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Original Article

ANTIMIGRAINE ACTIVITY OF METHANOLIC EXTRACT OF *ABROMA AUGUSTA* L. IN LABORATORY ANIMALS

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ABSTRACT

Objective: The present study aimed to evaluate of antimigraine activity of methanolic extract of *Abroma augusta* L. leaves in laboratory animals.

Methods: The antimigraine activity was evaluated against nitroglycerine (NTG, 10 mg·kg-1, i . p.) and bradykinin (BK, 10 µg, intra-arterial) induced hyperalgesia in rats. Rats were divided randomly into six groups: normal, control, standard (sumatriptan, 42 mg·kg–1, s . c.), and *Abroma augusta* L. (100,200 and 400 mg·kg-1, p . o.). In the nitroglycerin (NTG) induced hyperalgesia model, rats were pre-treated with standard drug sumatriptan and *Abroma augusta* L. for 0, 7 and 14 d and tail flick latency were recorded separately in 0-day, 7-day and 14-day pretreatment study. Brain serotonin concentration was also estimated by HPLC method at the end of the study. In bradykinin induced hyperalgesia model the number of vocalizations were recorded as a measure of hyperalgesia in rats.

Results: *Abroma augusta* L. showed a significant (P<0.001) elevation in the tail-flick latency (at dose 400 mg·kg–1) and body weight (at doses 100, 200, and 400 mg·kg–1) in NTG -induced hyperalgesia model in rats. Further, *A. augusta* L. (400 mg/kg) showed a significant (P<0.001) increase in brain serotonin concentration compared to NTG control group animal. It showed a significant (P<0.01, P<0.001) reduction in the elevated number of vocalizations at doses (200 and 400 mg·kg–1) in the bradykinin-induced hyperalgesia model in rats.

Conclusion: We concluded that the methanolic extract of *Aroma augusta* L. possessed an anti-migraine effect in nitroglycerine and bradykinin-induced hyperalgesia model in rats.

Keywords: Migraine, *Abroma augusta* L., Nitroglycerin, Bradykinin, Hyperalgesia model

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INTRODUCTION

Migraine is a common cause of chronic pain and the most prevalent neurologic disorder. It is an episodic brain disorder that affects 15-18% of the population worldwide each year [1, 2]. Previous studies showed that 6.5% of men and 18.2% of women were suffering from migraine in the year 2017 [3]. Migraine is understood to be a spectrum of illness, consisting of episodic and chronic forms. Chronic migraine typically progresses from episodic migraine. The emerging epidemiologic evidences supports the unique underlying physiology of the two migraine states [4]. Natural products are playing an important role in antimigraine therapy. We can now fulfil the demand of effective antimigraine drugs by sustained investigation in natural product [5]. The emerging scenario of widespread usage and therapeutic potential of phytomedicines prompted us to investigate *Abroma augusta* L. (*A. augusta* L.) as a potential therapy to treat migraine.

The drugs used in the treatment of migraine can be divided into two groups. The first group of agents abolish the acute migraine headache and the second group of agents aimed at its prevention. In the last decades, there has been tremendous progress in the acute therapy of migraine. Sumatriptan belonging to a new class of drugs, now known as 5 HT1B/D receptor agonists [6]. These agents have changed the life of number of patients suffering from migraine. Current prophylactic treatment for migraine includes calcium channel blockers, 5 HT2 receptor antagonist, beta blockers and GABA agonists [7, 8]. Unfortunately, many of these treatments are nonspecific and not always effective [9]. Most surveys confirmed that herbal remedies are the most prevalent therapies to treat headache or migraine. This is one of the most frequent reason to use plant-derived medications for the treatment of migraine [10].

