INHIBITION OF PHENYLEPHRINE- OR SEROTONIN-INDUCED RAT AORTA CONTRACTIONS BY MEXICAN FEVERFEW

(*Tanacetum parthenium* (L.) Schultz-Bip).

**ABSTRACT**

*Tanacetum parthenium* (L.) Schultz-Bip (Asteraceae) is widely distributed around the world. This plant has been used for centuries as a traditional herbal medicine for headache treatment. Previous evidence pointed out the relationship of this plant on vasoconstriction regulation. Therefore, the aim of this work was to evaluate the *T. parthenium* dichloromethane crude extract, the essential oil and three isolated compounds (reynosin, santamarin, and santin) against induced contractions by phenylephrine or serotonin, using isolated rat aorta rings model. The results obtained in this work showed that *Tanacetum parthenium* dichloromethane extract exhibited a noncompetitive inhibitory activity against contractions induced by serotonin and phenylephrine. But the essential oil did not inhibit phenylephrine contractions. Dichloromethane extract, and the isolated compounds santin, reynosin and santamarin showed a preference to antagonize the α1-adrenergic agonist phenylephrine. These results show that there is a preference for the components of the essential oil to antagonize serotonin receptors, whereas, the extract components show a preference for the adrenergic pathway, suggesting that both types of receptors are involved in the plant antagonistic vasoconstriction mechanism.

**Keywords:** pA2' (antagonist potential); vasoconstriction; Mexican Feverfew; antia-drenergic; antiserotonergic.

**RESUMEN**

*Tanacetum parthenium* (L.) Schultz-Bip (Asteraceae) es ampliamente distribuida alrededor del mundo. Esta planta ha sido utilizada por siglos como tratamiento herbolario para dolores de cabeza. Existe evidencia previa que indica una relación de esta planta en la regulación de la vasoconstrición, debido a esto, el objetivo de este trabajo fue evaluar el extracto crudo de diclorometano, el aceite esencial

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INTRODUCTION

Tanacetum parthenium (L.) Schultz-Bip (Asteraceae) has been used for centuries in different traditional medicine systems. This plant is native from Iran, Iraq and Eastern Europe but is widely distributed around the world and it has been used mainly for headache treatment. It is reported that people usually chew 2 to 3 leaves of fresh plant daily to reduce the frequency and severity of migraine attacks (Pareek, Suthar, Rathore, & Bansal, 2011). In Mexico, this plant is named Santa María (Rzedowski, J. and de Rzedowski, 1997; Vibrans H., 2009) and it is used to relieve menstrual disorders, as an emmenagogue, antispasmodic and childbirth accelerator. People use the infusion against digestive disorders, as an anti-inflammatory and to reduce fever (BDMT, 2009; Pareek et al., 2011).

In 1992 Barsby and collaborators obtained the T. parthenium chloroform extract from fresh leaves and evaluate its inhibition activity on contractile responses of various agonists, including serotonin (5-hydroxytryptamine, 5-HT), phenylephrine (α1-adrenoceptor agonist), thromboxane A2 and U46619, a mimetic of angiotensin II, on rabbit aorta rings. The bioactivity of T. parthenium has mainly ascribed to the sesquiterpene lactone parthenolide (Figure 1D) (Heptinstall et al., 1992), however, the T. parthenium growing in México contain a very low concentration of this compound (Avula, Navarrete, Joshi, & Khan, 2006). Previous works showed relaxant activity of other sesquiterpene lactones on the smooth muscle of the uterus and rat aorta (Campos et al., 2003), so, it is possible that these compounds shared action mechanisms due to structural similarities. This plant has a wide chemical biodiversity that could contribute to its diverse biological activities; the main compounds present in the extract are flavonoids, terpenoids, sesquiterpene lactones and other compounds (Pareek et al., 2011).

