reaction time was noted at 15, 30, 60, 90 minutes after the animals were treated orally with MEJC (100mg/kg and 200mg/kg) and with intraperitoneal injection of Pethidine (10mg/kg). Normal saline (0.1ml/10gm) served as control group.

Antagonism of the antinociceptive effect of extract with Naloxone

To characterize pharmacologically the antinociceptive effect of MEJC extract, naloxone (2mg/kg) a strong µ- receptor blocker was used intraperitoneally. After 15mins of the naloxone injection, MEJC at doses 100mg/kg and 200mg/kg were used orally and the animals were tested for tail flick response at 15 mins, 30 mins, 60 mins, 90 mins[23, 24]. Pethidine (10mg/kg) i.p. served as standard drug.

Statistical analysis

Data were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett's test using Graphpad Prism-5. The unpaired *t*- test was used to compare between 2 groups.

RESULTS

Acetic acid induced writhing test

The methanolic extract used orally at different doses (100mg/kg and 200mg/kg) showed significant($P<0.01$) and dose dependent inhibition of pain responses (52.39% and 56.55% respectively) as compared to control group (Table 1). The number of writhes during the 15mins period showed by the control group was 52.16 ± 1.740(n=6). The percentage inhibition of pain by 100mg/kg Acetyl salicylic acid was found to be 74.11% as compared to the control.

Formalin induced pain

The formalin induced test produces a dose dependent inhibition of pain responses. The extract with dose 200mg/kg produced significant ($P<0.05$) inhibition (12.6%) in the early phase of the pain. In the late phase, the extract at both the doses produced significant ($P<0.01$) inhibition of pain (Table 2). Acetyl salicylic acid (100mg/kg) produced significant reduction in pain response when compared with control.

Tail flick test

The antinociceptive activity by oral administration of the extract showed dose dependent characteristic (Table 3). The extract at both the doses (100mg/kg and 200mg/kg) exhibited significant ($P<0.01$) inhibition. Pethidine (10mg/kg) i.p. was found to be highly effective. The effect was found to be very significant after 30mins and it showed sustained efficacy till 60mins which was found to be reduced after 90mins.

Effect of antinociceptive antagonist

The Opioid antagonist, Naloxone caused a significant inhibition in the activity of pethidine. The activity of the extract was also inhibited which signifies its central antinociceptive component. (Table 4).

Table 1: Effect of MEJC and Aspirin on writhing response in mice induced by acetic acid injection

Treatment Groups	Dose(mg/kg)	Number of writhing	Inhibition (%)
Control	10	52.16±1.740	--
Aspirin	100	13.50±0.991*	74.11
MEJC	100	24.83±2.007 *	52.39
	200	22.66±2.186*	56.55

The animals were administered these extract and drug orally. Values are represented as mean± SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. *$p<0.01$ was considered as significant.

Table 2: Effect of MEJC and Aspirin in mice by formalin test

Treatment Groups	Dose (mg/kg)	Licking of hind paw (sec)			
		(0-5mins)	Inhibition%	(15-20mins)	Inhibition%
Control	10	95.16±2.725	--	95.20±3.98	--
Aspirin	100	96.66±3.00	--	27.0±1.81**	71.63
MEJC	100	85.66±3.46	9.98	50.60±0.81**	46.84
	200	83.16±1.53*	12.6	47.20±1.35**	50.42

The animals were treated orally. Values are represented as mean± SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. *$p<0.05$, **$p<0.01$was considered as significant.

Table 3: Effect of MEJC in mice by tail flick test

Treatment Groups	Dose (mg/kg)	Reaction Time(sec)				
		Basal Time	15mins	30mins	60mins	90mins
Control	10	3.95 ±0.25	4.26±0.27	4.68±0.65	4.06±0.48	4.86±0.26
Pethidine	10	4.267±0.29	9.06±0.15*	9.10±0.15*	9.13±0.12*	9.08±0.13*
MEJC	100	4.95± 0.32	5.53±0.16*	5.86±0.13	6.08±0.13*	5.88±0.14*
MEJC	200	4.78± 0.57	7.15±0.33*	7.63±0.26*	7.66±0.31*	7.41±0.17*

The extract was administered orally and pethidine was administered intraperitoneally. Values are represented as mean± SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. * $p<0.01$was considered as significant.

Table 4: Effect of MEJC and Naloxone in mice by tail flick test

Treatment Groups	Dose (mg/kg)	Reaction Time(sec)				
		Basal Time	15mins	30mins	60mins	90mins
Naloxone	2	3.56 ± 0.32	3.18±0.41	4.68± 0.65	4.06± 0.48	3.33± 0.38
Naloxone + Pethidine	2 10	3.40± 0.16	3.93±0.38	3.85±0.15	3.75± 0.20	3.86± 0.20
Naloxone + MEJC	2 100	3.18± 0.33	3.71±0.24	4.10± 0.21	4.31± 0.30	4.36± 0.21
Naloxone + MEJC	2 200	3.21± 0.20	3.70±0.08	3.86± 0.23	4.13± 0.26	4.25± 0.35

Naloxone and Pethidine were administered intraperitoneally and the extract was administered orally. Values are represented as mean± SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test.

DISCUSSION

The results obtained from the study revealed that the methanolic extract of *J. communis* produced a significant dose dependent inhibition of pain response. The standard drugs however showed greater effect than the extract. The acetic acid induced writhing is basically a revelation of peripheral pain[25, 26]. The acetic acid induces release of different endogenous chemical pain mediators like prostaglandin E2 (PG) [27, 28], substance P, serotonin, histamine, bradykinin which further causes stimulation of nociceptive neurons[22, 28]. The extract caused inhibition of this pain, which is thought to be due to inhibition of release of prostaglandins, the mechanism quiet similar to Aspirin and other non steroidal anti inflammatory drug (NSAIDS).

MEJC also exhibited activity in the formalin test which can differentiate between the central and peripheral pain component. Formalin induced pain is biphasic having an early phase (0-5mins) and the other late phase (15-20mins) [29]. The early phase is due to activation of C-fiber neurons and the late phase occurs due to release of inflammatory mediators which are initiated by the C-fibers [30]. Centrally acting drugs show good response in both the phases, but the peripherally acting drugs act only on the late phase [29, 31, 32] which is due to inhibition of prostaglandin synthesis.

The tail flick study is however a confirmatory test towards the centrally acting component of the pain mechanism[33], believed to be spinally mediated reflex. MEJC significantly increases the pain threshold which is revealed in this study. The central action of MEJC and pethidine was proved as its effect was abolished by the blocking action of naloxone, an antagonist to the μ- receptor [34, 22]. So this provides enough evidence towards the rationale in its use as an antinociceptive agent.

CONCLUSION

The present study on the analgesic activity of extract of *Juniperus communis* was confirmed to have promising role in pain management. We would initiate further study on isolation of the active compounds and their exact mechanism of action responsible for antinociceptive activity.

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