Bioactivity-Guided isolation of β-Sitosterol and Some Fatty Acids as Active Compounds in the Anxiolytic and Sedative Effects of Tilia americana var. mexicana

Abstract

Tilia species have been used as anxiolytics for many years. In a previous study anxiolytic-like effects of a hexane extract of Tilia americana var. mexicana inflorescences were observed in experimental models in mice. To get additional insights into the neuroactive actions of this particular Tilia species, in this study we report a bioactivity guided-fractionation of the extract and separation by column chromatographic methods to isolate three fatty acids and a triterpene identified as β-sitosterol as major constituents. Our results revealed that the crude extract at 10 and 30 mg/kg i.p. and some pooled fractions at the same dosages potentiated sodium pentobarbital-induced sleeping time and caused a significant increase in the time spent at the open-arm sides in the plus-maze test. A reduction in the exploratory behavioral pattern manifested as ambulatory activity, as well as head dipping and rearing tests was also observed. Further fractionation and purification yielded four major fractions containing fatty acids and β-sitosterol as the active compounds. A dose-response curve of β-sitosterol in the range 1 to 30 mg/kg doses indicated that this compound produced an anxiolytic-like action from 1 to 10 mg/kg and a sedative response when the dose was increased to 30 mg/kg, these effects resemble those produced by diazepam (0.1 mg/kg). Our results suggest that hexane extract of Tilia americana var. mexicana produces depressant actions on the central nervous system, at least in part, because of the presence of β-sitosterol and some fatty acids that remain to be identified.

Key words
Anxiety · β-sitosterol · central nervous system · sedative · Tilia americana var. mexicana · Tiliaceae

Introduction

Around the world, Tilia species have been used in traditional medicine for their bioactive properties [1], [2], [3]. Infusions of the inflorescences of Tilia species are widely used in Latin America and Europe as sedatives and tranquilizers [4], [5]. The inflorescences of Tilia americana L. var. mexicana (Schldl.) Hardin, are prominent in phytotherapy. The flower infusion has been used for treating flu, cough, migraine, nervous tension, and some types of spasms, as well as liver and gallbladder disorders. However, it is mostly used in several regions of Mexico as a nerve tranquilizer [3].

Phytochemical studies have demonstrated that Tilia species synthesize hydrocarbons, esters, aliphatic acids, flavonoids and triterpenoids [5], [6], [7]. In addition, mucilage (10%), including arabinogalactans, uronic acid, carbohydrates [8] and tannins (2%) have also been reported. The relation between tannin and mucilage content is very important and related to the plant's pleasant flavor [2]. Quercetin 3,7-D-dirhamnoside, quercitrin, isorhamnetin, kaempferol 3,7-O-dirhamnoside, astragalin and tiliroside have been reported as major components in polar extracts of Tilia species [7]. Scientific studies have attributed the central nervous system effect of Tilia species to flavonoid constituents.
because of their ability to interact with high affinity benzodiazepine receptors [5], [9]. Nevertheless, there is little information about the non-polar compounds from *Tilia* species and their possible pharmacological properties.

Concerning *Tilia americana* var. *mexicana*, information in the literature about its pharmacological and chemical aspects is lacking. In a previous work we demonstrated the sedative and anxiolytic properties of both methanol and hexane extracts of this species evaluated in experimental models in mice [10]. In this study, we report for the first time the bioactivity-guided fractionation of a hexane crude extract of *Tilia americana* var. *mexicana* inflorescences to determine the active fractions and compounds mainly involved in its anxiolytic and sedative effects using experimental models in mice and intraperitoneal administration to ensure the maximum bioavailability of the active compounds.

Materials and Methods

Animals

Male Swiss albino mice (25–30 g, from Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico) were used in pharmacological tests. Animals were kept at constant room temperature (22 ± 1°C) and maintained on a 12 h light/dark cycle (lights on from 7:00 to 19:00). Experiments were carried out in accordance with the Ethical Committee Guidelines laid down by the local committee regarding the care and use of animals for experimental procedures and in compliance with international rules on care and use of laboratory animals. The animals were fed *ad libitum* with standard food and water during the course of the study.