It is well known that the α1-adrenoceptors (Cotecchia, 2010) and serotonin receptors 5-HT_{2A} (Lu et al., 2008) are involved in vasoconstriction, and interactions between agonists and antagonists of serotonergic and adrenergic receptors are known by decades (de la Lande, 1992; Delbin, Silva, Antunes, & Zanesco, 2012; Kalsner, 1973; Murray & Purdy, 1985). Therefore, it is important to investigate the activity of T. parthenium on both receptor types. This study aimed to evaluate the antagonist effect of the di-
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chloromethane extract, essential oil and some of its isolated compounds (reynosin, santamarin and santin, Figure 1A-C) no previously studied on contractile responses stimulated by 5-HT or phenylephrine in rat aorta rings isolated model, contributing to figure out its biological action mechanism on vasoconstriction.

MATERIALS AND METHODS

Plant material
Tanacetum parthenium aerial parts were harvested in Tulyehualco, Mexico City. The specimens were identified by botanists from Universidad Autónoma Chapingo, Forestal Science Division Herbarium. A voucher specimen was deposited in the herbarium (No. 59516).

Drugs
Phenylephrine, norepinephrine, and serotonin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reynosin (A), santamarin (B) and santin (C) (Figure 1) were isolated from the dichloromethane extract prepared with the inflorescences and leaves of T. parthenium by silica gel column chromatography. These metabolites were identified by mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectra, compared with those reported (el-Feraly & Chan, 1978).

Dichloromethane extract preparation
The air-dried inflorescences and leaves (5 kg) were crushed and macerated with 20 L of dichloromethane three times for periods of 72 h each, at room temperature (22°C). The solvent was eliminated in a rotatory evaporator connected to a vacuum pump, obtaining 734 g of crude extract. This was kept in airtight and waterproof containers, stored at 4°C until its use.

Essential oil isolation
The essential oil was extracted from air-dried and ground fresh inflorescences and leaves (1.5 kg) by steam distillation for three
hours using a typical clevenger circulatory hydrodistillation apparatus, as is described in the Mexican Herbal Pharmacopoeia (Comisión Permante de la Farmacopea, 2013). It was obtained 2 mL of essential oil that was stored at 4°C until use.

**Essential oil composition (gas chromatography-mass spectrometry analysis)**

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oil was performed on an Agilent 6890N GC (Agilent Technologies, Palo Alto, CA, USA) coupled to a LECO 4D TOF (LECO Corporation, St. Joseph, MI, USA) mass spectrometer. Using a DB5 (5% phenyl methyl polysiloxane, 10 m x 0.18 mm i.d.; 0.18 μm film thickness, J & W Scientific, Folsom, CA, USA) column. With the following temperature program: 3 min at 40°C, subsequently 10°C/min up to 280°C at 5°C/min, held for 10 min, and finally raised to 340°C at 4°C/min for 20 min isothermally.Injector and transfer line temperatures were 300°C. Helium was the carrier gas, at a flow rate of 1.0 mL/min; injection volume: 1 μL of diluted oil in hexane (1:100); split mode (ratio: 1:75). The mass acquisition range was 45 - 600 m/z, in electron impact mode with an ionization voltage of 70 eV. A mixture of aliphatic hydrocarbons (C8 - C24) (Sigma-Aldrich, St. Louis, MO, USA) in hexane (J. T. Baker, Deventer, Netherlands) was directly injected into the GC injector under the above temperature program, to calculate the retention index (as the Kovats index) of each compound. The analysis was repeated three times. The analysis data were performed using MSD ChemStation software (Agilent, Version G1701DA D.01.00). The identification of the compounds found by gas chromatography-mass spectrometry was based on the comparison of their Kovats index and mass spectra with those obtained from the NIST 08, Wiley 275 libraries and by comparison with data in the literature (Adams, 1989) (See supplementary material, S1).

**Animals and aorta preparation**

Male Wistar rats of 180-220g body weight were used (n=6 per curve), obtained from Envigo (Envigo RMS S.A. de C.V., Mexico City), maintained at constant temperature (22 ± 1°C) with free access to water and food. The animal care and procedures were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and in compliance with international rules on the care and use of laboratory animals. Furthermore, clearance for conducting the study was obtained from the Bioethics Committee (ENMH-CB-149-2015).

