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Anti-hyperalgesic activity of *Ficus deltoidea* in an animal model of migraine

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Migraine is one of the most common primary headaches, causing significant morbidity to its sufferers. *Ficus deltoidea* (FD) is a plant that is traditionally used in Malaysia to treat headaches. This study evaluated the potentials of FD on attenuating hyperalgesia in nitroglycerin (NTG)-induced mice model of migraine and its related C-fos expression. This study was divided into phases. In Phase1, NTG-induced mice model of migraine was constructed by a single dose of NTG 10 mg/kg and validated through formalin and hot plate tests, followed by evaluation of the level of C-Fos in their trigeminal nucleus caudalis (TNC) through immunoblotting. In Phase 2, the effect of FD (50, 100, and 200 mg/kg) on hyperalgesic behaviours and levels of C-Fos in TNC of NTG-induced mice was evaluated and compared with NTG-induced mice. The results showed a significant increased (p <0.05) of nociceptive behaviours in both formalin and hot plate tests and increased (p <0.05) C-Fos expression in TNC were observed in NTG-induced mice when compared to control, sumatriptan and topiramate groups. However, treatments with aqueous extract of FD (100 and 200 mg/kg) significantly reversed all the observed changes. In conclusion, FD dose-dependently attenuated nociceptive behaviours in NTG-induced mice model of migraine and inhibition of C-Fos production in TNC of the mice. This shows the potential of FD to be used for future development as a natural therapeutic agent for migraines, supporting its traditional use in relieving headache.

Keywords: C-Fos, *Ficus deltoidea*, Hyperalgesia, Migraine, Nitroglycerin, Nociception

IPC code; Int. cl. (2021.01)−A61K 36/00, A61K 127/00, A61P 25/06

Introduction

Migraine is a highly prevalent disorder that affects people across the globe. It is experienced by 14% of the world's population and was reported as the most disabling disease[1]. It is characterised by a recurrent headache that is accompanied by certain features, including hypersensitivity to various stimuli such as light, sound, touch, and smell[2]. The overall symptoms that occur in migraineurs reflect migraine as a general disorder of sensory processing that includes altered cognition, emotion, and general homeostasis, other than those mentioned above[3]. A low pain threshold in migraineurs was first reported by Burstein *et al.*[3] where migraineurs experience pain resulting from both noxious and innocuous stimuli to the normal skin or scalp. This low pain threshold, or hyperalgesia, can be observed during migraine attacks[3,4] and even extend into periods in between attacks[5]. It is interesting to note that the observed hyperalgesia was not only reported in and around the trigeminal area (e.g., face, periorbital)[6] but was also reported throughout the body[7,8]. This may reflect the possibility of the peripheral mechanism involved in migraine, where primary afferent nociceptive neurons exhibit an increased response to external mechanical stimuli at the original site of injury or inflammation[9,10]. The molecular events that occur in peripheral sensitisation may lead to the generation of central sensitisation, which is hypothesised to be established in migraineurs. Thus, the development of pain hypersensitivity, including hyperalgesia, in the extracephalic region may be explained by the central sensitisation of third-order neurons in the thalamus[3].

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One of the most effective human models of migraine was constructed by administering nitroglycerin (NTG)[11]. Administration of NTG was able to induce migraine-like headaches exclusively in migraineurs regardless of the route of administration, which fulfilled the diagnostic criteria for migraine set by the International Headache Society (IHS)[11,12]. In addition, the spontaneous-like attacks induced by NTG are aborted by triptans and prevented by prophylactic use of valproate[13]. In animals, administration of NTG was able to produce behaviour similar to migraine, namely photophobia, hyperalgesia, and cutaneous allodynia. A single dose of NTG consistently produced hyperalgesia in the cranial as well as extracranial regions, tested using various behavioural responses to chemical, mechanical, and thermal stimuli[14]. Headaches that occur during migraine attacks are a result of the activation of the peripheral trigeminal nociceptor in the perivascular region as well as the trigeminal nucleus caudalis (TNC), which further transmits signals to the higher part of the brain[15]. The activation of these perivascular fibres causes an increase in C-fos expression, a marker for neuronal activation, in the trigeminal ganglion and TNC[16]. The sustained C-fos expression in the TNC was shown to be sufficient to enhance the activation of the central trigeminal, which may lead to central sensitization[17]. The upregulation of C-fos expression in TNC was inhibited by sumatriptan, demonstrating its specificity for migraine[18].

