

**EFFECTS OF A MIXTURE OF FATTY ACIDS FROM SUGAR CANE
(Saccharum officinarum L.) WAX OIL IN INFLAMMATION**

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Summary

A mixture of fatty acids obtained from sugar cane (*Saccharum officinarum* L.) wax oil, (FAM) in which the main constituents are palmitic, oleic, linoleic and linolenic acids, was evaluated in two models of inflammation: zymosan- induced arthritis and in the tail test for psoriasis, both on mice. In the first model, FAM significantly reduced zymosan-induced increase of β glucuronidase (DE_{50} 90 ± 7 mg/kg). Histopathological studies showed inhibition in cellular infiltration and reduction of synovial hyperplasia and synovitis, whereas in the second test, histopathological and ultrastructural studies showed that topical application of FAM induced orthokeratosis with the presence of keratohyalin granules in the previously parakeratotic adult mouse-tail, and without effects on epidermal thickness The ED_{50} of FAM in this model was 155 ± 10 mg. The results of our studies showed that topical application of FAM exerts an important anti-inflammatory activity in both tests without evidence of irritant effects. The antiinflammatory effects exerted by FAM may be due to its inhibitory effects on arachidonic acid metabolism. To our knowledge, this is the first report on the anti-inflammatory effect of sugar cane by-products in experimental models of arthritis and psoriasis.

Keywords: Antiinflammatory agent, Fatty acids, sugar cane, arthritis, zymosan, psoriasis.

Introduction

Recently, it was reported that a mixture of Fatty Acids from Sugar Cane (*Saccharum officinarum* L.) wax oil (FAM), which mainly contains palmitic, oleic, linoleic and linolenic acids, exerts anti-inflammatory action in various in vitro and in vivo experimental models. Particular composition of the fatty acids and ratio are unique in sugar cane.

Rheumatoid arthritis and psoriasis, are inflammatory diseases of unknown etiology. Several inflammatory mediators such as prostaglandins, leukotrienes, and interleukins are involved in psoriasis and rheumatoid arthritis (1) The effective therapies for both diseases are limited. (2). It has been shown that essential fatty acid deficiency induces inflammatory processes in the organisms, which are reversed by the cutaneous application of linoleic acid (3). Therefore, taking into account these findings, we decided to test FAM in arthritis induced by zymosan and to determine the potential antipsoriatic effect of local application of this mixture, in animal models that mimic some of acute inflammatory responses seen in both diseases (4).

Materials and Methods

Chemicals, materials and animals. Sugar cane wax oil was obtained from “Amancio Rodríguez” Cuban sugar factory. All reagents were purchased from Sigma Chemical (St Louis, MO). Animals from the National Center for Production of Laboratory Animals (CENPALAB, Havana, Cuba) were used in this study. The experiments were carried out in accordance with the ethical guidelines for investigations in laboratory animals. (EEC Directive of 1886 (86/609/EEC))

Chemical composition of mixture of fatty acids from sugar cane wax oil (FAM). FAM was obtained as is described by Ledon et al (5)

Zymosan-induced arthritis in mice. Female 6 week old OF1 mice (20-25g, n=10) were injected intra-articularly with 10 μ L of a 15 mg/ml sterile suspension of zymosan. Four days after, each group was treated either with FAM (53, 106, 212, and 425 mg/kg), or vehicle or triamcinolone (10 mg/kg), which was used as a reference drug. They were administered orally on a daily basis, from days 4 to 12. Thereafter, mice were then killed by cervical dislocation and the synovial fluid of knee joints was sampled in order to measure the level of β glucuronidase enzyme. The knee joints were then totally removed for histological studies.(6).

Determination of β glucuronidase activity. The patellar ligament was cut and the synovial cavity incised, the total fluid was then absorbed by means of small pieces of filter paper (No 1575-Prolabo). The paper tips were cut and deposited at the bottom of

tubes containing 0.9 ml of 50 mM acetate buffer, pH 4.5. The enzyme was measured as described previously by Folliard et al, 1992.(7).

Histological processing. The processing was made as described previously by Ramirez et al, 1999(5).

