Tephrosia toxicaria Pers. reduces temporomandibular joint inflammatory hypernociception: The involvement of the HO-1 pathway

D.R. do Val¹, M.M. Bezerra², A.A.R. Silva³, K.M.A. Pereira³, L.C. Rios², J.C. Lemos², N.C. Arriaga², J.N. Vasconcelos⁴, N.M.B. Benevides⁵, V.P.T. Pinto², G. Cristino-Filho², G.A.C. Brito⁶, F.R.L. Silva⁴, G.M.P. Santiago⁴, A.M.C. Arriaga⁴, H.V. Chaves³

¹ Master of Biotechnology Degree Program, Federal University of Ceará, Sobral, Brazil
² Faculty of Medicine, Master of Biotechnology Degree Program, Federal University of Ceará, Sobral, Brazil
³ Faculty of Dentistry, Master of Healthy Sciences Degree Program, Federal University of Ceará, Sobral, Brazil
⁴ Department of Chemistry, Federal University of Ceará, Fortaleza, Brazil
⁵ Department of Biochemistry and Molecular Biology, Federal University of Ceará, Fortaleza, Brazil
⁶ Department of Morphology, Federal University of Ceará, Fortaleza, Brazil

Correspondence
Heliadá V. Chaves
E-mail: heliadachaves@yahoo.com.br

Funding sources
This work was supported by Brazilian grants from Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Pesquisa (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Conflicts of interest
None declared.

Accepted for publication
16 February 2014

doi:10.1002/j.1532-2149.2014.488.x

Abstract

Background: We investigated both the efficacy and the sub-chronic toxicity of Tephrosia toxicaria Pers. in the zymosan-induced temporomandibular joint (TMJ) inflammatory hypernociception in rats evaluating the possible role of heme oxygenase-1 (HO-1).

Methods: Rats were pretreated with T. toxicaria (0.2, 2.0 or 20 mg/kg) 60 min before the intra-articular injection of zymosan (2 mg, 40 μL) in the left TMJ. In another series of experiments, rats were treated with ZnPP-IX (3 mg/kg), a specific HO-1 inhibitor, before T. toxicaria (20 mg/kg). Von Frey test was used to evaluate inflammatory hypernociception (g) 4 h after zymosan injection. Six hours after zymosan injection, the synovial lavage was collected for total cell count and myeloperoxidase (MPO) activity, and joint tissue for histopathological analysis and immunohistochemistry for HO-1. To evaluate the sub-chronic toxicity, mice received T. toxicaria (20 mg/kg) or saline once a day for 14 days to analyse body mass, organ weight and biochemical parameters.

Results: T. toxicaria partially reversed the zymosan-induced head withdrawal threshold, the number of cells and the MPO activity. T. toxicaria reduced the inflammatory cell influx in the synovial membrane. TMJ immunohistochemical analyses treated with T. toxicaria showed increased HO-1 expression. These effects of T. toxicaria were not observed in the presence of ZnPP-IX. T. toxicaria treatment for 14 days did not show significant signs of toxicity when administrated to mice.

Conclusions: T. toxicaria did not produce any signs of toxicity and effectively decreased zymosan-induced TMJ inflammatory hypernociception dependent, at least in part, upon the HO-1 pathway integrity.

1. Introduction

Temporomandibular joint (TMJ) disorders are a group of conditions that result in TMJ pain, which frequently limits talking, chewing and other basic daily activities with high levels of inflammatory pain-related disability (Cairns, 2010). Experimental models that allow the study of the mechanisms underlying these inflamma-


**What's already known about this topic?**
- Temporomandibular joint (TMJ) disorders are associated with high levels of inflammatory pain-related disability. *Tephrosia toxicaria* is a shrub that has been used in Amazonian countries traditional medicine to alleviate inflammation and pain.

**What does this study add?**
- *Tephrosia toxicaria* Pers. is effective and safe in zymosan-induced TMJ inflammatory hypernociception in rats. *T. toxicaria* Pers. efficacy depends upon the integrity of the heme oxygenase-1 (HO-1) pathway.

Tory and pain conditions are of great clinical relevance. In this regard, we have developed a rat model of TMJ inflammation using intra-articular (i.art.) injections of pro-inflammatory agent, zymosan (Chaves et al., 2011). Zymosan is a polysaccharide from yeast cell walls that produces a severe and erosive synovitis (Gegout et al., 1994; Bezerra et al., 2004) associated with inflammatory pain in animal models of knee arthritis (Bezerra et al., 2007).

