Methanolic Extract of Sikari Laharo (*Periploca calophylla*) has Analgesic Properties

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ABSTRACT

Pain is a manifestation of adverse pathophysiology during various clinical presentations. Periploca calophylla is a herbal plant used traditionally for multiple malaises in Nepal. However, studies on the analgesic property of this plant are scanty except empirical evidence reported by the traditional healers. This study aimed to determine the analgesic property of this plant, which is widely used for multiple conditions. Adult albino mice animal model was used for the in vivo assessment of the analgesic property. Three different doses of 80 % methanolic extract of the vine of P. calophylla (1.5 mg/kg, 2 mg/kg and 2.5 mg/kg), were administered intra-peritoneally to the test groups, once. The positive control group received indomethacin (25 mg/kg) and a negative control group received distilled water (3 ml/kg), via the same route of administration. The analgesic property was evaluated by the hot-plate test method, tail-flick test, acetic acid-induced writhing test and formalin-induced hind paw licking test. Extract of P. calophylla (1.5 mg/kg bd weight) significantly ($p<0.01$) inhibited pain sensation in all the pain reduction evaluation of animal models. The data obtained from this study indicate that the phyto-extract of P. calophylla possessed the analgesic property which rightly corroborated its traditional use.

Keywords: *Periploca calophylla, methanolic extract, analgesic, pain, animal*

INTRODUCTION

The International Association for Study of Pain defines pain, as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Tadiwos *et al*., 2017). The substance which alleviates painful sensation by elevating the threshold to external stimuli is defined as an analgesic (Khanum *et al*., 2019). Non-steroidal anti-inflammatory drugs (NSAIDs), opioid analgesics and co-analgesics such as corticosteroids, neuroleptics, benzodiazepines, local anaesthetics, capsaicin and clonidine are the commonly used analgesics (Sriraj *et al*., 2018). Regular use of NSAIDs causes adverse side effects such as inflammation of gastrointestinal tract, renal failure and liver toxicity (Khanum *et al*., 2019). Studies have shown that opiate analgesics cause physical dependence, tolerance and addiction (Hijazi *et al*., 2017). New analgesic compounds would be beneficial as most analgesics drugs available in the market possess multiple

limitations such as efficacy and undesired effects, which constraints their usefulness (Tadiwos *et al*., 2017). Phytoactive natural compounds from plants have been used in complementary and alternative medicine (CAM) to treat pain as well as other diseases that exacerbate pain since time immemorial. Traditional herbal medicine, the knowledge of which is handed down from generation to generation has been used for over centuries and has a strong potential for alternative medicines. Herbal medicines are a better choice due to their enhanced absorption rate, less toxicity and availability. It is noteworthy that most of the synthetic analgesic drugs such as aspirin and morphine are derived from plant products (Koech *et al*., 2017). Cragg *et al*., 1997 mention that approximately 70% of the analgesic compounds are synthetic drugs and most common ones like aspirin were also prototyped from the phytoactive chemicals. This emphasizes the importance and needs of studies on plants believed to have analgesic phytoactive natural compounds or used for such purposes (Nandani *et al*., 2018).

The usage of herbal medicine is gradually gaining recognition and momentum worldwide because these products have minimal or no side effects and are relatively cheaper than chemical compounds (Koech *et al*., 2017). *Periploca calophylla,* (Wight) Falconer of family *Asclepiadaceae* is a trailing shrub having lanceolate, long acuminate, leathery and shiny stalked leaves, 3.5-8.5 cm long and 0.3-1.7 cm wide. Flowers are pinkish in lax cymes and fruit is cylindrical. It flowers in April-May and fruits in November-January. It propagates by seeds and is distributed in shady places around the altitude of 1500-2000 meters above sea level throughout Nepal as well as northern India, Bhutan, Tibet and Central West China. Throughout the altitude range of 500-900 masl in Nepal, the *P. calophylla* (locally named as Sikari laharo) is one of the commonly used plants for treating multiple ailments (Bhuju 2005). In Nepal, the paste made from whole-plant of *P. calophylla* is used to treat bone fractures and muscle pains (Aryal *et al*. 2016). The objective of this study was to evaluate the analgesic properties of the stated plant. Despite its widespread usage, the evidence of proof is missing so this study would help evaluate the scientific merits of this empirical evidence.

