JABET
Journal of Advanced Biotechnology and Experimental Therapeutics

ORIGINAL ARTICLE

J Adv Biotechnol Exp Ther. 2024 Sep; 7(3): 498-506
eISSN: 2616-4760, https://doi.org/10.5455/jabet.2024.d43
Published by www.bsmiab.org

Noni (*Morinda citrifolia* L.) fruit juice reduces paw edema and protects gastric mucosal injury in rats

Alvira Firdausi Ali[1], Muhammad Mufaiduddin[1], Lingga Agustina[1], Desy Armalina[2,*]

[1]Department of Medicine, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
[2]Department of Anatomy and Histology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

***Corresponding author**
Desy Armalina
Department of Anatomy and Histology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
Email:desyarmalina@lecturer.undip.ac.id

Academic editor
Md Jamal Uddin, PhD
ABEx Bio-Research Center, Bangladesh

Article info
Received: 03 November 2023
Accepted: 11 January 2024
Published: 01 July 2024

Keywords
Gastric mucosal injury, *Morinda citrifolia*, Noni fruit juice, Paw edema.

ABSTRACT

Edema is a reliable parameter to evaluate the anti-inflammatory process. Noni (*Morinda citrifolia* L.) fruit has been shown to decrease inflammation. However, studies on the impact of noni juice on gastric mucosal injury are still limited. Thus, the current study aimed to assess the effect of noni fruit juice as an anti-inflammatory agent in rat paw edema and gastric mucosal injury. The rats were divided into five groups such as control, carrageenan only, carrageenan and diclofenac sodium, carrageenan and 90% noni, and carrageenan + 100% noni. Paw edema was measured, and histopathological examination of the gastric tissues was performed. The administration of noni juice significantly reduced paw edema in the carrageenan + 90% noni and carrageenan + 100% noni groups of rats. Compared to the carrageenan + diclofenac sodium group, the carrageenan + 90% noni and carrageenan + 100% noni groups were not significantly different in reducing paw edema. It suggests that the administration of noni juice can reduce paw edema with the same potential as diclofenac sodium. Gastric mucosal injury in the carrageenan + diclofenac sodium group (scores 2 to 3) was higher than in the carrageenan + 90% noni and carrageenan + 100% noni groups (scores 1 to 2), both of them had significant differences (p<0.05). In conclusion, noni juice can reduce paw edema and protect against gastric mucosal injury.

INTRODUCTION

Inflammation is a defense mechanism of the tissue against pathogens, damaged cells, toxic compounds, or irradiation [1]. However, chronic inflammation may cause tissue damage leading to the death of healthy cells [2]. The inflammatory reaction is caused by releasing various inflammatory mediators and is easily recognized as five cardinal signs such as fever, pain, redness, edema, and loss of function [3, 4]. A previous study mentioned that edema was reliable for evaluating the anti-inflammatory process [5].

The inflammatory process includes the regulatory role of prostaglandins and the primary cytokine mediators including cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [6]. The most frequently given anti-inflammatory drugs for both acute and chronic illnesses are non-steroidal anti-inflammatory drugs (NSAIDs) [7]. Unfortunately, NSAIDs altered the surface of the gastric mucosal layer, causing back diffusion of hydrogen ions, and pepsin resulted in cell damage. Consumption of NSAIDs increases aggressive factors and decreases defensive factors that can develop gastric ulcers [8].

Noni (*Morinda citrifolia* L.) fruit is safe to be consumed for an extended period and does not affect the gastric mucous [9]. Noni fruit is known for its bioactive compounds, including scopoletin, alizarin, rutin, asperulosidic acid, nonisade A, and tricetin which can potentially decrease inflammation [10, 11]. This decreased inflammation might be caused by inhibiting COX-2, reducing the expression of nitric oxide, reducing the

expression of IκB kinase (IKK) α/β, an inhibitor of kappa B alpha (I-κBα), and nuclear factor kappa B (NF-κB) p65 in lipopolysaccharide (LPS)-stimulated macrophages [10]. Noni fruit has a gastroprotective effect because of its polyphenol and metabolite content [12]. This research aimed to assess the effect of noni fruit as an anti-inflammatory agent in rat paw edema and gastric mucosal injury.

