

## Prophylactic effects of asiaticoside-based standardized extract of *Centella asiatica* (L.) Urban leaves on experimental migraine: Involvement of 5HT1A/1B receptors

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**[ABSTRACT]** The present study aimed at evaluation of prophylactic efficacy and possible mechanisms of asiaticoside (AS) based standardized extract of *Centella asiatica* (L.) Urban leaves (INDCA) in animal models of migraine. The effects of oral and intranasal (i.n.) pretreatment of INDCA (acute and 7-days subacute) were evaluated against nitroglycerine (NTG, 10 mg·kg<sup>-1</sup>, i.p.) and bradykinin (BK, 10 µg, intra-arterial) induced hyperalgesia in rats. Tail flick latencies (from 0 to 240 min) post-NTG treatment and the number of vocalizations post-BK treatment were recorded as a measure of hyperalgesia. Separate groups of rats for negative (Normal) and positive (sumatriptan, 42 mg·kg<sup>-1</sup>, s.c.) controls were included. The interaction of INDCA with selective 5-HT1A, 5-HT1B, and 5-HT1D receptor antagonists (NAN-190, Isamoltane hemifumarate, and BRL-15572 respectively) against NTG-induced hyperalgesia was also evaluated. Acute and sub-acute pre-treatment of INDCA [10 and 30 mg·kg<sup>-1</sup> (oral) and 100 µg/rat (i.n.)] showed significant anti-nociception activity, and reversal of the NTG-induced hyperalgesia and brain 5-HT concentration decline. Oral pre-treatment with INDCA (30 mg·kg<sup>-1</sup>, 7 d) showed significant reduction in the number of vocalization. The anti-nociceptive effects of INDCA were blocked by 5-HT1A and 5-HT1B but not 5-HT1D receptor antagonists. In conclusion, INDCA demonstrated promising anti-nociceptive effects in animal models of migraine, probably through 5-HT1A/1B mediated action.

**[KEY WORDS]** *Centella asiatica* (L.) Urban; Experimental migraine; Anti-nociception; Serotonin, 5HT1A and 5HT1B receptors

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### Introduction

Migraine is a disabling headache characterized by intermittent attacks with a number of physiological and emotional stressors associated with pain, tiredness, nausea, vomiting, photophobia, or phonophobia [1]. Migraine is multi-mechanistic disease and associated with a wide array of symptoms in-

cluding nausea, vomiting, and increased sensitivity to light and sounds. Migraine attacks can be unilateral throbbing headache, worsened by movements and routine daily activities, and lasting from 4 to 72 h. The underlying mechanisms of migraine interspersed with acute symptoms of attack appear to be increasingly complex. An important aspect of migraine heterogeneity is comorbidity with other neurological, cardiovascular, and psychiatric diseases [2]. Migraine associated aura is associated with peripheral sensitization is an acute, chemical-induced form of functional plasticity, which converts high-threshold nociceptors into low-threshold sensory neurons. This form of sensitization occurs in migraine attacks where the nerve terminals (meningeal nociceptors) of the neurons of the trigeminal ganglion are soaked with the "inflammatory" soup (prostaglandin E<sub>2</sub>, bradykinin, serotonin,

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and cytokines) along with the vasculature of the cerebral dura mater [3].

Pharmacological treatment for migraine falls into two main categories: symptomatic (acute) and prophylactic [4]. Despite the pharmacological advances for the treatment of migraine, patients are forced to endure symptoms until the medications take effect and so experience a poor quality of life despite an aggressive regimen of pharmacotherapy [5]. Current acute symptomatic therapy for migraine includes NSAIDs, and migraine specific therapies such as triptans and ergot derivatives play an important role in aborting the migraine attacks [6]. Such symptomatic treatments can be quick and effective for pain relief and disappearance of associated symptoms (nausea, photophobia, and phonophobia). However, inability to provide complete full pain relief and contraindications such as cardiovascular diseases [7] limit the use of symptomatic treatments. Therefore, prophylactic (preventive) treatment of migraine is recommended for patients with frequent and/or disabling attacks. Most of the drugs recently used in migraine prophylaxis have been identified by serendipity and they have been originally approved for other indications [8]. Most notable amongst them are antidepressants [9], beta-adrenergic blockers, calcium channel blockers, and antiepileptic drugs [8]. However, the use of these agents is limited by inconclusive evidences on targets of migraine prophylaxis (reduction in frequency and intensity of attacks and to decrease disability related to chronic headache) and dose limiting side-effects.