MATERIALS AND METHODS

Collection and identification of the test plant

Vine along with bark of locally available Sikarilahara (literally translates as hunter vines in English) was collected from Ghalegaun of Lamjung district at 1800 meters above sea level in Nepal. A specimen was submitted to National Herbarium, Godawari, Lalitpur, Nepal for species identification. It was identified as *Periploca calophylla* by the taxonomists (Ref No 076/77, Dispatch No: 142, National Herbarium and Plant Laboratory, Godavari, Lalitpur, Nepal).

Methanol extraction

The collected plant parts were washed with clean water and air-dried in shade, powdered and soaked in 80 % methanol for 48 hours in a capped conical flask inside a rotatory incubator. The plant-methanol mixture was sieved by using a double layer of fine muslin cloth and Whatmann® filter paper no. 1. The filtrate was evaporated dry at 55^0C in a rotary evaporator to get the methanolic extract. The extract was used as a trial drug for the downstream applications.

Animal experiments

Healthy male Swiss albino mice, with an individual weight of about 25-30 grams were used in this study. These animals were maintained as recommended by Acharya *et al*., (2019) but with slight modifications. They were housed at 23-25^0 C at a relative humidity of 60-70% with 12 hours of alternating light and dark cycle in standard-sized plastic cages at the lab animal house, Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Chitwan, Nepal. They were fed *ad libitum* on a commercial pellet diet and sterile filtered water. All the animal experiments were approved by the Animal Ethical Committee, Agriculture and Forestry University, Rampur, Chitwan, Nepal.

Experimental design

The animal model experiments were carried out in a completely randomized design (CRD). Experimental animals were randomly divided into three different treatment groups with five animals in each group and one positive and negative control groups with the same number of mice. Five replications were made for each treatment regimen. The animal equivalent dose was calculated based on body surface area. Animal equivalent doses (mg/kg) for rat and mouse were calculated by multiplying human equivalent dose as recommended by the traditional healers (mg/kg) by factor 6.2 and 12.3 respectively (Nair and Jacob, 2016). The extract of *P. calophylla (Pc)* was tested at doses of 1.5, 2.0 and 2.5 mg/kg given intraperitoneally (I.P), respectively. Indomethacin at the rate of 25 mg/kg, I.P. was used as the positive control, while only distilled water (3 ml/kg, I.P.) was used for the negative control. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) used as a prescription drug to reduce fever, pain, stiffness, and swelling due to inflammation. It inhibits the production of prostaglandins, (which cause the inflammatory responses) by inhibiting cyclooxygenase.

Evaluation of analgesic activity

Hot-plate test

The 'hot-plate' (thermal) analgesic test method using an analgesiometer (with adjustable temperature), described in an earlier publication by Acharya *et al*. (2019) was implemented with slight modification. The hot-plate was maintained at 55±0.5^{0}C and individual mouse's response like the licking of the forepaws and inclination to jump out of the beaker was noted by placing the animal in a beaker on top of the hot plate. The animal's response as shown by jumping out of the beaker was taken as an indicator of pain. The time taken for each mouse to jump out of the beaker (in seconds, referred to as reaction time) was recorded. Each mouse was taken as its control. Pre-treatment reaction time was estimated twice at an hourly interval. The mean of these two determinations constituted the 'pre-treatment reaction time'. The mean reaction times of all the mice in the experiment were pooled to obtain the final, 'control' mean reaction time (Tc). Each test mice were then treated intra-peritoneal (I.P.) with distilled water, different doses of plant extract or indomethacin. The reaction time was again evaluated once, twenty minutes post-I/P injection. This reaction time was pooled for all the mice used in each treatment group, and the final 'test' mean reaction time value (Tt) for each treatment group was calculated to represent 'post-treatment reaction time' for each group of treated mice. This Tt was finally used to determine the percentage of thermal pain relief for protection using the formula,