A. augusta L. is generally used in gynecological disorders. Leaves of A. *augusta* L are useful in treating uterine disorders, diabetes, rheumatic pain of joints, and headache with sinusitis. Leaves and stem of *A. augusta* L are demulcent in nature. Fresh leaves and stem infusion in cold water is very efficacious in gonorrhea. It is reported that different parts of methanolic extracts of *A. augusta* L. showed significant anti-inflammatory activity. It also possesses the analgesic activity, which evident in all the nociceptive models. It also suggested that *A. augusta* L. possess both central as well as peripherally mediated activities [11]. Different parts of the plant are useful in treating stomachache, dermatitis, leucorrhoea, scabies, gonorrhea, cough, leukoderma, jaundice, nerve stimulant, weakness, and hypertension [12]. The methanolic extract of *A. augusta* L. also showed a significant cardioprotective effect in some studies [13]. *A. augusta* L. extract inhibited the activity of pancreatic lipase which indicates its protective role in obesity-like diseases [14].

Since the treatments available on migraine are old, there is need to develop new acute and preventive therapy for the effective management of migraine disease [15-17]. The antimigraine activity of *A. augusta* L. has not been well explained in animals. Hence, current research work is an effort to reveal the antimigraine activity of methanolic extract of *A. augusta* L. leaves in experimental animal models of migraine. It is expected that the information may open a new dimension in the management of migraine in the near future.

MATERIALS AND METHODS

Collection and authentication of plant seeds

A. augusta L. is found in tropical Asia, South and eastern Africa, and Australia. Fresh leaves of *A. augusta* collected from hot and humid parts of India in month of October. Leaves of *A. augusta L.* Deposited at Green Heaven, Nagpur, India. After the authentication of the leaves, the voucher specimen was obtained at our institute (Voucher No.1012).

Drugs and chemicals

Bradykinin was purchased by Sigma-Aldrich chemical company Australia. Nitro-glycerin was purchased from New Medicon lab Pvt Ltd. India. Sumatriptan was procured from Sun Pharmaceuticals Ind.

Ltd, India. Methanol, Tween 80, Perchloric acid, EDTA and Picric acid were purchased from Thomas baker (Chemicals), Mumbai. Urethane was obtained from Hi-media Laboratories Pvt. Ltd (Mumbai). All solvents used were of HPLC grade.

Preparation of methanolic extract of *A. augusta* L. leaves

The air-dried crushed leaves (1000g) were soaked for 12 h. in methanol (3L) at room temperature. The residue was extracted with hot methanol under reflux for 3 times (each 1500 ml) after vacuum filtration. All the solvent was evaporated under a vacuum and the extract was then lyophilized to yield approximately 12% w/w of the residue, which was stored at 20 °C until use. The concentrate was suspended in 5% w/v Tween 80 and given at dose 1 ml/100 gm body weight to the animals.

Phytochemical standardization of plant extract

The extracts so processed for the presence of different phytoconstituent viz. carbohydrate, protein, amino acid, steroids, saponin glycosides, alkaloids, tannins as per the method given [18]. Standardization of plant material was carried out as per the WHO guidelines for quality control of medicinal plant materials.

Experimental animals

Adult female Wistar rats with body weights ranging from 200-250 gm of age 8 w were purchased from National Institute of Bioscience, Pune. Rats were housed in groups of six animals per cage at standard laboratory conditions with a temperature of 25±1 °C and relative humidity of 45-55%. Feed (Neutrivet Life, Pune) and water were provided *ad libitum* to all the animals. The 12 h light/dark cycle was maintained in the animal house.

Approval of the experimental protocol

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Sinhgad Institute of Pharmacy, Narhe, Pune, constituted as per the Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA). The IAEC-approved protocol number is SIOP/IAEC/2017/02/01.

Nitroglycerin (NTG) induced hyperalgesia in rats

Experimental design

Thirty-six female Wistar rats (200-250 g) were divided into the following six groups containing six rats in each group. First and second groups were used as normal (1 ml/kg saline) and NTG (10 mg/kg) treated groups respectively. Sumatriptan (42 mg/kg, s. c.) and *A. augusta* L. (100, 200 and 400 mg/kg, *p. o*) were administered to animals constituting Group-III, Group IV, Group V and Group VI, respectively [23].