Stress is reported to impact the migraine sufferer at many levels [10]. The stress related psychological factors such as mood and anxiety are known causes of poor response to migraine prophylaxis therapies [11]. These stress related disorders are often highlighted as trigger factors of migraine attacks, but may also appear as a psychological reaction to recurrent and severe migraine attacks [2, 12].

Serotonin (5-HT), a neurotransmitter, has been implicated in the pathophysiology of the migraine syndrome. Methysergide (nonspecific antagonist of 5-HT receptors) is reported to be effective for migraine prophylaxis [13]. Among a variety of identified serotonin receptors, 5-HT<sub>1A</sub> attracts particular attention due to its central role in the regulation of 5-HT-ergic neurotransmission and the data on its involvement in the mechanisms of stress response, aggressive behavior, anxiety, and depression [14-15].

One of the promising natural products having evidence of anxiolytic and mood elevating activity in clinic is the extract of leaves of *Centella asiatica* (Linn.) Urban (CAE) [16]. *Centella asiatica* (Linn) Urban has a long history of traditional use as a medicinal herb in traditional Chinese medicine (TCM), Ayurveda, and African medicine systems. Total triterpenes of *Centella asiatica* L. leaves (TTCA) are shown to have promising medical properties including antidepressant effects [17] in forced swim test (FST), an acute model of depression in laboratory animals. The Asiaticoside (AS) is a

major pentacyclic triterpenoid saponin component of CAE and has been shown to be bioactive component of CAE and TTCA. Amongst the most promising pharmacological activities of AS are antidepressant [18] and anxiolytic [19-20] activities. These effects are believed to be mediated through the hypothalamic-pituitary-adrenocortical (HPA) axis, relieving stress and increasing the contents of monoaminergic neurotransmitters [21]. Recently, we have reported promising antidepressant activity of AS-based standardized extract of *Centella asiatica* L. leaves (INDCA) against mood related parameters in chronic stress-medicated depression, in olfactory bulbectomy (OBX) rats [22] and suicidal behaviour related traits [23]. As the management of stress, anxiety and depression is an important component in the migraine prophylaxis, INDCA can be explored and developed as a promising agent. However, such studies have not been attempted. Therefore, the present study was undertaken with an objective to evaluate prophylactic efficacy INDCA in nitroglycerine (NTG) and bradykinin (BK)-induced hyperalgesia in rats. Both models are well-validated and produce acute hyperalgesia condition similar to migraine. Considering the important role of serotonergic (5-HT) receptor system in migraine prophylaxis, we also attempted to explore role of 5-HT<sub>1</sub> serotonergic receptor subtypes (5-HT<sub>1A</sub>, 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub>) in the mechanism of INDCA.

## Material and Methods

### Animals

Wistar rats (weighing 200–250 g) of either sex were purchased from National Toxicology Centre (NTC), Pune, India. The animals were housed at a temperature of 25 ± 1 °C and relative humidity of 45%–55% under 12 : 12 light: dark cycle. The animals had free access to feed pellets (Chakan oil mills Ltd., Sangli, Maharashtra, India) and tap water *ad libitum*. The experimental protocol (No: CPCSEA/51/2010) was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India. All observations were recorded between 8: 00 and 15: 00 and each rat was used only once. To avoid subjective bias, the observer was not aware of the administered treatment. Each experimental group consisted of 6 rats unless otherwise stated. The rats were transported from the animal house to the testing area in their own cages and were allowed to adapt to the new environment before testing.

### Drugs and chemicals

NTG (New Medicon Lab Pvt Ltd., Bangalore, India), sumatriptan succinate (ST) (Sun Pharmaceuticals Ltd, Mumbai, India) and pentazocine (Ranbaxy Laboratories Ltd., Ahmadabad, India) ampules were purchased from local suppliers. Bradykinin (BK) and NAN-190 (selective 5-HT<sub>1A</sub> receptor antagonist) were purchased from Sigma Aldrich, St Louis, MO, USA. Isamoltane hemifumarate (selective 5-HT<sub>1B</sub> receptor antagonist) and BRL-15572 (selective 5-HT<sub>1D</sub> receptor antagonist) were purchased from Tocris Cookson Ltd.,

Bristol, UK. The NTG solution was prepared in saline with the help of propylene glycol as reported earlier [24-25] and was injected by intraperitoneal (i.p.) route at a dose of 10 mg·mL<sup>-1</sup>. The test composition, INDCA was prepared from authenticated samples of *Centella asiatica* L. leaves per reported procedure [22] and provided by Indus Biotech Private Limited, (Pune, India). The authentication of plant sample was done by Dr A. S. Upadhye, a qualified taxonomist at Agharkar Research Institute, Pune (deposited voucher specimen-WP-80). The solution of INDCA was prepared in distilled water immediately before use and administered once daily orally in volume of 10 mL·kg<sup>-1</sup> to rats during the experiments.