In Malaysia, *Ficus deltoidea* (FD) is locally known as 'mas cotek'. It is an evergreen shrub native to Malaysia and other Southeast Asian countries[19]. It is used traditionally to treat ailments such as toothache, headache, wounds, sores, and pain. *Ficus* species possess a variety of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, and terpenes. Most of these phytochemical compounds have strong antioxidant potential that may aid their effect on certain health conditions[20]. A phytochemical study on FD itself revealed that the plant, from its leaves to the roots, contains saponins, flavonoids, tannins, polyphenols, triterpenoids, and proanthocyanins[21]. Toxicity studies showed that the LD50 of aqueous extract of FD was greater than 5000 mg/kg body weight[22], suggesting its non-toxic and safe therapeutic use. The folkloric use of the FD plant has been supported by various studies, as accumulating evidence has shown that FD has strong anti-inflammatory properties in some models of inflammation[23,24], antidiabetic activities[25], antiulcerogenic[26], and antinociception[24,27]. Our previous animal study also supports the antinociceptive effect of aqueous extract of FD. Further, the leaf extract of FD has been reported to exert some anti-inflammatory properties by suppressing the levels of PGE2 and IL-1β in rat models of osteoarthritis[23]. Additionally, aqueous extracts of three different varieties of FD showed different anti-inflammatory activities against 12-O-tetradecanoylphorbol 13-acetate- (TPA-), hyaluronidase and lipoxygenase-induced ear oedema, respectively[28].

Despite all these properties attributed to FD, there is a paucity of data on its effect on NTG-induced hyperalgesia and neuronal activation in a mouse model, supporting its usage in headaches, particularly migraine. Therefore, this study aimed to evaluate the anti-hyperalgesic effects of FD on the NTG-induced mice model. This study constructed and confirmed NTG-induced hyperalgesia in mice models under laboratory conditions, besides evaluating the levels of C-Fos in the TNC of the mice through immunoblotting.

Materials and Methods

Equipment, chemicals, and reagents

Antibodies for immunoblotting (C-Fos and beta actin) were purchased from Cell Signalling Technology (Danvers, MA, USA). Nitroglycerin, Sumatriptan, topiramate and formalin were purchased from Sigma-Aldrich (St. Louis, MO, USA), while a standardized aqueous leaf extract of *Ficus deltoidea* var. *trengganuensis* was obtained from Zach Biotech Sdn. Bhd. Malaysia. Hot plate (Ugo Basile, model-7280), gel documentation (Syngene, USA) perspex cylinder and Plexiglas were also used in this study.

Experimental animals

A total of 88 Male ICR mice (25–35 g), purchased from ASapphire Enterprise (Malaysia), were used in this study. The mice were kept at the animal house, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, under a 12-hour light/dark cycle with free access to food and water. The mice were acclimatised and habituated for one week before the experiment and were used only once throughout the experiments. All the experiments were conducted in accordance with the ethical guidelines on animal experimentation, approved by the Institutional Animal Care Use Committee (IACUC), Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM/ICAUC/AUP-R031/2015). Animals are acclimatised to the test environment 1 hour prior to each test.

Drug preparation and administration

A stock of 5.0 mg/mL of nitroglycerin (NTG) (Sigma) dissolved in 30% alcohol, 30% propylene glycol, and water was freshly diluted in 0.9% saline in a polypropylene tube. A dose of 10 mg/kg was administered intraperitoneally to each mouse. Control mice received normal saline via intraperitoneal (i.p.) injection at a volume of 10 mL/kg. Sumatriptan and topiramate (Sigma) were freshly diluted in normal saline and administered to the mice (i.p.). The standardised aqueous extract of *F. deltoidea* var. *trengganuensis* was prepared daily in normal saline (FDA) and administered to the mice via i.p. administration.

Experimental procedure

In this study, the experimental procedure consisted of two phases: the induction and validation phase (Fig. 1) of the NTG-induced mice model of migraine as previously described[29], followed by the treatment phase (Fig. 2) with FDA.