Percentage protection against thermal pain = [Test mean, (Tt) – Control Mean, (Tc)] x 100/Control Mean, (Tc)

Tail-flick test

Mice were held firmly and the tail was extended out. The tail end (approx. 5 cm) was then dipped into a hot water bath maintained at 55±0.5^{0}C. The time (in seconds) taken for withdrawal of the tail out of the water was recorded as the reaction time. The readings were taken after one, two, three and four hours of treatment as suggested by Palanichamy and Nagarajan, 1990; using the formula,
Percentage analgesic activity = (Tt-Tc) x 100/Tc, where, Tc, the average of the two values is the initial reaction time and Tt is the reaction time after the treatment.

Acetic acid-induced writhing test

This method recommended by Acharya *et.al*. 2019 was used to access the visceral pain. Intraperitoneal injection of 0.6% acetic acid at a dose of 0.1 ml/10 gram body weight elicits the writhing response. Visible abdominal constriction and total extension of the hind limbs indicates a positive writhe response. Test drugs were administered one hour before acetic acid injection after which the number of positive writhes was recorded for 20 min. The percentage of analgesic activity was calculated as,
Percentage analgesic activity = (N-N1) x 100/N, where, N represents the average number of the writhes of the control group and N1 is the average number of the writhing of the test group.

Formalin induced hind-paw licking test

The formalin test has two distinctive phases, indicating different types of pain. In this study, the method proposed by Hunskaar and Hole (1987) and Hossain *et al.*, (2015) was used with some modification. Mice were injected intra-peritoneally with treatment doses of the plant extract and after thirty minutes, 20 µl of 1% formalin was injected intra-dermally into the dorsal surface of the right hind paw. Mice were kept in the individual chambers and observed. The time (herein referred to as licking time) spent in licking the injected paw was recorded. Mice were observed immediately after treatment for five minutes (early phase) and at 20 minutes after treatment (for 10 minutes; late phase). The inhibition of licking, in percentage, was calculated based on,
Mean vehicle-treated group time – Mean drug-treated group time X 100/mean vehicle-treated group time

Data analysis

Data collection, management and analysis was done using Microsoft Office Excel 2010 and M-STAT version 3C. Descriptive statistical analysis was done using one way ANOVA. Data obtained from experiments were expressed as mean standard deviation (±SD). $P<0.05$ were considered significant.

RESULTS

Hot plate test

Inhibition percentage of pain in treatment groups in comparison to the control group of the same hour was statistically significant (Table 1). Inhibition percentage of pain in the group treated with indomethacin was statistically non-significant with the group treated with *Pc* 1.5 mg/kg but statistically significant with the groups treated with other doses of *Pc* at all the hours. Similarly, inhibition percentage of pain in the group treated with *Pc* 1.5 mg/kg was statistically non-significant with the group treated with other doses of *Pc* at 1, 3 and 4 hours. But, at 2 hours, inhibition percentage of the pain of the group treated with *Pc* 2.5 mg/kg was statistically non-significant with the group treated with other doses of *Pc*.

Table 1. Effect of *P. calophylla (Pc)* extract on inhibition percentage of pain in hot plate test

Treatments	Inhibition percentage			
	1 (hr)	2 (hr)	3 (hr)	4 (hr)
Pc (1.5 mg/kg)	$85.39^{ab} \pm 5.87$	$93.09^{ab} \pm 3.10$	$86.05^{ab} \pm 2.45$	$85.96^{ab} \pm 3.37$
Pc (2.0 mg/kg)	$83.38^{b} \pm 6.21$	$89.14^{b} \pm 6.21$	$84.16^{b} \pm 3.96$	$83.87^{b} \pm 1.48$
Pc (2.5 mg/kg)	$80.35^{b} \pm 3.14$	$84.20^{c} \pm 3.14$	$82.03^{b} \pm 4.50$	$82.02^{b} \pm 3.19$
Indomethacin (25 mg/kg)	$90.93^{a} \pm 6.33$	$97.21^{a} \pm 6.33$	$91.02^{a} \pm 5.89$	$90.12^{a} \pm 8.24$
Control	$0.00^{c} \pm 0.00$	$0.00^{d} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$
LSD	6.542	4.445	5.163	5.648
CV (%)	7.29	4.63	5.70	6.26
F- Value	296.981**	738.235**	484.513**	401.270**