#### *Acute oral toxicity (AOT) study*

The AOT testing was performed according to the Organisation for Economic Co-operation and Development (OECD guideline 425 [26]) on 10 healthy rats (6–8 weeks old, weighing 125–150 g, 5 males and 5 females) for each of the vehicle control and INDCA treated groups. The females were nulliparous and non-pregnant. The rats were administered with single oral dose of either vehicle (distilled water) or INDCA (2 000 mg·kg<sup>-1</sup>). The rats were observed for any clinical signs of toxicity, morbidity or mortality (for next 14-days) and gross pathology (after sacrifice on d 14).

#### *Grouping and treatment schedule*

The rats were divided into groups of 6 rats each and received drug treatment as follows: Group I was the “normal” control which received vehicle (distilled water). Group II was the NTG control (NTG) and received vehicle (distilled water) for 7-days before NTG (10 mg·kg<sup>-1</sup>, i.p.). Group III received single sub-cutaneous (s.c.) dose of positive control, sumatriptan, at a dose of 42 mg·kg<sup>-1</sup>, 15 min before NTG treatment. Groups IV and V received INDCA at oral doses of 10 and 30 mg·kg<sup>-1</sup>, respectively, 15 min before NTG treatment (acute schedule). Groups VI and VII received daily oral dose INDCA at doses of 10 and 30 mg·kg<sup>-1</sup>, respectively, 7 days before NTG treatment (sub-acute schedule). Groups VIII and IX received intranasal (i.n.) administration of INDCA (110 µg/rat) as acute (15 min) and sub-acute (once daily for 7 d) before NTG administration, respectively.

Additional treatment groups included groups of 6 rats each to study the interaction of 5HT1A receptor antagonist (NAN-190, 1 mg·kg<sup>-1</sup>, i.p.), 5HT1B receptor antagonist (Isamoltane hemifumarate, 4 mg·kg<sup>-1</sup>, i.v.), and 5HT1D receptor antagonist (BRL-15572, 0.3 mg·kg<sup>-1</sup>, i.v.) with NTG and/or INDCA. Those groups included the antagonist alone groups (X, XIII, and XVI), the antagonist+NTG groups (XI, XIV, and XVII) and the groups with subacute (7 d) pretreatment of INDCA (30 mg·kg<sup>-1</sup>, p.o.) + acute treatment with (antagonist + NTG) (XII, XV, and XVIII), respectively.

#### *NTG-induced hyperalgesia*

The effects of treatments on NTG-induced hyperalgesia were evaluated using thermal nociceptive stimulation as previously reported procedure [27]. The role of various serotoner-

gic receptors subtypes (5-HT1A, 1B and 1D) was assessed using previously reported procedure [28-29]. The effects of INDCA in presence and absence of specific antagonists of 5-HT1A, 1B, and 1D receptors, namely NAN-190, Isamoltane hemifumarate, and BRL-15572, respectively were measured using the tail flick latencies against NTG.

On the last day of the treatment period, the baseline tail-flick latency was recorded using a tail flick analgesiometer (UGO Basile, Italy). At 15 min after acute treatment or the last dose of sub-acute treatment, the rats were administered with NTG (10 mg·kg<sup>-1</sup>, i.p.) and the tail flick latencies were recorded at 30, 60, 90, 120, and 240 min post NTG treatment. The anti-nociception was quantified using area under curve (AUC) obtained from the graphs of tail flick latencies (s) versus time (min) based on trapezoid rules [30]. After the tail flick experiment, all the rats were sacrificed, brain tissues were immediately isolated, and tissue homogenates were prepared. Brain serotonin concentration (5-HT) was analyzed using a spectrofluorometer (Shimadzu, Japan) at excitation and emission wavelengths of 385 nm and 485 nm, respectively as reported previously [31].

#### *Bradykinin (BK) induced vocalization*

The effects of treatments on BK-induced vocalizations were evaluated using a previously reported procedure [28] in separate groups of rats. Wistar rats were divided into following groups (*n* = 6): Group I was the sham control which received vehicle (distilled water); Group II was the BK control group and received vehicle (distilled water); Groups III and IV received positive control, ST (42 mg·kg<sup>-1</sup>, s.c.) and INDCA (30 mg·kg<sup>-1</sup>, p.o.) respectively.

