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Evaluation of anti-inflammatory activity of Aqueous Extract of Philippine *Morinda citrifolia* (Noni) juice on Albino rats (*Rattus norvegicus)* using Carrageenan induced paw edema

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Abstract: The study investigates the anti-inflammatory activity in aqueous juice of *Morinda citrifolia* L. using carrageenan induced paw edema in albino rats. Traditional healers used the plant for the treatment of various diseases and there have been literature supporting that it contains anti-inflammatory property. Pure noni juice was lyophilized and two concentrations (50mg and 100mg/mL) were made. The anti-inflammatory test was performed by carrageenan induced paw edema method. Mefenamic acid was used as the standard drug. Four test group received carrageenan + mefenamic acid, carrageenan + distilled water, carrageenan + 50mg/mL noni juice and 100mg/mL noni juice and changes of paw volume hourly was noted. In comparison to the anti-inflammatory activity between both concentration, the 100mg/mL of noni juice showed a higher inhibition percentage compared to the 50mg/mL concentration and the standard drug mefenamic acid. However, the reduction of the edema over the course of the experiment can be seen on the Mefenamic acid and 50mg/mL concentration.

Key Words: *Morinda citrifolia* L.; anti-inflammatory activity; carrageenan induced paw edema

1. INTRODUCTION

Over one-third of the population in developing countries lack access to essential medicines and these places use traditional medicine to help in the primary health care need of their people (Macabeo and Aguinaldo, 2008). Because the pharmaceutical treatments are often expensive, others have sorts into using alternative traditional herbal medicine, regardless with its criticism on effectivity and safety.

Morinda citrifolia L. known as "noni", is a plant that grows widely Polynesia where its parts have been traditionally used as folk medicine for the treatment of various diseases (Wang et al., 2002). Currently, there are two recognized varieties of *M. citrifolia* (*M. citrifolia* var. citrifolia and *M. citrifolia* var. bracteate) and one cultivar (*M. citrifolia* cultivar Potteri). The most important and commonly found is the *M. citrifolia* var. citrifolia, the variant that has been recognized by traditional healers having

medicinal properties (Pawlus and Kinghorn, 2007). Its roots, stems, bark, leaves, flowers, and fruits has been used for over 40 recorded herbal remedies (Bruggnecate, 1992), including diabetes, high blood pressure, cancer and other various illnesses (Abbott and Shimazu, 1985). There are also many pharmacological activities documented including antiviral (West et al., 2012), antifungal and antihelminthic (Jayaraman et al., 2008; Brito et al., 2009), antioxidant (Su et al., 2005), anti-inflammatory (Su et al., 2001; Dussossoy et al., 2011), wound healing (Palu et al., 2010) and anticancer (Chan-Blanco et al., 2006; Akihisa et al., 2009). In recent years, there has been a tremendous growth of botanical dietary supplements of noni in North America, Western Europe and elsewhere and is its widely available in stores, pharmacies, and the internet (Pawlus and Kinghorn, 2007). Even with the absence of specific mechanisms of action for the plant, the market sales of the product claimed to reach up to US $ 1.3 billion (Potterat and Hamburger, 2007).

In traditional medicine (TM), the leaves of the plant have been used occasionally for direct application to the skin to relieve pain and swelling (McKoy et al., 2002) because of its inflammatory effect. Although there have been studies on the anti-inflammatory properties of the Noni fruit juice itself (Su et al., 2001; Mckoy et al., 2002; Akihisa et al., 2007; Dussossoy et al., 2010), particularly the Tahitian and Costa Rican, very little information is conducted on the Philippine variant. Evidence has presented that the chemical compositions and concentration are related significantly not just by the Noni plant parts but also its country of origin (Deng et al., 2010).

2. METHODOLOGY

A. Preparation of fruit juice

Two (2) liters of pure Noni Juice was obtained by the researcher and the collected juice was freeze-dried or lyophilization until it reaches into a hard-candy state. The lyophilized juice was prepared into two aqueous concentration extracts; 50 mg/ml and 100 mg/ml.

B. Experimental Animals

Sixteen (16) male albino rats weighing 20-30 g was the test subjects for this experiment. The animals were grouped in separate, standard-sized cages and maintained under standard laboratory conditions (temperature 25+ 2 ℃) and relative humidity (50+ 5%). They were allowed free access to standard pellet diet and water ad libitum. The rats were acclimatized to laboratory condition for 7 days to adjust to the 12 h light, 12 h dark cycle before commencement of experiment. The proper handling and maintenance of the rats was observed and the experimental use was approved by the Institutional Animal Care and Use Committee of DLSU.