** $p<0.01$ compared with control at the same hour. Treatment means superscripted with the same letter of the same column are not statistically significant.

Tail flick test

Pain latency increased with the intraperitoneal application of *Pc* across the entire treatment groups. The pain latency period at all hours was statistically significant in the groups treated with different doses of *Pc* and indomethacin, at 0 hours of the same group. Similarly, the latency period of the groups treated with various doses of plant extract at 4 hours and indomethacin was significantly different from other hours of the same treatment. Regarding the control group, the latency period at all hours was non-significant to each other (Table 2).

Table 2. Effect of *P. calophylla* (*Pc*) extract on pain induced by tail immersion test

Time (hr)	Latency period (in seconds)				
	Pc (1.5 mg/kg)	*Pc* (2.0 mg/kg)	*Pc* (2.5 mg/kg)	Indomethacin (10 mg/kg)	Control
0	$2.56^{c} \pm 0.29$	$2.78^{d} \pm 0.13$	$2.74^{c} \pm 0.26$	$2.80^{c} \pm 0.07$	$2.68^{a} \pm 0.39$
1	$5.10^{a} \pm 0.16$	$4.88^{b} \pm 0.13$	$4.84^{a} \pm 0.11$	$5.34^{a} \pm 0.05$	$2.70^{a} \pm 0.32$
2	$5.26^{a} \pm 0.21$	$5.08^{a} \pm 0.13$	$4.98^{a} \pm 0.16$	$5.26^{a} \pm 0.11$	$2.74^{a} \pm 0.15$
3	$5.08^{a} \pm 0.15$	$4.96^{ab} \pm 0.18$	$4.84^{a} \pm 0.11$	$5.14^{a} \pm 0.11$	$2.72^{a} \pm 0.18$

4	$4.760^{b} \pm 0.11$	$4.68^{c} \pm 0.13$	$4.54^{b} \pm 0.11$	$4.80^{b} \pm 0.16$	$2.58^{a} \pm 0.33$
LSD	0.2538	0.1866	0.2168	0.2044	0.3801
CV (%)	4.24	3.18	3.73	3.30	10.72
F- Value	171.091**	227.743**	163.157**	238.008**	0.234**

** $p<0.01$ compared to the same group at 0 hours. Treatment means with the same superscript letter within the column are not statistically significant.

Maximum inhibition percentage of pain was recorded for the group treated with indomethacin, at all the hours followed by the group treated with *Pc* 1.5 mg/kg. However, the difference in inhibition percentage of pain in the groups treated with indomethacin and *Pc* 1.5 mg/kg was not statistically significant from two hours onwards (Table 3). The inhibition percentage of pain treated with all doses of *Pc* and indomethacin were statistically significant at all hours compared with the controls. At one hour, the inhibition percentage of the pain of the group treated with indomethacin was significantly different from the pain of groups treated with all the doses of *Pc.* Inhibition percentage of the pain of the group treated with *Pc* 1.5 mg/kg was also significantly different from the groups treated with its remaining doses. The inhibition percentage of the pain of the group treated with indomethacin was significantly different with the inhibition percentage of the pain of the group treated with *Pc* 2.5 mg/kg at two, three and four hours.