All the rats were anaesthetized with urethane (125 mg·kg<sup>-1</sup>, i.p.) and surgically prepared before BK administration. The common carotid artery and a femoral vein were exposed and cannulated with indwelling polyethylene catheters; three needle electrodes were subcutaneously implanted on the chest and connected to an 8-channel recorder POWER LAB (AD instruments, New Delhi, India) for the recording of the ECG. A microphone was placed at the approximate distance of 3 cm over the snout of the rat, and connected to the POWERLAB for the recording of vocalization. BK was bolus injected into the arterial catheter at the dose of 10 µg, in the volume of 10 µL. The choice of dose of BK was based on preliminary experiments when the chosen dose induced vocalization and tachypnea in 100% of rats. Vocalization and ECG were recorded for 5 min after BK administration. Sumatriptan and INDCA (30 mg·kg<sup>-1</sup>, p.o.) were administered 10 and 30 min before BK injection, respectively.

#### *Statistical analysis*

The data were expressed as mean ± SEM. The data obtained from NTG experiments (AUC) and brain 5-HT concentration levels were analyzed by one way ANOVA, followed by Dunnett's multiple comparison test using software Prism<sup>®</sup> version 5 (GraphPad Inc, La Jolla, CA, USA). The data obtained from BK-induced vocalization experiments

were analyzed by Kruskal-Wallis ANOVA, followed by Mann-Whitney U test using SPSS v20.0 (SPSS Inc, Chicago, IL, USA).

## Results

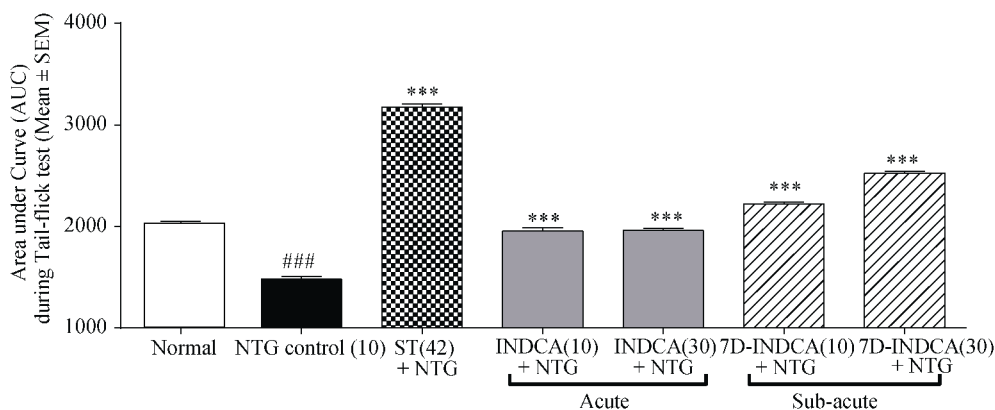
### Acute oral toxicity (AOT)

During the AOT study, all the rats (male and female, vehicle and INDCA treated) survived for a period of 14 days. Therefore, acute lethal oral dose (LD<sub>50</sub>) of INDCA was found to be greater than 2 000 mg·kg<sup>-1</sup>. Gross pathological examination did not reveal any abnormalities attributable to the INDCA treatment.

### Effects of INDCA on NTG induced hyperalgesia

The effects of acute treatment of INDCA (10 and 30 mg·kg<sup>-1</sup>, p.o.) and sumatriptan (42 mg·kg<sup>-1</sup>, s.c.) with and

without the NTG treatment during tail-flick test are presented as Fig. 1. The normal rats showed a mean AUC of 2034.67. The rats from NTG control showed an AUC of 1480.83 (22.27% reduction), suggesting hyperalgesia. The AUC value of the group with acute pre-treatment with ST (42 mg·kg<sup>-1</sup>, s.c.) with acute dose of NTG (10 mg·kg<sup>-1</sup>) was significantly greater than that of the NTG control ( $P < 0.001$ ). Similarly, the AUC values of the rats with acute or subacute (7 d) pre-treatment of INDCA (10 or 30 mg·kg<sup>-1</sup>, oral) were significantly greater than that of the NTG control ( $P < 0.001$ ). The seven-day pre-treatment of INDCA (10 or 30 mg·kg<sup>-1</sup>, oral) before NTG (7D-INDCA + NTG groups) showed not only the preventive effects on the NTG-induced hyperalgesia, but also a 24% higher AUC than that of the normal rats.