Mouse tail test for psoriasis. Tails of mice (n=10) were treated locally on its proximal part with 0.1 ml of FAM at doses of 27, 53, 106, 212 or 425 mg or with vehicles (saline or paraffin) or non treated. Animals were treated twice daily, for 3 weeks. Another group of animals was orally treated per day with a suspension of retinoic acid in water(0.1 mg/kg) and used as positive control group. At the end of the treatment, animals were killed by cervical dislocation and longitudinal sections of tails of about 5 mm thickness were prepared and stained with hematoxylin-eosin for histological examination. Smaller pieces were taken for ultrastructural studies.(8)

Histological examination. Ten sequential scales were examined for the presence of a granular layer induced in the previously parakeratotic skin areas. The induction of orthokeratosis in those parts of the adult mouse tail, which have normally a parakeratotic differentiation, was quantified measuring the length of the granular layer (A) and the length of the scale (B). The proportion (A/B) x 100 represents the % orthokeratosis per scale, and the drug activity (DA) was calculated as follows:

$$DA = \frac{\text{mean OK of treated group} - \text{mean OK of control group}}{100 - \text{mean OK of control group}} \times 100$$

where OK=orthokeratosis

The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit (8). The measurement of epidermal thickness was obtained by measuring the distance between the dermoepidermal borderline and the beginning of the horny layer. Five measurements per animal were made in every 10 scales and the mean of different animals was calculated.

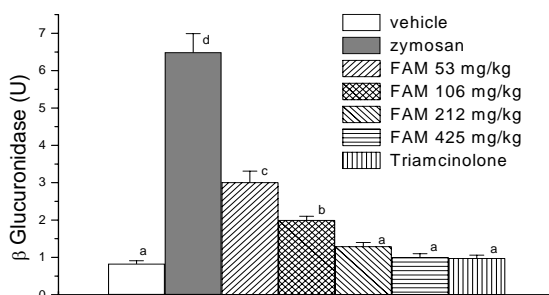
Ultrastructural processing. The processing was made as described previously by Casacó et al (8).

Statistical analysis. Data are presented as means \pm standard deviation. Mean differences between groups were compared by one way ANOVA and a Duncan's multiple comparison test. Due to a non-gaussian distribution of orthokeratosis values (100% is the maximal effect) the Kruskal-Wallis test was used on psoriasis tail test. Values of $p < 0.05$ were considered to be significant. The ED₅₀. Value was determined from the best-fit regression line of a dose-response curve.

Results

In zymosan-induced arthritis, complement is activated via alternative pathway and the secretion of lysosomal enzymes into the knee joint synovial fluids is induced. This activity correlates with histomorphological changes observed in the joint, such as vasculitis, synovitis and sometimes pannus formation. Arthritis induced by zymosan resulted in a significant increase of β glucuronidase levels, which were decreased by FAM with a $DE_{50} = 90 \pm 7$ mg/kg. This effect was dose-dependent (Figure 1). Triamcinolone almost completely abolished enhanced β glucuronidase activity in the synovial fluid of zymosan treated animals.

Fig 1. Effect of FAM on β glucuronidase activity in synovial fluid of knee joint in mice



Different letters means significant differences among the experimental groups $p < 0.05$ ($\bar{X} \pm S.D$, $n=10$ mice/group) .

In agreement with these findings, histological evaluation revealed that the group treated with zymosan showed severe destruction of cartilage with loss of the general architecture and pannus formation. There was erosion of bone structure accompanied by severe inflammation of articular tissues (grade 4) (Figure 2A). FAM treatment revealed a marked and dose dependent decrease of histology score (grade 2 and 1), the inflammatory response was less severe, and there was no destruction of general joint architecture or pannus formation. Also, after treatment the reduction of bone erosion was pronounced (Figure 2B, FAM 425 mg/kg).

