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Evaluation of antipyretic, sedative and hypnotic activities of methanol extract of *Tabebuia hypoleuca* (C. Wright ex Sauvalle) Urb. stems

[Evaluación de la actividad antipirética, sedante e hipnótica del extracto metanólico de los tallos de *Tabebuia hypoleuca* (C. Wright ex Sauvalle) Urb.]

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Abstract: Species of the genus Tabebuia are used in traditional medicine and are reported in the literature for their properties against various diseases. The objective of this study was to evaluate the antipyretic, sedative and hypnotic activities of methanol extract of Tabebuia hypoleuca stems (THME) using the Brewer's yeast induced pyrexia, Open field and Sodium thiopental-induced sleeping time tests, respectively. In the Brewer's yeast induced pyrexia test, THME at 500 mg/kg produced a significant ($p<0.001$) decrease of the fever as from the first hour after administration and was sustained for 4 h. In the Open-field test, THME did not cause any significant change in the number of crossings, rearing, preening and defecation, and either in the time of immobility. Moreover, THME did not produce changes in neither the sleeping latency nor the sleeping time induced by sodium thiopental. These results showed that THME administered orally at 500 mg/kg exerts antipyretic activity, probably mediated by the inhibition of the enzyme cyclooxygenase-2. This study also showed that THME does not exert sedative and hypnotic effects at the doses tested.

Keywords: *Tabebuia hypoleuca*, antipyretic, sedative, hypnotic, methanol extract

Resumen: Especies del género Tabebuia se utilizan en la medicina tradicional y se reportan en la literatura por sus propiedades contra diversas enfermedades. El objetivo de este estudio fue evaluar la actividad antipirética, sedante e hipnótica del extracto metanólico de los tallos de Tabebuia hypoleuca (THME) utilizando las pruebas de pirexia inducida por levadura de cerveza, campo abierto y tiempo de sueño inducido por tiopental sódico respectivamente. En el ensayo de pirexia inducida por levadura de cerveza, THME a 500 mg/kg produjo una reducción significativa ($p<0.001$) de la fiebre a partir de la primera hora después de la administración y se mantuvo durante cuatro horas. En el ensayo de campo abierto, THME no causó ningún cambio significativo en el número de cruces, levantamientos, acicalamientos y defecación, ni en el tiempo de inmovilidad. Además, THME no produjo cambios ni en la latencia de sueño, ni en el tiempo de sueño inducido por tiopental sódico. Estos resultados mostraron que THME administrado oralmente en dosis de 500 mg/kg posee actividad antipirética, mediado probablemente a la inhibición de la enzima ciclooxigenasa-2. Este estudio también demostró que THME no posee actividad sedante e hipnótica en las dosis ensayadas.

Palabras clave: *Tabebuia hypoleuca*, antipirético, sedativo, hipnótico, extracto metanólico

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INTRODUCTION

Pyrexia or fever is the increase in body temperature above normal physiological range (36.5 - 37.5 °C). It is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. The infected or damaged tissue initiates the increase formation of pro-inflammatory mediators such as cytokines, interleukin, interferon and tumor necrosis factor α (TNF-α) which increase the synthesis of prostaglandin E_2 (PGE_2) near the peptic hypothalamus area and the prostaglandin in turn act on the hypothalamus to elevate the body temperature (Sultana *et al.,* 2015).

The elevated body temperature is reduced by non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit cyclooxygenase (COX) expression thereby inhibiting prostaglandin synthesis. However, these synthetic antipyretic agents inhibit the COX-2 with high selectivity but they have toxic effects on other organs like glomeruli, cortex of the brain, hepatic cells and heart muscles (Vane & Botting, 1995; Bhowmick *et al.,* 2014). This fact explains the need to search for new safer and more effective therapeutic options.

Tabebuia spp. (Bignoniaceae) includes approximately 100 species, known as strictly woody, found in tropical rain forest areas throughout Central and South America (Olmstead *et al.,* 2009). Species of the genus *Tabebuia* have been traditionally used and are reported in the literature for their properties to treat syphilis, malaria, cutaneous infections, stomach disorders, fever, cancer, inflammation, pain, bacterial and fungal infections, anxiety, poor memory, irritability, depression and others (De Miranda *et al.,* 2001; Higa *et al.,* 2011; Sadananda *et al.,* 2011; Gómez *et al.,* 2012, Lee *et al.,* 2012; Franco *et al.,* 2013; Freitas *et al.,* 2013; Cragg *et al.,* 2014; Ferreira *et al.,* 2014).