Over the past few years, numerous studies have led to the appreciation of heme oxygenase 1 (HO-1). To date, three isoforms of HO (HO-1, HO-2 and HO-3) have been identified. HO-1 is inducible (Bednarz et al., 2007; Bezerra et al., 2008), whereas HO-2 and HO-3 are constitutively expressed (Bezerra et al., 2008). HO-1 is induced in a variety of cells including endothelial cells, monocytes/macrophages, neutrophils and fibroblasts by heme, endotoxins, cytokines, nitric oxide (NO) and other mediators produced during inflammatory responses (Botros et al., 2005; Bressan and Tonussi, 2008; Choi et al., 2008), and its induction would provide a negative feedback for cell activation and the production of inflammatory mediators, beyond its role in the antioxidant defence system, which could modulate, at least in part, the inflammatory pain process (Alcaraz et al., 2003; Grangeiro et al., 2011; Vanderlei et al., 2011).

Thus, the present study was aimed at investigating the unexplored antinociceptive and anti-inflammatory efficacy of *T. toxicaria* in the model of zymosan-induced TMJ inflammatory hypernociception in the rat. Additionally, considering our previous findings showing that the inhibition of HO-1 pathway is associated with worsening of inflammatory response (Grangeiro et al., 2011), we also investigated whether *T. toxicaria* efficacy depends upon the integrity of the HO-1 pathway. Further, a systemic evaluation of the sub-chronic toxicity of *T. toxicaria* was also performed.

### 2. Methods

#### 2.1 Plant materials

*T. toxicaria* Pers. was collected in Guaraciaba do Norte, Ceará State, Brazil. A voucher specimen (# 32.139) was deposited at the Prisco Bezerra Herbarium, Federal University of Ceará, Brazil.

#### 2.2 Extraction

The air-dried and powdered roots (710.0 g) of *T. toxicaria* were extracted with ethanol at room temperature to afford a crude extract (78.9 g) in 11.1% yields on fresh weight basis.

#### 2.3 Animals

Male Wistar rats (160–220 g) (*n* = 60) and Swiss mice (20–25 g) (*n* = 20) were housed in standard plastic cages with food and water available *ad libitum*. They were maintained in a temperature-controlled room (23 ± 2 °C) with a 12/12-h light–dark cycle. All experiments were designed to minimize animal suffering and to use the minimum number of animals required to achieve a valid statistical evaluation. This study was conducted in accordance with the Institutional Animal Care and with the approval of the Ethics Committee of the Federal University of Ceará, Fortaleza, Brazil (CEPA No. 57/10).
2.4 Induction of TMJ inflammatory hypernociception

Rats were anaesthetized with tribromoethanol (1 mL/100 g i.p.) and received an i.art. injection of 2 mg zymosan (40 μL total volume) dissolved in sterile saline into the left TMJ using a 30-gauge needle and a 0.5-mL syringe. Sham animals received only saline i.art. Before zymosan or saline injections, the TMJ skin region was carefully shaved, the postero-inferior border of the zygomatic arch was palpated, and the needle was inserted inferior to this point and advanced in a medial and anterior direction until the needle made contact with the condyle. This contact was verified by moving the mandible, and the puncture of the needle into the joint space was confirmed by the loss of resistance. Gentle aspiration ruled out intravascular placement, after which the specified volume of zymosan or saline was injected.

As shown by our group previously (Chaves et al., 2011) during the time course of zymosan TMJ inflammatory hypernociception development, it is maximal at 4 h of arthritis whereas cell influx peaks at 6 h. Based upon these results, we used these time points to assess both nociceptive (head withdrawal threshold) and inflammatory parameters [total cell counting and myeloperoxidase (MPO) assay].

2.5 Evaluation of inflammatory hypernociception

Inflammatory hypernociceception in the TMJ was evaluated by measuring the threshold of force intensity that needed to be applied to the TMJ region until the occurrence of a reflex response of the animal (e.g., head withdrawal). The measurements were performed by an examiner unaware of the treatments and used a digital device (Insight, Ribeirão Preto, SP, Brazil) that consisted of a rigid filament linked with an electronic device that measured the response threshold in grams (g) when the filament was applied to the surface of the tested region (Denadai-Souza et al., 2009). The facial areas to be tested around the TMJ were carefully shaved, and the animals were placed into individual plastic cages 45 min before the beginning of the tests. The animals were submitted to a conditioning session of head withdrawal threshold measurements in the testing room for 4 consecutive days under controlled temperatures (23 ± 2 °C) and low illumination. On the fifth day, the basal force threshold value was recorded (in triplicate) before the i.art. injections of either zymosan or vehicle and after 4 h.

