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Research Paper

Anti-inflammatory activity of animal oils from the Peruvian Amazon

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ABSTRACT

Ethnopharmacological relevance: Animal oils and fats from the fishes *Electrophorus electricus* and *Potamotrygon motoro*, the reptiles *Boa constrictor*, *Chelonoidis denticulata* (*Geochelone denticulata*) and *Melanosuchus niger* and the riverine dolphin *Inia geoffrensis* are used as anti-inflammatory agents in the Peruvian Amazon. The aim of the study was to assess the topic anti-inflammatory effect of the oils/fats as well as to evaluate its antimicrobial activity and fatty acid composition.

Materials and methods: The oils/fats were purchased from a traditional store at the Iquitos market of Belen, Peru. The topic anti-inflammatory effect was evaluated by the mice ear edema induced by arachidonic acid (AA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) at the dose of 3 mg oil/ear. Indomethacine and nimesulide were used as reference anti-inflammatory drugs. The application resembles the traditional topical use of the oils. The antimicrobial effect of the oils/fats was assessed by the microdilution test against reference strains of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enteritidis*. The fatty acid composition of the oils/fats (as methyl esters) was determined by GC and GC–MS analysis after saponification.

Results: All oils/fats showed topic anti-inflammatory activity, with better effect in the TPA-induced mice ear edema assay. The most active drugs were *Potamotrygon motoro*, *Melanosuchus niger* and *Geochelone denticulata*. In the AA-induced assay, the best activity was found for *Potamotrygon motoro* and *Electrophorus electricus* oil. The oil of *Electrophorus electricus* also showed a weak antimicrobial effect with MIC values of 250 μg/mL against *Escherichia coli* ATCC 25922 and *Salmonella enteritidis*-MI. The main fatty acids in the oils were oleic, palmitic and linoleic acids.

Conclusions: Topical application of all the oils/fats investigated showed anti-inflammatory activity in the mice ear edema assay. The effect can be related with the identity and composition of the fatty acids in the samples. This study gives support to the traditional use of animal oils/fats as ant-inflammatory agents in the Peruvian Amazon. However, new alternative should be encouraged due to the conservation status of several of the animal sources of the crude drugs.

1. Introduction

Latin American traditional medicine is a blend of the practices from Amerindian cultures and the introductions from the European conquerors and settlers. Healers and users, mainly in medium and large human settlements employs plants and herbal drugs that are easily accessible, can be collected from the wild, grown in small gardens or be purchased in markets or shops. Much less is known on the use of animals in therapeutic practices. The use of animal drugs is often overseen in Latin America. This fact is associated with the habitat loss of several animal species formerly integrated in the colonial or pre-hispanic tradition, the extinction of many of them and the substitution of their former indications of use for other drugs. The use of animal-derived drugs for human and veterinary medicine has been revised by several authors in Brazil (Alves and Rosa 2006; Alves and Rosa, 2007; Alves and Alves, 2011; Souto et al., 2011a, 2011b; Alves et al., 2012). Much less is known for other South American countries, including Peru.

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Studies on the use of natural resources in healing practices in the Peruvian Amazon include the assessment of antimalarial activity of plants from the Nanay river (Loreto Department) against *Plasmodium falciparum* and on the ferriprotoporphyrin inhibition assay (Ruiz et al., 2011). An ethnobotanical survey was carried out at Suni Miraño, in the same Department (Jovel et al., 1996). The authors focused the work on plants and tested the extracts on different microorganisms using the disk diffusion assay. The shamanic practices in Iquitos and nearby areas was described by Luna (1984), including the diet restrictions associated with the apprenticeship to become a shaman and the process of plant teaching. At present, ayahuasca still play a relevant role in the healing practices of Peruvian Amazon. Other studies undertaken with plants collected near Iquitos, the Loreto Department or Pucallpa, comprises the screening for wound healing plants (Villegas et al., 1997) and the evaluation of risks associated with the use of oje, an anthelmintic preparation obtained from *Ficus insipida* latex (Hansson et al., 2005). On spite of the diversity of medicinal resources used in the Peruvian Amazon, little has been done on the therapeutic practices involving animal drugs. A visit to the Mercado Belén in Iquitos, offers a challenging perspective on the use of natural resources in the area.

Fig. 1. Plant macerates in alcohol, aphrodisiac preparates and animal oils on sale at a traditional shop in the Belen Market, Iquitos, Peru.

Fig. 2. Animal oils selling place at the Belen Market, Iquitos, Peru.

The Belen market at Iquitos (3°45′07″ S latitude, 73°16′06.1″ W longitude) is a commercialization center for all kind of local food and traditional medicine resources produced, collected and gathered from the Peruvian Amazon area. Along with aromatic and medicinal plants, wildlife meat, turtle eggs, plant and animal drugs are sold in small stands (Figs. 1 and 2).

As we did not find information on the use of animal drugs in Amazonian Peru, a survey was undertaken to identify the most common animal drugs commercialized in the traditional market of Iquitos. The animal oils and fats are trading products from fishers and hunters and are sold without controls regarding environmental issues. The oils are stored either in plastic or glass bottles and kept at room (environmental) temperature, which means an average of 30 °C or higher during the summer months of December through February.

Following our studies on South American pharmacopeias, we now report the bioactivity and composition of the most commonly traded animal oils at the Iquitos market.

2. Materials and methods

2.1. Animal oils

Sellers of animal oils and animals used as medicine at the Iquitos Belen market were interviewed by two different individuals (G S-H and G. V.-A) and the information was compared. Sellers were asked about the products offered, their uses, origin, obtention/preparation of the products, administration and storage. The responses for the general uses were consistent for all six sellers, including the larger shops with medicinal plants preparations and animal oils as well as for small shops offering mainly unprocessed animals or its parts. The oils were purchased by one of the authors (G. V.-A.), accompanied by a biologist (Javier del Águila Chávez, Universidad Científica del Perú, Iquitos) for the association of the oil sources with the taxonomic identity of the animals. The oil/fat samples were purchased at the "Pasaje Paquito", Mercado Belén, Iquitos, Peru, in October, 2012, Puesto de Venta el Otoronguito. Small samples were obtained as follows: *Electrophorus electricus* (6.96 g), *Potamotrygon motora* (7.15 g), *Boa constrictor* (6.93 g), *Chelonoidis denticulata* (*Geochelone denticulate*) (6.99 g), *Melanosuchus niger* (6.94 g) and *Inia geoffrensis* (3.15 g). The oil samples for analysis were stored in amber glass containers and used for fatty acid analyses, antimicrobial activity and topical anti-inflammatory effect in animals.

2.2. Antiinflammatory activity

Two inflammatory agents, namely arachidonic acid (AA) and phorbol 12-myristate 13-acetate (TPA) were used to estimate the probable anti-inflammatory action mechanism of the oils under study. The reference drugs used were indomethacin and nimesulide against TPA and AA, respectively.

All animal experiments were performed according to the ethical guidelines suggested by the "International Norms for the Biomedical Investigation with Animals", elaborated by the Council of International Organizations (1990) and the bio-ethics norms of the Commission of the Chilean Public Health Institute and Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Adult male CF-1 mice (20–25 g), obtained from a stock maintained at the Chilean Public Health Institute, were used to assess the anti-inflammatory effect. All animals were housed in a climate- and light-controlled room with a 12 h light-dark cycle, fasted overnight before the day of the assays, with free access to water. For each of the samples under study, the anti-inflammatory