Tabebuia hypoleuca (C. Wright ex Sauvalle) Urb. commonly known as "Roble macho" is an species endemic of Cuba and native to Sierra Maestra and Guantanamo. Regalado et al. (2015) demonstrated the anti-inflammatory activity of the methanol extract of *T. hypoleuca* stems (THME) using the carrageenin-induced paw edema models and the croton oil induced auricular edema in mice. Subsequently, Regalado *et al.* (2017) demonstrated the antinociceptive activity of this extract using several models (chemical and thermal) of nociception in mice and suggested that the effect was mediated by the participation of both peripheral and central antinociceptive mechanisms.

The present study was carried out to evaluate the antipyretic activity of THME administered orally in rats. Moreover, this study aimed to investigate sedative and hypnotic effect in order to exclude the possibility that the antinociceptive action shown in previous studies could be related to non-specific disturbances in the locomotor activity of the animals, affecting motor coordination as a consequence of sedation, hallucination, anxiety or fear.

MATERIAL AND METHODS

Plant material and extraction

T. hypoleuca stems were collected at the National Botanical Garden (JBN), Havana province, Cuba. The identification of the plant was confirmed by Dr. R. Eldis Becquer and the sample was deposited in the herbarium of the experimental station with the number HFC-88204. The total extract was obtained from 330 g of *T. hypoleuca* stems by solid-liquid extraction in Soxhlet apparatus with methanol (Merck®). The methanol extract was filtered and concentrated by rotary evaporation.

Drugs and chemicals

The extract and all drugs were diluted in 0.9% saline solution (NaCl diluted in distilled water). The drugs and chemicals used were: paracetamol (SOLMED, Havana, Cuba), thiopental sodium (Quimefa, Havana, Cuba), diazepam (AICA, Havana, Cuba) and methanol (Merck, Germany).

Animals

Male Sprague-Dawley rats (180-200 g) and Male Balb/c mice (20-25 g) were supplied by the National Center for Laboratory Animal Production (CENPALAB, Santiago de Las Vegas, Havana, Cuba). Animals were raised under standard conditions of 23 ± 2 °C with 40-60% relative humidity under a 12/12 h light-dark cycle, and were given food and water *ad libitum* for seven days. All experimental procedures were performed in accordance with International Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Ethical Committee of the National Center for Animal and Plant Health (CENSA),

Havana, Cuba. The number of animals and the intensity of the noxious stimuli were the minimum necessary to obtain reliable data.

Antipyretic activity: Brewer's yeast induced pyrexia method

The antipyretic activity of THME was evaluated using Brewer's yeast induced pyrexia in rats as described by Asongalem *et al.* (2004). Male rats were divided into five groups of six rats each. Hyperthermia was induced by subcutaneous injection of 25% Brewer's yeast (1 mL/100 g body weight). Before yeast injection, basal rectal temperature of rats was recorded. At the18h after yeast injection, the rectal temperatures were recorded again, and the animals were treated orally with paracetamol (150 mg/kg), distilled water (10 ml/kg), and THME (150, 300 and 500 mg/kg). Rectal temperature was recorded by digital thermometer at 0^{th}, 1^{st}, 2^{nd}, 3^{rd} and 4^{th} h after drug administration. The percent reduction in pyrexia was calculated by the following formula:

$$\text{Percent reduction} = \frac{\text{Pyrexia induction temperature} - \text{Post-treatment temperature}}{\text{Pyrexia induction temperature} - \text{Normal body temperature}} \times 100$$

Sedative activity: Open-field test

The apparatus consisted of a plastic box measuring 45×45×20 cm, with the floor divided into nine equal squares 15×15 cm (Spindola *et al.*, 2012). Each mouse was gently placed at the centre of the open field and the following parameters were registered for 6 min: crossing (the number of squares crossed with all paws), rearing (rising on hind paws), preening, defecation and time of immobility (De Mattos *et al.*, 2007). For this purpose, male mice were divided into five groups of six mice each. The animals were treated orally with diazepam (4 mg/kg), distilled water (10 ml/kg), and THME (150, 300 and 500 mg/kg) 60 min beforehand. The floor of the open field was cleaned with ethanol after each session.

