

Antinociceptive and anti-inflammatory effects of thiamine in Long Evans rats

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Abstract

Background: Thiamine along with other B vitamins has been prescribed since long for treatment of various painful conditions, though individual effects of thiamine on nociception and inflammation are yet to be clearly demonstrated. **Objective:** To assess the effects of increasing doses of thiamine supplementation against pain and inflammation. **Methods:** This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2014 to February 2015. Total twenty four male Long Evans rats weighing (200±20 gm) were treated with three different doses of thiamine hydrochloride (THCL 100, 200, 250, mg/kg/day; experimental) or normal saline (5 ml/kg/day; control) intraperitoneally for 7 consecutive days. To evaluate the thiamine's effect on nociceptive pain, early phase (0-5 minutes) and on inflammatory pain, late phase (16-60 minutes) of the formalin test, were observed. In both phases, total frequency of jerking and total duration of flexing and licking of the right hind paw were counted after administration of subcutaneous formalin (50 µl, 2.5%) injection. After formalin test, all the rats were also subjected to formalin induced paw edema test using a water plethysmometer to observe the anti-inflammatory effect of thiamine. Statistical analysis was done by ANOVA, followed by Bonferroni post hoc test. In the interpretation of results, $p \leq 0.05$ was considered as significant. **Results:** In formalin test, thiamine lowered frequency of jerking (after all 3 doses, in both phases) and duration of flexing and licking (200 mg/kg - $p < 0.001$, in late phases; 250 mg/kg ($p < 0.001$), in both phases) significantly. Additionally, thiamine lowered paw edema significantly ($p < 0.001$) in higher 2 doses. **Conclusion:** This study concludes that, thiamine may have dose dependent antinociceptive and anti-inflammatory effects.

Key words: Thiamine, antinociceptive, anti-inflammation, formalin test, paw edema test.

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Introduction

Among the water soluble vitamin B complex, Thiamine (B_1) was isolated in 1930s and was one of the first organic compounds to be recognized as a vitamin¹. It was first named "Aneurin" (anti-neuritic vitamin)² and is one of the most important medication needed in basic health

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system³ in the present world. It is used in biosynthesis of different neurotransmitters, such as, acetylcholine and gamma-aminobutyric acid (GABA) in addition to its role as a coenzyme⁴. Thiamine deficiency may cause insomnia, fatigue, constipation, gastrointestinal distress, muscular atrophy, cramps in addition to beriberi (Wernicke-Korsakoff syndrome) with central as

well as peripheral nervous and cardiovascular symptoms leading to death⁵.

Thiamine with B₆ and B₁₂, have been prescribed for long, either alone or with other analgesics, for treatment of various painful conditions, which are not associated with their deficiencies, such as polyneuropathy, neuritis, painful vertebral syndrome, rheumatic diseases, carpal tunnel syndrome as well as premenstrual tension⁶⁻⁸. However, it has been proposed that, high therapeutic doses of B vitamins might be needed to be administered to exert their complete analgesic, antinociceptive or anti-inflammatory effects⁹⁻¹¹. Several studies have shown that high doses of thiamine with vitamin B₆ and B₁₂, potentiate the antinociceptive effect of diclofenac, ketorolac, ketoprofen as well as acetaminophen¹²⁻¹⁵. Furthermore, in several animal studies combined thiamine, B₆ and B₁₂ have been found effective to reduce nociceptive and inflammatory pain, both after single (acute) as well as after chronic administrations (for 3-7 consecutive days)^{9,13,15-19}.

It is noteworthy that, rats treated with high doses of B vitamins have not shown any toxic effects²⁰. Moreover, repetitive (chronic) administrations of the B vitamins produced long term inhibition of both severity and duration of pain²⁰. Recently, the analgesic and anti-inflammatory effects of thiamine have been demonstrated in different types of animals and the acute and chronic antinociceptive effect of thiamine was evaluated by hot plate test in mice model^{21,22}. In addition, 125 mg/kg/body weight of thiamine decreased acute inflammation in the animals' ear induced by xylene²¹. However, Franca et al. have shown that, thiamine at chronic (for 3-7 consecutive days administration) doses with 50 and 100 mg/kg/body weight, reduced pain in the second phase (16th to 30th minutes) of formalin test¹⁶. Similar trend of decrement in the inflammatory pain after administration of different doses of combination of thiamine, B₆ and B₁₂ have been reported in several studies^{13,15,17,18}.