2.6 Synovial lavage collection and cell counting

Six hours (6 h) after zymosan injections, the rats were sacrificed under anaesthesia and exsanguinated. The superficial tissues were dissected, and the TMJ cavity was washed to collect the synovial lavage by a pumping and aspiration technique using 0.05 mL of ethylenediaminetetraacetic acid (EDTA) in neutral phosphate buffered saline (PBS). This procedure was repeated twice. The total number of white cells in the synovial lavage was counted using a Neubauer chamber.

2.7 MPO activity analysis

MPO is an enzyme found primarily in the azurophilic granules of the neutrophils and has been used extensively as a biochemical marker of granulocyte infiltration into various tissues. The MPO activity assay has been described previously by Bradley et al. (1982). In our study, the MPO assay was conducted on the collected synovial lavage 6 h after zymosan injection. Briefly, the synovial lavage was centrifuged at 4500 rpm for 12 min at 4 °C. MPO activity was assayed by measuring the change in absorbance at 450 nm using o-dianisidine dihydrochloride and 1% hydrogen peroxide. The results are reported as the MPO units/joint fluid. A unit of MPO activity was defined as the conversion of 1 μmol of hydrogen peroxide to water in 1 min at 22 °C.

2.8 Histopathological analysis

After sacrifice at 6 h after zymosan-induced TMJ inflammatory hypernociception, the TMJ was excised. The specimens were fixed in 10% neutral buffered formalin for 24 h, demineralized in 10% EDTA, embedded in paraffin and sectioned along the long axis of the TMJ. Sections of 5 μm, which included the condyle, articular cartilage, articular disc, synovial membrane, periarticular tissue and the skeletal muscle periarticular tissue, were evaluated under light microscopy.

For the specimens processed for routine haematoxylin-eosin (H&E) staining, histological analysis considered a 0–4 score grade based upon the cell influx in the synovial membrane (SM).

2.9 Immunohistochemistry for HO-1

Immunohistochemistry for HO-1 was performed using the streptavidin-biotin (labelled streptavidin biotin – LSAB) method in formalin-fixed, paraffin-embedded tissue sections (5 μm thick), mounted on glass slides previously prepared with an organosilane-based adhesive (3-aminopropyltriethoxysilane). Briefly, it consisted of the following steps: the sections went through two baths in xylol, each lasting 10 min. After this, they were immersed in three passages of absolute alcohol, then washed in running water, and immediately after this, a passage in distilled water.

Antigen recovery was performed with citrate at pH 6.0 for 30 min at 99 °C. After returning to ambient temperature, the sections were immersed in a 3% hydrogen peroxide blocking solution for 10 min. After returning to ambient temperature, the sections were incubated overnight (4 °C) with a primary rabbit anti-HO-1 antibody (ab13243, ABCAM®, England, UK), at the dilution of 1:200, and afterwards washed with PBS.
The samples were incubated with the secondary antibody LSAB Kit for 10 min at ambient temperature. Next, incubation was performed in a chromogen solution prepared with 3’3’ diaminobenzidine for 10 min in a dark chamber. Afterwards, the specimens were washed in running water and then in distilled water. Counter-staining was performed with haematoxylin, and afterwards the specimens were dehydrated in alcohol and diapathanized in xylol. Lastly, they were mounted on glass slides, which were examined under optical microscope. The negative control sections were performed, excluding the application of the primary antibody. The parameter of positivity for the immunohistochemical marking of the antigen in all the specimens included in the sample consisted of the cells that exhibited brown staining in their cytoplasm, irrespective of the intensity of the immunomarking.

2.10 Treatment

*T. toxicaria* (0.2, 2 or 20 mg/kg) was diluted in 10 μL of Tween 80 and the final volume of solution was adjusted using 0.9% sterile saline. *T. toxicaria* (0.2, 2 or 20 mg/kg; 1 mL/200 g) was injected (*per os*) 1 h prior to zymosan i.art. Zymosan group consisted of rats that received (*per os*) *T. toxicaria* vehicle (0.9% sterile saline; 1 mL/200 g) 1 h prior to zymosan i.art. To validate the data, a positive control group of rats was pretreated (s.c.) with indomethacin (5 mg/kg) 1 h before zymosan injection. Sham group received (*per os* and i.art.) 0.9% sterile saline.