0 day acute study

Animals in this study were pretreated with vehicle, sumatriptan (42 mg/kg, s. c.) and *A. augusta* L. (100, 200 and 400 mg/kg, p. o.). Then after 15 min NTG was administered to all groups (except normal group animals) to induce migraine. Tail flick latency were recorded at 30, 60, 90,120 and 240 min. after the NTG treatment.

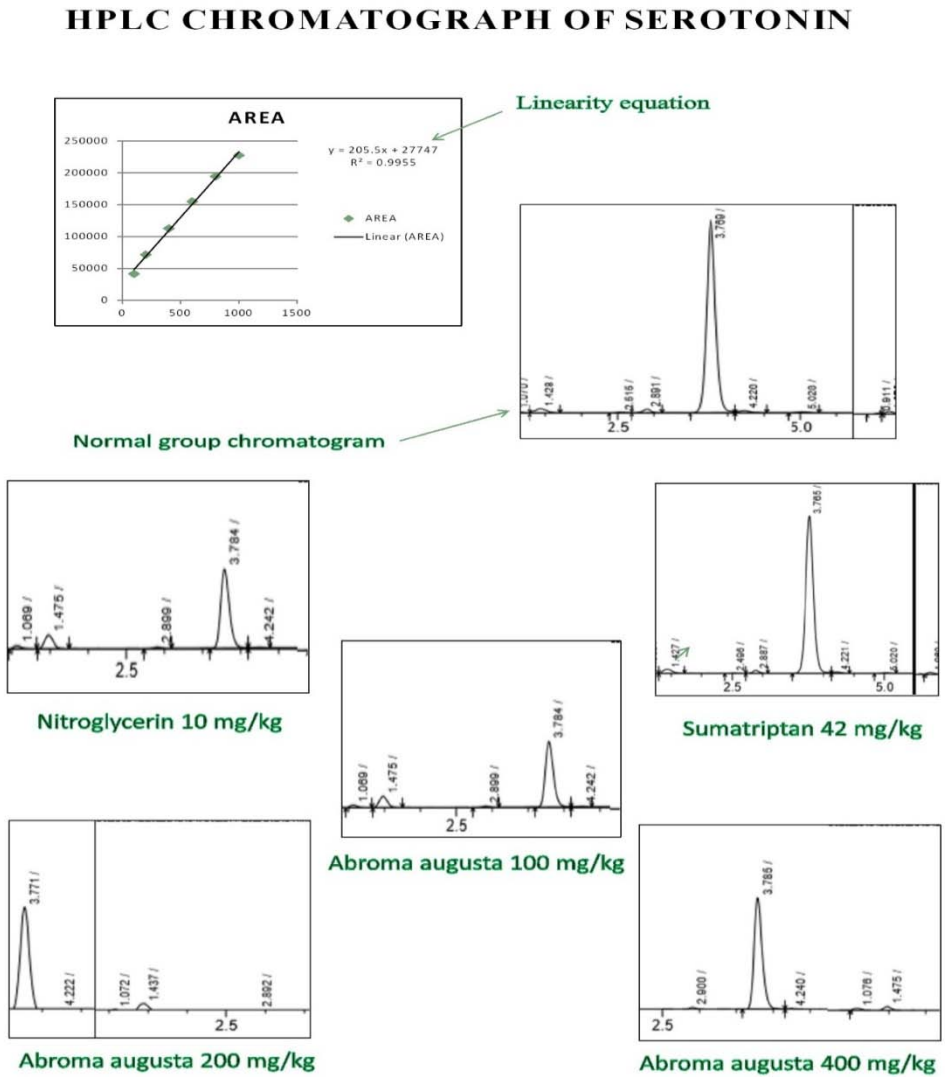

Fig. 1: Estimation of Brain-serotonin concentration by HPLC method

7 days pretreatment study

Seven days washout period was maintained between 0-day acute study and 7-days pretreatment study. After 7 d of washout period, same experimental design was followed as previous. In which control standard and test groups were pretreated with vehicle, sumatriptan (42 mg/kg, s. c.) and *A. augusta* L. (100, 200 and 400 mg/kg, p. o.) respectively for 7 d. On day 7 NTG were administered to induce migraine and Tail flick latency were recorded at 0, 30, 60, 90,120 and 240 min. after the NTG treatment.