Table 3. Effect of *P. calophylla (Pc)* extract on inhibition percentage of pain in the tail-flick test

	Inhibition percentage of pain			
Treatments	1 (hr)	2 (hr)	3 (hr)	4 (hr)
Pc (1.50 mg/kg)	$88.89^{b} \pm 5.86$	$91.97^{a} \pm 7.57$	$86.76^{a} \pm 5.45$	$84.50^{a} \pm 4.42$
Pc (2.00 mg/kg)	$80.74^{c} \pm 4.83$	$85.40^{ab} \pm 4.76$	$82.35^{ab} \pm 6.68$	$81.40^{ab} \pm 5.05$
Pc (2.50 mg/kg)	$79.26^{c} \pm 4.22$	$81.75^{b} \pm 6.00$	$77.94^{b} \pm 4.19$	$75.97^{b} \pm 4.42$
Indomethacin (25 mg/kg)	$97.78^{a} \pm 2.03$	$91.97^{a} \pm 4.16$	$88.97^{a} \pm 4.19$	$86.05^{a} \pm 6.13$
Control	$0.00^{d} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$
LSD	5.263	6.809	7.092	5.963
CV (%)	5.75	7.35	6.96	6.89
F- Value	489.190**	292.820**	326.395**	332.544**

** $p<0.01$ compared with control at the same hour. Treatment means followed by the same superscript in the same column are not statistically significant.

Acetic acid-induced writhing test

Acetic acid-induced writhing was significantly inhibited in all treatment groups as compared to control. Inhibition percentage of the writhing of the group treated with Indomethacin was statistically non-significant only in comparison with the group treated with *Pc* 1.5 mg/kg. Inhibition percentage of the pain of the group treated with *Pc* 1.5 mg/kg was statistically non-significant with the group treated with *Pc* 2 mg/kg but significant with 2.5 mg/kg. Inhibition percentage of pain was observed at maximum in the group treated with Indomethacin followed by *Pc* 1.5 mg/kg (Table 4).

Table 4. Effect of *P. calophylla (Pc)* extract on the acetic acid-induced writhing

Treatments	No. of writhing	Inhibition percentage
Pc (1.5 mg/kg)	$7.2^{bc} \pm 1.30$	$84.75^{ab} \pm 2.76$
Pc (2.0 mg/kg)	$8.2^{bc} \pm 0.84$	$82.63^{bc} \pm 1.77$
Pc (2.5 mg/kg)	$9.4^{b} \pm 1.14$	$80.08^{c} \pm 2.42$
Indomethacin (25 mg/kg)	$6.0^{c} \pm 1.58$	$87.29^{a} \pm 3.35$
Control	$47.2^{a} \pm 2.95$	$0.00^{d} \pm 0.00$
LSD	2.277	3.112
CV (%)	11.07	3.52
F- Value	2.980**	1264.597**

** $p<0.01$ compared with control at the same hour. Treatment means followed by the same superscript in the same column are not statically significant.

Formalin induced hind paw licking test

Inhibition percentage of pain in treatment groups as compared to the control group of the same phase was statistically significant. Inhibition percentage of pain in the groups treated with *Pc* 1.5 mg/kg and 2 mg/kg were statistically non-significant with other groups in both phases. Whereas, inhibition percentage of pain in the groups treated with *Pc* 2.5 mg/kg and indomethacin were statistically non-significant. In both phases, maximum inhibition of pain was observed in the group treated with *Pc* 1.5 mg/kg (Table 5).

Table 5. Effect of *P. calophylla* (*Pc*) extract on inhibition percentage of pain in formalin-induced hind paw licking test