Formalin test is one of the valid and reliable animal models to assess both the nociceptive as well as inflammatory form of pain and is sensitive to both centrally as well as peripherally acting analgesics^{23,24}. Here, subcutaneous injection of formalin at hindpaw induces inflammation, which leads to a response characterized by jerking, flexing followed by licking of the affected hindlimb^{23,25,26}. Pain intensity is measured by converting these behavioral responses into numerical values^{24,25,26,27}, assessed in early phase (first 5-7 minutes, results from direct chemical stimulation of the nociceptive afferent fibers mainly C fibers)²⁸, while the late phase (last 40-45 minutes, results from the action of locally released inflammatory mediators and also by the facilitation of synaptic transmission in the spinal cord)²⁹.

In addition, the formalin induced paw edema test in rodents is a very simple, accurate and one of the common models for the assessment of inflammation in animals^{30,31,32}, where the characteristic hindpaw edema has been associated with increased amount of chemical mediators in tissue fluids³¹.

In recent years, various researchers have intended to find out alternatives to the conventional analgesics in order to replace them with natural substances, or to decrease their doses to minimize their adverse effects. Though antinociceptive and anti-inflammatory effects of thiamine with different doses and duration were reported but there is lack of information about the potency of aforementioned effects with higher doses along with chronic administration of this vitamin. Therefore, this study was designed to evaluate the antinociceptive as well as anti-inflammatory effects of chronic (single daily dose for 7 consecutive days) supplementations of thiamine in increasing doses (100, 200 and 250 mg/kg of body weight) by formalin test and formalin induced paw edema test, in rats.

Methods

This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2014 to February 2015, with prior protocol approval from the Institutional Review Board of the University.

Procurement and maintenance of animals^{33,34}:

Twenty four (24) male Long Evans rats weighing 180 to 220 gm were obtained from animal house of Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka, and were kept under a 12/12 hour light/dark cycle in a room with constant temperature (28±50C). The animals were acclimatized for 7 consecutive days prior to the experiments and had free access to standard laboratory food and cooled boiled water. The experiments were performed during the day time between 8:00 to 14:00 hours, to avoid any circadian influences. All experiments and animal care were performed according to the guidelines set in the 'Manual for Care and Use of Laboratory Animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

Dose schedule^{5,16,21,35,36}:

Experimental solutions of thiamine (100, 200, 250 mg/kg) were prepared by dissolving desired amounts of thiamine hydrochloride (THCL) in 5 ml/kg of normal saline (NS). All compounds (THCL for experimental animals and NS for control animals) were administered intraperitoneally for 7 consecutive days and the last dose was administered 1 hour prior to the experiments.

Formalin test^{16,20,22,30,37-38}:

The rats were acclimatized in the experimental laboratory environment, in the observation cage of the plexiglass formalin box (30×30×30 cm³) for 1 hour daily (within 8:00 to 14:00 hours) for 7 consecutive days prior to the formalin test. Then on the day of experiment (Day 7), 1 hour after last dose of (i.p.) injection of THCL or equal

volume of NS, the rats were restrained by a thick towel and 50 µl of dilute formalin (2.5%) was injected subcutaneously into the plantar aspect of the rats' right hindpaw with an insulin syringe. Immediately thereafter, the animal was placed in the observation cage of the formalin box and the pain behaviors were observed for 60 consecutive minutes. Within this time the first 5 minutes (1st – 5th) was considered as the early phase, and last 45 minutes (16th – 60th) as the late phase. Observation was made by counting the total frequency of jerking and total duration of flexing plus licking of the injected paw during this time through a mirror fixed below the formalin box at 45° angle. A stop watch was used to note the time.