Plant material

Inflorescences of *Tilia americana* L. var. *mexicana* (Schltdl.) Hardin (Tiliaceae) were collected in Omiltemic, Chilpancingo, Gro. in México in June 2005. Abigail Aguilar identified the specimen and a voucher (IMSSM-15 069) of the plant was deposited in the herbarium of the Instituto Mexicano del Seguro Social, Mexico, Federal District, for future reference.

Extraction and isolation

Air-dried, powdered ground inflorescences (1,865 g) were successively extracted with hexane (3x4 L) by maceration at room temperature (22°C). The solvent was separated from the residue by gravity filtration and then evaporated under vacuum. A yellow greasy hexane extract (25.8 g, 1.38% with respect to dried material plant) was finally obtained. This extract was separated on column chromatography (CC) using an open column packed with silica gel 60 GF254 Merck in a 1:15 proportion with respect to the extract and using an increasing amount of hexane gradually enriched with ethyl acetate (EtOAc). The elution started with hexane folowed by hexane-EtOAc mixtures (9:1, 8:2; 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), EtOAc (100%), EtOAc-methanol (MeOH) mixtures (9:1, 8:2 and 7:3), and ended with MeOH (100%). A total of 75 fractions, 100 mL each, was collected from the CC; those with similar thin layer chromatography (TLC) profiles were combined. Fractions F1–4 (P1); F5–12 (P2); F13–15 (P3), F17–19 (P4), F27–29 (P5); F31–37 (P6) and F38–42 (P7) were selected for showing the major yield (Fig. 1). These fractions were also tested in all experimental models (Tables 1 and 2). From the seven pooled fractions (P1 to P7) eluted with hexane-EtOAc mixtures (9:1, 8:2 and 7:3), four compounds were isolated and purified: three fatty acids and one triterpene (Fig. 1). The triterpene was purified as a white solid [15 mg, melting point (m.p.) 132°C] and identified as β-sitosterol, with 90% purity, as determined by using gas chromatography/mass spectrometry analysis (GC/MS).

Drugs

Sodium pentobarbital and diazepam were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Sodium pentobarbital was dissolved in saline solution (s.s.). The extract, fractions and diazepam (reference drug) were resuspended in the vehicle (0.5% tween 80 in s.s.). All drugs and extracts were freshly prepared on the day of the experiments and administered by intraperitoneal (i.p.) route in a volume of 10 mL/kg body weight. Control animals received the same volume of the vehicle and in the same route of administration.

Pharmacological evaluations

All mice were adapted to manipulation through a daily s.s. injection for 5 days before treatment administration. For each experimental procedure, animal groups consisted of mice without prior knowledge of the experimental conditions of the tests. Five or six animals were used for the bio-guided-fractionation, whereas ten animals for each of the four doses were used to plot the dose-response curves of β-sitosterol. The extract, fractions, and pure compounds were evaluated 60 min after administration. The results of the fractions at 10 and 30 mg/kg doses were compared with the sedative and anxiolytic-like effects of the same dosage of the hexane crude extract obtained from *Tilia americana* var. *mexicana* inflorescences. The effect of β-sitosterol was compared to that produced by diazepam (ED₅₀ = 0.1 mg/kg, previously determined in [11]) as reference drug. Experiments were performed blind in a quiet room by a skilled researcher. During the observations of mouse behavior, the investigator stood next to the apparatus occupying always the same place.

Sodium pentobarbital-induced hypnosis

For this test, mice received 42 mg/kg, i.p. of sodium pentobarbital. Each mouse was observed for the onset of uncoordinated movements (sedative phase) and the loss of righting reflex (hypnosis), but also for duration of sleep (the criterion for sleep is defined as the loss of righting reflex). The time between loss and recovery of the righting reflex was recorded as sleeping time [12, 13].

Ambulatory activity

Sixty minutes after the administration of hexane extract, fractions or pure compounds and prior to the anti-anxiety test, each mouse was placed into a cage divided into 12 squares (6 cm x 6 cm). The number of squares explored by each mouse in a 2 min interval was registered as ambulatory activity.