	Time of licking (in seconds)		Inhibition percentage	
Treatments	First phase	Second phase	First phase	Second phase
Pc (1.5 mg/kg)	$12.80^{d} \pm 2.59$	$21.20^{d} \pm 2.59$	$84.50^{a} \pm 3.13$	$92.13^{a} \pm 0.96$
Pc (2.0 mg/kg)	$21.00^{c} \pm 5.52$	$37.80^{c} \pm 6.14$	$74.58^{b} \pm 6.69$	$85.97^{b} \pm 2.28$
Pc (2.5 mg/kg)	$21.90^{c} \pm 3.01$	$53.60^{bc} \pm 3.51$	$69.01^{bc} \pm 6.44$	$80.10^{c} \pm 1.30$
Indomethacin (25 mg/kg)	$30.40^{b} \pm 5.46$	$58.20^{b} \pm 7.29$	$63.19^{c} \pm 6.61$	$78.40^{c} \pm 2.71$
Control	$82.60^{a} \pm 8.20$	$269.4^{a} \pm 25.62$	$0.00^{d} \pm 0.00$	$0.00^{d} \pm 0.00$
LSD	7.064	16.33	6.974	2.297
CV (%)	15.87	14.06	9.07	2.59
F- Value	136.891**	342.271**	200.825**	2383.794**

The values are shown as mean ± SD

** $p<0.01$ compared with control at the same hour. Treatment means followed by the same superscript in the same column are not statistically significant.

DISCUSSION

P. calophylla (locally called Sikari laharo) has been used extensively since the historical times in Nepal for treating different ailments in animals and humans by traditional healers and village elders. The extracts of this plant are used as a concoction in joint pains and aches (Sapkota, 2009). Acetic acid-induced writhing reflex is a model for the assessment of visceral pain and commonly

used in the screening of peripherally acting analgesic drugs (Acharya *et al*, 2019). The constriction response of the abdomen involves the release of arachidonic acid (AA) from tissue phospholipids via cyclooxygenase (COX), elevated levels of prostaglandin E2 (PGE2) and prostaglandin F2-alpha (PGF2α) and lipo-oxygenase (LOX) products in the peritoneal fluids. Prostaglandins (PGs) activate and sensitize the peripheral chemo-sensitive nociceptors, causing inflammatory pain and abdominal constriction (Tadiwos *et al*., 2017). The acetic acid administered intraperitoneally provokes abdominal contractions, total-body movements, twisting of the dorso-abdominal muscles, and suppression of motor activity and coordination in mice (Bars *et al*., 2001). Acetic acid elevates the release of endogenous stimulus via mediators to activate the nociceptive neurons. It is sensitive to NSAIDs, narcotics as well as some centrally acting drugs (Reichert *et al*., 2001). The release of cytokines, such as TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells contribute to the nociceptive activity of acetic acid (Ribeiro *et al*. 2000). The administration of indomethacin, that suppresses the COX-1 and COX-2, also inhibits the algogenic action of acetic acid by scavenging the inflammatory mediators of pain in peripheral tissues (Tadiwos *et al*., 2017).

In this study, the test drug, at all doses attenuated the writhing response of mice inflicted by intraperitoneal acetic acid administration. Inhibition percentage of the test extract at 1.5 mg/kg was statistically non-significant as compared to the indomethacin. Inhibition of local peritoneal receptors, AA pathways, involving COX and/or LOX could have mediated the observed analgesic effect. Although different agents ameliorate the pain due to acetic acid, substances that inhibit the writhing response preferably work by inhibiting the synthesis of PG (Muhammad *et al*., 2012). Thus, it could be inferred that our plant's extract possibly inhibited the pain through the peripheral action, involving inhibition of PG synthesis. Some flavonoids have been shown to produce analgesic action by interfering with the PG synthesis (Tadiwos *et al*., 2017). The results of our test indicate that the anti-nociceptive action of *Pc* in the acetic acid-induced writhing test could be due to the inhibition of the release of TNF-α, interleukin-1β and interleukin-8 and the analgesic effect is probably mediated via its peripheral effect. The central analgesic action of the plant extract could also play an additional role in preventing the pain in this model because it has been reported that analgesics with central actions diminish the writhing response (Tadiwos *et al*., 2017).