**Fig. 1** Effects of oral pre-treatment of INDCA on the NTG-induced pain latencies during tail-flick testing. It was analyzed by One-way ANOVA, followed by Dunnett's multiple comparison test, ###  $P < 0.001$  vs the normal control, \*\*\*  $P < 0.001$  vs the NTG control ( $n = 6$ , mean  $\pm$  SEM)

The effects of sub-acute (7 d) pretreatment of INDCA (30 mg·kg<sup>-1</sup>, p.o.) in presence and absence of specific 5-HT receptor antagonist(s) during NTG-induced hyperalgesia are presented as mean AUC  $\pm$  SEM in Table 1. The acute pretreatment of NAN-190, isamoltane, and BRL-15572 did not cause significant changes in AUC, compared with the untreated (normal) rats. Similarly, NAN-190, isamoltane, and BRL-15572 pretreatment before NTG (NAN-190 + NTG, Isamoltane + NTG and BRL-15572 + NTG groups) did not show significant changes in AUC, compared with the NTG control rats.

When sub-acute (7 d) pretreatments of INDCA (30 mg/kg, oral) with either NAN-190 or isamoltane were administered, the AUC did not show significant change, compared with that of the NAN-190 + NTG or isamoltane + NTG groups, respectively. However, sub-acute pretreatment of INDCA (30 mg·kg<sup>-1</sup>) with BRL-15572 + NTG schedule showed significant increase in AUC (38.04%), compared with that of the NTG control rats.

### Effects of INDCA on brain 5-HT concentrations during NTG-induced hyperalgesia

The effects of sub-acute (7 d) pretreatment of INDCA (10

and 30 mg·kg<sup>-1</sup>, p.o.) and acute treatment of sumatriptan (42 mg·kg<sup>-1</sup>, s.c.) on brain serotonin concentrations during NTG-induced hyperalgesia are presented in Fig. 2. The mean 5-HT concentration in the normal rats was 901.73 ng·g<sup>-1</sup> of brain tissue). The NTG control group's 5-HT concentration (449.64 ng·g<sup>-1</sup>) was significantly lower than that of the normal controls by 49.86% ( $P < 0.001$ ). The brain 5-HT concentration levels of the acute pretreatment of ST and sub-acute pretreatment of INDCA (10 and 30 mg·kg<sup>-1</sup>) groups (805.98, 603.37, and 786.25 ng·g<sup>-1</sup>, respectively) were increased by 79.25%, 34.18%, and 74.86%, respectively, compared to that of the NTG control rats (all  $P < 0.001$ ).

### Effects of intranasal INDCA treatment on NTG-induced hyperalgesia

As shown in Fig. 3, acute and sub-acute (7 d) administration of INDCA by intranasal route showed significant reversal of NTG-induced reduction in AUC in tail-flick latency ( $P < 0.001$ ). The mean AUC in the NTG control group was 1 480, whereas the same figure in the INDCA (i.n.) +NTG group was increased to 2197 (48.44%) and 2490 (68.2%) with acute and sub-acute pre-treatment with INDCA, respectively.

**Table 1** Effects of pre-treatments of INDCA on NTG induced pain latencies during tail-flick in presence and absence of selective 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptor antagonists (NAN-190, Isamoltane hemifumarate, and BRL-15572, respectively) ( $n = 6$ , mean  $\pm$  SEM)

Treatment	Dose (mg·kg <sup>-1</sup> )	AUC in Tail Flick
Normal	-	2034.67 $\pm$ 14.04
NTG control	10	1480.83 $\pm$ 27.68 <sup>###</sup>
7D-INDCA + NTG	30 + 10	2525.33 $\pm$ 17.54 <sup>***</sup>
5-HT <sub>1A</sub> receptor interaction		
NAN-190	1	1732.50 $\pm$ 76.18 <sup>ns</sup>
NAN-190 + NTG	1 + 30	1399.00 $\pm$ 61.73 <sup>ns1</sup>
7D-INDCA + NAN-190 + NTG	30 + 1 + 10	1296.50 $\pm$ 111.60 <sup>ns1</sup>
5-HT <sub>1B</sub> receptor interaction		
Isamoltane	4	1906.00 $\pm$ 75.16 <sup>ns</sup>
Isamoltane + NTG	4 + 10	1547.83 $\pm$ 124.60 <sup>ns1</sup>
7D-INDCA + Isamoltane + NTG	30 + 4 + 10	1427.33 $\pm$ 119.50 <sup>ns1</sup>
5-HT <sub>1D</sub> receptor interaction		
BRL-15572	0.3	1795.83 $\pm$ 74.25 <sup>ns</sup>
BRL-15572 + NTG	0.3 + 10	1311.00 $\pm$ 55.96 <sup>ns1</sup>
7D-INDCA + BRL-15572 + NTG	30 + 0.3 + 10	2044.17 $\pm$ 72.72 <sup>***</sup>