Phase 1: Induction and validation of NTG-induced mice model of migraine

In this set of experiments, nociception and C-fos expression were investigated in an NTG-induced acute mice model of migraine (Fig. 1). The effect of NTG on nociception was evaluated via the formalin-induced paw licking test (Protocol 1) and the hot plate test (Protocol 2). The effect of NTG on c-fos expression in TNC was evaluated via immunoblotting (Protocol 3). In Protocol 1, the induction of an acute model of migraine was done by a single dose of NTG (Fig. 1a). The mice were randomly divided into 3 groups (n=8). The control group received physiological saline (10 mL/kg, i.p.), the NTG group received NTG (10 mg/kg, i.p.), and the SUMA group received sumatriptan (5 mg/kg, i.p.), an approved drug for the treatment of migraine, and NTG (10 mg/kg, i.p.). The formalin-induced paw-licking test was carried out 4 hours after the administration of physiological saline or NTG. In Protocol 2, evaluation of the acute model of NTG-induced migraine in mice was done through the hot plate test (Fig. 1b). The mice were randomly divided into two groups (n=8). The control group received physiological saline (10 mL/kg, i.p.), and the NTG group received NTG (10 mg/kg, i.p.). In Protocol 3, evaluation of the acute model of NTG-induced migraine in mice was done through the evaluation of the level of C-Fos expression in the TNC by

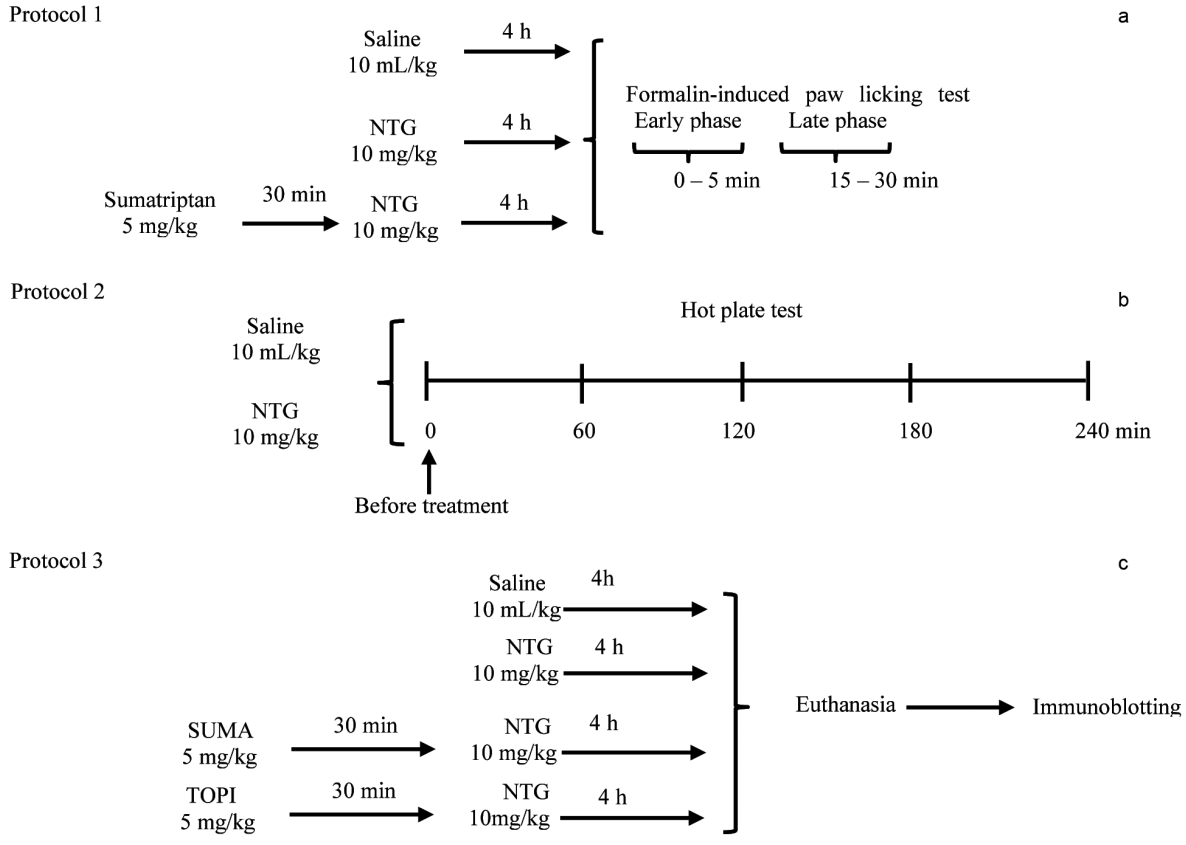

Fig. 1 — Experimental design for phase I.