14 days pretreatment study

After 7 d of washout period, same experimental design was followed as previous. In which control, standard and test animals were pretreated with vehicle, sumatriptan (42 mg/kg, s. c.) and *A. augusta* L. (100, 200 and 400 mg/kg, p. o.) respectively for 14 d. On day 14, NTG was administered to induce migraine and tail flick latency were recorded at 0, 30, 60, 90,120 and 240 min. after the NTG treatment. At the end of the study period, brain was collected for the measurement of brain serotonin concentration.

Estimation of brain-serotonin concentration by HPLC method

At the end of experimental period, brain samples were suspended in 10 mlg^{-1} of tissue in ice-cold 0.1 $molL^{-1}$ perchloric acid containing 1.34 $mmolL^{-1}$EDTA and 0.05%, w/v sodium bisulfite and were sonicated. Then, the homogenates were centrifuged at 35000 rpm for 20 min at 4 °C. The supernatant was then filtered with 0.25 filters and injected into the chromatographic system for detection of brain-serotonin concentration at 280 nm wavelength according to previously reported method [19] (fig. 1).

Bradykinin induced hyperalgesia in rats

Rats were anaesthetized with Urethane (125 mg/kg *i. p.*), and surgically prepared for the study. A common carotid artery was exposed and cannulated with indwelling polyethylene catheter. A microphone was placed a few centimeters over the mouth of rat for the recording of vocalization. Vocalization was recorded for 5 min after BK injection. BK were dissolved in water and administered to the rats intra-arterial using an arterial catheter at the dose of 10 µg, in the volume of 10 µl on 14th day of *A. augusta* L. treatment. The BK control rats received water as a vehicle. After the administration of the last dose on 14th day the number of vocalization were recorded [20].

Statistical analysis

The data were expressed as mean±standard error mean (SEM). Analysis of the data was performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA). The tail flick latency and body weight data were analyzed by two-way analysis of variance (ANOVA) and Bonferroni's test was applied for *post-hoc* analysis. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of *A. augusta* L. on NTG induced hyperalgesia at 0 d

Treatment with NTG 10 mg/kg i. p significantly reduced tail flick latency at 30, 60, 90, 120 and 240 min as compared to normal group rats. Treatment with Sumatriptan (42 mg/kg) increased tail flick latencies significantly (P<0.001) at 90, 120, 240 min. However, the treatment of *A. augusta* L.(100, 200 and 400 mg/kg) increased tail flick latencies non-significantly at all the time intervals as compared to NTG treated group animals (table 1).

Table 1: Effect of sumatriptan (42 mg/kg) and *A. augusta* L. (100, 200 and 400 mg/kg) in nitroglycerine induced hyperalgesia at 0 d

	Tail flick latency (s)					
Time interval (min)	**Normal**	**NTG control (10 mg/kg)**	**NTG+sumatriptan (42 mg/kg)**	**NTG+*A. augusta* (100 mg/kg)**	**NTG+*A. augusta* (200 mg/kg)**	**NTG+*A. augusta* (400 mg/kg)**
Baseline	8.8±0.06	8.2±0.07	8.7±0.08	8.4±0.06	8.4±0.05	8.3±0.08
30	8.6±0.14	6.7±0.10$^{\#\#}$	6.4±0.10	5.7±0.09	5.7±0.04	5.6±0.06
60	7.8±0.11	5.8±0.07$^{\#\#}$	7.3±0.28	5.6±0.04	5.5±0.05	5.9±0.13
90	7.8±0.14	5.0±0.06$^{\#\#\#}$	9.2±0.36***	6.3±0.08	5.3±0.02	6.0±0.13
120	7.0±0.08	5.1±0.10$^{\#\#}$	11.13±0.52***	5.5±0.05	5.4±0.05	6.6±0.15
240	6.6±0.09	4.8±0.03$^{\#\#}$	12.30±0.36***	5.4±0.04	5.35±0.10	5.8±0.09

The data represents mean±SEM for n=6 per rats. The data was analyzed by Two-way ANOVA followed by Bonferroni's test*P<0.05, **P<0.01, ***P<0.001 as compared with NTG control group, ###P<0.001 compared to normal group animals.