It was analyzed by One-way ANOVA, followed by Dunnett's multiple comparison test, ns: not significant, <sup>###</sup> $P < 0.001$  vs Normal, ns1: not significant, <sup>\*\*\*</sup> $P < 0.001$  vs NTG control

#### Effects of INDCA treatment on BK-induced vocalization

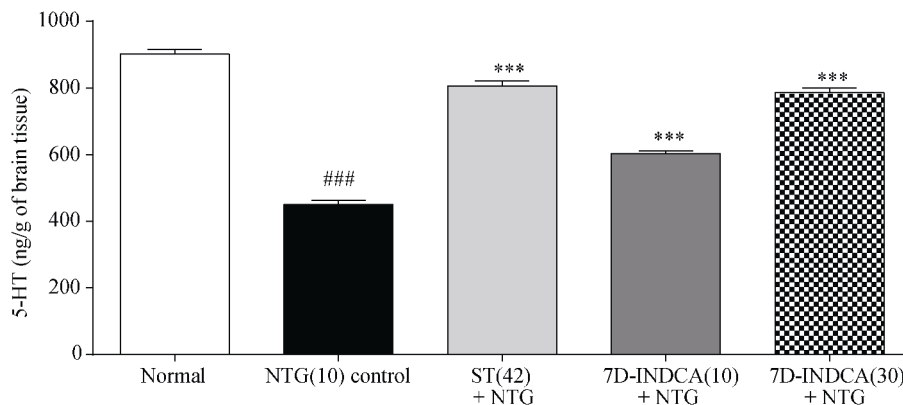
The effects of INDCA treatments on the BK-induced vocalization are presented as median number of vocalization and range in Fig. 4. Intraarterial administration of BK induced vocalizations (median = 183) in the rats. Acute treatment of ST (42 mg·kg<sup>-1</sup>, s.c.) before BK resulted in a significant reduction in the median number of vocalization (3.5, 98.8%

decrease), compared with the BK control group ( $P < 0.01$ ). Subacute (7 d) pretreatment of INDCA (30 mg·kg<sup>-1</sup>, p.o.) also showed a significant reduction in the median number of vocalization (median = 72, 60.65% reduction), compared with the BK control group ( $P < 0.01$ ).

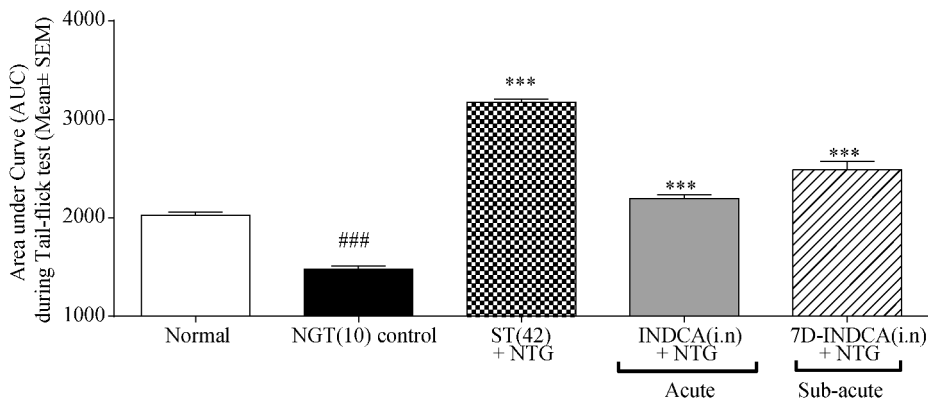
## Discussion

The goals of migraine prophylaxis are to reduce the frequency, painfulness, and/or duration of migraines and to increase the effectiveness of abortive therapy [32]. However, monotherapy often does not offer benefits in sizable proportion of migraineurs. Therefore, combination treatments with nutritional supplements, lifestyle alterations, and avoidance of migraine triggers or surgery may be needed. Combination treatment is also used to avoid medication overuse headache (MOH) or rebound headache. MOH is a common problem among chronic migraineur cases [33-34]. Therefore, the search for better drugs for migraine prophylaxis is still ongoing.