C. Inflammation Method Design

Carrageenan was prepared as a 1% W/V solution in 0.9% saline and were use no more than 24 hours. Male albino rats will be randomized into 4 groups (4 rats per group) and was marked with a dye for identification. Pro-inflammatory agents were provided by injection of 0.1 ml of 1.0% carrageenan solution subcutaneously into the plantar region of the left hind paw using a 25-gauge needle syringe. After 30 minutes, one (1.0) ml of the test compounds: A. Control, B. Mefenamic Acid, C. Distilled water, D. Low Dosage: 50 mg/ml aqueous Noni juice and E. High Dosage: 100 mg/ml aqueous Noni Juice were administered to the animals via oral route using a sterile syringe without the needle. Changes in paw volume was measured hourly as required from 1-5 hours with the use of a digital caliper and again at 24th hour. The edema inhibitory activity was calculated according to the following formula below:

$$\text{Percent Inhibition} = [(X - Y)/X] \times 100 \quad \text{(Eq. 1)}$$

Where,

X – represents the rate without inhibitor
Y – represents the rate with inhibitor (increased paw volume after administration of treatment)

D. Statistical Analysis

The statistical analysis was use via Anova followed by Dunnett's t test and expressed as means

± SEM. Significant differences between the mean of treated animals and control groups was considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

Inflammation is a response triggered when there is damage on the living tissues. This defense mechanism is a response in order to protect against infection and injury. The carrageenan-induced paw edema as an *in vivo* model of inflammation has been used to assess the anti-edematous effect of the contribution of mediators involved in vascular changes associated with acute inflammation. In this experiment shown in Table 1, the extracts exhibited statistically significant in doses of the 50mg/ml and 100mg/ml within the 30 minutes of administration. Their difference was compared at the different hours to that of the control for the inhibition percentage of the paw volume. Group A showed no difference in the initial paw volume and the paw volume in each hour, Group B showed an elevated paw volume as it reached the 2nd hour but decline on the remaining time, giving out the lowest paw volume value. Group C showed elevation on the 2nd hour, decline on the 3rd and 4th but increased on the 5th. Group D and E showed the highest paw volume on the 2nd hour and proceeded to decline on the 3rd, 4th, and 5th hours respectively.

Table 1. Anti-inflammatory activity of *Morinda citrifolia* fruit juice in experimented albino rats

Group	Initial Paw Volume	Paw volume after induction					
		1st hr	2nd hr	3rd hr	4th hr	5th hr	24th hr
A	1.20 ± 0.13	1.20 ± 0.13	1.20 ± 0.13	1.20 ± 0.13	1.20 ± 0.13	1.20 ± 0.13	1.20 ± 0.13
B	4.61 ± 0.13	4.42 ± 0.01	4.43 ± 0.01	4.43 ± 0.01	4.41 ± 0.01	3.70 ± 0.29	3.87 ± 0.29
C	5.05 ± 0.02	5.03 ± 0.01	5.03 ± 0.01	4.47 ± 0.01	4.47 ± 0.01	4.63 ± 0.15	4.43 ± 0.02
D	5.05 ± 0.02	4.89 ± 0.14	4.75 ± 0.16	4.46 ± 0.01	4.47 ± 0.01	4.46 ± 0.01	4.45 ± 0.01
E	5.04 ± 0.01	4.99 ± 0.36	5.27 ± 0.25	4.61 ± 0.15	4.60 ± 0.13	4.60 ± 0.16	4.41 ± 0.02

Legend: *Values are mean ± SD of four samples in each group. A: Control B: Mefenamic Acid, C: Distilled water, D: 50mg/ml Noni Juice E: 100mg/ml Noni Juice. Significant differences between the mean of treated animals and control groups was considered significant at P < 0.05.*

Table 2. Percentage of Inhibition

Group	Initial Paw Volume	5th hour (mm)	Difference in Paw	Inhibition Percentage
A	1.20 ± 0.13	1.20 ± 0.13	0.00	100
B	4.61 ± 0.13	3.70 ± 0.29	0.91	75.41
C	5.05 ± 0.02	4.63 ± 0.15	0.42	90.93
D	5.05 ± 0.02	4.46 ± 0.01	0.59	86.77
E	5.04 ± 0.01	4.60 ± 0.16	0.44	90.43