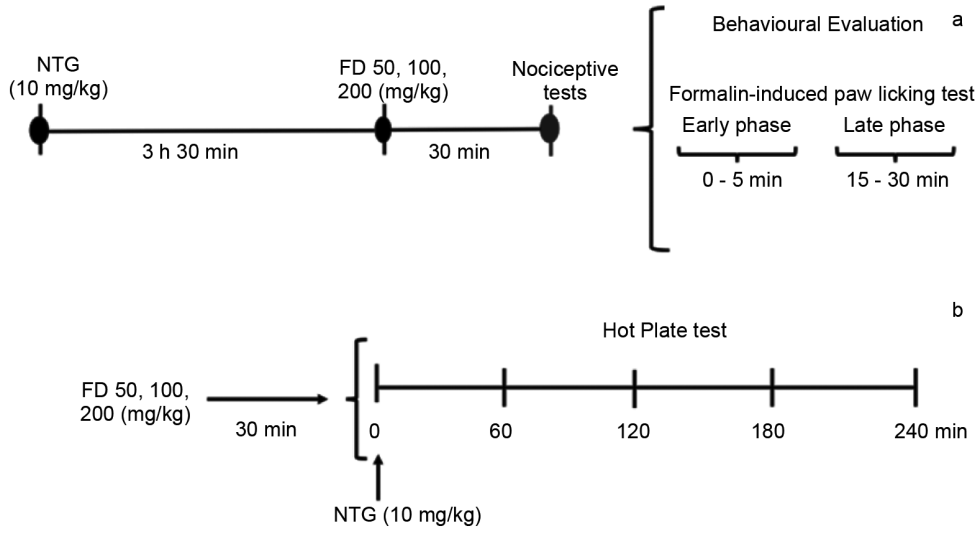

Fig. 2 — Experimental design for phase II.

immunoblotting[29] (Fig. 1c). The mice were randomly divided into 4 groups (n=6). The control group received physiological saline (10 mL/kg, i.p.), the NTG group received NTG (10 mg/kg, i.p.), the SUMA group received sumatriptan (5 mg/kg, i.p.) 30 minutes before NTG administration and NTG (10 mg/kg, i.p.) and the TOPI group received topiramate (80 mg/kg, i.p.) 30 minutes prior to NTG administration and NTG (10 mg/kg, i.p.). The euthanasia was done 4 hours after the administration of NTG, and immunoblotting was carried out.

Phase 2: Effects of FD on NTG-induced mice model of migraine

In phase 2, the effect of FD on the NTG-induced migraine model was evaluated via a formalin-induced paw-licking test and expression of C-fos in TNC (Fig. 2). In these tests, the animals were divided into 4 groups. The NTG group received NTG (10 mg/kg, i.p.), the FD50 group received NTG (10 mg/kg, i.p.) and treated with FDA (50 mg/kg, i.p.), the FD100 group received NTG (10 mg/kg, i.p.) and treated with FDA (100 mg/kg, i.p.) and the FD200 group received NTG (10 mg/kg, i.p.) and treated with FDA (200 mg/kg, i.p.). The doses of FD used were based on our previous study and a subchronic toxicity study of FD extract[22]. In the nociceptive test (Fig. 2a), the formalin-induced paw licking test was carried out 30 minutes after FDA administration. For the c-fos expression test (Fig. 2b), euthanasia was done 4 hours after the administration of NTG and immunoblotting was carried out.

The formalin-induced paw licking test

After administering physiological saline, NTG or FD, mice were placed in a Plexiglas chamber for behavioural observation. The formalin test was performed according to the procedure described by Dubuisson and Dennis[30]. Mice were placed in a Plexiglas observation chamber equipped with an angled mirror to facilitate observation of the injected paw. After 4 hours of physiological saline and NTG administration, 30 µL of 2.5% formalin solution was injected subcutaneously into the plantar surface of the left hind paw of the mice. The behavioural responses to nociception, including licking and biting the injected paw, were observed and recorded. After formalin injection, the time spent was recorded in 2 phases, 0 to 5 minutes (early phase) and 15 to 30 minutes (late phase). The data obtained were recorded and kept for later evaluation.

The hot plate test

The hot plate test was conducted using the method described by Sulaiman *et al.*[27] with slight modifications. Briefly, the hot plate (Ugo Basile, model-7280) was maintained at 53.5±2°C. Mice were placed into a perspex cylinder on a heated surface, and the time between placement and licking of the hind paws or jumping movements was recorded as response latency. FD was administered 30 minutes before the beginning of the test, and the response latencies of the mice to the heat emanating from the hot plate were observed immediately before (0 minute) and at 60, 120, 180, and 240 minutes after administration of physiological saline or NTG. The maximum time the mice spent on the hot plate was 20 seconds before they were removed to avoid heat-related injuries that could be caused by longer exposure to the hot plate.

