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In vivo antinociceptive potential of ethanolic extract of leave, catkin and cone of *Alnus nitida* (Spach.) Endl.

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Abstract

Alnusnitida (Spach.) Endl. (Betulaceae) is used to cure pain and inflammation in traditional medicine. In present study acetic acid induced writhing assay in mice was used to validate its traditional use as pain reliever. The extracts at all doses showed highly significant ($p<0.001$) percent reduction in pain compared to control. The crude ethanolic extracts (70%) of leave showed highly significant ($p<0.001$) and highest pain reduction of 77.87±1.01% and 59.44±1.70% at 200 and 100mg/kg doses respectively, followed by 52.98±1.01% and 42.84±1.70% reduction by cone extract at 200 and 100mg/kg doses respectively. While, 36.84±1.66% and 35.01±1.18% reductions were shown by the leave and cone extracts respectively at dose of 50mg/kg each. The catkin extract showed 15.19±2.83%, 27.17±1.17% and 33.62±1.59% reduction in pain at doses 50, 100 and 200mg/kg respectively. Our results justified the folkloric use of the leave of *A. nitida* as pain reliever and also revealed the pain reducing potency of catkin and cone extracts.

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Introduction

Pain is unpleasant emotional as well as sensory experience linked with damage of tissues. Pain is protective in nature and acts as warning sign however; it results in distress and numerous side effects (Raquibul, *et al.*, 2010). Drugs used for curing pain or reducing its intensity are called Analgesics. The traditional analgesic drugs particularly the NSAIDs (nonsteroidal anti-inflammatory drugs) and opiates are derived from natural resources. However, several synthetic compounds developed with same mechanism of action have severe side effects like respiratory discomfort, vomiting, ulceration, drowsiness and gastrointestinal bleeding etc., (Laurence *et al* 1997, Mate., 2008). Development of novel synthetic compounds with analgesic effects is very expensive and may again have severe side effects. On the other hand, several effective medicines of plant origin are in use for years, without any serious side effects (Kumar *et al.*, 2010). Thus, exploration of novel biologically active compounds with analgesic effects is necessary from natural resources; mainly from medicinally important plants to minimize the side effects.

Many plant extracts are reported for in vivo analgesic activity including *Acacia hydaspica, Glaucium grandiflorum, Adhatodavasica and Boswellia serrate* (Morteza-Semnani *et al.*, 2004; Sharma *et al.*, 2010; and Mulla *et al.*, 2010 and Afsar *et al.*, 2015). Members of the Genus Alnus contains a variety of plant secondary metabolites i.e. flavonoids, phenols, tannins, diarylheptanoids, steroids and terpenoids (Sati *et al.*, 2011), and different parts of *Alnus* species have shown antidiarrheal, antipyretic, anti-inflammatory, antioxidant, antimicrobial, antitumor, anti-adipogenic, anti-atopic, insecticidal, antiviral and hepatoprotective activities (Ren *et al.,* 2017). *Alnus nitida* (Spach.) Endl. is found in temperate Himalayas and usually occurs at lower elevations (Ranges between 1,000m & 3,000m asl). This species is native to Pakistan, Afghanistan, India and Nepal (Shaw *et al.*, 2014). Catkins of this plant are expectorant, sedative and diuretic (Hazrat *et al.*, 2011). Poltice of *Alnusnitida* leaves is used for relieving body pain while leaves decoction is applied to cure sour feet (Ilyas *et al.*, 2013).

The traditional use of A. nitida bark to cure pain and inflammation is also experimentally verified in animal model (Sajjid *et al.* 2017) but no such work is done on the leave of *A. nitida* to verify its local use as pain reliever. The catkin and cone of *A. nitida* are also not investigated for analgesic activity. The aim of the present work was to evaluate the leave, catkin and cone extracts of this plant for analgesic potential.