MATERIALS AND METHODS

Animal study

Twenty male rats, aged 2-3 months and weighing 150-200 g, underwent 7 days of adaptation. Rats were kept in an environment with a consistent temperature and humidity level, a twelve-hour light/dark cycle with *ad libitum* access to food and water. The Medical and Health Research Ethics Commission, Faculty of Medicine, Diponegoro University, provided its ethical permission (No. 66/EC/H/FK-RSDK/VI/2018).

Noni juice preparation

The fruits were collected from Jepara, Indonesia, and brought to the Animal Laboratory of Diponegoro University, Indonesia. The seeds and skin at 390 g of noni fruits were removed before processing. 130 g of noni juice was centrifuged at 3000 rpm for 20 min until the supernatant was clear. The supernatant (60 cc) was used at 100% and 90% concentrations (diluted with physiologic saline). The juice was given at 1 cc orally right after the carrageenan injection.

Carrageenan-induced paw edema

The rats were divided into five groups such as control, carrageenan only, carrageenan and diclofenac sodium, carrageenan and 90% noni, and carrageenan + 100% noni. Carrageenan (Gumindo Perkasa Industry, Indonesia) was used to induce paw edema in rats. 30µl of 1% carrageenan (diluted in distilled water) was injected into four groups except the control group as previously described [5]. A plethysmometer was used to measure paw edema volume (ml) for 0, 15, 30, and 60 min post-injection. We defined paw edema volume changed as Δn (n = 1-4; 1 = reducing paw edema volume from 0 to 15 min; 2 = reducing paw edema volume from 15 to 30 min; 3 = reducing paw edema volume from 30 to 60 min; and 4 = reducing paw edema volume from 0 to 60 min).

Gastric mucosa examination

Animals were terminated, and the stomach was removed for further examination. For 24 hours, the stomach was fixed in 10% buffered. Gastric mucosal integrity was examined through a histopathological examination using hematoxylin-eosin (HE) staining and the severity of mucosal injury was assessed using the Barthel Manja score [3]. We used an Olympus CX23 microscope (Olympus, Japan) for histopathological examination of the gastric mucosa.

Statistical analysis

The paw edema volume showed normal distribution using the Saphiro-Wilk method except in the carrageenan + 100% noni group. Repeated ANOVA was used to compare

paw edema volume in 0-, 15-, 30-, and 60-minute post-carrageenan injection in control, carrageenan + diclofenac sodium, and carrageenan + 90% noni groups, while the carrageenan + 100% noni group were analyzed using the Friedman test. The one-way ANOVA was used to assess **Δ**2 and **Δ**4 to find differences across groups and a post hoc test was used to find differences between groups. The Kruskall-Wallis and Mann-Whitney test was used to examine gastric mucosa's **Δ**1, **Δ**3, and histopathological features. The results were presented as the mean ± SD. When, $p< 0.05$, the differences were deemed significant. The SPSS software application was used to conduct statistical analysis.

RESULTS

All groups were observed for their behavior during the research. Indicators such as activity, the excess daily volume of water, and viability were observed. All those indicators were assessed semi-quantitatively. All groups were at the same activity levels and survived until the experiment was completed.

Effect of noni fruit juice on paw edema volume in rats

The results showed decreasing volume edema from 0 to 60 min after treatment in the carrageenan + diclofenac sodium group, carrageenan + 90% noni group, and carrageenan +100% noni group (Figure 1). Paw edema volume in the group carrageenan was only adjusted based on the previous study [13]. The largest paw edema volume after being induced by 30 µl sub–plantar 1% carrageenan (0 min) was shown in the carrageenan + 90% noni group (1.30 ± 0.23 cc). At the last minute (60 min), the smallest paw edema volume occurred in the carrageenan + diclofenac sodium group (0.65 ± 0.23 cc). All groups showed a decrease in paw edema volume over time. We used repeated ANOVA and the Friedman test to assess these results, and the results revealed a significant difference ($p< 0.05$). These results proved that both groups with diclofenac sodium and noni juice administration have the potential to be anti-inflammatory with a decrease in paw edema volume.

Effect of noni fruit juice on paw edema volume in rats

The previous results showed differences in paw edema volume in the first minute (0 min) after carrageenan injection, so further analysis was needed as a comparison among groups. We analyzed the comparison with **Δ**n. **Δ**n was used as a more accurate parameter in comparing the effectiveness of reducing paw edema volume among groups. Figure 2 compares decreasing paw edema volume from 0 to 60 min. We performed **Δ**n outcome analysis to compare among groups, the Kruskall-Walli's test and Mann-Whitney test were used. Based on the analysis, almost all comparisons among groups showed no significant differences except for **Δ**3, the comparison between carrageenan + diclofenac sodium group and carrageenan + 90% noni group ($p = 0.005$). These results indicated no significant difference in the administration of diclofenac sodium with noni juice in reducing paw edema volume.