Immunoblotting for C-Fos expression

After 4 hours of administration of physiological saline, NTG or FD, the mice were transcardially perfused with ice-cold saline. The TNC segment of their brain (1 to 5 mm from the obex) was immediately harvested and stored at -80°C[31]. Total protein was extracted using ice-cold sucrose lysis buffer supplemented with protease inhibitors, and the Bradford method was used to determine the protein concentration of the supernatant. Samples (40 µg of total protein each) were denatured separately on a 10% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane using an electroblotting apparatus. The membrane was blocked in 5% skimmed milk for 1 hour, then incubated with the C-Fos primary antibody (1:500). β-actin (1:5000) served as a housekeeping protein for the equal loading of the lanes. After washing with Tris-buffered saline and Tween 20 (TBST), the membrane was incubated with horseradish peroxidase (HRP). Finally, the immunoblots were detected using the ECL kit (Western Bright), viewed using Gel Documentation, and the image of the protein of interest was obtained. Densitometric analyses were performed using ImageJ software.

Statistics

All data obtained were analyzed through one-way or two ANOVA as the case might be (GraphPad Prism 8 software), followed by Dunnett's multiple comparison tests where applicable. The results were also expressed as the mean±S.E.M. to show variation in groups. A probability level of less than 0.05 was considered significant. Data obtained on immunoblotting from both phases of the experiments were normalized for each reading by dividing the mean intensity of the protein of interest (C-Fos) with the housekeeping protein β-actin. The protein levels were presented as percentage changes compared to the control sample, designated as 100%.

Results

NTG-induced hyperalgesia - formalin test

The intraplantar administration of formalin solution on the left hind paw of the mice was evaluated in two phases of nociceptive behaviour. There were no significant changes noted in the early phase of the formalin test in the NTG group compared to the control (Fig. 3a). However, Fig. 3b showed that i.p. administration of NTG (10 mg/kg), 4 hours prior to the formalin test, produced significant hyperalgesia manifested by an increase in nociceptive behaviour in the late phase of the formalin test. Sumatriptan, a specific antimigraine drug used in acute attacks of migraine, significantly inhibited nociceptive behaviour induced by NTG only in the late phase of the formalin test but not in the early phase.

NTG-induced hyperalgesia – hot plate test

Administration of saline (10 mL/kg) produced a stable response latency over time up to 240 minutes after its administration, as shown in Fig. 4.

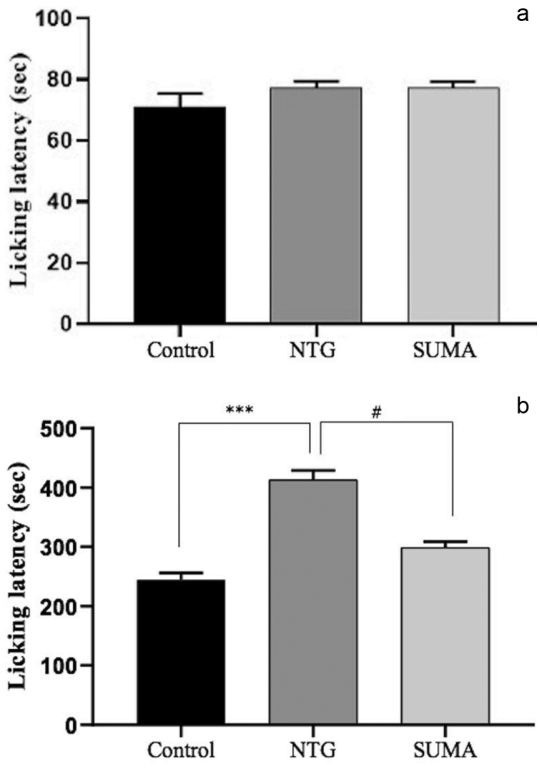

Fig. 3 — The effect of nitroglycerin (NTG), a) early phase; b) late phase of the formalin-induced paw licking test. Each column represents the mean±SEM of the total licking latency (sec) of eight mice. ***p<0.001 when compared with the control group receiving saline and #p<0.001 when compared with the SUMA group receiving sumatriptan.