Hypnotic activity: Sodium thiopental-induced sleeping time test

The experiment was conducted following the method described by Ferrini *et al.* (1974). Male mice were divided into five groups of six mice each. The animals were treated orally with diazepam (1 mg/kg), distilled water (10 ml/kg), and THME (150, 300 and 500 mg/kg). Sixty minutes later, thiopental sodium (40 mg/kg, i.p) were administered to each mouse to induce sleep. The animals were observed and the following parameters were registered: sleeping latency (time between thiopental administrations to loss of righting reflex) and sleeping time (time between the loss and recovery of righting reflex).

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS (version 21.0). The data were expressed as mean ± SEM. One-way ANOVA, followed by Dunnett's post hoc test was applied to determine the significant differences between the control and treated groups. P-values < 0.05 were considered statistically significant.

RESULTS

Antipyretic activity

In this test the animals showed a marked increase of rectal temperature after 18 h of subcutaneous injection of Brewer's yeast. Subsequently, the oral administration of THME at 500 mg/kg and paracetamol at 150 mg/kg, caused a significant reduced ($p < 0.001$) yeast induced pyrexia in rats as from the first hour after administration and was sustained for 4 h, while control group and THME at 150 and 300 mg/kg showed no antipyretic activity in the entire period of experiment (Table 1). The maximum antipyretic effect of THME and paracetamol was observed at 2 h, with a maximum inhibition percentage of 54.76% and 72.59% respectively

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Table 1
Effect of the oral administration of THME (150, 300, 500 mg/kg) and paracetamol (150 mg/kg) on Brewer's yeast induced pyrexia test in rats.

Treatment	Dose	Temperature in ºC					
		Initial	18 h	1 h	2 h	3 h	4 h
Control	10ml/kg	37.22 ± 0.07	39.73 ± 0.08	39.68 ± 0.08	39.52 ± 0.21	39.75 ± 0.11	39.70 ± 0.08
Paracetamol	150mg/kg	37.23 ± 0.10	39.55 ± 0.09	38.02 ± 0.21*** (68.5%)	37.90 ± 0.12*** (72.6%)	37.95 ± 0.18*** (71.8%)	38.00 ± 0.09*** (67.7%)
THME	150mg/kg	37.12 ± 0.17	39.78 ± 0.08	39.42 ± 0.11 (13.3%)	39.28 ± 0.29 (20.6%)	39.53 ± 0.11 (8.8%)	39.72 ± 0.08 (2.5%)
THME	300mg/kg	37.22 ± 0.12	39.68 ± 0.10	39.35 ± 0.15 (13.5%)	39.10 ± 0.13 (22.4%)	39.47 ± 0.17 (8.6%)	39.37 ± 0.21 (13.8%)
THME	500mg/kg	37.17 ± 0.08	39.43 ± 0.11	38.58 ± 0.23*** (37.3%)	38.22 ± 0.11*** (54.8%)	38.37 ± 0.09*** (47.6%)	38.30 ± 0.06*** (50.5%)

Values are expressed as mean ± SEM (n = 6).
****** P < 0.001 vs. control group by one-way ANOVA followed by the Duncan´s Test.***

Sedative activity

The oral administration of THME (150, 300 and 500 mg/kg) did not cause any significant change in the crossing (Figura 1A), rearing (Figura 1B), preening (Figura 1C), defecation (Fig. 1D) and time of immobility (Figura 1E) compared to the control group over a 5-min period. However, the group treated with diazepam (4 mg/kg, p.o.) showed a significantly diminution ($p < 0.01$, $p < 0.001$) of these parameters in comparison with the control group (Figuras 1A, B, C, D, E).