Anti-anxiety and sedative effect

For testing anti-anxiety and sedative responses, hexane extract, fractions and pure compounds were qualified in the following tests:

The elevated plus-maze: The elevated plus-maze used in this study was modified from Lister [14]. The apparatus consists of
two open arms (25×5 cm), facing each other, and two closed arms (25×5×15 cm) with an open roof and walls 40 cm high. After the mice had been placed in the centre of the maze, an observer took the total time spent in open-sided arms during a 5 min period test. An increase in this measure was taken as an anxiolytic-like response.

Hole board test: Mice were individually placed in the centre of a perforated board and the number of head-dips was registered during a 3 min trial. The perforated board test was made by using a plastic floorboard, in which 16 evenly spaced holes (3 cm in diameter) were made; the board was delimited by a glass box having 20 cm in height and 20 cm in deep [15]. The number of explored holes provides a measure of the number of head-dips.

Exploratory rearings: Each mouse was individually placed on a filter paper-covered floor of a glass cylinder (16 cm in height, 11 cm in diameter). The number of spontaneous rearings on its posterior limbs is counted during the first 5 min. Reduced exploratory rearing showed by naive mice after placement in an unfamiliar environment reveals a sedative effect [16]. Control animals received vehicle. Diazepam was used as positive control in all the tests.

**Statistical analysis**
Results are expressed as mean ± SEM. Statistical differences were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's test. A value of $P < 0.05$ was considered significant (we report the exact $P$ value). A SIGMA STAT version 2.3 software program was used.

**Results**
As shown in Table 1, the hexane extract of *Tilia americana* var. *mexicana* inflorescences did not modify the latency to the onset of sedation or hypnosis induced by sodium pentobarbital; however, the sleeping time was significantly increased in comparison to the control group ($F_{13,6} = 3.2, P = 0.001$). This effect was maintained or even potentiated when the extract was separated in fractions ($F_{13,6} = 3.2, P = 0.001$), which were previously chromatographed to produce pooled fractions P1 to P7. These were evaluated at 10 and/or 30 mg/kg i.p. dosages in the experimental models. According to the pharmacological trials, a depressant effect was produced by the extract and all pooled fractions tested, being significant in fractions P2 to P7 (Table 1).

From 87 mg of pooled fraction P6, 15 mg of a compound identified as $\beta$-sitosterol were purified (Fig. 1). This compound did not
Table 1  Effect of the pooled fractions obtained on the bioactivity guided-fractionation of the hexane extract from *Tilia americana* var. *mexicana* inflorescences on the sodium pentobarbital-induced hypnosis in mice

<table>
<thead>
<tr>
<th>Extract/fraction</th>
<th>Dose (mg/kg)</th>
<th>Latency of sedation (min)</th>
<th>Latency of hypnosis (min)</th>
<th>Duration of hypnosis (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>1.6 ± 0.3</td>
<td>3.7 ± 0.4</td>
<td>10.6 ± 1.2</td>
</tr>
<tr>
<td>Hexane</td>
<td>10</td>
<td>1.9 ± 0.3</td>
<td>4.4 ± 0.5</td>
<td>25.3 ± 4.9</td>
</tr>
<tr>
<td>30</td>
<td>1.2 ± 0.2</td>
<td>3.6 ± 0.5</td>
<td>19.2 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>P1 (F1−4)</td>
<td>10</td>
<td>1.7 ± 0.3</td>
<td>4.4 ± 0.4</td>
<td>20.6 ± 2.8</td>
</tr>
<tr>
<td>30</td>
<td>2.2 ± 0.5</td>
<td>3.3 ± 0.7</td>
<td>30.4 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>P2 (F5−12)</td>
<td>10</td>
<td>1.3 ± 0.2</td>
<td>2.9 ± 0.4</td>
<td>26.2 ± 2.8</td>
</tr>
<tr>
<td>30</td>
<td>1.3 ± 0.1</td>
<td>2.9 ± 0.3</td>
<td>45.0 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>P3 (F13−15)</td>
<td>30</td>
<td>1.5 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>24.5 ± 1.6</td>
</tr>
<tr>
<td>P4 (F17−19)</td>
<td>30</td>
<td>1.8 ± 0.2</td>
<td>5.0 ± 0.6</td>
<td>36.8 ± 3.6</td>
</tr>
<tr>
<td>P5 (F27−29)</td>
<td>10</td>
<td>1.3 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>37.3 ± 3.0</td>
</tr>
<tr>
<td>30</td>
<td>1.6 ± 0.2</td>
<td>3.6 ± 0.9</td>
<td>38.2 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>P6 (F31−37)</td>
<td>10</td>
<td>1.2 ± 0.0</td>
<td>3.1 ± 0.4</td>
<td>38.5 ± 1.0</td>
</tr>
<tr>
<td>30</td>
<td>1.4 ± 0.1</td>
<td>3.2 ± 0.3</td>
<td>32.6 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>P7 (F38−42)</td>
<td>30</td>
<td>1.5 ± 0.1</td>
<td>3.0 ± 0.6</td>
<td>37.6 ± 3.0</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M for 5–8 trials. ANOVA followed by Dunnett’s test.