In psoriasis' model, the induction of a granular layer by topically administered drugs was measured in previously parakeratotic scale regions in the mouse-tail. In the tail skin samples of negative control group, lack of granular layer in epidermal stratum was observed, as occurred normally in scale regions of adult mouse tail (Figures 3A and 4A). FAM induced a significant and dose-dependent increase in orthokeratosis in the mouse tail epidermis ($DE_{50} = 145 \pm 10$ mg) (Figure 3B, Table 1) with a percentage of orthokeratosis induction ranging from 23.8 to 60.1%. In the interfollicular regions of tail skin specimen removed from fatty acid mixture-treated mice, granular cells with prominent nucleus and cytoplasm with the typical keratohyalin granules were found in the epidermal stratum (Figure 4B). FAM did not produce significant changes on the epidermal thickness (Table 1).

Fig 2 A Histological section of knee joint injected with zymosan. Acute inflammation is present. The surface of the cartilage is eroded and there is pannus formation (p)(grade 4). Hematoxilin –eosin, 10x. B. Mice treated with zymosan and FAM (425 mg/kg). Histology of arthritic knee joints shows restoration of articular cartilage and absence of pannus formation. Hematoxilin –eosin, 10x

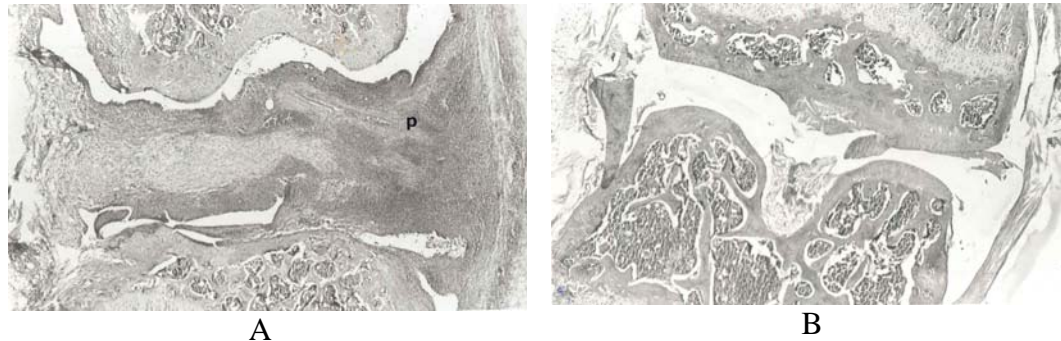


Table 1 Effect of the fatty acids mixture (FAM) from sugar cane wax oil on epidermal differentiation.

Treatment	Orthokeratosis (%)	Activity (%)	Change in epidermal thickness (%)
Non-treated	27.0 ±2.0	-----	-----
Saline	27.1±2.5	-----	-----
Paraffin	29.3 ± 2.1	-----	-----
Retinoic acid (0.1 mg/kg)	75.6±3.1*	66.5	5
FAM 27 mg	44.4 ±3.7*	23.8	0
FAM 53 mg	53.2 ± 3.3*	35.8	3
FAM 106 mg	60.0 ±2.4*	45.2	6
FAM 212 mg	67.0±3.4*	54.7	7
FAM 425 mg	70.9±4.5*	60.1	7

Values are means ± SD *p<0.05 in regard to non treated.(n=10 animals/group). ED₅₀ 145 ± 10 mg

Fig.3.A Histopathology of a mouse tail scale after treatment with saline for 3 weeks (negative control). The granular layer is in the neighboring of the hair follicle. Hematoxylin-eosin staining. 50X. B Histopathology of a mouse tail scale after topical treatment with the fatty acid mixture from sugar cane wax oil for 3 weeks. The granular layer covers the whole scale (g). Hematoxylin-eosin staining. 50X