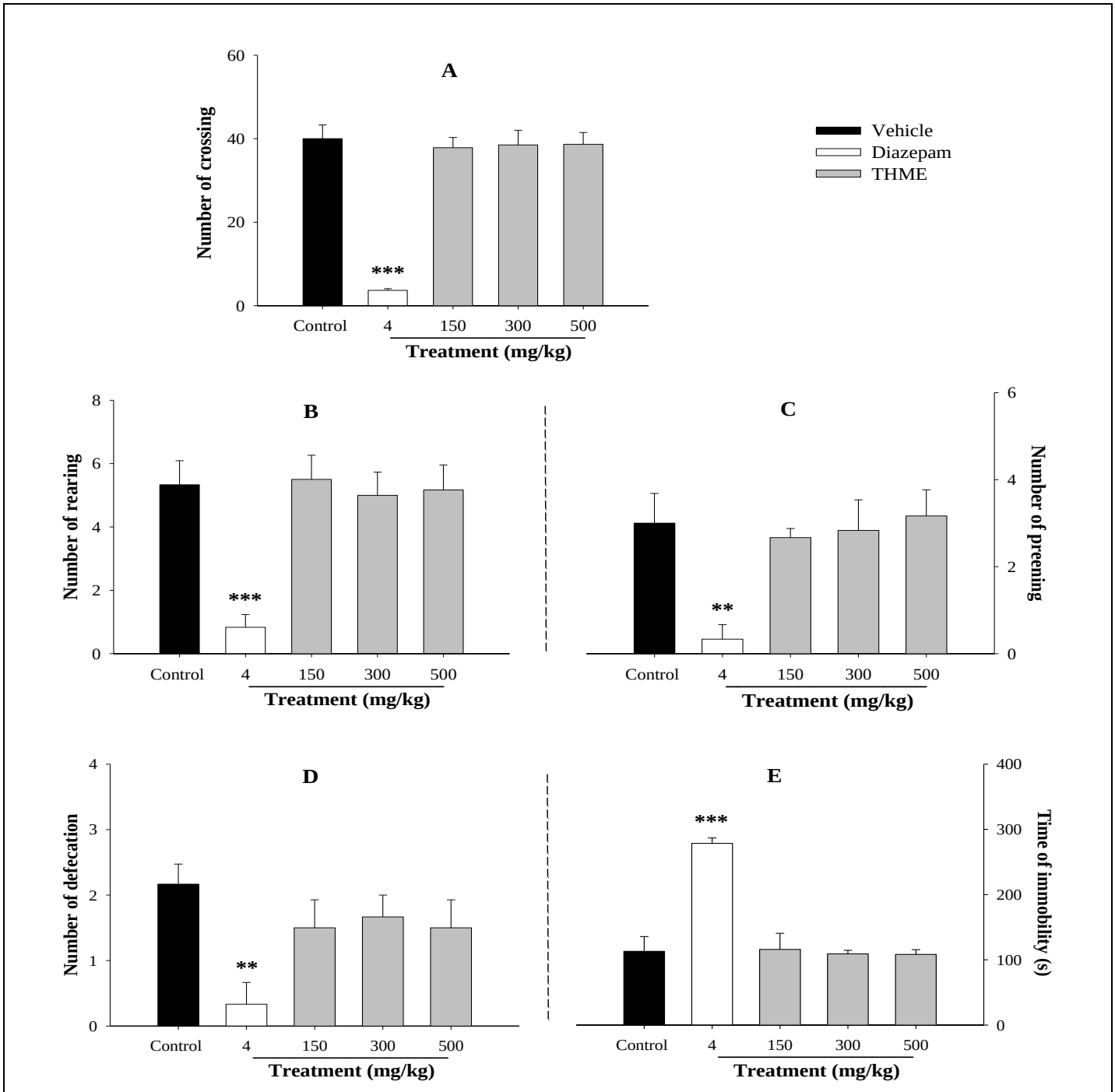

Figura 1. Effect of the oral administration of THME (150, 300, 500 mg/kg) and diazepam (4 mg/kg) in the open-field test. Crossing (A), rearing (B), preening (C), defecation (D) and time of immobility (E) were recorded. The results are presented as mean ± SEM (n=6). **$p < 0.01$, ***$p < 0.001$ vs. control group (one-way ANOVA followed by Dunnett test).