To analyse the possible effect of HO-1 pathway on antinociceptive and anti-inflammatory efficacy of *T. toxicaria* in the model of zymosan-induced TMJ inflammatory hypernociception in the rat, another series of experiments was performed. Animals were pretreated (s.c.) with ZnPP IX (3 mg/kg), a specific HO-1 inhibitor, alone or followed by an injection (*per os*) of *T. toxicaria* (20 mg/kg) 30 min later. After 1 h, zymosan (2 mg) was injected (i.art.), and on the fourth hour of inflammatory hypernociception in the TMJ was evaluated as described above.

2.11 Sub-chronic toxicity of *T. toxicaria*

Body mass, organ weight changes and blood levels of the biochemical parameters alanine amino transferase (ALT) and aspartate amino transferase (AST) were evaluated after once-daily treatment of 20 mice with *T. toxicaria* (20 mg/kg) or 0.9% sterile saline for 14 days. Mice were weighed daily, and after 14 days of treatment, samples of peripheral blood were collected for biochemical analysis (determined by enzymatic and colorimetric tests – LABTEST). After sacrificing the animal, the liver, kidney and heart were removed and weighed, and possible ulcerative lesions or haemorrhaging were quantified and macroscopically measured.

2.12 Statistical analysis

The data are presented as means ± SD or medians, where appropriate. Differences between means were compared using a one-way analysis of variance, followed by the Student’s *t*-test or the Bonferroni test. The Kruskal–Wallis test, followed by Dunn’s test was used to compare medians. A probability value of *p* < 0.05 indicated significant differences.

3. Results

3.1 Efficacy of *T. toxicaria* on the zymosan-induced TMJ inflammatory hypernociception

The i.art. 2 mg injection of zymosan resulted in inflammatory hypernociception as measured by a clear decrease in the mechanical threshold for head withdrawal at 4 h (Fig. 1A). Further, a 2 mg injection of zymosan resulted in a significant increase in the number of polymorphonuclear cells (Fig. 1B). This increase in neutrophils was certified by the increase of MPO activity in the TMJ synovial lavage following zymosan injection (Fig. 1C). In the intra-articular saline-injected animals (sham), no significant changes in withdrawal thresholds, polymorphonuclear cells counts or MPO activity were observed (Fig. 1A–C). *T. toxicaria* (0.2, 2 or 20 mg/kg) injected (*per os*) 1 h prior to zymosan i.art. injection significantly (*p* < 0.05) partially reversed zymosan-induced head withdrawal threshold (Fig. 1A). Further, *T. toxicaria* (0.2, 2 or 20 mg/kg) significantly (*p* < 0.05) decreased the number of polymorphonuclear cells (Fig. 1B). This decrease in neutrophils was certified by the decrease of MPO activity in the synovial lavage (Fig. 1C).

3.2 Effect of zinc protoporphyrin IX (ZnPp IX), a specific HO-1 inhibitor, on the *T. toxicaria* efficacy on the zymosan-induced TMJ inflammatory hypernociception

To investigate the role of HO-1 activity in the anti-inflammatory effect of *T. toxicaria*, the animals were pretreated (s.c.) with ZnPp IX (3 mg/kg), a specific HO-1 inhibitor. The effects of *T. toxicaria* (20 mg/kg) on the zymosan-induced TMJ inflammatory hypernociception (Fig. 2A) and inflammation [polymorphonuclear cells counts (Fig. 2B)] and MPO activity in the TMJ synovial lavage (Fig. 2C) were not observed in the presence of ZnPp-IX (3 mg/kg).

3.3 Histopathological analysis

Six hours after zymosan-induced TMJ inflammatory hypernociception, an inflammatory cell influx was observed in the SM (Fig. 3B) compared to the sham group (Fig. 3A). The cell types were predominantly
neutrophils, which characterized acute inflammation. Oedema was also observed in the synovium (Fig. 3B). Table 1 shows the scores attributed to TMJ’s histopathological analysis and compares the values between the TMJs of sham and zymosan groups. A significant ($p < 0.05$) increase in the inflammatory parameters was observed in the zymosan group.