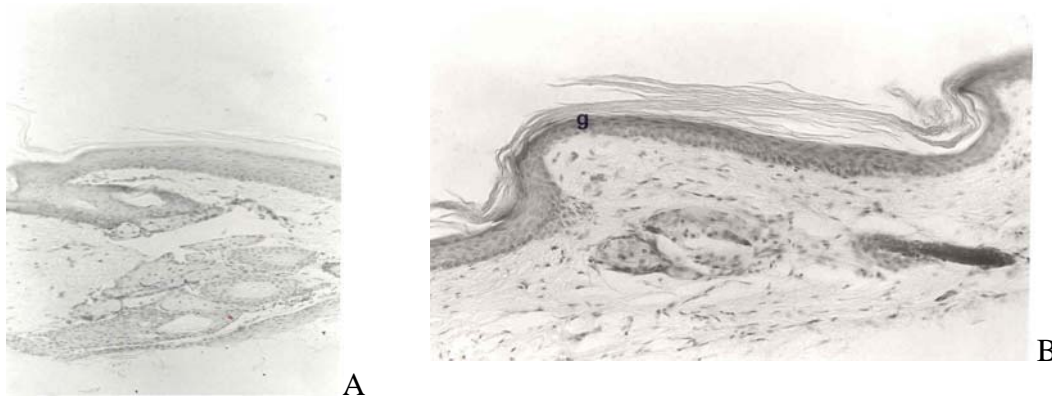
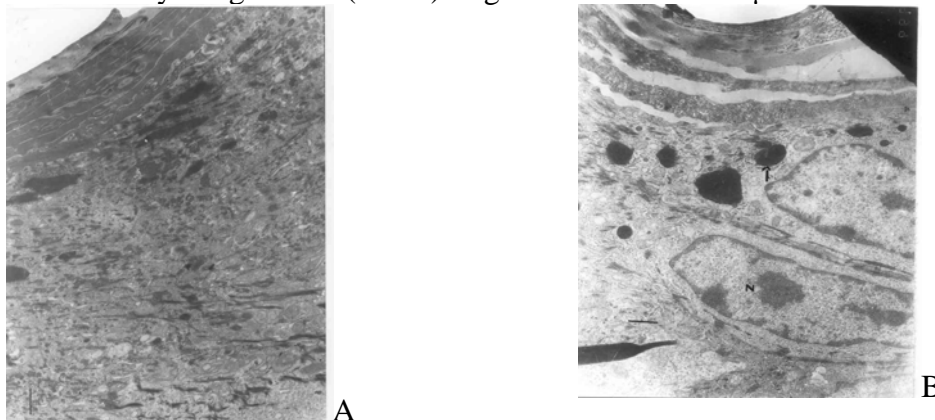


Fig.4.A. Control animal. Ultrastructure of interfollicular region of mouse tail skin. Observe lack of granular layer in the epidermal stratum. Bar: 1 μ m. B. Animal treated with the fatty acid mixture. Ultrastructure of interfollicular region showing nucleus (N) and keratohyalin granules (arrow) of granular cells. Bar: 1 μ m.



Discussion

Zymosan is well known as a powerful releaser of arachidonic acid metabolites which are very important for vasodilation and swelling as well as the release of chemotactic factors that recruit polymorphonuclear cells to the affected zone and that contribute actively to the early phase of inflammatory reaction (6).

For the treatment of these illnesses, inhibitors of arachidonic acid metabolism such as fatty acids are commonly used (9). Fatty acids are important for cutaneous eicosanoids metabolism since they can exert remarkable effects on epidermal phospholipid fatty acid composition, as well as on the release of arachidonic acid and further biosynthesis of the inflammatory eicosanoids. The incorporation of other fatty acids in the tissue is paralleled by elevated levels of biosynthesis of metabolites with potent anti-inflammatory effects or with minor inflammatory effects than those derived from arachidonic acid. (10).

In this context, FAM inhibited degranulation of cells in hypersensitivity models such as ovalbumin induced sensitization and oxazolone induced sensitization in mice. The latter is a model of delayed type hypersensitivity in which it is known that lymphocytes play a major role as also occurs in rheumatoid arthritis (11). Also, FAM inhibited the chemotaxis of neutrophils (12). These results are in agreement with those observed by other authors that had described previously the beneficial effects of different oils, mixtures of fatty acids and fatty acids isolated from different sources for the treatment of rheumatoid diseases and psoriasis (10). On the other hand, the fact that FAM did not affect on epidermal thickness, is an indicator of its negligible irritant effect.

These data suggest that FAM may be a valuable approach in order to control inflammation in arthritis and psoriasis. However, further studies will be needed to elucidate the mechanism(s) involved in this effect of fatty acid mixture of *Saccharum officinarum* L.) wax oil.

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