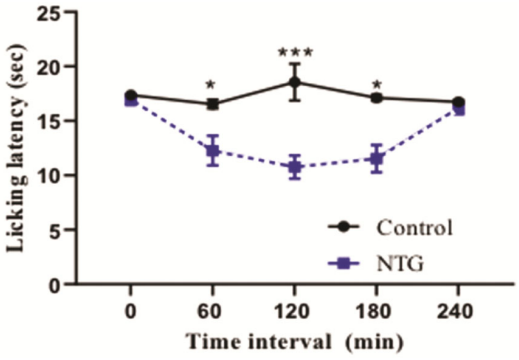

Fig. 4 — The effect of nitroglycerin (NTG) on the hot plate test. Each point represents the mean±SEM of the total licking latency (sec) of eight mice. ***p<0.001 and *p<0.05.

Administration of NTG (10 mg/kg) produced significant, clear-cut hyperalgesia, manifested by reduced response latency, compared to the control group receiving saline. The effect was observed at the 60th minute and peaked at the 120th minute after the administration of NTG. This effect persisted until the 180th minute, and a near recovery result was apparent by the 240th minute.

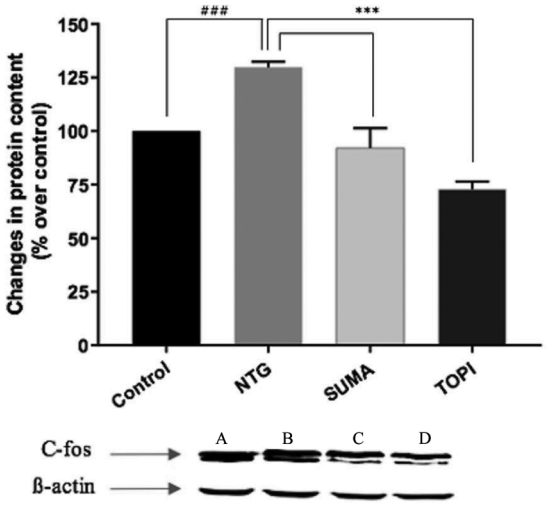

Fig. 5 — The effect of nitroglycerin (NTG) on C-fos expression in trigeminal nucleus caudalis (TNC). The upper panel represents the densitometric quantification by ImageJ, and the lower panel represents a western blot of rat C-Fos or β-actin. A=Control group; B=NTG group; C=SUMA group; D: TOPI group. ###p<0.001 as compared with the Control group. ***p<0.001 as compared with the NTG group.

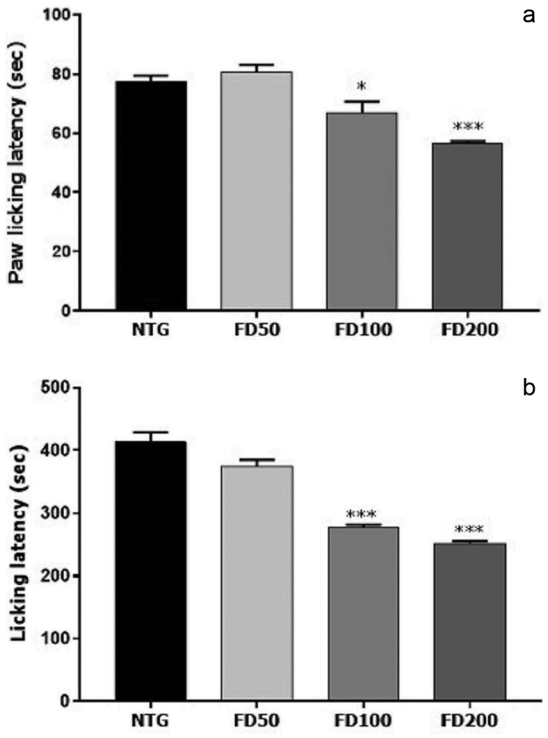

Fig. 6 — The effect of FD. a) early phase; b) late phase of the formalin-induced paw licking test. Each column represents the mean±SEM of the total licking latency (sec) of eight mice. *p<0.05, ***p<0.001.

NTG-induced C-Fos production- immunoblotting

Administration of NTG (10mg/kg) produced a significant increase in C-Fos expression in TNC as compared to the control group receiving physiological saline (p <0.001), as shown in Fig. 5. Sumatriptan administration did not significantly reduce C-Fos expression induced by NTG while topiramate administration significantly reduced NTG-induced C-Fos expression (p <0.001).

Effect of FD on nitroglycerin-induced hyperalgesia - formalin test

The lowest dose of FD (50 mg/kg, i.p.) did not produce any significant inhibition of the nociceptive behaviour in both the early and late phases of the formalin test. Administration of extract at doses of 100 and 200 mg/kg produced significant inhibition observed in both early (16.05 and 56.67%, respectively) (Fig. 6a) and late phases (31.95 and 37.98%, respectively) (Fig. 6b).