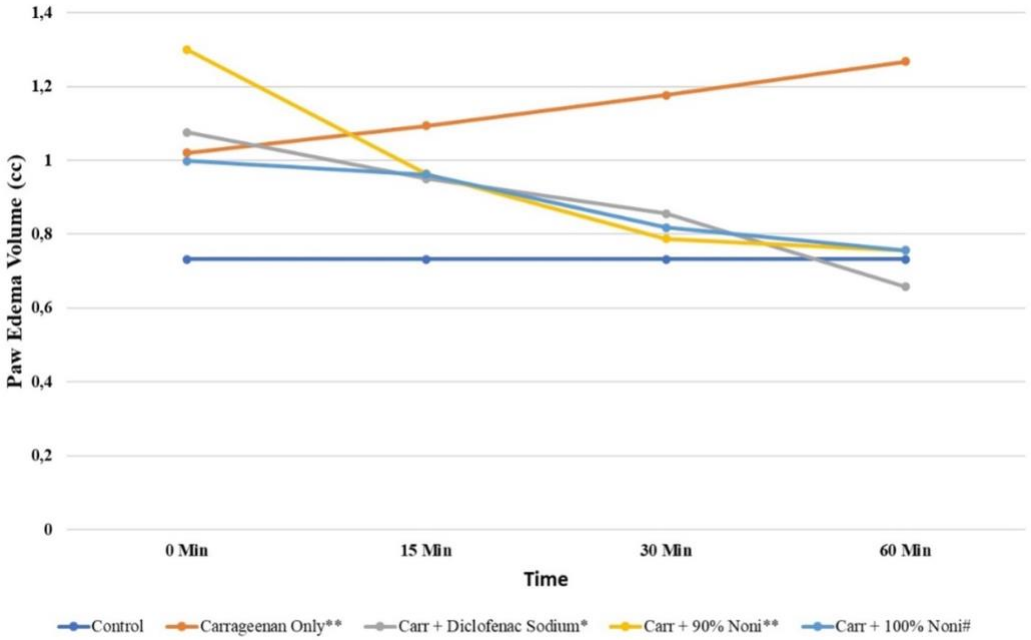

Figure 1. Paw edema volume decreased from 0 to 60 min. Control, carrageenan only (Carr), carrageenan and diclofenac sodium, carrageenan and 90% noni, and carrageenan + 100% noni. The mean is used to represent the values. $^{*}p$=0.002; with repeated ANOVA test, $^{\#}p$=0.002; with Friedman test, and $^{**}p$<0.0001; with repeated ANOVA test.

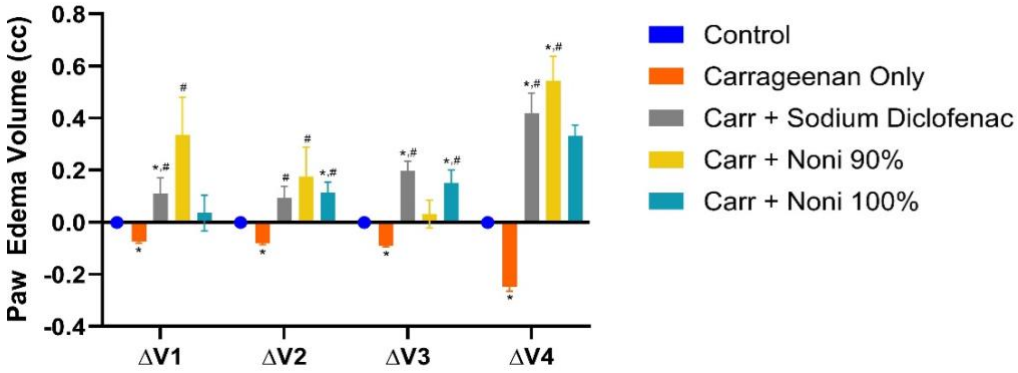

Figure 2. Comparison of decreasing paw edema volume among groups. Control, carrageenan only (Carr), carrageenan and diclofenac sodium, carrageenan and 90% noni, and carrageenan + 100% noni. **Δ**V1, decreasing paw edema volume from 0-15 minutes; **Δ**V2, decreasing paw edema volume from 15-30 minutes; **Δ**V3, decreasing paw edema volume from 30-60 minutes; and **Δ**V4, decreasing paw edema volume from 0-60 minutes. The mean is used to represent the values. The min-max is used to represent the values. (*) p<0.05 compared with control group, (#) p<0.05 compared with carrageenan + diclofenac sodium group.