• $F_{13,54} = 3.2$, $P < 0.001$.

modify the latency to the onset of sedation (Fig. 2A) or hypnosis (Fig. 2B), but it produced an increase in the sleeping time starting from 1 mg/kg, this effect was significant ($F_{5,54} = 5.49$, $P < 0.001$) from 3 mg/kg and maintained up to 30 mg/kg dosage resembles the effect produced by diazepam at 0.1 mg/kg as positive control (Fig. 2C).

A significant reduction ($F_{13,54} = 8.3$, $P < 0.001$) in the ambulatory activity of mice was produced by the extract and all pooled fractions tested (Table 2). In the test for anxiety behaviour, a significant increase ($F_{13,54} = 5.7$, $P < 0.001$) in the time spent in the open-arm sides of a plus-maze was observed for hexane at both doses tested (10 and 30 mg/kg), whereas fractions P1, P4, P5 and P7 (F38−42) had a significant effect ($F_{13,54} = 10.4$, $P < 0.001$).
Table 2  Effect of the pooled fractions obtained from the bioactivity guided-fractionation of the hexane extract of *Tilia americana* var. *mexicana* inflorescences on the sedative and anti-anxiety response in mice

<table>
<thead>
<tr>
<th>Extract/fraction</th>
<th>Dose (mg/kg)</th>
<th>Ambulatory activity (counts/2 min)</th>
<th>Time in open-sided arms (s)</th>
<th>Head-dipping (counts/3 min)</th>
<th>Rearings (counts/5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>68 ± 4</td>
<td>38 ± 2</td>
<td>74 ± 3</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Hexane</td>
<td>10</td>
<td>34 ± 5</td>
<td>157 ± 35</td>
<td>50 ± 8</td>
<td>14 ± 4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>35 ± 5</td>
<td>153 ± 21</td>
<td>56 ± 10</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>P1 (F1 - 4)</td>
<td>10</td>
<td>43 ± 6</td>
<td>109 ± 19</td>
<td>37 ± 9</td>
<td>9 ± 2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>32 ± 4</td>
<td>67 ± 15</td>
<td>54 ± 5</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>P2 (F5 - 12)</td>
<td>30</td>
<td>33 ± 2</td>
<td>47 ± 6</td>
<td>41 ± 5</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>P3 (F13 - 15)</td>
<td>30</td>
<td>24 ± 7</td>
<td>48 ± 9</td>
<td>34 ± 4</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>P4 (F17 - 19)</td>
<td>30</td>
<td>16 ± 4</td>
<td>144 ± 7</td>
<td>36 ± 6</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>P5 (F27 - 29)</td>
<td>30</td>
<td>31 ± 7</td>
<td>105 ± 22</td>
<td>52 ± 7</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>P6 (F31 - 37)</td>
<td>30</td>
<td>37 ± 7</td>
<td>142 ± 23</td>
<td>44 ± 6</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>P7 (F38 - 42)</td>
<td>30</td>
<td>32 ± 5</td>
<td>115 ± 9</td>
<td>23 ± 7</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M for 5–6 trials. ANOVA followed by Dunnett’s test.

