Pharmacological study of *Syagrus oleracea* (Martius) Beccari and *Mauritia vinifera* Martius fruit extracts

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ABSTRACT: Pharmacological study of *Syagrus oleracea* (Martius) Beccari and *Mauritia vinifera* Martius fruit extracts. The species of Palmae family are very interesting from a chemical and pharmacological point of view. However, the oil from *Mauritia vinifera*’s fruit has being used in the treatment of burn without any previous pharmacological studies. The fruits from *Syagrus oleracea* haven’t being studied either. This work aims to evaluate the anti-inflammatory and analgesic activities of the ethanolic and hexanic extracts of fruits from those two Palmae species. The extracts were tested for the analgesic activity by three methodologies: writhing, tail flick and hot plate. They have being tested too for anti-inflammatory activity by formaline and ear oedema induced by croton oil methodologies. Each test group consisted of five Swiss 44 male mice that received 0.1ml of an extract solution, with doses of 100 or 400mg/kg, by p.o. The essays evaluated the ethanolic extracts from epicarp/mesocarp of *S. oleracea*, epicarp/mesocarp of *M. vinifera* and mesocarp/endocarp of *M. vinifera*; the hexanic extract from endosperm of *S. oleracea*; the hexanic fractions from epicarp/mesocarp of *S. oleracea* and epicarp/mesocarp of *M. vinifera*. In the used doses, only the hexanic extracts from epicarp/mesocarp and from endosperm of *S. oleracea* were significantly active. These results suggest that the compounds responsible for the analgesic and anti-inflammatory activities of *S. oleracea* are concentrated in the lipophilic extracts. For *M. vinifera*, in a general way, the mesocarp/endocarp extract had the best results for analgesic and anti-inflammatory activities, suggesting that compounds of the endocarp from the fruits are those responsible for activities.

Key words: *Syagrus oleracea*, *Mauritia vinifera*, Palmae, pharmacological studies.

INTRODUCTION

The species of Palmae family are very interesting from a chemical and pharmacological point of view; such species can be found widespread in the world, mainly in tropical and temperate areas (Cruz, 1965; Zofemler, 1994). Chemically, the plants of this family are generally not cianogenics; the alkaloids (occasionally pirimidynics) and proantocianidins can be present or not; the flavonoids are rare, but, when present, are derived from kaempferol, quercetin, tricin and luteolin; saponins and sapogenins are occasionally found; triterpene methyl eters have been isolated from fruits of some palm species (Heim de Balsac, 1931; Shimokomaki et al., 1975; Harborne et al., 1994; Lubrano et al., 1994; Brotons, J.A. et al., 1995; Garcia et al., 1995; Lubrano & Robin, 1997; Lewis & Zona, 2000). Many palm species are economically important, for example, *Cocos nucifera* Martius, *Copernicia cerifera* Martius and *Serenoa repens* (Bartram) Small (Ferreira & Yokomizo, 1978; Ichimaru & Sales, 1980; Cambie & Ash, 1994; Prance et al., 1995; Cruz, 1995; Plosker & Brogden, 1996; Joly, 1998). Two species belonging to this family were chosen to initialize the chemical and pharmacological approach of their fruits: *Syagrus oleracea* (Mart.) Becc. and *Mauritia vinifera* Mart. In Brazil, these two fruits are respectively known as Guariroba and Buriti. They can be found in several regions of Brazil, especially at the northeast and southeast of the country (Lorenzi, 2000; Silva, 2001). The following pharmacological tests were done with the two studied fruits: analgesic (by writhing, tail flick and hot plate methodologies) and anti-inflammatory (by formaline and ear oedema induced by croton oil methodologies). The objective of this work was to contribute for the pharmacological study of palm species, evaluating the analgesic and anti-inflammatory activities of the extracts obtained from the fruits of *Syagrus oleracea* and *Mauritia vinifera*, using different pharmacological models.
MATERIAL AND METHODS:

Plant Material

The fruits of Syagrus oleracea (Martius) Beccari were collected in the campus of Barra Mansa University, Rio de Janeiro, Brazil, in May 2002. The plant was identified by Professor Mârcia Simões, from Biology Museum of Barra Mansa University (UMB), and it was deposited under voucher number UBM 5963. The fruits of Mauritia vinifera Martius were collected in Bananeira farm, Teresina, Piauí, Brazil, in November 2002. The plant was identified by Professor Roseli Farias Melo de Barros, from Federal University of Piauí (UFPI), where there was an exemplar deposited under voucher number TEPB 18930.