Effect of FDA on nitroglycerin induced hyperalgesia - hot plate test

Administration of the FD (50, 100 and 200 mg/kg) produced a significant prolongation in the latency response time to the heat stimulus (Table 1). The lowest dose of FD (50 mg/kg) produced significant inhibition starting from the 120th minute up to the 180th minute. FD at doses of 100 and 200 mg/kg produced significant inhibition, which began early at 60th minute and lasted up to 240th minute.

Table 1 — Effect of FD on the hot plate test compared to the NTG group

Treatment	Dose		Latency Time (s)			
	(mg/kg)	0	60	120	180	240
NTG	10	16.95	12.27	10.76	11.53	16.18
		±0.48	±1.36	±1.07	±1.25	±0.56
FD	50	14.65	16.14	16.54*	16.15*	15.83
		±0.99	±0.66	±1.00	±0.88	±0.87
	100	15.51	18.16*	20.08#	17.01#	16.83
		±0.36	±1.63	±1.72	±1.86	±1.66
	200	16.21	18.98#	23.81#	21.05#	21.33*
		±0.63	±0.96	±1.32	±1.53	±1.36

Each point represents mean±SEM of the total licking latency (sec) of eight mice. *p<0.001, #p<0.0001

Effect of FD on NTG-induced C-Fos expression- immunoblotting

Administration of FD at doses of 100 and 200 mg/kg significantly reduced the number of C-Fos expressions in TNC (Fig. 7). There was no significant effect of FD 50 mg/kg on C-Fos protein expression.

Discussion

The present study revalidated the preclinical use of NTG to construct animal models of hyperalgesia and neuronal activation. The NTG-induced mice model showed significant hyperalgesia in both formalin and hot plate tests, besides the increased expression of C-Fos in their TNC. The revalidation of the model in the present study is necessary in order to overcome differences in laboratory setups that might alter behavioural studies. Nevertheless, the measured outcomes in the present study are in consonance with several rodent studies[29,32]. Further, the present study demonstrated that administration of FD to NTG-induced mice model attenuates hyperalgesia and decreases the level of C-Fos in their TNC. To the best of the literature searched so far, this is the first study demonstrating the therapeutic role of an aqueous extract of FD in an NTG-induced mice model.

There are several theories on migraine pathophysiology. However, none of the hypotheses can explain the various symptoms that occur in migraineurs[33]. The throbbing headache in migraineurs is recognized as a result of activation of the trigeminovascular pathway, which conveys inputs from the meninges, which are innervated by afferent nerves that originate in the trigeminal ganglion[34]. These fibres convey information from intracranial structures and synapse with second-order neurons within the trigemino cervical complex (TCC), which consists of the TNC and the upper two cervical divisions. The neurons transmit information to the third-order neurons in the contralateral thalamus. During the activation of these peripheral meningeal nociceptors (first-order TGVS neurons), there is a release of ions, protons, and inflammatory agents sensitizing the peripheral nociceptor, resulting in peripheral sensitization. Peripheral sensitization refers to an increased responsiveness or sensitivity of primary afferent neurons to external mechanical or thermal stimuli. The exact inflammatory mediators participating in this activation and sensitization are still unknown[35]. In migraine attacks, throbbing pain and its aggravation during routine physical activities, such as coughing, sneezing or bending over, were thought to be mediated by peripheral sensitization[36]. In sensitization of the peripheral nociceptors, there is a development of spontaneous activity manifested by the continuous bombardment of impulses to the second-order neurons. The dorsal horn neurons that receive an increased number of signals from the periphery become hyperexcitable and begin to respond to previously non-noxious stimuli. This further sensitization of second- and third-order neurons led to their sensitization, which is termed central sensitization. The course of third-order neurons explains extracephalic pain hypersensitivity during a migraine attack.

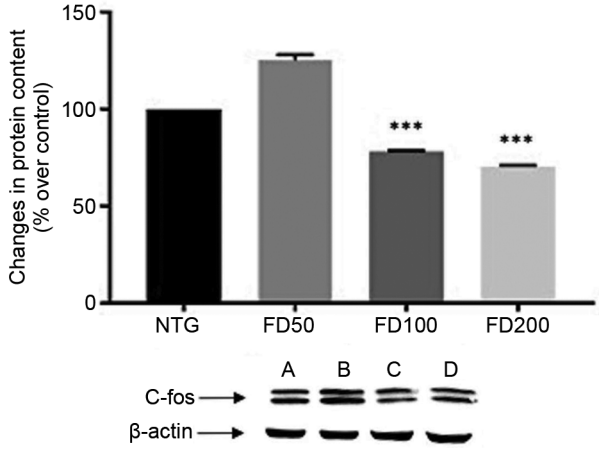

Fig. 7 — Effect of FD on NTG-induced C-fos expression. The upper panel represents the densitometric quantification by ImageJ, and the lower panel represents the western blot of rat c-fos or β-actin.A=Control group; B= FD150 group; C= FD100; D=FD200group.***p<0.001.