Formalin induced paw edema test^{16,31,39}:

Anti-inflammatory effect of thiamine was determined by the formalin induced paw edema model, where the amount of paw edema resulted from intra-planter injection of 50 µl of 2.5% formalin was used an indicator of inflammation severity. Following 60 minutes of recording the pain behaviors (about 120 minutes after the i.p administration of THCL or NS), the animal was sacrificed. Then the volumes of the animal's right and left hind paws were measured by using a water plethysmometer. Left paw volume was subtracted from right paw volume to attain the net edema volume.

Data were expressed as mean±SEM and statistically analyzed by SPSS (version 17.0) using analysis of variance (ANOVA), followed by a Bonferroni Post hoc test. In the interpretation of results, p value ≤ 0.05 was considered as significant.

Results

In this study, THCL significantly reduced the jerking frequency ($p \leq 0.001$, in all doses) and flexing and licking duration ($p \leq 0.001$, in 250mg/kg) in the early phase of formalin test (Figure 1).

In addition, this vitamin also reduced the jerking frequency ($p \leq 0.001$, in all doses) as well as the duration of flexing and licking ($p \leq 0.001$, in 200

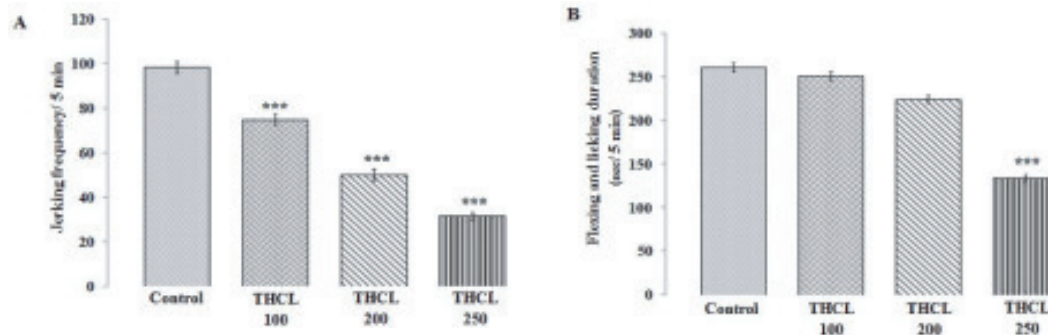


Figure 1: Antinociceptive effects of thiamine hydrochloride (THCL) in the early phase of formalin test (1st-5th minutes). THCL (at 100, 200 and 250 mg/kg doses) lowered both jerking (A) and flexing and licking (B) responses. Each bar symbolizes for mean \pm SE for 6 rats. ***= $p < 0.001$, compared to control.

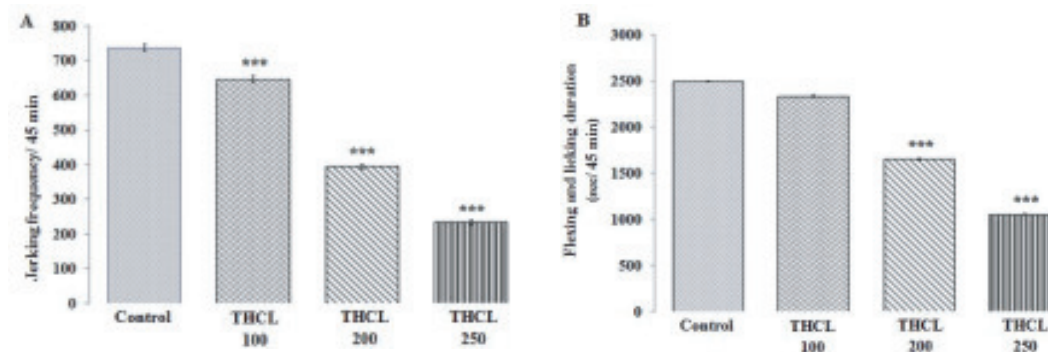


Figure 2: Antinociceptive effects of thiamine hydrochloride (THCL) in the late phase of formalin test (16th-60th minutes). THCL (at 100, 200 and 250 mg/kg doses) lowered both jerking (A) as well as flexing and licking (B) responses. Each bar symbolizes for mean \pm SE for 6 rats. ***= $p < 0.001$, compared to control.