*T. toxicaria* (20 mg/kg) reduced the inflammatory parameters to a normal status with a lower inflammatory cell influx in the SM (Fig. 3C). Table 1 shows the scores attributed to TMJ’s histopathological analysis and compares the values between the TMJ of zymosan and *T. toxicaria* (20 mg/kg) groups. A significant ($p < 0.05$) reduction in the inflammatory parameters was observed. Further, the effects of *T. toxicaria* (20 mg/kg) on the TMJ’s histopathological analysis (H&E) in the SM was not observed in the presence of ZnPP-IX (3 mg/kg) (Fig. 3D).

### 3.4 Immunohistochemical analysis

An immunohistochemical analysis for HO-1 showed an increase in HO-1 expression that was characterized by brown-coloured cells in the SM of the zymosan-induced TMJ inflammatory hypernociception and treated with *T. toxicaria* (20 mg/kg) (Fig. 4C). The cell types that expressed HO-1 were the synovial cells in the synovial membrane. Decreased immunoreactivity to HO-1 in synoviocytes and neutrophils of the zymosan-induced TMJ inflammatory hypernociception was also observed (Fig. 4B). The negative control group sections were composed of zymosan-induced TMJ inflammatory hypernociception that were not treated with an anti-HO-1 antibody (Fig. 4A). None of the negative controls showed HO-1 immunoreactivity.

### 3.5 Sub-chronic toxicity of *T. toxicaria*

Repeated injections (*per os*) of *T. toxicaria* (20 mg/kg) over 14 consecutive days did not produce any signs of toxicity in mice. The overall body mass and the weights of the liver, kidney and heart (wet basis) were normal. Serum levels of the enzymatic markers of hepatic function (ALT and AST) did not differ from the respective controls (Table 2).
4. Discussion

In this work, we demonstrated the antinociceptive and anti-inflammatory efficacy of *T. toxicaria* Pers. in the model of zymosan-induced TMJ inflammatory hypernociception in rats. We also found evidence that, at least in part, the antinociceptive and anti-inflammatory effects of *T. toxicaria* in zymosan-induced TMJ inflammatory hypernociception depends upon the integrity of the HO-1 pathway.

Zymosan has been used as a pro-inflammatory agent for some time (Gadó and Gigler, 1991). To our knowledge, we performed the first demonstration of a zymosan-induced TMJ inflammatory hypernociception describing the histopathological inflammatory parameters involving the articular and periarticular tissues in an experimental TMJ arthritis (Chaves et al., 2011). Acute rat knee joint zymosan arthritis is characterized by an increase in vascular permeability, cell migration and pain. Later, a progressive synovitis occurs with mononuclear cell infiltration and a fibroblast reaction (Keystone et al., 1977; Rocha et al., 1999). Our group has also demonstrated articular cartilage and subchondral bone degradation during the chronic stages of zymosan arthritis in the rat knee joint (Bezerra et al., 2004).

In the present study, the i.art. 2 mg injection of zymosan decreased the head withdrawal threshold, which was partially increased by *T. toxicaria* treatment. Regarding the inflammatory parameters, *T. toxicaria* administration decreased the number of polymorphonuclear cells, which was certified by the decrease of the MPO activity in the synovial lavage. Further, the TMJ’s histopathological analysis from rats treated with *T. toxicaria* correlated with the decrease in both leukocyte influx and MPO activity, since this treatment reduced the inflammatory parameters to a normal status with a lower inflammatory cell influx in the synovial membrane.

Beyond larvicidal activity of *T. toxicaria* against *Aedes aegypti* – the main vector of dengue fever – presented
lately by our group (Vasconcelos et al., 2012), some authors have shown that extracts obtained from plants of Tephrosia genus have different actions such as inhibition of inflammatory total leukocytes and neutrophil recruitment induced by a variety of inflammatory stimuli in mice (Martinez et al., 2012), wound healing (Lodhi et al., 2006), hepatoprotective effects (Khatri et al., 2009), cancer chemopreventive action (Jang et al., 2003), reduction of oxidative stress in rats (Khan et al., 2001) and inhibition of delayed-type hypersensitivity reaction in vivo (Damre et al., 2003).

Table 1 Histopathological analysis of temporomandibular joint in zymosan-induced inflammatory hypernociception: the effects of Tephrosia toxicaria Pers.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cell influx in the synovial membrane</th>
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</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Zymosan</td>
<td>3 (1–4)*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0 (0–1)**</td>
</tr>
<tr>
<td>T. toxicaria (20 mg/kg)</td>
<td>1 (0–3)**</td>
</tr>
<tr>
<td>T. toxicaria (20 mg/kg) + ZnPP-IX</td>
<td>3 (2–4)*****</td>
</tr>
</tbody>
</table>

*p < 0.05 versus sham.