The animals were sacrificed in a CO₂ chamber and immediately, the thoracic aorta was dissected out and transferred to a petri dish containing Krebs-Henselet solution (KHS) at 37°C. The aorta was trimmed free of connective tissue and then cut into ring segments of 2-3 mm length. Each aorta ring was placed in a 10 mL organ bath chamber containing KHS with next composition (mM): NaCl 118, KCl 4.7, NaH₂PO₄ 1.2, MgSO₄·7H₂O 1.2, CaCl₂·2H₂O 2.5, NaHCO₃ 25, glucose 11.1. The solution was maintained at 37°C and bubbled with 5% CO₂-95% O₂. Tissues were placed under 4.0 g resting tension and let them stabilize for 60 min. They were washed with fresh KHS solution three times at intervals of 20 min before starting the experiments. After the stabilization period, the aorta rings were contracted with norepinephrine (10⁻⁸M) three times, let them rest for 30 min intervals each time before the next stimulation. The tissues were washed with fresh KHS after each contraction. The isometric tension was recorded by an eight-channel Biopack System polygraph MP100A-CE via a TSD 125C force transducer (Biopac System Inc., Goleta, CA). The data were digitalized and analyzed by the software Acknowledge 3.7.3.
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**The cumulative concentration-response curves, using serotonin (5-HT) (1x10^{-7} to 3x10^{-4} M) or phenylephrine (1x10^{-11} to 3x10^{-5} M) were constructed to establish the 100% of contraction in each tissue. After that, the aorta rings were washed with fresh KHS to stabilize. Then, an individual concentration of each treatment was added to the tissue and incubated for 10 min. Concentrations tested for the extract and the essential oil were 75, 100, 150, 200 or 250 µg/mL; and for the isolated compounds were 17.7, 30, 57, 100, 177 or 300 µg/mL. The water not soluble drugs, dichloromethane extract and the essential oil were suspended in distilled water with Tween 80 (0.5%), whereas, the other drugs were dissolved in distilled water; the controls were tested with the respective vehicle solution.

After that, a cumulative concentration-response curve was performed with 5-HT or phenylephrine, respectively, using the same concentrations mentioned above. The percentage contraction for the second curve was calculated taking the first curve as 100% contraction. When the vehicle was used for the incubation, there was no statistical difference between the first and the second concentration-response curve for both agonists.

**Data analysis**

All the values are shown as the mean ± standard error of the mean (SEM) of at least six experiments. The experimental data were analyzed using two-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test. The differences were considered statically significant for p < 0.05. The antagonist effect of the essential oil, compounds or extracts on the cumulative concentration-response curve of serotonin or phenylephrine, were expressed as pA2’ value. These values were calculated according to the equation: pA2’ = pB2’ + Log (X-1). Where pB2’ is the negative logarithm of the concentration (-Log Bx) of antagonist (essential oil, compounds or extract) and X is the ratio of the maximal effect (E_{max}) of the agonist (in absence of antagonist) between the maximal effect shown after incubation of the antagonist (essential oil, compounds or extract). The pA2’ value is the abscissa to the origin of the graph Log (X-1) vs. pB2’ when X = 2 (Ko et al., 2002).

**RESULTS**

The *T. parthenium* dichloromethane extract and the essential oil of this plant inhibited the 5-HT-induced contractions in a non-competitive way (*Figure 2*); the effect observed was concentration-dependent. On the other hand, phenylephrine-induced contractions were inhibited only by dichlo-
romethane extract, while the essential oil was not able to inhibit them (Figure 2). It is important to mention that the essential oil showed a reversible effect, while the dichloromethane extract effect was irreversible for both 5-HT and phenylephrine-induced contractions agonist. The isolated compounds from the dichloromethane extract reynosin, santamarin and santin also, showed an inhibitory effect on the 5-HT- or phenylephrine-induced contractions in a non-competitive way (Figure 3). The lactones reynosin and santamarin showed an irreversible effect, whereas santin (flavonoid) showed a reversible inhibitory effect. It is important to mention that, an “irreversible effect” was established when the tissue was not able to get a maximum contraction again, after the first contact with the compound or extract.