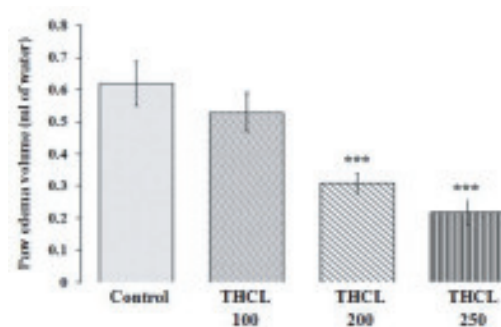


Figure 3: Anti-inflammatory effects of thiamine hydrochloride (THCL at 100, 200 and 250 mg/kg doses) in formalin induced paw edema model. Each bar represents for mean \pm SE for 6 rats. ***= $p < 0.001$, compared to control.

and 250 mg/kg) in the late phase of formalin test (Figure 2).

Moreover in the outcome of the paw edema test, THCL showed statistically significant ($p < 0.001$) decrement in edema volume after 200 and 250 mg/kg in the paw edema model (figure 3).

Discussion

In this study, thiamine supplementation in chronic (consecutive 7 days) administration elicited potent antinociceptive effects, as evidenced by significant lowering of jerking frequency as well as duration of flexing and licking in both phases of formalin test in experimental rats. Similar trend

of findings has been observed by Franca et al.¹⁶, though they investigated the effect of administration of individual thiamine as well as combined (20:20:1) thiamine, pyridoxine and cyanocobalamine (TPC), both at 50 and 100 mg/kg (i.p.). Furthermore decrement of pain response was also reported by other researchers after single oral administration of TPC at different doses in different pain models^{13,18,19}.

Again, in this study, chronic supplementation of B₁ (200 and 250 mg/kg) significantly lowered the edema in the formalin induced paw edema test. Similar observations were reported by Moallem and his colleagues after single supplementation of B₁ alone²¹ and also by Franca et al. after chronic supplementation of combined B₁, B₁₂ and B₆ in mice models in different dosage from this study¹⁶.

The exact possible mechanisms regarding these observed effects cannot not be revealed directly from the present study. However, several investigators of different countries proposed various suggestions on these aspects, which might be cause of our present findings. It has been suggested that, antinociceptive effect of THCL may be due to increase inhibitory control of afferent nociceptive neurons at the spinal cord along with decrease response of thalamic neurons to nociceptive stimulation^{40,41}. Moreover, thiamine supplementation may also increase the availability or effectiveness of serotonin as well as noradrenaline in the CNS^{24,26,27}. Besides the central effects in lowering nociception, it has been proposed that peripheral anti-inflammatory effect of this vitamin may be mediated through regulation of arachidonic acid pathway in macrophage, where it can block the expression of any of the enzymes (cyclooxygenase-2, lipoxygenase-2, thromboxane B synthase, prostacycline synthase) as well as transcription factor nuclear factor kappa²⁸. In addition, this vitamin may also decrease the oxidative stress and production of free radicals²⁹.

As, all 3 doses of THCL(100, 200 and 250 mg/kg) in our study significantly lowered the early and late phases of formalin test, it is suggestive

that all those doses possess both the central and peripheral antinociceptive effects. Along with this, significant reduction of formalin induced hind paw edema after THCL administration, suggested its probable role in amelioration of inflammation and thereby inflammatory pain with higher two doses of (200 and 250 mg/kg). In addition all these effects of B₁ on lowering pain and inflammation in the present study were related with the dose of supplementation, as evidenced by more decrement of all the study variables in the higher dose (250 mg/kg/day) group. It is suggested that, chronic supplementation at progressively higher doses might cause more accumulation of B₁ in the serum which probably exerted a cumulative effect for greater decrement of pain and inflammatory variables in our study. Strikingly, the lowest dose (100 mg/kg) of THCL used in our study did not lower the paw edema significantly, whereas it inhibited the jerking frequency significantly in the late phase of formalin test. These findings incite our thinking that B₁ may possess any other mechanism of decrement of inflammatory pain other than anti-inflammation.

Conclusion

In conclusion, it is recommended that, chronic thiamine supplementation may diminish both the acute and chronic forms of nociception and may have potent role against inflammation, which are dose dependent. However, further experimental study is recommended to elucidate the exact mechanism responsible for these effects.

Conflict of interest: none

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