Effect of sumatriptan (42 mg/kg) and *A. augusta* L. on NTG induced hyperalgesia after 7 d pretreatment

In present study the treatment with NTG 10 mg/kg *i. p.* significantly (P<0.001) reduced the tail flick latencies at 30, 60, 90,120 and 240 min as compared to normal group rats. The treatment with standard drug sumatriptan (42 mg/kg) increased tail flick latencies significantly (P<0.001) at all the time intervals. Further *A. augusta* L. (200 mg/kg and 400 mg/kg) increased tail flick latencies significantly (P<0.001) at 240 min compared to NTG treated control group animals (table 2).

Table 2: Effect of sumatriptan (42 mg/kg) and *A. augusta* L. (100, 200 and 400 mg/kg) in nitroglycerine induced hyperalgesia after 7 d pretreatment study

	Tail flick latency (s)					
Time interval (min)	**Normal**	**NTG Control (10 mg/kg)**	**NTG+Sumatriptan (42 mg/kg)**	**NTG+*A. augusta* (100 mg/kg)**	**NTG+*A. augusta* (200 mg/kg)**	**NTG+*A. augusta* (400 mg/kg)**
Baseline	8.1±0.07	8.0±0.05	7.8±0.14	8.0±0.10	8.1±0.05	8.1±0.03
30	8.6±0.14	5.6±0.07$^{\#\#\#}$	8.0±0.16***	4.7±0.09*	4.9±0.10	5.4±0.15
60	7.9±0.11	4.8±0.08$^{\#\#\#}$	7.2±0.12***	4.7±0.04	4.7±0.05	5.0±0.07
90	7.8±0.14	4.0±0.01$^{\#\#\#}$	8.0±0.06***	5.1±0.06**	4.6±0.08	5.3±0.13***
120	7.0±0.08	4.0±0.08$^{\#\#\#}$	7.1±0.07***	4.2±0.05	4.7±0.05	5.6±0.09***
240	6.6±0.09	3.8±0.03$^{\#\#\#}$	6.7±0.25***	4.4±0.04	5.0±0.10***	5.1±0.06***

The data represents mean±SEM for n=6 per rats. The data was analyzed by Two-way ANOVA followed by Bonferroni's test*P<0.05, **P<0.01, ***P<0.001 as compared with NTG control group, ###P<0.001 compared to normal group animals.

Effect of sumatriptan (42 mg/kg) and *A. augusta* L. on nitroglycerine-induced hyperalgesia after 14 d pretreatment

Treatment with NTG significantly (###P<0.001) reduced tail-flick latencies at 30, 60, 90,120 and 240 min as compared to normal group rats. Treatment with sumatriptan (42 mg/kg) and *A. augusta* L. (400 mg/kg) increased tail-flick latencies significantly (***P<0.001) at all the time intervals as compared to NTG-treated group animals (table 3).

Table 3: Effect of sumatriptan (42 mg/kg) and *A. augusta* L. (100, 200 and 400 mg/kg) in NTG-induced hyperalgesia 14 d pretreatment study

	Tail flick latency (s)					
Time interval (min)	**Normal**	**NTG Control (10 mg/kg)**	**NTG+Sumatriptan (42 mg/kg)**	**NTG+*A. augusta* (100 mg/kg)**	**NTG+*A. augusta* (200 mg/kg)**	**NTG+*A. augusta* (400 mg/kg)**
Baseline	7.9±0.05	7.6±0.07	7.8±0.09	7.9±0.08	7.9±0.05	7.8±0.06
30	8.6±0.14	4.1±0.04 ###	7.7±0.08 ***	4.6±0.06	5.1±0.07*	5.5±0.07 ***
60	7.8±0.11	3.8±0.02 ###	8.4±0.17 ***	4.6±0.05	4.8±0.09*	5.6±0.10 ***
90	7.8±0.14	3.8±0.02 ###	8.3±0.18 ***	4.8±0.08**	4.6±0.07*	5.7±0.11 ***
120	7.0±0.08	3.8±0.03 ###	8.1±0.21 ***	4.3±0.05	4.5±0.04	5.6±0.08 ***
240	6.6±0.09	3.8±0.03 ###	8.1±0.35 ***	4.4±0.04	4.7±0.06*	6.0±0.03 ***