Figure 3. In superior panel it is shown the concentration-response contraction curves for 5-HT alone (●) or in the presence of A: santin 17.7 µg/mL (△), 30 µg/mL (▲), 57 µg/mL (■), 100 µg/mL (□), 177 µg/mL (♦), 300 µg/mL (○). B: reynosin 17.7 µg/mL (△), 30 µg/mL (▲), 57 µg/mL (■), 100 µg/mL (□), 177 µg/mL (♦), 300 µg/mL (○). C: santamarin 17.7 µg/mL (△), 30 µg/mL (▲), 57 µg/mL (■), 100 µg/mL (□), 177 µg/mL (♦), 300 µg/mL (○). In the lower panel is shown the concentration-response contraction curves for phenylephrine alone (●) or in presence of D: santin 17.7 µg/mL (△), 30 µg/mL (▲), 57 µg/mL (■), 100 µg/mL (□), 177 µg/mL (♦). E: reynosin 17.7 µg/mL (△), 30 µg/mL (▲), 57 µg/mL (■), 100 µg/mL (□). F: santamarin 17.7 µg/mL (△), 30 µg/mL (▲), 57 µg/mL (■), 100 µg/mL (□). Each point represents mean ± SEM of 6 repetitions.* p<0.05 vs control (Two ways ANOVA, followed by Dunnett’s test).
Figure 4. Modified Schild plot for noncompetitive antagonism effect of the dichloromethane extract (A and B) or essential oil (C) on phenylephrine-induced contractions (A) or serotonin-induced contractions (B and C). Each point represents mean ± SEM of at least six experiments.

Figure 5. Modified Schild plot for noncompetitive antagonism effect of santin (A), reynosin (B) or santamarin (C) on 5-HT-induced contractions; or santin (D), reynosin (E) or santamarin (F) on phenylephrine-induced contractions. Each point represents mean ± SEM of at least six experiments.
evaluated, despite several washes and time resting. The quantitative antagonist effect was estimated by pA$_2'$ values using the modified Schild plot for extract and essential oil (Figure 4) and pure compounds (Figure 5). All the tested compounds showed higher antagonist potency to inhibit the contractions induced by phenylephrine than those caused by 5-HT (Table 1); with exception of the essential oil that did not show activity on phenylephrine-induced contractions.

**DISCUSSION**

In this study, was demonstrated that crude extract, essential oil and the pure compounds from *Tanacetum parthenium* antagonized the contractions induced by serotonin (5-HT) in rat aorta. However, only the extract and the compounds isolated from it, presented an antagonistic activity on phenylephrine-induced contractions. The antagonistic potential (pA$_2'$ values), showed that dichloromethane extract and essential oil inhibited the contractions induced by 5-HT with the same potency order (Table 1), whereas, the dichloromethane extract also showed non-competitive inhibition of phenylephrine-induced contractions (Figure 2). The last is in agreement with the reported by Barsby et al. (1992), who described this effect for *T. parthenium* chloroform extract on rabbit aorta contractions. It is important to mention that in our phytochemical analysis, it was not possible to isolate parthenolide (main active metabolite reported, Figure 1D); the low concentration or absence of this metabolite in Mexican feverfew has been previously documented (Avula et al., 2006). However, other sesquiterpene lactones, reynosin and santamarin, and one flavonoid, santin, were isolated and tested to inhibit the serotonin- or phenylephrine-induced contractions. These three compounds showed a preference to inhibit the phenylephrine-induced contractions, according to the pA$_2'$ calculated (Table 1); suggesting more affinity of these metabolites for α1-adrenergic receptors. On the other hand, the essential oil did not show any effect against phenylephrine contractions (Figure 2), this denotes a kind of selectivity of the essential oil components to antagonize the 5-HT activity. The results obtained suggest that the *T. parthenium* vascular activity depends on the interaction between its secondary metabolites presents in the essential oil and the extract, which confirms the existence of other active compounds besides parthenolide. Moreover, previous studies have reported a relaxant activity of sesquiterpene lactones on smooth muscle (Campos et al., 2003). This activity was related to a methylene group in the alpha position of γ-lactones parthenolide and cyanaropicrin (Figure 1D and 1E) (Hay et al., 1994).