**p < 0.05 versus zymosan.

***p < 0.05 versus T. toxicaria (20 mg/kg); n = 6 (Kruskal–Wallis, Dunn’s).

Figure 3  Photomicrographs of the histopathological analysis of temporomandibular joints (TMJs): (A) sham group TMJ (40x); (B) TMJ inflammatory hypernociception induced by zymosan 2 mg (40x) showing inflammatory cell influx in the synovial membrane; (C) TMJ of rats pretreated (per os) with Tephrosia toxicaria (20 mg/kg) and injected (i.art.) with zymosan 2 mg (40x) showing reduction of the inflammatory parameters to a normal status with a lower inflammatory cell influx in the synovial membrane; and (D) TMJ of rats pretreated (s.c.) with ZnPP-IX (3 mg/kg), a specific HO-1 inhibitor, followed by an injection (per os) of T. toxicaria (20 mg/kg) and injected (i.art.) with zymosan 2 mg (40x). ZnPP-IX reverted the effects of T. toxicaria (20 mg/kg) on the TMJ histopathological analysis (H&E) in the synovial membrane. C, condyle; AC, articular cartilage; AD, articular disc; SM, synovial membrane; PAT, periarticular tissue. Haematoxylin and eosin (H&E) staining.

Figure 4  Photomicrographs of the immunostaining for HO-1 in the temporomandibular joint (TMJ) and periarticular tissues of zymosan-induced TMJ inflammatory hypernociception in rats. (A) Negative control (sections in the absence of anti-HO-1 antibody) (400x). (B) TMJ inflammatory hypernociception induced by zymosan 2 mg showing immunostaining for HO-1 synoviocytes (black arrow) and neutrophils (white arrow) (400x). (C) TMJ inflammatory hypernociception induced by zymosan 2 mg and pretreated with Tephrosia toxicaria showing strong immunostaining for HO-1 synoviocytes (black arrow) (400x).
Animals were weighed and injected once daily with T. toxicaria over 14 consecutive days. After 14 days of treatment, peripheral blood samples were collected for biochemical dosage (AST, ALT). After that, mice were sacrificed and the wet weight of organs determined. Values are reported as mean ± SD (Student’s t-test). ALT, alanine amino transferase; AST, aspartate amino transferase.

Although the mechanisms of Tephrosia genus remain to be determined, one study provided pre-clinical evidence that anti-inflammatory activity of an extract of T. toxicaria is related to inhibition of pro-inflammatory cytokine (tumour necrosis factor-α and interleukin-1β) production and NO-dependent inhibition of leukocyte recruitment in mice. Additionally, in vitro data demonstrated that the extract inhibits oxidative stress by scavenging free radicals, iron chelating activity and inhibition of iron-dependent and iron-independent lipoperoxidation (Martinez et al., 2012).

In the present study, histopathological analyses of immunohistochemical stains showed HO-1 expression in the synovial cells and neutrophils of the zymosan-induced TMJ inflammatory hypernociception. Heme oxygenase (HO) is the rate-limiting enzyme that catalyses the degradation of heme to liberate carbon monoxide (CO), biliverdin (BVD) and free iron in mammalian cells (Alcaraz et al., 2003). HO-1 is induced by oxidative or nitrosative stress, cytokines and other mediators produced during inflammatory processes, likely as part of a defence system in cells exposed to stress to provide a negative feedback for cell activation and the production of mediators, which could modulate the inflammatory response (Alcaraz et al., 2003). Over the last few years, numerous studies have demonstrated that HO-1 expression and the concomitant production of its metabolites, CO and BVD have anti-inflammatory consequences (Vane and Warner, 2000; Soares et al., 2004; Majewska et al., 2008). Considering this, we demonstrated that the HO/BVD/CO pathway plays antinociceptive effects during acetic acid-evoked nociception (Grangeiro et al., 2011). In fact, heme-induced HO-1 was reported to result in a reduction of cell migration, exudation and pro-inflammatory mediators release in a zymosan-induced air pouch inflammation model (Vicente et al., 2003). There is evidence that CO stimulates soluble guanylate cyclase activity and increases the cellular levels of cyclic guanosine monophosphate (cGMP) (Dearden et al., 1989; Morita et al., 1995). Ferreira et al. (1991) provided experimental support to suggest that elevated levels of cyclic GMP are associated with inhibition of nociceptor hyperexcitability. In this regard, our research group demonstrated increased antinociceptive response produced by the combination of agents that increase intracellular cyclic GMP concentrations (Bezerra et al., 2008).