Hypnotic activity

Treatment with THME (150, 300 and 500 mg/kg, p.o.) did not produce changes in neither the sleeping latency (Figura 2A) nor the sleeping time induced by sodium thiopental (Fig. 2B), while diazepam (1 mg/kg, p.o.) significantly reduced ($p < 0.001$) the sleeping latency (Figura 2A) and the sleeping time (Figura 2B) compared with control group.

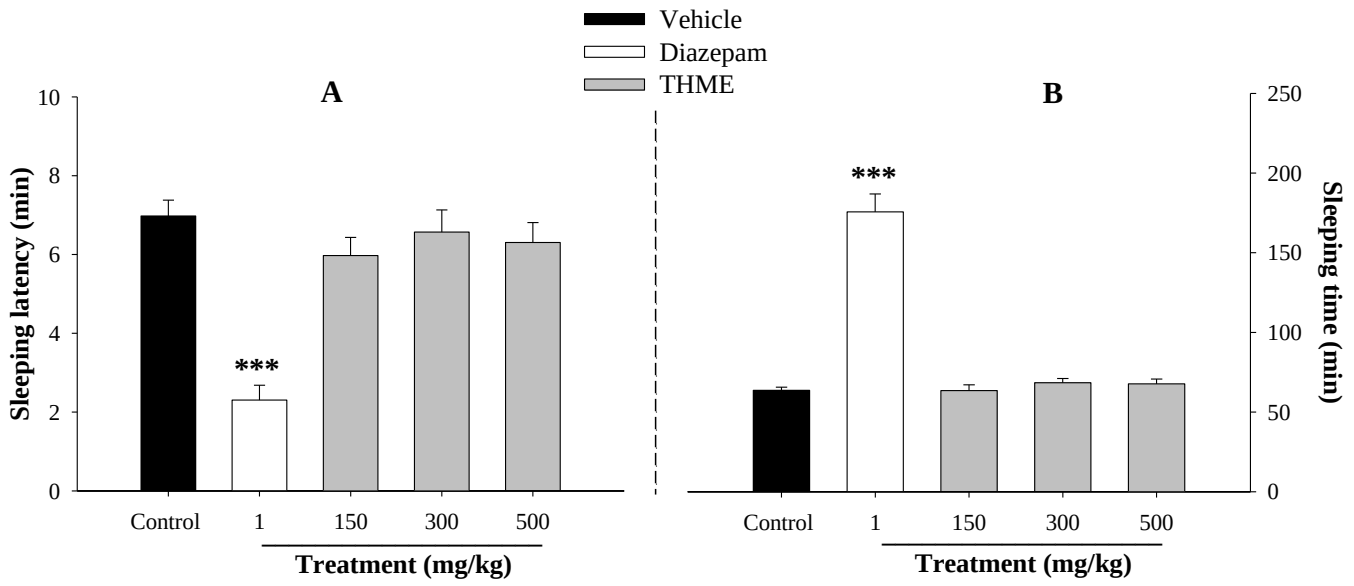

Fig. 2. Effect of the oral administration of THME (150, 300, 500 mg/kg) and diazepam (1 mg/kg) in the Sodium thiopental (45 mg/kg) induced sleeping time test. Sleeping latency (A) and sleeping times (B) were recorded. The results are presented as mean ± SEM (n=6). ***$p < 0.001$ vs. control group (one-way ANOVA followed by Dunnett test).

DISCUSSION

The World Health Organization (WHO) estimates that 80% of population depends on herbal preparations for their health care. In recent years interest in the WHO has grown in traditional medicine and ways are being sought to add traditional medicines to the International Classification of Diseases (ICD). Traditional medicines could become an effective resource for linking the Western and Eastern hemispheres (Kawahara *et al.*, 2010).

The present study was designed to determine the antipyretic, sedative and hypnotic activities of THME. The dose levels used to assess these pharmacological effects were selected based on a previous report of anti-inflammatory and antinociceptive activities for this extract (Regalado *et al.*, 2015; Regalado *et al.*, 2017).