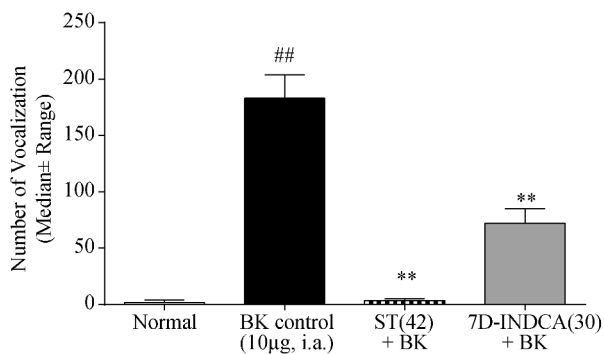
In the present study, asiaticoside (AS)-based standardized extract of *Centella asiatica*, INDCA, was studied for its anti-migraine activity using NTG and BK induced experimental migraine models in rats. NTG is a marketed anti-anginal agent and induces migraine like symptoms such as headache attacks [27, 35] which can be attenuated with anti-migraine drugs [28, 36]. NTG is a highly lipophilic organic nitrate that easily penetrates blood-brain barrier (BBB) and releases nitric oxide (NO) by enzymatic and non-enzymatic reactions [35]. Therefore, NTG-induced pain is considered a good experimental animal model to evaluate anti-migraine medications. In the present study, INDCA showed significant reversal of NTG-induced hyperalgesia after acute and subacute treatments. The similar effects were seen with positive control, ST, which was in accordance with the previous result [28].



**Fig. 2** Effects of subacute oral administration of INDCA on brain serotonin (5-HT) concentration during NTG-induced pain. It was analyzed by One-way ANOVA followed by Dunnett's multiple comparison test, <sup>###</sup> $P < 0.001$  vs the normal control, <sup>\*\*\*</sup> $P < 0.001$  vs the NTG control ( $n = 6$ , mean  $\pm$  SEM)



**Fig. 3** Effects of intranasal (i.n.) treatment of INDCA on NTG-induced pain latency during tail-flick. It was analyzed by One-way ANOVA, followed by Dunnett’s multiple comparison test, ###*P* < 0.001 vs the normal control, \*\*\**P* < 0.001 vs the NTG control (*n* = 6, mean ± SEM)



**Fig. 4** Effects of oral INDCA treatment on BK- induced vocalization. It was analyzed by non-parametric Kruskal-Wallis ANOVA, followed by Mann-Whitney U test, ###*P* < 0.001 vs the normal control, \*\**P* < 0.001 vs the NTG control (*n* = 6, mean ± SEM)

As nausea and vomiting are the major symptoms in severe migraine patients, intranasal administration is a preferable route in migraine management [37]. Therefore, we evaluated intranasal administration of INDCA against NTG-induced hyperalgesia. In the present study, subacute (7 d) intranasal pretreatment of INDCA significantly prevented the NTG induced hyperalgesia.

Migraine is often associated with psychopathological disorders, particularly anxiety, depression [38], and suicidal tendencies [39]. Amongst many neurotransmitters in the brain, the serotonergic (serotonin, 5-HT) system from the brainstem raphe nucleus has been most convincingly implicated in migraine pathophysiology [40]. In the present study, NTG resulted in a significant decrease in brain 5-HT concentration, compared to normal (control) rats, whereas INDCA pretreatment showed protection against NTG-induced decrease in brain 5-HT concentration. Therefore, anti-nociceptive effects of INDCA against NTG-induced hyperalgesia are suggested to be mediated through enhancement of central 5-HT neurotransmission. This notion is further supported by antidepressant effects shown by INDCA against stress-induced depres-

sion [22] and suicidal behaviour related traits [23].

Migraine is known to be associated with a relative hypersensitivity of central 5-HT1A receptors. Therefore, the role of 5-HT receptor (especially 5-HT1A) subtypes in the possible association between migraine with anxiety/ depression is crucial [41]. Furthermore, the effects of buspirone (a 5-HT1A agonist), a known anti-anxiety [42-43] and anti-depressant [43] agent, showed clinical promise migraine prophylaxis [44-45]. Therefore, we studied anti-nociceptive effects of INDCA in presence of 5-HT1A, 1B and 1D specific antagonist namely NAN-190, Isamoltane hemifumarate, and BRL-15572 against NTG induced hyperalgesia.

In the present study, INDCA did not produce anti-nociceptive effects in presence of NAN-190 and Isamoltane hemifumarate. On the other hand, BRL-15572 did not block the anti-nociceptive effect of INDCA. These results suggested 5-HT1A and 5-HT1B but not 5-HT1D receptor were involved in the mechanism of anti-nociceptive action of INDCA. Therefore, INDCA can be termed as selective 5HT1A/ 1B agonist or so called “serenics” [46].

