Cyclooxygenase Enzyme Inhibitory Property of Anoxe®

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Abstract: *Laboratory assay to determine the cyclooxygenase inhibitory activity of a new SOD-like compound, in a powder and treated mixture, which is extracted from a combination of herbs with recognized and studied antioxidant properties. At the National Food Safety and Toxicologic Center, Michigan State University, in USA, under the supervision of the associate Professor Muralee G. Nair, was applied a standardized method to measure an aqueous extract and a methanolic extract of the herbal extract mixture over the ciclooxygenase-1 and cyclooxygenase-2. COX-1 activity was recorded using an enzyme preparation from ram seminal vesicles and COX-2 activity with a preparation of human prostaglandins H synthase isozyme 2 (hPGHS-2) cloned in insect cells. Concomitantly and for comparison the inhibition activity of aspirin and NSAIDs were also measured, which permitted to advent some conclusions. One of the most interesting conclusions, when analyzing the results, was to verify that the aqueous extract, NR-w, presents inhibitory properties over COX-1 and COX-2 comparable or even superior to aspirin and to the most known NSAIDs, which foresees a good advantage on using a natural product that avoids the known gastric problems attributed to aspirin, ibuprofen and naproxen or the cardiovascular problems that can occur with the use of a coxib, like Vioxx (rofecoxib). Finally, the confirmation of the good inhibitory activity over the cyclooxygenase mechanism of inflammation, points to the need of more in vitro studies that might determine if Anoxe® can be also used as a cancer chemoprotective supplement, giving scientific sustainability to the clinic work already performed at the Holiterapias Institute.*

Introduction – Anoxe® is a trademark product of the Company Natiris Centro Dietético, Basically, Anoxe® is a developed and combined mixture of herbs with recognized antioxidant properties, processed in a powder compound of low molecular weight antioxidants that is being used in the Holiterapias Institute as a SOD-like antioxidant in human ROS activity. Anoxe® is simply a non-toxic natural health food product made from a variety of herbal extracts (soy bean, Japanese daikon radish, rice bran, green tea, wheat germ, Japanese yzu orange, sesame seed and hatomugi). The manufacturing method for obtaining the mixture powder of Anoxe® follows some very important steps, like to submit the herbal extracts to infrared rays, steaming and brewing to cut the polymer structures where antioxidants and enzymes are trapped (Niwa et al., 1988)[1]. The purpose of such method, is in fact to extract the large quantities of antioxidants contained in all these plants, but poorly absorbed by the digestive tract once they are trapped in big and not easy absorbed polymer structures. At the time such polymer structures are cut the powder mixture becomes rich in very active and low molecular weight antioxidants components, which include gluthation, catechins, catalase, tannin, riboflavin, carotenoids, flavonoids, polyphenols, vitamin C and vitamin E. The great impact of this innovative compound with a very effective antioxidant activity and easily absorbed, contrary to the known absorption difficulties of the Cu/Zn-SOD, led researchers to inquiry themselves about the possible activity of Anoxe® over the mechanism of inflammation, giving subsequence to what other authors had stated, describing Anoxe® as a delivery system highly effective in ROS activity and inflammatory processes, when compared with SOD oral tablet (Niwa and al., 1986)[2]. Following this undeniable knowledge and many suggestive results obtained in treated clinical cases, performed at the Holiterapias Institute, it was pertinent for these

researchers to evaluate this product in a study that could suggest inhibitory properties over the cyclooxygenase enzymes. Consequently a bioassay was performed at the Bioactive Natural Products Laboratory in the Department of Horticulture and National Food Safety and Toxicology Center at the Michigan State University, under the supervision of the associate professor, Dr. Muralee G. Nair.

Objectives *- The conversion of arachidonic acid to prostaglandins, mediated by cyclooxygenase enzymes, is a prerequisite for many inflammation stimulus. Cyclooxygenase enzymes are produced in the body in two or more forms, termed COX-1 and COX-2, for different purposes. COX-1 is built in many different cells to create prostaglandins, which are used for basic "housekeeping" messages throughout the body. The second enzyme, COX-2, is built only in special cells and is used for signaling pain and inflammation. Some pain relief medication works by blocking the messages carried by COX-1, COX-2, or both, and thus the body does not feel pain or inflammation. In the present investigation, Anoxe® compound was analyzed for its inhibitory effect on COX enzymes. The inhibitory effect of Anoxe® compound on Cox enzymes is determined by measuring the O_2 uptake during the endoperoxide formation by arachidonic acid and COX enzymes:*

1- Inhibition of Ciclooxygenase-1 enzyme activity.
2- Inhibition of Ciclooxygenase-2 enzyme activity.

Methods and results

Anoxe® extract preparations

Anoxe® was provided by Natiris - Centro Dietético, S.A. The product received, in sealed bags, was not completely soluble in water, DMSO or organic solvents. Therefore, the product was extracted separately with water and methanol to yield aqueous and organic extracts.