To evaluate the antipyretic activity of THME, Brewer's yeast induced pyrexia test in rat were used. It is considered as a valuable *in vivo* screening test for the assessment of antipyretic potential (Rauf *et al.*, 2014). Brewer's yeast induces release of pyrogens which are subsequently phagocytized by Kupffer cells, monocytes and macrophages. Enhanced release of pro-inflammatory cytokines from these cells causes an increase in the synthesis of prostaglandins (Okokon *et al.*, 2012). The increased production of PGE2 stimulates the hypothalamus to generate responses to raise the body temperature (Shah & Seth, 2010). Although pyrexia causes unnecessary suffering and discomfort, it is considered as a natural defense mechanism that creates an environment where infectious agent or damaged tissue cannot survive (Mbiri *et al.*, 2016).

Studies have established that at least two COX isoenzyme exist, COX-1 and COX-2. The COX-1 is expressed constitutively and generally produces PGs to modulate physiological processes, synthesizes prostaglandins that protect the stomach and the kidney from damage; whereas COX-2 is

inducible and typically produces proinflammatory PGs in response to physiological stresses such as infection and inflammation; stimulates PGE_2 production that contributes to fever induction (Vane & Botting, 1998; Lee *et al.*, 2007).

NSAIDs such as aspirin, paracetamol, diclofenac and ibuprofen are the commonly used in the medication of fever (Warden, 2010). Most of the antipyretic drugs including the paracetamol (reference drug in this study) usually inhibit the cyclooxygenase (COX-2) expression and decreased the body temperature by reducing the synthesis of prostaglandins. Induction of toxicity to hepatic and renal glomerulus is evident with paracetamol during clinical treatment and in animal models (Jan & Khan, 2016). Continuous search for alternative therapeutic agents with minimum adverse effects is therefore necessary.

In this test, the oral administration of THME at 500 mg/kg, caused a significant reduced of yeast induced pyrexia in rats as from the first hour after administration and was sustained for 4 h. The results of this study suggest that the antipyretic activity of THME in Brewer's yeast induced pyrexia in rat could be attributed to the inhibition of the enzyme COX-2 responsible for the production of PGE_2.

In addition, THME at the same doses was studied for evaluated the central nervous system depressant effect using rodent behavioral models, such as Open field and Sodium thiopental-induced sleeping time tests in mice.

Initially, the open field test was performed in order to exclude the possibility that the antinociceptive action of THME shown in previous studies could be related to non-specific disturbances in the locomotor activity of the animals (Prut & Belzung, 2003; Spindola *et al.*, 2012). Treatment with THME (150, 300 and 500 mg/kg, p.o.) did not cause any significant change in the locomotor coordination activity of mice when tested in the open field. The results therefore, eliminate the possibility of locomotor impairment in the antinociceptive activity of the extract. In open-field tests when the animals are taken out from their home cage and placed in a new environment, they express anxiety and fear by showing alteration in all or some parameters, such as decreases in ambulation and exploration, immobilization or ''freezing'', reduction in normal rearing and in grooming behavior, and increased micturition and defecation due to augmented autonomic activity (Novas *et al.*, 1988). The oral administration of THME (150, 300 and 500 mg/kg) did not cause any significant change in the ambulation, rearing, preening, defecation and time of immobility compared to the control group over a 5-min period in mice. This study indicate that the THME did not exert sedative effects at any of the doses tested.

The Sodium thiopental-induced sleeping time test in mice, is a classic method used to evaluate drugs with sedative-hypnotic properties. "Thiopental" is basically a hypnotic agent given at appropriate dose, induced hypnosis by potentiating GABA mediated postsynaptic inhibition through allosteric modification of GABAergic receptors. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both (Raihan *et al.*, 2011). In this test, the treatment with THME (150, 300 and 500 mg/kg, p.o.) did not produce any significant change in the sleeping latency nor the sleeping time induced by sodium thiopental in mice. These results indicate that the THME did not exert hypnotic activity at any of the doses tested.

CONCLUSIONS

The present study demonstrated antipyretic activity of methanol extract of stems from *Tabebuia hypoleuca* administered orally in animal model, and suggest that this effect could be attributed to the inhibition of the enzyme COX-2. However, further investigations are required to identify the bioactive components responsible for the observed effect, and to determine the mechanism of action by which these compounds exert their antipyretic properties. Moreover, this study showed that THME no possesses sedative and hypnotic activities. This result therefore, demonstrated that antinociceptive activity of THME shown in previous studies has no relationship with CNS depression or sedation, eliminating the possibility of locomotor impairment in the antinociceptive activity of the extract.

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