Effect of noni fruit juice on gastric mucosa integrity

The results of gastric mucosa integrity after being treated with noni fruit juice and diclofenac sodium can be seen in Table 1 and Figure 3.

Table 1. Barthel Manja scores in experimental animals.

Groups	Barthel Manja Score	
	Mean ± SD	Max - Min
Control group, only given standard feed	0.20 ±0.447	1 - 0
Induced group, 30 µl 1% carrageenan, and given diclofenac sodium orally at 9 mg/kg BW	2.20 ± 0.548	3 - 2
Treatment group, induced 30 µl 1% carrageenan, and given one cc 90% noni juice	1.40 ± 0.548	2 - 1
Treatment group, induced 30 µl 1% carrageenan, and given one cc 100% noni juice	1.20 ± 0.447	2 - 1

The control group showed no or less pathological findings on the gastric mucosa (score 0 to 1). The carrageenan only group was not evaluated for the gastric mucosa histopathology. Based on Szandruk-Bender et al, administration of carrageenan only does not damage the gastric mucosa (score 0 = no damage) [14]. The carrageenan + diclofenac sodium group showed injury at the muscular level (score 2 to 3). The carrageenan + diclofenac sodium group score was higher than the carrageenan + 90% noni or carrageenan + 100% noni groups. The results of the carrageenan + 90% noni and carrageenan + 100% noni groups showed epithelial desquamation (score 1 to 2). The comparisons of all groups revealed significant differences according to the Kruskal-Walli's test (p=0.002). Additionally, the Mann-Whitney test was used to determine the differences between groups. Except for the carrageenan + 90% noni and carrageenan + 100% noni groups, which did not demonstrate significant differences with p>0.05, the Mann-Whitney test revealed significant differences between groups with p<0.05. These results could prove that the effect of noni juice administration as an anti-inflammatory agent in rat's paw edema was safer on the gastric mucosa integrity than diclofenac sodium administration.