The test drug was evaluated by the hot plate test to access its anti-nociceptive effect via the supra-spinal mechanism. Heat-induced nociceptive pain in the paw of mice is a highly sensitive model for evaluating the therapeutic agents for the analgesic property (Acharya *et al*. 2019). During the inflammation process arachidonic acid (AA) pathway significantly contributes towards the generation of inflammatory response such as pain. AA generated from membrane phospholipids by the action of phospholipases, is, later on, degraded by cyclooxygenase (COX) to potent inflammatory mediators such as the prostaglandins. This hot-plate test is used in elucidating the centrally mediated analgesic effects of the test drug. Generally, heat induces low pain reaction threshold (latency time) that is elevated by the use of analgesic agent (Jan and Khan, 2016). In our study, the test extract (at all doses) elevated the mean reaction time and the pain was inhibited significantly. While comparing the pain inhibition percentage of test drug at the dose rate of 1.5mg/kg with indomethacin, it was statically non-significant.

In the tail-flick test that assesses the response to a thermal stimulus, increased reaction time is regarded to be an important criterion for evaluating central analgesic property (Rujjanawate *et al*.,

2003). This test can differentiate between central and peripheral analgesics (Asongalem *et al*., 2004). Increase in the reaction time in this experiment indicates the central analgesic property of the test extract. Inhibition of thermal pain induced by hot plate test by the plant extract is characteristic of the central analgesic effect while peripheral analgesia is known to be inactive on this kind of painful stimuli (Yin *et al*., 2003).

The formalin-induced hind paw licking test is useful for assessing analgesic drugs as well as in elucidating the mechanism of analgesia (Zeashan *et al*., 2009). The inhibition of both early and late phases is due to the action on central analgesic effect while the inhibition of the late phase (only) is due to peripheral analgesic effect just like the action of non-steroidal anti-inflammatory drugs (Hossain *et al*., 2015). In our experiments, the effect oftest extract on the late phase of the formalin test indicates that the result is due to its peripheral action when compared with indomethacin activity. The anti-nociceptive effect of test agent could be attributed to inhibition of prostaglandin release and other mediators and also by modulation of pain transmission via μ and Κ receptors (Perianayagama *et al*, 2004).

In all the analgesic test models, the lower dose (1.5 mg/kg) was found to be more effective than higher doses. This possibly shows that 1.5 mg/kg dose of the extract is an optimum dose for inducing both central and peripheral analgesia. Lack of increase in potency at higher doses of the extracts suggests that at the dose beyond 1.5 mg/kg is not necessarily effective for the required analgesic property. This phenomenon is explained by the hypothesis that some of the active constituent(s) of the plant extract at high concentrations may exhibit pro-inflammatory (Rezazadeh *et al*., 2005). On the other hand, some explain that the plant extract has multiple compounds acting differently via anti- or pro-inflammatory responses (Tadiwos *et al*., 2017).

CONCLUSION

The analgesic effect of *P. calophylla* has been evaluated using a mouse model. This study validates the traditional use of plant parts as an analgesic agent and provided scientific evidence of its effectiveness. The pain was significantly inhibited by 86.76±5.45, 82.35±6.68 and 77.94±4.19 percentage at the dose level 1.5, 2.0 and 2.5 mg/kg of *Pc* respectively in the tail-flick test. The analgesic property of *Pc* in superficial pain was also supported by the similar results of significant inhibition of pain in hot plate test. Inhibition percentage of pain (84.75±2.76) at the dose level of 1.5mg/kg in acetic acid writhing test at 1.5 mg/kg suggests the centrally acting mechanism of pain inhibition, which was also supported by formalin-induced paw licking test. However, the active phytochemicals present in the extract needs to be purified and further tested for their exact physiological mechanism of action at the cellular level along with the sub-acute and chronic toxicity as well as the LD_{50} and ED_{50} of the plant.

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Conflict of Interests

All the authors declare that there are no conflicts of interests that might affect the findings of this study.

Authors Contribution

JA and NP conceptualized, designed, executed the experiments, collected & analyzed the data and drafted the manuscript, ST located the plants and provided the experiments guidelines, MKS and SS provided the mentorship on animal experiments, JA and NP designed the experiments, and NP finalized the draft. All authors have read and agreed upon the submission of this manuscript.

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