Materials and methods

Plant parts were collected from Shamozai area of district Swat, Pakistan. Plant was properly pressed, dried, mounted on herbarium sheet, and identified by Ghulam Jilani, curator department of Botany university of Peshawar, Pakistan. Further confirmation of the plant species was made through Flora of Pakistan. The plant specimen was kept for reference in the herbarium of Department of Botany, University of Peshawar, Pakistan with voucher number (Bot.20151-PUP).

i) Preparation of plant extracts

Cleaned, washed and shade dried plant parts were powdered in electric grinder. 10g powder was two times extracted with 70% ethanol (200ml) at room (25±2°C) temperature. The extracts were evaporated in rotatory evaporator to dryness and then used for evaluation of analgesic potential.

ii) Chemicals

Aspirin was used as +ve control. Normal saline (sterile) was used as -ve control and all extract solutions were prepared in normal saline.

iii) Animals

BALB/c mice (either sex), purchased from National Institute of Health (NIH) were provided standard laboratory conditions (12 hours light /dark cycles and 25±2°C), standard food and water. One day before experiment food was withdrawn from the animals but free water access was provided to them. The experimental procedure used was approved by the ethical committee of University of Peshawar, Pakistan.

iv) Antinociceptive activity

Animals were divided into XI groups (six animals each group). Group I was administered normal saline

which served as -ve control. Aspirin (10mg/kg) was injected into group II and served as +ve control. Group III-XI was injected with doses 50, 100 and 200mg/kg i.p. of the leave, catkin and cone extract. After 30 minutes the animals were injected 1% acetic acid and 5 minutes later the number of writhes (abdominal constriction) were counted. Percent reduction in pain was calculated by the following formula (Muhammad *et al*., 2012).

% Reduction in pain=Wc-Wt / Wc× 100
Wc = No. of writhings in control.
Wt = No. of writhings in tested animals.

Statistical analysis

All experimental results were statistically analyzed by usingone-way ANOVA followed by Tukey's multiple comparison test. Differences at $p<0.05$ were considered significant. Graph pad prism version 6.4 was used for statistical analysis

Results

The effect of the ethanolic extracts of the leave, catkin and cone of *Alnus nitida* (Spach.) Endl. at doses of 50, 100 and 200mg/kg on the acetic induced abdominal constrictions in mice is represented in table 01. Leave extract at dose of 200mg/kg showed highest and highly significant reduction in abdominal constriction and showed 77.87±1.01% reduction in pain which was higher than Aspirin (73.10±0.93%), followed by 59.44±1.70 and 52.98±1.01% reduction in pain by Leave and cone extract at 100 and 200mg/kg dose respectively. Leave extract sowed 36.84±1.66% reduction in pain at 50mg/kg while, % pain reduction of cones at 100 and 50mg/kg were 42.84±1.70%, and 35.01±1.18% respectively. The % reduction in pain by catkin extract at doses 50, 100 and 200mg/kg were 15.19±2.83%, 27.17±1.17% and 33.62±1.59% respectively (Fig. 1). All treatments showed highly significant reduction in pain compared to saline (-ve control) (Fig. 2). The order of% reduction in pain was Leave> Cone> Catkin.

Table 01. Antinociceptive effect of different doses of ethanolic extracts of Leave, Catkin and Cone of *Alnus nitida* (Spach.) Endl. on acetic acid induced writhing in mice.

Treatments	Dose (mg/kg)	No. of writhings (mean±SEM)	% Reduction in pain (mean±SEM)
Saline (-ve control)		36.17±0.32	
Aspirin (+ve control)	10	9.667±0.33**	73.10±0.93
L	50	23.00±0.63**	36.84±1.66
	100	14.67±0.61**	59.44±1.70
	200	8.00±0.36**	77.87±1.01
SC	50	30.67±28.04**	15.19±2.83
	100	26.33±0.42**	27.17±1.17
	200	24.00±0.58**	33.62±1.59
PC	50	23.50±0.45**	35.01±1.18
	100	20.67±0.61**	42.84±1.70
	200	17.00±0.36**	52.98±1.01

Table.01. Values presented in table are mean±SEM (n=6), showing significance at**p<0.001 compared to saline (-ve control). L= Leave, SC=Staminate catkin, PC=Cone (Pistillate catkin with seeds).

