Original Article

COX 2 Inhibitory effect of Morinda citrifolia leaf extract- an In-Vitro study

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ABSTRACT

Introduction :A popular treatment for chronic pain and inflammation involves the use of nonsteroidal anti-inflammatory drugs (NSAIDs). While NSAIDs have been effective in reducing inflammation and pain associated with it, NSAIDs have a number of adverse side effects. As researchers studied the COX inhibitory activity of NSAIDs, they discovered that there are in fact two different COX enzymes: COX-1 and COX-2. Morinda citrifolia is a tree in the coffee family, Rubiaceae. Among some 100 names for the fruit across different regions are the common English names; great morinda, Indian mulberry, noni, beach mulberry, and cheese fruit. Although Morinda is considered to have biological properties in traditional medicine, there is no confirmed evidence of clinical efficacy for any intended use. Materials and Methodology: The Morinda citrfolia leaf extract was obtained from Greenchem Herbal extracts and formulations, Banglore. The COX Inhibitor Screening Assay directly measures PGF2α by stannous chloride reduction of COX-derived PGH2 produced in the COX reaction. Results: Different concentrations (15.625, 31.25, 62.5, 125, 250, 500, 1000µg/ml) of Morinda fruit extract was evaluated for the inhibitory effect on the activity of COX. The plant extracts exhibited potent inhibition of the COX – 2 enzymes. Concentration based inhibition was observed against COX – 2 activities. The IC50 was found to be 112.1µg/ml. Maximum inhibition was found to be 94.36% at 1000µg/ml. Conclusion :The Morinda citrifolia plant, and especially its fruit, has been used for centuries in folk medicine. It has been shown by various studies, some of which with controversial methodologies, that this fruit includes several nutritional and functional compounds without assessing the amount of them. Anti-microbial, anti-cancer, antioxidant, anti-inflammatory, analgesic and cardiovascular activity of noni has been proven through research previously. Therefore, the extract can be functional as better anti-inflammatory medicine rather than the synthetic one.

Keywords: Inflammation, COX Inhibitor, morinda citrifolia, NSAIDS, anti-inflammatory, analgesic.

Introduction

A popular treatment for chronic pain and inflammation involves the use of nonsteroidal anti-inflammatory drugs (NSAIDs) [1]. NSAIDS are particularly useful in treating joint pain, muscle pain, and joint Swelling [2]. There are many different types of NSAIDs, including aspirin and other Salicylates. Examples include ibuprofen, (e.g., Advil(R), Motrine, Nuprince) naproxen, Sulindac, diclofenac, piroxicam, ketoprofen, diflunisal, nabumetone, etodolac, oxaprozin, and indomethacin. Popular NSAIDs include: ibuprofen, naproxen and aspirin [3, 4]. While NSAIDs have been effective in reducing inflammation and pain associated with it, NSAIDs have a number of adverse side effects [5]. The major side effects of NSAIDs are gastrointestinal related [6]. For example, between 10 and 50 percent of the patients being treated with NSAIDs, suffer the side effects such as diarrhea, heartburn, increased abdominal pain, and upset stomach [6, 7]. A significant percentage of these patients also develop ulcers in the Stomach and upper GI tract, which can cause internal bleeding and other complications [8].

Since significant numbers of patients taking NSAIDs were suffering from an increased risk of ulceration in the Stomach, researchers began investigating the mechanisms by which NSAIDs inhibit and prevent inflammation [9]. Researchers knew that in most instances, inflammation in human tissues (and the pain associated with it) is related to the conversion of arachidonic acid (a molecule present in the majority of human body cells) into a prostaglandin in the cells of the tissue [10, 11]. The

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Varusha Sharon C, Lakshmi Thangavelu, Anitha Roy. COX 2 inhibitory effect of morinda citrifolia leaf extract- an in-vitro study. J Adv Pharm Edu Res 2017;7(4):469-472.
Source of Support: Nil, Conflict of Interest: None declared.

conversion arachidonic acid to a prostaglandin requires the presence of an enzyme known as cyclooxygenase (COX) [12]. NSAIDS were known to inhibit the COX enzyme and thereby prevent or reduce inflammation [13].