Water extract: the product (2.5g) was stirred with R.O. water (25ml) and for 24h and centrifuged. The supernatant was removed, evaporated to dryness under reduced pressure (39°C) to yield a powdered material (500mg), NR-w.

Methanol extract: the product (2.5g) was extracted with methanol (25ml) and filtered. The greenish extract was dried under vacuum (39°C) to yield methanol extract (520mg), NR-m

Cyclooxygenase enzyme inhibitory assay

COX-1 activity was recorded using an enzyme preparation from ram seminal vesicles and COX-2 activity with a preparation of human prostaglandins H synthase isozyme 2 (hPGHS-2) cloned in insect cells. COX assays were performed by monitoring the initial rate of O_2 uptake in an *Instech* micro chamber with an oxygen electrode attached to a *YSI model 5300* biological oxygen monitor as reported earlier[3,4]. Each assay mixture contained 12ml of 0.1M Tris/1 mmol phenol buffer (pH 7) and 340mg hemoglobin. The test samples and the enzyme (20µl) were allowed to incubate for 10 min before the addition of 10 µl of arachidonic acid (0.25mg/0.5ml Tris buffer). Data was recorded using Quicklog for Windows data acquisition and control software (*Strawberry Tree Inc*., Sunnyvale, CA). All extracts and fractions were tested at 250ppm, whereas the pure compounds were tested at 83ppm. For comparison

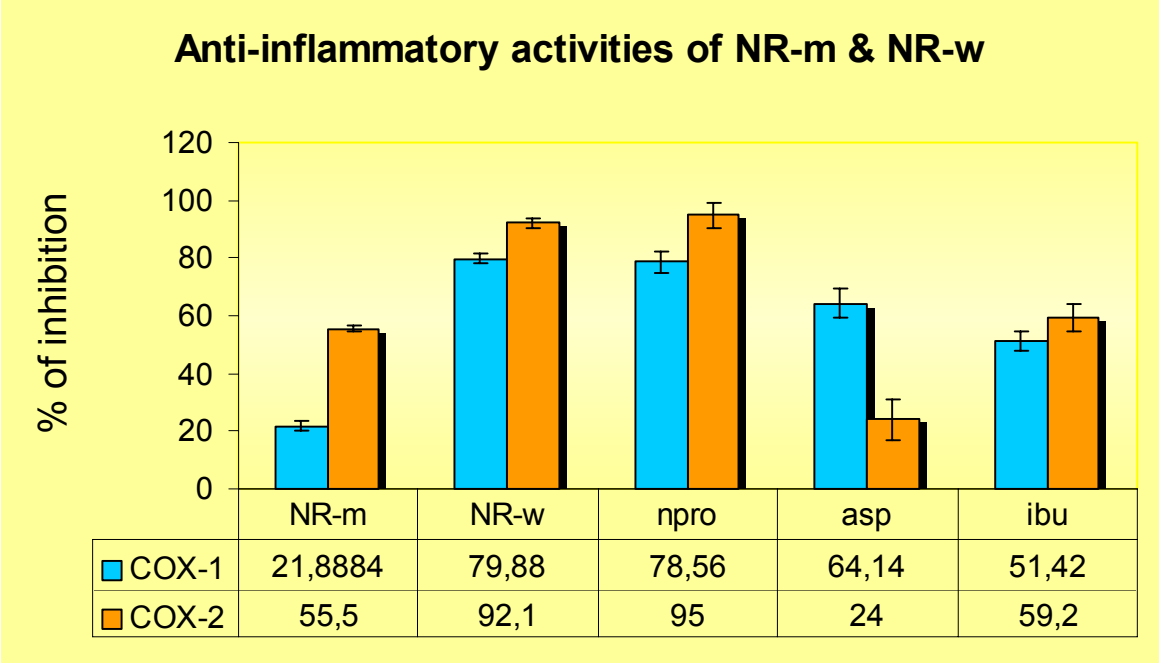
purposes, the activities of commercial NSAIDs, rofecoxib (*Vioxx,* at 1.67ppm), ibuprofen (at 2.06ppm), naproxeno (at 2.52ppm) and of aspirin (at 180ppm) were also carried out.

Cyclooxygenase enzymes (COX-1 & COX-2) inhibitory assay was performed at 250ppm concentration for both NR-w and NR-m extracts as *per* published methods[5].

The obtained results express the percentage of inhibition of the Anoxe® extracts (aqueous and methanolic) against the solvent control, and comparatively with known nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin.