Preparation of Plant Extracts

The fruits of S. oleracea were separated in epicarp/mesocarp and endosperm, while the fruits of M. vinifera were separated in epicarp/mesocarp and mesocarp/endocarp. Dried and powdered parts of fruits of S. oleracea and of M. vinifera were submitted to successive extraction with ethanol at room temperature. Only the endosperm of S. oleracea was extracted with hexane. The extracts obtained were evaporated separately to dryness under reduced pressure. The liquid-liquid partition of the dried ethanolic extracts, after suspension in water, lead to fractions obtained with different solvents, but only the hexanic partitions were used in this study.

Drugs

Indomethacin, dexametason and phentanyl were purchased from Sigma (St Louis, USA), dimethylsulfoxide from Fisher Biotech (Fisher Scientific). All drugs were dissolved in phosphate buffer saline (PBS) just before use. Extracts were dissolved in dimethylsulfoxide (DMSO) in order to prepare a saline (PBS) just before use. Extracts were dissolved in DMSO from Fisher Biotech (Fisher Scientific). All drugs were dissolved in phosphate buffer saline (PBS). All extracts were stored at 8°C. In the day of the experiment the testing solutions were diluted in PBS. In all experiments, the final concentration of DMSO did not exceed 0.5% at the concentration of 100 mg/ml and the reaction time was recorded when the animals withdrew their fore and hind paws and/or jumped. The reaction time, in seconds, was measured 40 and 20 minutes before and 20, 40, 60, 80, 100 and 120 minutes after administration of AA. In the positive control groups the animals were pretreated with indomethacin (10 mg/kg, p.o.).

Analgesic activity

Acetic acid-induced abdominal writhing: Mice were used according to Costa et al. (2003). Briefly, the total number of writhing following intraperitoneal (i.p.) administration of 2% (v/v) acetic acid (AA) was recorded over a period of 20 minutes, starting 5 minutes after AA injection. The animals were treated orally with partitions or PBS (0.1 ml), 60 minutes before administration of AA. In the positive control groups the drugs were used according to Costa et al. (2003). Briefly, mice tails were immersed on a water bath set at temperature of (50 ± 1)°C. The time (in seconds) necessary for the mice to withdraw their tails from the water was registered. The reaction time, in seconds, was measured 40 and 20 minutes before and 20, 40, 60, 80, 100 and 120 minutes after treatment. Baseline was considered as the mean of values from 40 and 20 minutes before injection. In the positive control groups the animals were pretreated with phentanyl (300 µg/kg, p.o.).

Hot Plate Test: Mice were tested according to the method described by Franzotti et al. (2000). The animals were placed on a hot plate set at (55 ± 1)°C and the reaction time was recorded when the animals withdrew their fore and hind paws and/or jumped. The reaction time, in seconds, was measured 60 and 30 minutes before and 30, 60, 90, 120 and 150 minutes after treatment. In the positive control groups the animals were pretreated with phentanyl (300 µg/kg, p.o.).

Anti-inflammatory activity

Formalin Test: Mice groups were pretreated orally with 0.1 ml of the saline solution (PBS), extracts or standard drug (indomethacin 10 mg/kg and phentanyl 0.3 mg/kg). One hour later the mice were anestesics with ether and were injected with 0.05 ml of formalin 0.2%, prepared in solution saline PBS into the room with controlled temperature (22 ± 2°C) for 12-h light/dark cycle with free access to food and water. Twelve hours before each experiment animals received only water, in order to avoid food interference with substances absorption. All extracts were taken orally at doses of 100 or 400 mg/kg in a final volume of 0.1 ml. The animals were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). All the experimental procedures applied in this study were approved by an experimental animals research ethical guidelines from ICB number 3078.
subplantar of the right hind paw. The duration of paw licking, an index of nociception, was measured for 1-5min (early phase) and 15-30min (late phase) after formalin administration (Hunskaar & Hole, 1987).

Ear oedema induced by croton oil: Mice groups were pretreated orally with 0.1 ml of the saline solution (PBS), extracts or standard drug (indomethacin 10mg/kg and dexametason 10mg/kg). One hour later each mouse received 10µl of a croton oil solution (2.5% v/v in acetone) on the intern surface of the right ear and the same volume of acetone on the left ear. Four hours after, discs of 6mm of diameter were cut off from the ears and weighed in analytical balance. The results were expressed as the mean of the difference of weight between the ear treated with croton oil and the ear treated only with acetone (Schianterelli et al., 1982).

**Statistical analysis**

The results are presented either as the mean ± S.D (n = 5) in acetic acid induced writhing and ear oedema induced by croton oil, as the increase of permanence time (%) in tail-flick and hot plate tests or as the decrease of reaction time (%) in formalin test. Statistical significance between groups was performed by the application of analyses of variance.
ANOVA followed by Bonferroni's test. The p values less than 0.05 (p < 0.05) were used as the significant level.