Administration of an aqueous extract of FD was able to inhibit hyperalgesia induced by NTG in both the formalin and hot plate tests. The formalin test is one of the best methods being extensively used to evaluate nociception in animal models such as mice and rats[37]. The nociceptive effect of formalin injection in the hind paw of a rodent consists of two phases characterized by different mechanisms. The early phase (neurogenic pain) starts immediately after formalin administration and involves direct stimulation of sensory afferent C-fibers by formalin. The late phase (inflammatory pain) begins 10 minutes to 1 hour after formalin administration and involves peripheral inflammatory processes. It is suggested that NTG exerts its activity through a direct effect on nitric oxide (NO) production and pro-inflammatory mediators at the central level or indirectly via inflammation mediated by NO-dependent mechanisms at the periphery as a consequence of trigeminovascular sensitization[38]. Therefore, it is suggestive that the anti-hyperalgesia effects of FD in

the present study could be due to its interaction at the central level or the periphery.

In the hot plate test, maximum thermal hyperalgesia was observed at 120 minutes post NTG administration, which was a bit later when compared to studies reported by Bates *et al.*[29] or Farkas *et al.*[32]. The variation observed could be due to differences in the strain of mice used. However, the findings of complete or partial recovery by 4 hours after NTG administration are in line with previous research findings. As NTG is a vasodilator, the changes in blood pressure after NTG administration may influence nociceptive behavior[39]. However, it was previously reported that NTG, when given intraperitoneally at 10 mg/kg, produced no significant changes in blood pressure or heart rate at any of the experimental points[29].

The involvement of FD in neuronal activation induced by NTG administration was also evaluated in the present study. Sensitization of the trigemino-vascular system (TGVS), which is comprised of the TNC, trigeminal nerve, and intracranial arteries, via both peripheral and central pathways is considered one of the neuropathogenic mechanisms of migraine[40]. The central projections of TGVS and second-order neurons are contained within TNC. The TNC and its rostral connections are involved in nociceptive transmission and modulation. C-Fos is an immediate early transcription factor that is a sensitive marker of neuronal activation in response to various stimuli. Its expression is increased by NTG administration in rats[18]. In the present study, the anti-hyperalgesic effect of FD was paralleled with the inhibition of NTG-induced neuronal activation in TNC. The administration of FD was able to suppress the production of C-Fos induced by NTG, suggesting its ability to reduce neuronal activation through its mechanism of action.

Previous studies isolated two main phytochemical constituents in FD, which are vitexin and an isomer of vitexin, isovitexin. These two compounds have a wide range of pharmacological effects, namely, anti-nociceptive[41], anti-inflammatory[42], anti-oxidant activity[43], anti-Alzheimer's disease[44] and so on. The ability of FD to abolish acute attacks of migraine may be due to its anti-nociceptive activity. Furthermore, the anti-inflammatory effect of FD may contribute to the suppression of neurogenic inflammation in migraines, which leads to the inhibition of activation of TGVS and further abolishing migraines. Taken together, the results of this study provided evidence that an aqueous extract of FD with the active compounds vitexin and isovitexin possessed anti-hyperalgesic activity in the NTG-induced mice model of migraine. The effects of FD could be due to its ability to prevent neuronal activation by suppressing C-fos production.

Conclusion

In conclusion, FD demonstrated significant anti-hyperalgesic activity in nitroglycerin-induced mice model of migraine, with the doses of 100 mg/kg and 200 mg/kg significantly reversing nociceptive behaviours in the formalin and hot plate tests (p <0.05). Additionally, FD markedly reduced C-Fos expression in the TNC, which is a key marker of neuronal activation in pain pathways. The significant reduction in pain-related behaviours and C-Fos levels accentuates FD's potential as a natural remedy for migraines. Future research should focus on elucidating the underlying mechanisms of FD's effects in chronic migraine models. Additionally, examining the active compounds within FD may lead to the development of targeted migraine therapies.

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Conflicts of interest

The authors declare no conflict of interest.

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