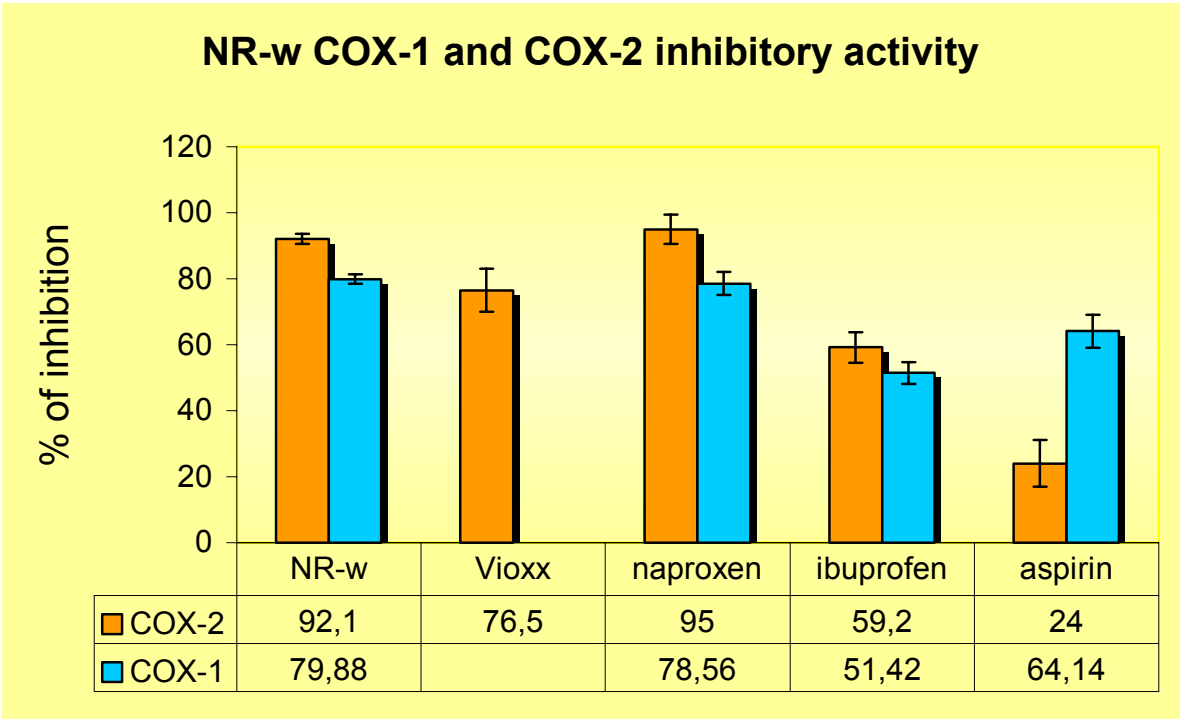
In a first approach the inhibitory assay began for measuring the percentage inhibition of both extracts, NR-w and NR-m, over the COX-1 and COX-2 enzymes, in comparison with aspirin, ibuprofen and naproxen. The results (graph I) were encouraging and showed that the aqueous extract of the studied compound showed a percentage of inhibition of 78.88% over COX-1 and of 92.10% over COX-2, comparatively identical to the ones obtained with naproxen, 78.56% and 95% respectively, but superior to the ones obtained with aspirin (64.14% and 24%) and with ibuprofen (51.42% and 59.2%).

Graph I



The graph results analysis shows that the aqueous extract of the Anoxe® have an inhibition activity over both cyclooxygenase enzymes, comparable or even superior to aspirin and to the most known NSAIDs. More remarkable is the fact that the activity of the NR-w is even higher over the COX-2, which turned indispensable to introduce a new parameter for evaluating and comparing the inhibition activity of the aqueous extract against a nonsteroidal anti-inflammatory of second generation, the refocoxib (Vioxx™), which only inhibits the COX-2. The result shown in graph II, for this new parameter, indicates that the verified inhibitory activity of the NR-w (92.1%) over the COX-2 is superior to the one obtained with Vioxx, only 76,5%.

Graph II



	NR-w	Vioxx	naproxen	ibuprofen	aspirin
COX-2	92,1	76,5	95	59,2	24
COX-1	79,88		78,56	51,42	64,14

Conclusions - The evidence of the results in this cyclooxygenase enzymes inhibitory assay is suggestive that the Anoxe®, a low molecular compound which results from a mixture of herbal extracts, and thus considered as a natural product, in a form of a Food Supplement, when used in nutrition supplementation, can have a beneficial anti-inflammatory activity, similar to Aspirin or to NSAIDs of first and second generation. The great advantage on doing a nutritional supplementation with Anoxe®, is probably to avoid the secondary effects provoked by the aspirin and the NSAIDs of first generation at gastric level, or the known cardiovascular problems that can occur when using NSAIDs of second generation.

This tested inhibitory activity of Anoxe®, together with the antioxidant activity of this compound, evaluated *in vitro* over the tyrosinase enzyme and conducted at the Applied Chemistry Department, Faculty of Science and Technology of the New University of Lisbon, as well as the evaluation of the effect of Anoxe® over redox potential and free radical generation in venous and blood plasma[6], correlates the defended idea of Serge Jurasunas that Anoxe® might inhibit cancer cell lines. Thus besides the Nutritional advantage of using a easily absorbed SOD-like compound in general ROS activity and anti-ageing, is to pursuit *in vitro* studies to determine if Anoxe® can be also used as a cancer chemoprotective supplement, giving scientific sustainability to the clinic work already performed at the Holiterapias Institute.

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