Table 2. Effect of the pooled fractions obtained from the bioactivity guided-fractionation of the hexane extract of *Tilia americana* var. *mexicana* inflorescences on the sedative and anti-anxiety response in mice.

P6, produced an increase, mainly at a 10 mg/kg dosage. This increase was less pronounced in these fractions when the dose was raised to 30 mg/kg. Even in fractions P1 and P6 it was reduced more than 50% in comparison to the previous dose tested (Table 2).

The hexane extract and all pooled fractions tested produced a significant reduction (F_{13,54} = 4.9, P < 0.001) in the number of headdips at 10 and/or 30 mg/kg dosages (Table 2). This effect was observed in a dose-dependant manner in pooled fractions P5 and P6. Whereas a significant diminution (F_{13,54} = 5.0, P < 0.001) in the rearing behavior was observed in mice treated with fractions P3, P5, P6 and P7 tested at a 30 mg/kg dosage (Table 2).

Regarding β-sitosterol (1 to 30 mg/kg, i.p.), a significant reduction (F_{5,54} = 22.8, P < 0.001) in the ambulatory activity was exhibited by mice; this effect was more pronounced when a dosage of 30 mg/kg was tested (Fig. 3A). A significant reduction in the ambulatory activity was also produced by 0.1 mg/kg of diazepam (Fig. 3A). The time spent in open-arm sides of a plus-maze by mice receiving β-sitosterol was significantly increased in a dozedependent manner from 1 to 10 mg/kg (F_{5,54} = 34.6, P < 0.001), but similar to that observed with fraction P6. When the β-sitosterol dose was increased to 30 mg/kg, the activity spent in the open arm region was markedly diminished (Fig. 3B). The significant anti-anxiety response observed resembled the effect exerted by diazepam at 0.1 mg/kg (Fig. 3B).

In the exploratory rearing (Fig. 3C) and head dipping (Fig. 3D) tests, mice receiving β-sitosterol showed a significant reduction (F_{5,54} = 15.9, P < 0.001 and F_{5,54} = 21.6, P < 0.001, respectively) mainly at a 30 mg/kg dosage. The effect of β-sitosterol observed in the head-dipping test was similar to that observed in mice treated with 0.1 mg/kg diazepam (Fig. 3D).

Discussion

In this study, a bioactivity-guided fractionation resulted in the isolation of three unidentified fatty acids and a triterpene obtained from the active fractions of a *Tilia americana* var. *mexicana* hexane extract, which were further analyzed in experimental models demonstrating their participation in the anxiolytic and sedative activities of this plant species.

At the beginning of this study, a screening was made using the highest doses (10 and 30 mg/kg) tested in the extract in order to analyze the presence of one or more active fractions. Since no potentiation in the response was observed after separation, this suggests that the pharmacological effect of the extract is the result of the activity of more than one constituent. However, purification of the fractions in our study allowed the identification of one compound with a major yield, a triterpene, which contributes to the effect produced by the extract, as can be observed in the dose-response curve in Fig. 3. The triterpene was purified and identified as β-sitosterol by direct comparison on TLC, m.p. 132°C and by using 1H-NMR and mass spectral analysis with authentic β-sitosterol (Sigma). In Fig. 3, the effect of 1 and 3 mg/kg of β-sitosterol was very similar or higher to that produced by the extract at a 30 mg/kg dosage. This demonstrates that β-sitosterol, as a pure compound, participates in the activity of the extract.