As researchers studied the COX inhibitory activity of NSAIDs, they discovered that there are in fact two different COX enzymes: COX-1 and COX-2 [14]. COX-1 and COX-2 are isoform of cyclooxygenase and both of which catalyze the first two steps in the biosynthesis from arachidonic acid to the prostaglandins [15]. The difference is that COX-1 is constitutive and COX-2 is inducible [16]. COX-1 presents in nearly all parts of body at a constant level to produce the prostaglandins to line the stomach, maintain normal renal function, prevent platelet aggregation [17]. Contrarily, COX-2 does not normally exist in body, and it is incited at the infected sites by which associates with inflammation like bacterial polysaccharide and cytokines, interleukin-1, -2, and tumor necrosis factor [18]. Once induced, COX-2 fabricates large amount of prostaglandins which decreases the pain threshold (causes pain), increases the set point of the temperature-regulating center (causes fever), and leads to peripheral vasodilatation with local redness and edema formation [19]. Therefore, the inhibition of COX-1 will cause a Series of Side effects such as gastrointestinal ulceration and bleeding, renal damage, and platelet dysfunction, while the selective inhibition of COX-2 offers advantage of inhibition of inflammatory without disturbing normal body functions [20].

Morinda citrifolia is a tree in the coffee family, Rubiaceae. Among some 100 names for the fruit across different regions are the more common English names, great morinda, Indian mulberry, noni, beach mulberry, and cheese fruit. In traditional Chinese medicine, the roots, known as ba ji tian, have been used for abdominal pain, impotence, and menstrual disorders [21, 22]. Although Morinda is considered to have biological properties in traditional medicine, there is no confirmed evidence of clinical efficacy for any intended use [23].

Selective COX-2 inhibitors are a type of nonsteroidal anti-inflammatory drug (NSAID) that directly targets cyclooxygenase-2, COX-2, an enzyme responsible for inflammation and pain [24]. Targeting selectivity for COX-2 reduces the risk of peptic ulceration, and is the main feature of celecoxib, rofecoxib and other members of this drug class [25].

After several COX-2 inhibiting drugs were approved for marketing, data from clinical trials revealed that COX-2 inhibitors caused a significant increase in heart attacks and strokes, with some drugs in the class having worse risks than others [26]. Rofecoxib (commonly known as Vioxx) was taken off the market in 2004 because of these concerns and celecoxib and traditional NSAIDs received boxed warnings on their labels [27]. Many COX-2-specific inhibitors have been removed from the U.S. market. Since December 2011, only Celebrex (generic name is celecoxib) is still available for purchase in the United States [28]. Naturally derived COX 2 inhinbitors will have less side effects, so this can be used as a great alternative [29].

The aim of the study is to assess the COX 2 inhibitory effect of Morinda fruit.

Materials and Methods

The Morinda citrfolia leaf extract was obtained from Greenchem Herbal extracts and formulations, Banglore. The COX Inhibitor Screening Assay directly measures PGF2α by stannous chloride reduction of COX-derived PGH2 produced in the COX reaction. The reaction system consists of reaction buffer, haem, enzyme and plant extract pre-incubated at 37 ˚C for twenty minutes with background and enzyme controls. First, arachidonic acid was added to initiate the reaction, then it was incubated for two minutes at 37°C. After that, the saturated stannous chloride solution was added to stop the reaction, and was put at room temperature. EIA was used to quantify the prostaglandins. An aliquot of these reactions were added to the pre - coated plates in triplicates together with AChE tracer and antiserum and incubated for 18hours at room temperature on an orbital shaker. Finally, the plate was then developed with Ellman's Reagent and preserved on an orbital shaker in the dark at room temperature for 60 minutes. The absorbance was read at 420 nm. The data was plotted as %B/B0 (Standard Bound / Maximum Bound) versus log concentration using a 4-parameter logistic curve fit. The concentration of each sample was determined from a standard curve with appropriate dilutions and used to calculate the percent inhibition based on the formula given below:

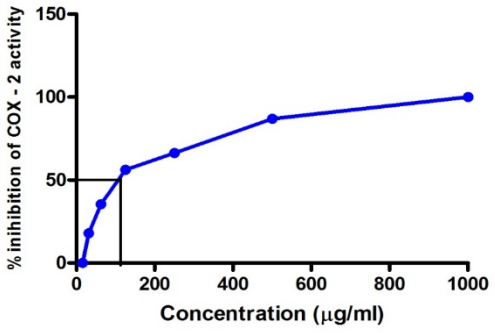
Percent Inhibition (%) = (Activity of Control – Activity of Test)/ Activity of Control x 100

The percent inhibition was plotted against the inhibitor concentration to determine the IC50 value (concentration at which there was 50% inhibition).

Results

Different concentrations (15.625, 31.25, 62.5, 125, 250, 500, 1000µg/ml) of Morinda fruit extract was evaluated for the inhibitory effect on the activity of COX. The plant extracts exhibited potent inhibition of the COX – 2 enzyme. Concentration based inhibition was observed against COX – 2 activity. The IC50 was found to be 112.1µg/ml. Maximum inhibition was found to be 94.36% at 1000µg/ml.

Figure 1: Inhibitory effect of the extract



Discussion

In the present study, it is observed that the inhibition level of Morinda citrifolia is about 94.36% which can be used instead of synthetic NSAIDs which can cause side effects like peptic ulcer. Hence this proves to be a better alternative.

The Morinda citrifolia plant, and especially its fruit, has been used for centuries in traditional medicine. Although different studies, some of them with controversial methodologies, showed that this fruit contains several nutritional and functional compounds, most of them have not been quantified [30].

With the help of a pro-inflammatory agent (bradykinin), the anti-inflammatory activity of an aqueous extract from leaf-extracts of Morinda citrifolia was discerned by inducing a locally acute inflammatory response. It was demonstrated that the formation of rat paw edema was inhibited by the oral administration of the extract rapidly. This effect may be the result of interference with the B2 receptor-mediated mechanism by which bradykinin induces rat paw edema [31].

Another research observed a selective inhibition effect on some cyclo-oxygenase enzymes involved in breast, colon and lung cancer, and also in anti-inflammatory activity of commercial noni juice [32].

Epidemiological observations stimulated interest in cancer chemoprevention with COX-2 inhibitors that the use of aspirin and other non-steroidal inflammatory drugs is connected to the decreased occurrence of colon and breast cancer [33].

The most important compounds identified in noni fruit are phenolics, such as damnacanthal and scopoletin, organic acids (caproic and caprylic acid), vitamins (ascorbic acid and provitamin A), amino acids such as aspartic acid, and minerals [34]. Another compound named xeronine, supposedly an alkaloid, has been reported, but its structure has never been published.

Contrarily, scientific researchers have depicted some new and interesting horizons in this area, but the nutritional or medical values of this plant have not been decisively proved [35]. The main proven functional properties of noni fruit are attributed to the control of several diseases [36]. In vitro research and limited experiments with lab animals have proven the anti-microbial, anti-cancer, antioxidant, anti-inflammatory, analgesic and cardiovascular activities of noni [37].

Conclusion

In the present market, basically centering on the Polynesian noni and more specifically on the Tahitian one, the leaf extract is of a unique and authentic appeal [38, 39]. Other countries may decide to start noni production and supplant the original producers in the future [40]. Although more researches are needed to discern the nutritional and functional compounds of this leaf extract, and explain its mechanisms of action in order to identify the real potential of it and the technological processes that preserve these properties, a bright future is predicted in market interest for this leaf extract.

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