The data represents mean±SEM for n=6 per rats. The data was analyzed by Two-way ANOVA followed by Bonferroni's test *P<0.05, **P<0.01, ***P<0.001 as compared with NTG control group, ###P<0.001 compared to normal group animals

Effect of sumatriptan (42 mg/kg) and *A. augusta* L. on body weight

Body weight of NTG control group animal was significantly reduced at 7th day and 14th day compared to the normal group. Treatments with Sumatriptan (42 mg/kg) and *A. augusta* (100, 200 and 400 mg/kg) significantly (p<0.001) prevented the weight loss at 7th day and 14th day (table 4).

Effect of sumatriptan and *A. augusta* L. on brain serotonin concentration

In NTG control group, there was a significant (###P<0.001) reduction in brain serotonin concentration as compare to normal. Treatment with the standard drug sumatriptan showed a significant (P<0.05) increase in brain serotonin concentration, *A. augusta* L. (400 mg/kg) significantly (P<0.001) increased brain serotonin concentration as compared to NTG control group. However, A. *augusta* L. (100 and 200 mg/kg) showed a nonsignificant increase in brain serotonin concentration (fig. 2).

Effect of sumatriptan (42 mg/kg) and *A. augusta* L. on the number of vocalizations in BK-induced hyperalgesia in rats

In BK control group, there was a significant (P<0.001) increase in number of vocalizations as compare to normal. Treatment with sumatriptan (42 mg/kg) significantly (P<0.001) reduced the number of vocalizations, *A. augusta* L. (200 and 400 mg/kg) significantly (P<0.05, P<0.001) reduced the number of vocalizations as compared to BK control group (fig. 3).

Table 4: Effect of sumatriptan (42 mg/kg) and *A. augusta* L. (100, 200 and 400 mg/kg) on body weight (g) of animal in NTG-induced hyperalgesia model

Day	**Normal**	**NTG control (10 mg/kg)**	**NTG+sumatriptan (42 mg/kg)**	**NTG+*A. augusta* (100 mg/kg)**	**NTG+*A. augusta* (200 mg/kg)**	**NTG+*A. augusta* (400 mg/kg)**
0	215±1.6	217±1.6	215±1.24	210±0.71	212±0.83	207±2.38
7	249±3.7	209±0.9###	220±1.8 **	210±2.2***	216±3.3***	203±0.91***
14	252±2.9	198±0.45 ###	227±3.8 **	213±2.29***	226±2.59**	214±1.04***

The data represents mean±SEM for n=6 per rats. The data was analyzed by Two-way ANOVA followed by Bonferroni's test *P<0.05, **P<0.01, ***P<0.001 as compared with NTG control group, ###P<0.001 compared to normal group animals.

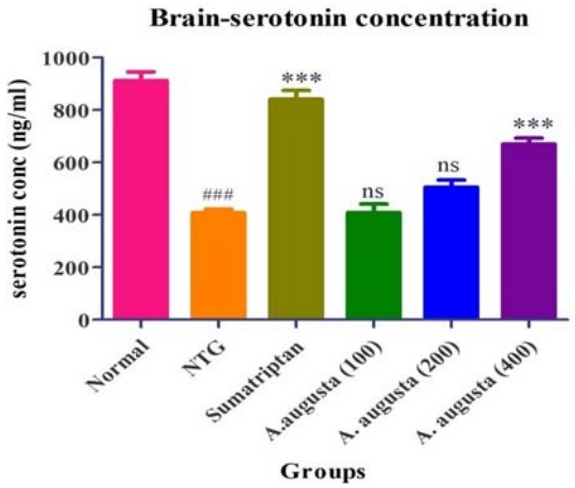

Fig. 2: Effect of sumatriptan (42 mg/kg) and *A augusta* L. on brain serotonin concentration, The data represents mean±SEM brain serotonin concentration, n=6 per group. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. *P<0.05, **P<0.01, ***P<0.001 as compared with NTG control group, ###P<0.001 compared to normal group animals

