

# The toxic effect of the hydroalcoholic extract biology essay

[Science](#), [Biology](#)



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## **INTRODUCTION**

The inflammatory reaction, typically characterized by redness, swelling, heat, and pain, is one of the most common manifestations of many diseases afflicting millions of people worldwide (Rang et al. 2011). The inflammation is the main of host defense mechanisms against invading pathogens. However, persistent or over inflammation leads to tissue damage and possibly failure of organs. Numerous studies have been carried out to develop more powerful anti-inflammatory drugs with lesser side effects, and the interest researches on herbal medicines for producing drugs that are more efficient has been intensified in recent years (Rates 2001, Castardo et al. 2008). Approximately 48% of drugs used in therapy come directly or indirectly from natural products, especially medicinal plants (Balunas and Kinghorn 2005). *Piptadenia gonoacantha* (Leguminosae - Mimosoideae) is a common tree species native to Atlantic Forest in southern and southeastern Brazil, commonly used in folk medicine to treat inflammatory disorders, and popularly known as pau jacaré (alligator stick). This species is easily identified by its bark with longitudinal wings filled with woody spines present on the trunk and branches. Phytochemical studies have be performed on the

composition of *P. gonoacantha*, and a number of compounds have been identified such as triterpenes (friedelin, lupenone, lupeol, cycloartenone, cycloartan-25-26-en-3-one, 24-methylene-cycloartanone, and 24-methylenecycloartanol), steroids (sitosterol, campesterol, and stigmasterol), saponin (sitosterol-3-O- $\beta$ -D-glucopyranoside), flavonoids (apigenin, 5-methylapigenin, 7, 4'-dihydroxy-3', 5-dimethoxyflavone, vitexin, and isovitexin), asperphenamate and methyl gallate (Carvalho et al. 2010). However, no research has been investigated on the analgesic and anti-inflammatory activity of *P. gonoacantha* yet. In this study, we investigated the analgesic and anti-inflammatory activities of the hydroalcoholic extract of *P. gonoacantha* (EPG) following oral administration in experimental animal models. The analgesic activity was evaluated by acetic acid-induced writhing response and hot plate test. Anti-inflammatory activity was determined by using carrageenan-induced paw edema model.

## **MATERIALS AND METHODS**

**Plant material and extract preparation** Fresh leaves of *Piptadenia gonoacantha* were collected in Viçosa, MG, Brazil. The plant material was identified and the voucher specimen (no. 35530) is deposited in the herbarium of Federal University of Viçosa. The leaves of *P. gonoacantha* were air dried at 40 °C and the dried material was then powdered using a knife mill (Marconi, Brazil). The hydroalcoholic extract was prepared according to Carvalho et al. (2011). Briefly, 200g of dried powder was submitted to ultrasound-assisted extraction in aqueous ethanol (80%) for 1h at 40 °C. The extract was filtrated and evaporated to dryness using a rotavapor Q344B1

(Quimis, Brazil). The solutions of hydroalcoholic extracts of *P. gonoacantha* leaves (EPG) was prepared by dissolving a given quantity of the ethanolic extract in a small volume of dimethylsulfoxide (DMSO) and made up to the appropriate volume with physiological saline. Phytochemical group tests The ethanolic extracts of *P. gonoacantha* were screened by using standard procedures of phytochemical group tests (Simões et al. 2004). The extracts were screened for the presence of alkaloids, anthraquinones, cardiotonic heterosides, coumarins, flavonoids, phenolic compounds, saponins, tannins, and terpenoids. The reagents were first tested by using standard drugs of corresponding groups available in market. Brine shrimp lethality assay In vitro preliminary cytotoxicity assay was performed according to the method of Meyer et al. (1982) adapted by Carvalho et al. (2009). Briefly, brine shrimp (*Artemia salina*) nauplii were hatched in sterile brine solution (prepared using sea salt 38 g/L and adjusted the pH to 8.5 using 1N NaOH) under constant aeration at 25 °C for 48 hours. After hatching, 10 nauplii were placed in each vial and various concentrations of EPG (250, 500, 1000, 1500, 2000 and 2500 mg/L) were added to each vial in a final volume of 5 mL and maintained at 37°C for 24 hours. The mortality end point of the bioassay was determined as the absence of controlled forward motion during 30 seconds of observation. Negative and positive controls were simultaneously performed using the same concentrations of the working solvent and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (0.33 mM), respectively. The assays were performed in triplicate and calculate LC<sub>50</sub> (the concentration of extract which caused 50 per cent mortality) based on Litchfield & Wilcoxon method (Litchfield and Wilcoxon 1949). Animals Female albino Wistar rats (190-210 g) were obtained from the