**Table 1.** Noncompetitive antagonist potential (pA$_2'$) values for extract, essential oil and pure compounds of *Tanacetum parthenium* to inhibit the contractions induced by 5-HT or phenylephrine.

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-HT</th>
<th>Phenylephrine</th>
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<tbody>
<tr>
<td></td>
<td>pA$_2'$</td>
<td>B (µg/mL)</td>
</tr>
<tr>
<td>Extract</td>
<td>-2.15 ± 0.09</td>
<td>142.29 ± 1.24</td>
</tr>
<tr>
<td>Essential oil</td>
<td>-2.21 ± 0.03</td>
<td>162.29 ± 1.02</td>
</tr>
<tr>
<td>Reynosin</td>
<td>-2.48 ± 0.34</td>
<td>306.35 ± 2.23</td>
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<tr>
<td>Santamarin</td>
<td>-2.23 ± 0.18</td>
<td>170.56 ± 0.65</td>
</tr>
<tr>
<td>Santin</td>
<td>-2.57 ± 0.26</td>
<td>372.52 ± 1.85</td>
</tr>
</tbody>
</table>

NA= no activity; B= antagonist concentration to inhibit 50% of agonist effect (Emax).
lactones reynosin and santamarin have this methylene group in the same position of the lactone ring (Figure 1A and 1B), which could be related to the inhibition of the contraction induced by serotonin and by the adrenergic agonist. It is well known that the methylene group, attached to the alpha position in the γ-lactones ring, leads to Michael-type additions forming covalent bonds, principally to nucleophile groups like exposed thiol groups, such as cysteine residues (Hohmann et al., 2016; Towers et al., 1976). This covalent addition deactivates many enzymes, structural proteins or essential peptides that could compromise the cells and tissues functionality (Hohmann et al., 2016). In addition, this structural characteristic is possibly related to the irreversible inhibitory effect shown on serotonin- or phenylephrine-induced contractions of lactones and the extract, which contains some other lactones.

On the other hand, the flavonoid santin showed a reversible inhibition of 5-HT- and phenylephrine-induced contractions, which is in accordance with previous reports about the flavonoids relaxant effect on rat aorta contracted by phenylephrine or norepinephrine (Ajay et al., 2003; Rodríguez-Ramos et al., 2011). The action mechanism of flavonoids has been related to phosphodiesterases inhibition (Rodríguez-Ramos et al., 2011) nitric oxide release from endothelium and inhibition of Ca+2 influx or calcium release from intracellular stores (Ajay et al., 2003).

These results are the first report about the effect of reynosin, santamarin, and santin to inhibit the contractions induced by serotonin or phenylephrine in rat aorta smooth muscle.

CONCLUSIONS

The results in this work showed that T. parthenium dichloromethane extract, santin, reynosin, and santamarin have a preference to inhibit phenylephrine aorta contractions, which could mean more affinity to α1-adrenoceptors. In contrast, the essential oil only antagonized the serotonin-induced contractions, which suggest more affinity to these receptors. Therefore, these results suggest that both receptor pathways (α1-adrenoceptors and 5-HT receptors) might be involved in the biological action mechanism of T. parthenium. In addition, this study confirmed that there are other compounds besides parthenolide, with biological activity that could be involved in the diverse therapeutic effects of Feverfew.

DECLARATION OF INTEREST

There is no conflicts interest related to the present investigation.

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