RESULT AND DISCUSSION

The writhing test was used in order to evaluate the peripheral analgesic response from the ethanolic extracts of S. oleracea and M. vinifera (Graphic 1). At a dose of 100 mg/kg, it was observed that none of the extracts significantly decreased the number of writhing when compared with the negative control. However, as the dose was increased, so the effect for all ethanolic extracts showed the same result, especially for the M. vinifera extracts, which showed a percentage number of writhing inhibition higher than the positive control. These results point out the presence of compounds with peripheral analgesic action in the fruits of M. vinifera. The central analgesic response from extracts was evaluated by tail-flick (spinal analgesia) and hot plate (supra-spinal analgesia) tests. In the tail-flick test, only the hexanic extract from endosperm and the hexanic fraction from epicarp/mesocarp of S. oleracea increased the time of response with a dose of 100 mg/kg. With a higher dose, the hexanic fraction from epicarp/mesocarp of S. oleracea showed almost the same response observed to the positive control one hour after treatment. The ethanolic extract from mesocarp/endocarp of M. vinifera proved to develop some spinal analgesic activity with a dose of 400 mg/kg (Graphic 2). The probable mechanism of action of this inhibitory effect noted for these three extracts could be a direct decrease on the activity evoked by the C fibers in ascendant axons or for a decrease in the production of PG's responsible for the C fiber stimulation, according to Rossi et al. (1993). The same samples from S. oleracea and M. vinifera, with doses of 100 mg/kg and/or 400 mg/kg, increased the latency time of animals in hot plate model in a similar way from that observed in the tail-flick test. It indicates that the lipophilic extracts from S. oleracea and the ethanolic extract from the mesocarp/endocarp of M. vinifera have compounds with supra-spinal analgesic activity (Graphic 3). Thus, the mechanism of action for these extracts could be the direct action in the µ receptors in the spinal medulla, as it occurs with the morphine-like compounds.

In order to evaluate the anti-inflammatory response of the extracts it was used the formalin and the ear oedema tests. The percentage of response reduction in phase I of the formalin test was 34.09% for hexanic extract from endosperm of S. oleracea, 17.44% for hexanic fraction from epicarp/mesocarp of S. oleracea, 15.03% for ethanolic extract from the mesocarp/endocarp of M. vinifera (both with a dose of 400 mg/kg), while it was 62.48% and 12.14% for Phentanyl and Indometacin, respectively (Graphic 4). In this way, we could conclude that those extracts have a direct action on the nociceptors from the mice's paw.
The percentage of response reduction in phase 2 of the formalin test was 33.02% for hexanic extract from endosperm and 22.78% for hexanic fraction from epicarp/mesocarp of *S. oleracea* (100 mg/kg), 17.99% for ethanolic extract from the mesocarp/endocarp of *M. vinifera* (400 mg/kg), 38.78% for Phentanyl and 47.36% for Indometacin (Graphic 5). These results indicate that the extracts may be acting by inhibition of prostaglandin's synthesis, via cyclooxygenase 1 and/or 2. The percentage of oedema reduction in the ear oedema test was 28.35% for hexanic extract from endosperm and 7.64% for hexanic fraction from epicarp/mesocarp of *S. oleracea* (100 mg/kg), 32.71% for ethanolic extract from the mesocarp/endocarp of *M. vinifera* (400 mg/kg), 89.10% for dexametasone and 53.89% for indometacin (Graphic 6).
As the dose of the hexanic fraction from epicarp/mesocarp of S. oleracea is increased, so the effect in both phases of the formalin test and in the ear oedema test shows the same result. It is important to remember that the extracts used are not pure drugs and they have different composition and concentration of several compounds. That is why the IC\textsubscript{50} values from extracts are so different from the classical analgesic and anti-inflammatory drugs. All obtained results suggest that the compounds with analgesic and anti-inflammatory activities are concentrated in the lipophilic extracts of the fruits from Syagrus oleracea. Probably, that is the reason why the ethanolic extract from epicarp/mesocarp of S. oleracea had no significant activity with a dose of 100 mg/kg. So, it is necessary to evaluate the extracts with higher doses. It might be concluded that the analgesic and anti-inflammatory activities of the ethanolic extract from the mesocarp/endocarp of M. vinifera are due to the constituents from the endocarp of the fruits. The search for pharmacological activities of plant extracts can make the design of less expensive therapies possible. The substances responsible for the activity of the fruits from S. oleracea and M. vinifera must be investigated.

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