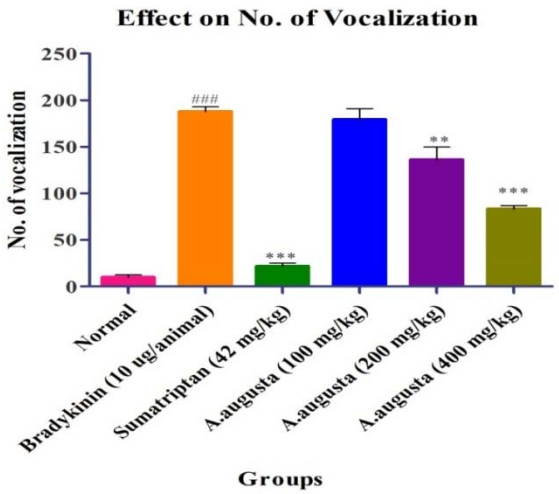

Fig. 3: Effect of sumatriptan (42 mg/kg) and *A. augusta* L. on the number of vocalizations in BK-induced hyperalgesia in rats, The data represents mean±SEM vocalization count, n=6 per group. The data was analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. *P<0.05, **P<0.01, ***P<0.001 as compared with BK control group, ###P<0.001 compared to normal group animals

DISCUSSION

The plant selected for the investigation was based upon literature survey and the medicinal uses. Leaves of *A. augusta* L. are recommended for the treatment of rheumatic pain of join and headache related to sinusitis [12]. Although the selected plant was mentioned for the treatment of migraine in Ayurveda, its scientific evaluation using pharmacological models were not performed, hence the *A. augusta* L. was selected for the study. The selected plant material was authenticated to confirm the identity of plant. Methanolic extract of *A. augusta* L. was prepared and its phytochemical study and standardization were carried out. The prepared leaves extract was then subjected to pharmacological screening using different models.

In the present study NTG induced hyperalgesia and bradykinin-induced hyperalgesia were used to induce migraine in wistar rats. NTG-induced hyperalgesia is a well-known migraine model in rats. NTG is a highly lipophilic organic nitrate which releases nitric oxide (NO) by enzymatic and non-enzymatic reactions [21]. NO is an oxygen free radical which acts as a smooth muscle relaxant and as a neuronal messenger with diverse signaling tasks in both the central and peripheral nervous system. Systemic nitroglycerin activates neuronal groups in selected areas of the rat brain involved in nociception [22, 23]. Several reports showed that nitroglycerin induces spontaneous-like headache attacks in migraine sufferers [24]. Further, the NO derived from nitroglycerin exerts a biological effect on neuronal activity. In addition, it is reported that systemic nitroglycerin increases the level of the neuronal NO synthase (NOS) in the rat medulla [25]. Hunter *et al.*, have reported that the tail-flick test shows an effect on the normal sensory nociceptive function which could be mediated by spinal and supraspinal mechanisms of nociception [26]. Tassorelli and his co-workers in 2006 reported that nitroglycerin induced a significant decrease in the latency of tail flick compared to baseline [23]. After the systemic administration of nitroglycerin there was an increased in the expression of neuronal NOS in the cervical cord. Further this increased neuronal NO may interfere with the ion channel activity and released the transmitters. In the present study, we observed that *A. augusta* L. (400 mg·kg–1) showed significant ($P<0.001$) increase in the tail-flick latency. Which suggest that the *A. augusta* L. may possess antimigraine activity by inhibiting neuronal NOS.