central animal house of the Federal University of Viçosa - UFV. They were kept at standard environmental conditions (12/12-h light/dark cycle) at the animal house of the School of Biological and Health Sciences - UNIVIÇOSA and were allowed free access to food and water. Before each test, the animals were fasted for 12 h with free access to water. The rats were randomly divided into tests and controls groups (n = 6) as will be described further below. The experimental protocol used in this study was approved (0006/2011-I) by the Ethics Committee of the School of Biological and Health Sciences - UNIVIÇOSA, and conforms with the Brazilian College for Animal Experimentation (COBEA) and with actual Brazilian legislation.

**Writhing test**  
The antinociceptive activity of EPG was performed according to the method of Dutra et al. (2006). Acetic acid solution 0.6% (0.25 mL/animal) was injected intraperitoneally, and the constriction of the abdominal muscles together with stretching of the hind limbs was counted over a 20 min period starting 5 min after acetic acid injection. EPG (50, 100, and 150 mg/kg, P. O.), indomethacin (10 mg/kg, P. O.) as the standard drug (positive control), and vehicle (1 mL of 1% DMSO in saline, P. O.) as the negative control were administered 30 minutes before the acetic acid injection. The results were expressed as the mean number of writhing accumulated over 20 min experimental evaluation.

**Hot Plate Test**  
The hot plate test was used to measure response latencies according to the method previously described by Eddy and Leimbach (1953). The hot plate was maintained at  $54 \pm 1^\circ\text{C}$ . The time taken (in seconds) to cause a discomfort reaction (licking paws or jumping) was recorded as the response latency 30 min after administration of EPG (50, 100, and 150 mg/kg, P. O.), indomethacin (10 mg/kg, P. O.) as

positive control, and vehicle (1 mL of 1% DMSO in saline, P. O.) as the negative control. A latency period of 10 sec was defined as complete analgesia. To avoid tissue injury, the experiment was stopped if the latency period was exceeded. Paw edema tests The anti-inflammatory effects of EPG were evaluated using the carrageenan-induced paw edema test in rats, according to the method of Ceccherelli et al. (1996). Briefly, rats were injected subplantarily into the right hind paw with 50  $\mu$ L of carrageenan suspension (1% in saline). The left hind paw was injected with 50  $\mu$ L of saline and used as a control. The paw diameters of each group were measured prior to and 1, 2, and 3 hours after carrageenan administration using a digital caliper (Marbeg). EPG (250, 500, and 750 mg/kg, P. O.) was administered 30 minutes prior to carrageenan injection. The control group received an equivalent volume of water. Indomethacin (10 mg/kg, P. O.) was used as the reference drug. The percent inhibition of edema was calculated. Statistical Analysis All data are expressed as Mean  $\pm$  SEM. The result was tested by One-Way ANOVA and Dunnett post test (GraphPad Prism 5. 0, USA).  $P < 0. 05$  was considered statistically significant.

## **RESULTS AND DISCUSSION**

The hydroalcoholic extracts of *P. gonoacantha* (EPG) submitted to phytochemical screening revealed the presence of flavonoids, phenolic compounds, and tannins. The resulting data are summarized in Table 1. In vitro cytotoxic activity of EPG using brine shrimp lethality assay revealed that these compounds inhibited the *Artemia salina* nauplii in a dose dependent manner (fig. 1). The LC<sub>50</sub> values of EPG was found to be 1, 508