Activation of 5-HT1A receptors is known to release of adrenocorticotrophic hormone (ACTH) from HPA axis. In the past, triterpenes of *Centella asiatica* (including AS, a marker compound of INDCA) were shown to reduce serum corticosterone levels through modulating HPA axis functions [21]. Furthermore, aberrant 5-HT function and dysregulation of the HPA axis, involving elevated corticotropin-releasing hormone (CRH) activity, plays a major role in stress-related illnesses [47-48] such as migraine [10]. The relationships between stress and migraine strongly suggest that stress management is an important component in migraine prophylaxis in adults [10, 49-51] and children [52]. Therefore, prevention of stress induced HPA axis dysregulation probably through activation of 5-HT1A receptor can be possible mechanism behind the observed anti-nociceptive effects of INDCA in the present study.

The contribution of 5HT1A and 5HT1B receptors mediated actions in analgesia and migraine has been well documented [53-54]. 5-HT1A receptor subtype is suggested to have a

major role in cognitive or integrative functions, as well as in emotional stress related states such as anxiety and depression [46]. The 5-HT<sub>1B</sub> receptor, is found on both endothelial cells and smooth muscle cells of many human blood vessels [55]. Intracranial arteries (important in migraine) appear to contract exclusively through 5-HT<sub>1B</sub> receptors [55]. Therefore, INDCA might act as a post-sympathetic 5HT<sub>1A</sub> receptor agonist, enhancing synaptic 5-HT concentration and stimulating 5HT<sub>1B</sub> receptor to cause vasoconstriction of the cranial blood vessels and anti-nociception effects.

Abnormal neuronal excitability (firing) and subsequent changes in vascular events are implicated in migraine pathophysiology [8, 56]. The visual aura experienced by some migraineurs is known to arise from cortical spreading depression (CSD), a neuronal event that activate perivascular nerve afferents, and cause vasodilation, neurogenic inflammation of the meningeal blood vessels and, throbbing pain [56]. The role of inflammatory and pain related endogenous mediators in neuronal excitability and subsequent vasoconstriction in development of CSD and aura in migraine is reported [56]. Therefore, the peripheral and central sensitizations due to release of inflammatory and pain related nociceptive endogenous mediators [56], such as BK [28, 57] are considered as important targets for development of agents for migraine prophylaxis. Endogenous BK facilitates the release of neuromodulators such as substance P, CGRP, neurokinin A and glutamate from sensory neurons [58-59] to induce edema and intense acute vascular pain during migraine [57]. Intra-arterial injection of BK has shown to cause intense vocalization, thus mimicking acute pain similar to migraine attack. Among the many responses evoked by nociception, vocalization is the only response associated with both central (nociception) and peripheral (perception) component of pain [60]. Therefore, in the present study, we examined the possible vasoconstriction effect of INDCA against BK induced vocalization in rats.

The peripheral form of sensitization occurs when the nerve terminals (meningeal nociceptors) of the neurons of the trigeminal ganglion are soaked with the "inflammatory" soup (prostaglandin E<sub>2</sub>, BK, 5-HT and cytokines) along the vasculature of the cerebral dura mater [3]. As a result, peripheral trigeminal meningeal nociceptors become sensitized due to cranial vasodilation during migraine attacks. In addition, the central sensitization causes an altered processing of sensory input in the brainstem [61]. Central sensitization is considered to be involved in many temporal and symptomatic features of migraine [62]. In the present study, significant prevention of BK-induced vocalization by INDCA and positive control ST confirmed the vasodilatory effects. Therefore, INDCA is suggested to act both central and peripheral sensitization in relieving migraine attack. Our results were in line with previous reports of antinociceptive potential of total triterpenes from *Centella asiatica* [63-64], AS (a marker compound in INDCA) [65] and asiatic acid (a endogenous metabolite of AS) [66] in animal models of peripheral and central mediated pain

through sensory neuronal function improvement [65] or inhibition of pro-inflammatory mediators [67]. However, to provide more direct evidence for the role of serotonergic neuronal excitability in the anti-migraine action of INDCA will require a detailed study involving electrophysiological experiments.

## Conclusion

Our results indicated promising prophylactic efficacy of INDCA in experimental migraine models (NTG and BK induced hyperalgesia) in rats, possibly through the 5HT<sub>1A/1B</sub> agonist action.

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