According to a study conducted by Taylor ad his coworkers in 2008 weight gain should be a concern in the patients suffering from migraine [27]. Further, he observed that overweight and obese patients with migraine are having a risk of the increased frequency and severity of migraine attacks. According to previous report methanolic extract of *A. augusta* L was showed the effective results in hyperlipidemic condition [13]. In present study we observed that the *A. augusta* L. Significantly inhibited the gain in body weight at the doses (100, 200 and 400 mg/kg). Hence, we can assume that *A. augusta* L. decreased the severity of migraine by inhibiting weight gain in NTG induced migraine in Wistar rats.

The serotonergic system in the brain has its origin in the raphe nuclei of the brainstem. From here, serotonergic neurons project to every region of the CNS, including the primary sensory cortex. Serotonin acts through several different receptor subtypes and is involved in many psychophysiological functions such as sleep, mood, appetite and pain modulation. A low level of serotonin interictally could result in disinhibition of pain signals from peripheral nociceptors, thus lowering the threshold for the induction of headache [28]. Kimball and friedman in 1960 have reported that serotonin and their precursor like 5-Hydroxytryptophan (5-HTP) cure sudden migraine attack by injecting intravascularly in patient with migraine [29]. Reduction in serotonin concentration is responsible for the induction of the migraine attack and its replenishment may relive migraine [30]. In the present study, *A. augusta* L. showed significant ($P<0.001$) increase in reduced serotonin level at dose (400 mg/kg). Therefore, *A. augusta* L. may shows antimigraine activity by enhancing central 5-HT neurotransmission.

Antimigraine activity of *A. augusta* L. was further confirmed by BK induced hyperalgesia model. BK is one of the chemical agents involved in the generation of pain. It is a powerful chemical irritant that mediates the inflammatory response by causing blood vessel dilation and reducing the neuronal pain threshold. BK is well known to be of primary relevance for cerebral circulation, either under normal or pathological conditions [31, 32]. Moreover, BK is one of the most potent algogenic mediators and the regulator of the noxious sensitivity of nociceptors [33]. BK through the activation of BK B2 receptors constitutively expressed on sensory terminals [34]; further, it facilitates the release of neuromediators such as substance P, calcitonin gene-related peptides (CGRP), neurokinin A and glutamate from sensory neurons [35, 36].

Several years ago, it was considered that bradykinin is a possible pathogenetic factor in migraine [37, 38]. The injection of a few micrograms of bradykinin into a common carotid artery of rabbits was shown to cause intense vocalization and flight; vocalization was observed also following the intracarotid injection of bradykinin into rats under general anesthesia (in the absence of verbal reporting). Vocalization has long been accepted as a signal of pain in animals; indeed, among the many responses evoked by nociception, vocalization is the only one uniquely associated with nociception on the one hand, and the perception of pain on the other [39]. Bobade and her co-workers reported that endogenous BK facilitates the release of neuromodulators such as substance P, CGRP, neurokinin A and glutamate from sensory neurons to induce edema and intense acute vascular pain during migraine. Intra-arterial injection of BK has shown to cause intense vocalization, thus mimicking acute pain similar to a migraine attack. Among the many responses evoked by nociception, vocalization is the only response associated with both the central (nociception) and peripheral (perception) component of pain [40, 41]. In present investigation *A. augusta* L. showed significant reduction in increase number of vocalizations at doses 200 and 400 mg/kg which explain its antimigraine activity in BK induced model.

CONCLUSION

We concluded that methanolic extract of *A. augusta* L. possessed antimigraine effect in nitroglycerine and bradykinin induced hyperalgesia model in rats. It is thus suggested that antimigraine effect of *A. augusta* L. may be due to inhibition to activation of neuronal NOS and reactive oxygen species, increasing serotonin and prostacyclin level and inhibiting the degradation of bradykinin, encephalin and substance P.

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Nil

AUTHORS CONTRIBUTIONS

AM contributed in collecting plant sample, identification, a confection of the herbarium, running the laboratory work and analysis of the data. SHS supervised the laboratory work, drafted the paper and contributed to critical reading of the manuscript. Both the authors have read the final manuscript and approved the submission.

CONFLICT OF INTERESTS

Declared none

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