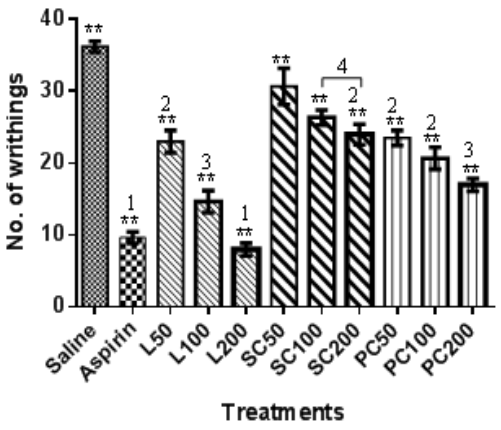

Fig. 1. Bars represent number of writhings (mean±SEM)(n=6). One-way ANOVA with Tukey's multiple comparision test was used to find significant difference between groups. Asteriks represent significance vs Saline (-ve control) at **P<0.001. Bars with different superscript number represent significant difference at p <0.05. L=Leave, SC= staminate catkin. PC=Cone (Pistillate catkin with seeds).

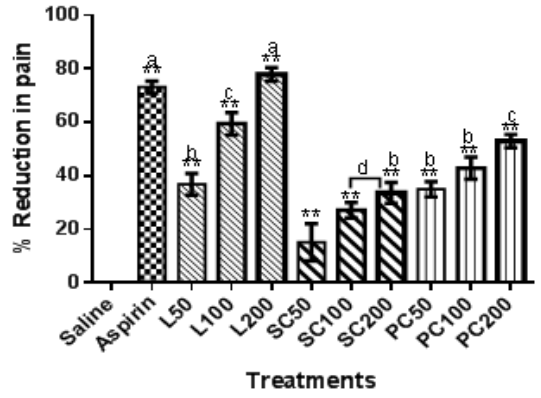

Fig. 2. Barsrepresent% reduction in pain (mean±SEM) (n=6). One-way ANOVA with Tukey's multiple comparision test was used to find significant difference between groups. Asteriks represent significance at **p<0.001 vs Saline (-ve control). Bars with different superscript letters have significant difference at P<0.05. L=Leave, SC= staminate catkin. PC=Cone (Pistillate catkin withseeds).

Discussion

All types of pain start from inflammations (Omoigui, 2007) during which many proinflammatory mediators are released such as cyclooxygenase-2 (COX-2), interferon (INF-γ), inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF) and interleukin 6 (IL-6), IL-12 (Chiu, 2012; Moncada, 1991).

Researchers throughout the world use primarily acetic acid induced writhing test to evaluate antinociceptive potential of natural compounds (Okokon & Nwafor, 2010; Ahmed *et al.*, 2011). Acetic acid trigger release of endogenous detrimental mediators like histamine, bradykinin substance P and serotonin (Mazid *et al.*, 2010; Dellai, 2012) which results in pain symbolized by abdominal muscle contraction, accompanied by forelimbs extension and elongation of the body. Peripheral nociceptive fibers are sensitive to both NSAIDs and narcotic analgesic drugs (Khan *et al.*, 2011; Khan *et al.*, 2009; Muhammad *et al.*, 2012). Inhibition of COX enzyme is necessary for reduction in writhings (Kumar *et al.*, 2015). Researchers have also shown that any agent that induces reduction in writhings will cause analgesic effect preferably by peripheral pain inhibitory mechanism through inhibition of prostaglandins synthesis (Ferdous *et al.*, 2008). In *Alnus* species frequently found phenolic compounds are recognized as inhibitors of prostaglandins synthesizing enzymes (Mohammad *et al.,* 2015).

In our present study the leave, catkin and cone extracts showed highly significant reduction in pain. It is suggested that these extracts may contain pharmacologically active constituents which can interfere with or inhibit the release or action of pain inducing mediators. Our results are similar to Sajjid *et al.*, (2017) who reported significantly higher antinociceptive effect of the *Alnus nitida* bark chloroform extract as compared to aspirin in acetic acid induced pain model.

Similar results were reported for other species of the genus Alnus that revealed the presence of active constituents in bark, leave, cones and seeds, which inhibited the release, synthesis or action of inflammatory mediators (O'Rourke *et al.*, 2005; Kuo, *et al.*, 2008; Choi *et al.,* 2011 *Sati et al.,* 2011; Sajjid *et al.*,2017). Our results justified the folkloric use of the plant leave for analgesic effect and explored the pain relieving potency of the catkin and cone extract.

Conclusion

The presents study on *A. nitida* revealed that not only leave but also the catkin and cone extract of *Alnus nitida* possess highly significant analgesic activity. Further studies should be carried out to isolate the active constituent(s) for the observed effects.

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