mg/L, that indicates a low toxicity of the extract, the World Health Organization (WHO) considers a toxic substance that has  $LC_{50} < 1000$  mg/L for this test (WHO 1965, Meyer et al. 1982). Anyway the  $LC_{50}$  was 10 times greater than the greatest dose used in antinociceptive and anti-inflammatory assays, the EPG did not induce lethality or any other signs of toxicity at doses up to 250 mg/L. The high  $LC_{50}$  values also suggest that EPG were relatively safe and nontoxic to the animals. Two different antinociceptive testing methods were employed in the current investigation, with the objective of identifying possible peripheral (acetic acid-induced writhing test) and central (hot plate test) effects of the EPG. Oral administration of 0.6% acetic acid produced a substantial number of writhes in control rats pretreated with vehicle (1 mL of 1% DMSO in saline) (Fig. 2). EPG (50, 100, and 150 mg/kg) significantly decreased the number of acetic acid-induced writhes (Fig. 2). 100 mg/kg of EPG showed best results reducing the writhes by 71.8%. 50 mg/kg and 150 mg/kg of EPG produced 61.3% and 64.9% reduction in writhes, respectively. These results were found to be highly significant compared with the control group. However, oral administration of EPG (50, 100, and 150 mg/kg) failed to prolong significantly the latency time compared with control animals in the hot plate test (Fig. 3). EPG exhibited significant antinociceptive activity against chemical stimuli (acetic acid tests), but not against thermal stimuli, suggesting that EPG predominantly inhibits peripheral, rather than central, pain mechanisms. Indomethacin (10 mg/kg) the positive control used in this study produced inhibition of writhing movements, about 43.1% and prolongs the latency time significantly (Fig. 3). Acetic acid indirectly triggers the release of nociceptive endogenous

mediators (such as bradykinin, serotonin, and prostaglandin) to cause painful sensation (Liao et al. 2012). Since EPG also attenuated acetic acid-induced writhing, it is probable that the plant species may be producing its antinociceptive activity by affecting the prostaglandin system. Carrageenan (1%) injected into the subplantar of the right hind paws of the rats pretreated with vehicle (1% DMSO in saline) caused an acute inflammatory response. The edema reached its maximum intensity 3h after injection after administration of the phlogistic agent. The oral administration of EPG at doses of 50, and 100 mg/kg significantly inhibited paw edema at 3rd h after the injection (Fig. 4 and Table 2). EPG at the concentrations of 50 and 100 mg/kg had about equal amount of inhibition as 34. 5% and 37. 5%, respectively. Indomethacin (10 mg/kg) profoundly reduced the carrageenan-induced edema in the right hind paws of rats, around 51. 4% at 3rd h of measurement (Fig. 4). Carrageenan-induced edema is an experimental animal model for acute inflammation and it is a biphasic response. In the early phase (1–2h) of this assay is mainly mediated by histamine, serotonin and an increasing synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release (Antonio and Souza Brito 1998). Our data showed that EPG demonstrated antinociceptive activity against chemical stimuli and inhibited the formation of the carrageenan-induced rat paw edema, these effects might be partially related to inhibition of prostaglandin synthesis. Prostaglandins (PGs) are responsible for inflammation features, and cyclooxygenase (COX) enzymes catalyze the first steps in their biosynthesis (Teather et al. 2002). COX-2 is referred to as the inducible isoform of COX, since levels of COX-2 increase in



response to several stimulus, including inflammation process (Berenguer et al. 2002, Teather et al. 2002). Substances present in the plant extracts may interfering with the initiation of the inflammatory response (Castardo et al. 2008). In EPG was identified presence of flavonoids like apigenins, vitexin, and isovitexin (Carvalho et al. 2010), which have previously demonstrated to possess antinociceptive and anti-inflammatory activities (Mills and Bone 2000, Jeong et al. 2009, Alves et al. 2012). Some flavonoids may interfere with inflammatory response by inhibiting COX-2 (Havsteen 2002). Therefore, based on the results of this study, we can suggest that the antinociceptive and anti-inflammatory effect of EPG may be attributed to inhibition of COX-2 and prostaglandin release. In conclusion, the data obtained show that *P. gonoacantha* has both antinociceptive and anti-inflammatory activities, and that it has been used in folk medicine to treat inflammatory disorders, the obtained results corroborate the folkloric use this plant. In addition, this plant would be a good candidate for further development of a new phytotherapeutical medicine. Also, the obtained results in this work contribute significantly to the pharmacological validation for the safe use of *P. gonoacantha*. However, more studies are needed to further elucidate the mechanism of the antinociceptive and anti-inflammatory actions of *P. gonoacantha*.