In the present study, after the pretreatment with ZnPP IX, a specific HO-1 inhibitor, the antinociceptive and anti-inflammatory efficacy of T. toxicaria in zymosan-induced TMJ inflammatory hypernociception in rats was not observed, suggesting that HO-1 activity is involved in the inhibitory effects of T. toxicaria, corroborating other data showing that the inhibition of HO-1 pathway is associated with worsening of inflammatory response (Alcaraz et al., 2003; Vicente et al., 2003; Bednarz et al., 2007). In this regard, we have recently demonstrated that ZnPP IX treatment potentiated the effect of acetic acid by increasing the number of writhes (Grangeiro et al., 2011). Further, analyses of immunohistochemical stains showed an increase in HO-1 expression in the synovial cells in the SM of the zymosan-induced TMJ inflammatory hypernociception and treated with T. toxicaria. In fact, HO-1 activity results in the inhibition of oxidative damage and apoptosis, with significant reductions in inflammatory events including oedema, leukocyte adhesion and migration, and production of inflammatory cytokines (Alcaraz et al., 2003).

Although there has been growing interest in the use of natural products in recent years, especially those derived from plants, most of them have been used broadly by local populations without having adequate information on the efficacy and probable toxicity associated with their use. Thus, research concerning both efficacy and toxicity profile of these acclaimed medicinal plants must be largely encouraged (Calixto et al., 2001). Therefore, in the present study, we also evaluated the sub-chronic toxicity of T. toxicaria administration.

The administration of any substance may bring about significant changes in the structure, function and even metabolic pathways. These alterations may produce clinical and laboratory findings that can be used to determine the extent of deleterious effect of foreign compound, including plant extract on the blood. Such laboratory investigations have been reported to be highly sensitive, accurate and reliable (Okonkwo et al., 2004). In this regard, in the present

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<th>Saline</th>
<th>T. toxicaria 20 mg/kg</th>
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<tr>
<td>Body mass (g) before</td>
<td>25.58 ± 0.35</td>
<td>25.08 ± 0.43</td>
</tr>
<tr>
<td>Body mass (g) after</td>
<td>29.42 ± 0.51</td>
<td>27.75 ± 0.49</td>
</tr>
<tr>
<td>Liver (g)/body mass</td>
<td>5.03 ± 0.09</td>
<td>4.79 ± 0.08</td>
</tr>
<tr>
<td>Heart (g)/body mass</td>
<td>0.50 ± 0.02</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>Kidney (g)/body mass</td>
<td>1.66 ± 0.03</td>
<td>1.63 ± 0.05</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>242.0 ± 54.01</td>
<td>247.4 ± 75.07</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>35.17 ± 10.71</td>
<td>33.12 ± 7.09</td>
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Table 2: Systemic effects of Tephrosia toxicaria Pers. (20 mg/kg) in mice.
study, we performed biochemical analysis in peripheral blood from animals treated with T. toxicaria. The analysis did not reveal any toxicity signals after T. toxicaria treatment. This finding may be very stimulating since T. toxicaria was as effective as indomethacin, used as positive control, without showing any toxicity signals. To the best of our knowledge, this is the first report to explore the T. toxicaria toxicity profile.

In conclusion, we demonstrated the antinociceptive and anti-inflammatory efficacy of T. toxicaria in the model of zymosan-induced TMJ inflammatory hypernociception in rats. Additionally, our results strongly suggest that T. toxicaria efficacy, at least in part, depends upon the integrity of the HO-1 pathway. Further, we verified that T. toxicaria in the effective dose did not produce any signs of toxicity. Taking into account the well-demonstrated antinociceptive and anti-inflammatory efficacy of T. toxicaria, the designing of alternative compounds to classical anti-inflammatory and analgesic agents is very encouraged to define new pharmacological targets for the inflammatory TMJ painful condition treatment.

Author contributions


Acknowledgements

The authors thank Adalberto Nascimento de Lima Júnior, Angela Magalhães Vieira and Jordânia Marques de Oliveira for the technical assistance.

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