β-Sitosterol is one of several plant sterols which are structurally similar to cholesterol. Sterols are not synthesized in the human body, however they are ubiquitous throughout the plant kingdom. Although poorly absorbed, they appear to have important beneficial activities in human physiology when administered in the diet [17], [18], [19]. A few studies in the literature concerning β-sitosterol refer to its effect on the central nervous system. Depressant-like actions have been demonstrated in presence of this.
sterol isolated from Mallotus peltatus [20]. Additionally, a bioactivity-guided fractionation and chromatographic separation of a crude hexane extract of Annona cherimolia led to the isolation of \( \beta \)-sitosterol as one of its major constituents producing an anxiolytic-like effect on the mouse avoidance exploratory behavior and burying behavior tests, both known as animal models of anxiety [21]. In our study, \( \beta \)-sitosterol produced a significant increase in the pentobarbital-induced sleeping time. It has been reported that increase in the pentobarbital-induced sleeping time by combination with plant extracts could be associated with the diminution in the metabolism of barbiturates caused by enzymatic inhibition [22]. On the other hand, potentiation of the barbiturate response has been used to demonstrate depressant effects of plant extracts [12], [13], since their inhibitory actions involve activation of the GABAergic system [23]. To our knowledge, this is the third study demonstrating central nervous system depressant properties of \( \beta \)-sitosterol. In this case, the compound was isolated from the inflorescences of Tilia americana var. mexicana crude hexane extract and exhibited anxiolytic and sedative activities.

On the other hand, for this study we decided to use the i.p. route to ensure the maximum bioavailability of the active constituents of Tilia americana var. mexicana, since our aim is not only to demonstrate the activity of the fractions but also to identify and purify the active compounds. Although the activity of the extract may be ascribed to various components, the significant anxiolytic-like effect observed at minimum doses, such as 1 mg/kg \( \beta \)-sitosterol i.p., suggests that this compound is responsible, at least in part, for these effects. It is important to mention that for the administration of the extract, fractions, and pure compounds, it was necessary to use Tween-80, a non-ionic surfactant, to obtain a uniform suspension. It has been described that surfactants improve the absorption of substances [24], [25] and Tween-80 is commonly used as an intestinal absorption enhancer generally regarded as safe for the development of oral drug delivery systems [26]. Thus, absorption of the constituents such as \( \beta \)-sitosterol in the extract might be increased by the vehicle. Nevertheless, and given the known low bioavailability of phytosterols, in future experiments it will be interesting to compare the effect of the active compounds of Tilia americana var. mexicana by the...
oral route, which is the traditional route of application of this plant species, as well as to determine the pharmacokinetic profile of β-sitosterol alone and in combination with Tween-80.

Diazepam, a well-known benzodiazepine, was used as a sedative and anxiolytic reference drug. Pharmacological and therapeutic actions of benzodiazepines such as anxiolytics, sedatives/hypnotics and anticonvulsants [27] are explained by enhancement of the inhibitory effect of γ-aminobutyric acid (GABA) acting on its type A receptor [28]. In fact, they are the most widely prescribed class of psychoactive drugs in current therapeutic use despite their important unwanted side-effects, such as myorelaxation, ataxia, amnesia, ethanol and barbiturate potentiation, as well as the related problems of tolerance and dependence after chronic use [29]. Phytochemical studies from *Tilia* species, have reported the presence of flavonoids as major components in polar extracts [7], which possess a selective and relatively mild affinity for GABA_A/benzodiazepine receptors and a pharmacological profile compatible with a partial agonistic action [5]. Nevertheless, other components, such as non-polar constituents may play an important role in the pharmacological activity of this genus. In our study, β-sitosterol and some fatty acids, not yet characterized, in the active fractions of *T. americana* var. *mexicana* produced a significant diminution in the ambulatory activity, rear- ing behaviour and in the number of head-dips, but also in the time spent in the open-arm region of a plus-maze at high doses (30 mg/kg i.p.), indicating a sedative effect. In the range 1 to 10 mg/kg of β-sitosterol, a dose-dependent anxiolytic-like effect was predominantly observed. Sedative and anxiolytic-like effects of β-sitosterol resemble those observed with 0.1 mg/kg of diazepam. It has been described that some steroids are neuromodulators of GABA_A receptors, producing an anxiolytic-like effect similar to that of benzodiazepines [30]. Although the effects of β-sitosterol on GABA/benzodiazepine receptors are unknown, further studies to elucidate the precise mechanism of action of β-sitosterol to produce its depressant effects are desirable.

In conclusion, our present results indicate that β-sitosterol is one of the active compounds partly responsible for the central activity of *Tilia americana* var. *mexicana*. Moreover, administration of β-sitosterol i.p. at low doses causes anxiolytic-like effects in mice without generating marked depressive actions on the central nervous system. However, this compound at higher doses might produce also sedative effects.

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