Legend: *Inhibition of carrageenan-induced rat paw edema by B: mefenamic acid, C: distilled water and Aqueous Noni juice (D: 50mg/ml and E:100 mg/ml) measured at 1, 2, 3, 4, and 5 h after carrageenan injection*

Table 2 showing the percentage of inhibition of the treated group of rats. Both the 100mg/ml of the aqueous noni fruit juice and distilled water showed the highest inhibition percentage effect on the carrageenan-induced inflammation, but by contrast, the low dosage (50mg/ml) and the Mefenamic acid displayed inhibition of the inflammatory response of 86.77% and 75.41% respectively. And although the inhibition of the paw edema was higher to the highest aqueous solution than that of the standard drug Mefenamic acid, the effectiveness of the reduction of the edema can be found more on the standard drug as seen on the fifth hour of the investigation.

The rate of development of edema reached its maximum level at the 2nd hour (*Fig. 2*) and after that it started to decline. The graph demonstrate that the low dose of the aqueous noni juice showed a continuous reduction of the paw volume while the high dose showed an increase as it reached the 2nd hour and then followed by reduction. Figure 1 shows the time course of the anti-inflammatory effect of the concentrations – how the reduction of the edema and the coloration of the paw from reddish to pink. There was no evidence of toxic effect on the rats within the 24th hour of oral administration. Dunnett's t test was done to compare the statistical significant changes

between control and the aqueous extract(s) and based on ANOVA result, the data is not significant.

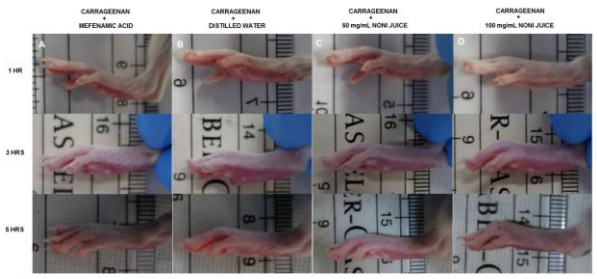

Figure 1. Typical representative macroscopic photographs of paw from the (A) carrageenan + mefenamic acid, (B) 1.0% carrageenan + distilled water, (C) 1.0% carrageenan + 50 mg/mL aqueous noni juice and (D) 1.0% carrageenan + 100 mg/mL aqueous noni juice groups.

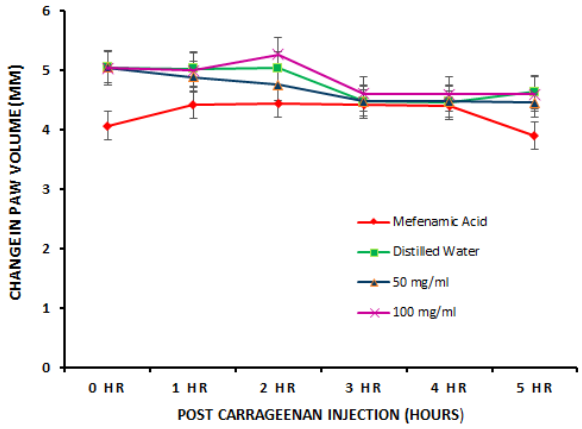

Fig. 2. Effects of Mefenamic acid and aqueous Noni juice extract (50mg and 100mg/ml, orally) on the development of carrageenan-induced edema on the rat paw. Values are means ± SEM for 4 animals in each group.

The characteristic swelling of the paw is caused by the formation of edema and the endogenous mediators of inflammation includes histamine and prostaglandin (Bowman and Rand, 1980). In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine and serotonin on vascular permeability. Anti-inflammatory drugs, such as mefenamic acid, works by blocking the effect of chemicals called cyclo-oxygenase (COX) enzymes. These enzymes help to make other chemicals in the body, called prostaglandins. Some prostaglandins are produced at sites of injury or damage, and cause pain and inflammation. By blocking the effect of COX enzymes, fewer prostaglandins are produced, which means pain and inflammation are eased. In the experiment, the low dosage partially showed anti-inflammatory effects compared to the high dose however the standard drug shows much effectiveness.

4. CONCLUSIONS

The result has shown that *Morinda citrifolia* (noni) aqueous juice does possess some anti-inflammatory effect induced by carrageenan but the specific mechanism on the juice against carrageenan-induced edema is not determine. Even though with the results of the study, further investigation of the anti-inflammatory effect of the fruit extract especially the identification and isolations of the bioactive components should be performed. The researcher also recommends the use of a digital plethysmometer for a more effective measurement of inflammatory agents and a histopathological assessment of the effects of each solution.

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