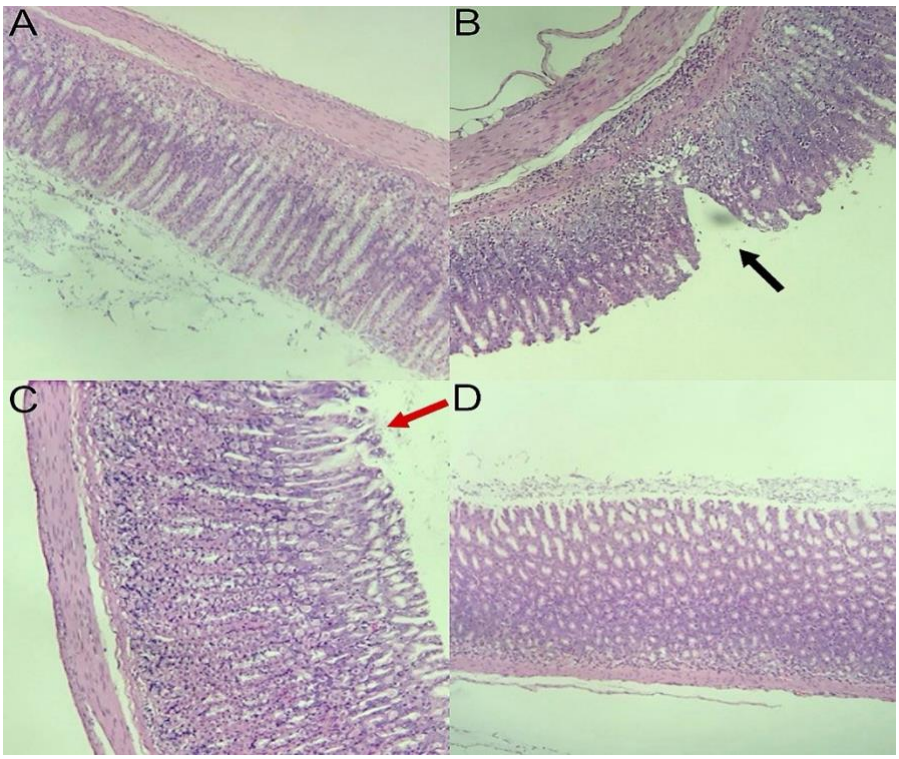

Figure 3. Histopathology examination of gastric mucosa epithelial in A) control group; B) carrageenan + diclofenac sodium group; C) carrageenan + 90% noni group; D) carrageenan + 100% noni group. Gastric epithelial ulcer (black arrow); epithelial desquamation (red arrow).

DISCUSSION

Noni is famous for its chemical compounds that have various health benefits. Besides its nutrition, noni contains antioxidant, antimicrobial, anticancer, and anti-inflammatory properties [11]. Noni fruit contains scopoletin, alizarin, rutin, asperulosidic acid, nonisade A, and tricetin. The bioactive compound of noni fruit exhibited potential anti-inflammatory activity [10, 11]. Another study showed that RAW264 cells activated by LPS showed anti-inflammatory effects from *Morinda citrifolia* [15]. The anti-inflammatory effect was also demonstrated in extensive studies regarding the biological activity reported in Noni fruit juice, showing the strongest nuclear erythroid-related factor 2 (Nrf2) activation and NF-κB inhibitory activity [16]. This evidence is consistent with our results, which demonstrated a significant decrease in paw edema, an inflammation parameter in our study. Edema is a form of inflammatory response [3, 4]. Therefore, our study used carrageenan induction to produce edema as a form of inflammation. It was demonstrated in this study that administering noni juice reduced edema as a form of anti-inflammatory. These results also followed a previous study proving that topical noni leaf extract could reduce rat paw edema induced by carrageenan [17].

Noni fruit containing rutin, asperulosidic acid, nonisade A, and tricetin may have therapeutic and preventative effects on inflammatory diseases. In anti-inflammatory tests, the active compounds in noni fruit prevented LPS-stimulated macrophages from producing nitric oxide (NO), a pro-inflammatory mediator. Furthermore, other mechanisms of anti-inflammatory action include down-regulating the expression of IKKα/β, I-κBα, NF-κB p65 in LPS-stimulated macrophages, and down-regulating the expression of nitric oxide synthase and COX-2 [10]. More than that, antioxidants help to prevent inflammatory responses. Noni juice ameliorated oxidative stress by up-regulating the expression of related antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) and attenuating reactive oxygen species (ROS) and malondialdehyde (MDA) [18]. Manavalan et al. demonstrated the antioxidant potential of Noni fruit juice [16]. Scopoletin in noni fruit can reduce oxidative stress by increasing Nrf2 activation and inhibiting NF-kB [16, 19].

The difference in the reduction of paw edema defined as **Δ**n between noni juice and diclofenac sodium groups was insignificant. It can be assumed that the potential anti-inflammatory effect between noni juice and diclofenac sodium is not much different. Carrageenan sub–plantar injection promotes histamine release, serotonin, prostaglandin, and bradykinin, whereas, after 1-hour injection, neutrophil infiltration, free radicals produced by neutrophils, NO, and pro-inflammatory cytokines such as tumor necrosis factor (TNF-α) were released [20]. According to reports, noni juice decreased carrageenan-induced paw edema by preventing COX-2 activity, preventing NO generation, and triggering prostaglandin E [10]. This mechanism is not too different from diclofenacsodium; it is through the inhibition of COX-1 and COX-2 relatively equally [21].

We examined the safety of using noni juice on the stomach because the anti-inflammatory side effects often cause gastric ulcers [22]. Our study showed that the noni juice group did not cause gastric mucosal injury. These results were significantly different from the group given diclofenac sodium. Although both anti-inflammatory mechanisms are not too other, the effect of noni juice is more selective as a COX-2 inhibitor. A previous study proved that Noni fruit acts as a COX-2 inhibitor [10]. Acute mucosal injury caused by diclofenac sodium as NSAID agent was commonly found and reversible [23]. The damage caused by selective COX-2 inhibitors was possible, even infrequent, depending on the subject's condition. Changes in gastrointestinal bacteria and free radicals increase the aggressive effect on the stomach, leading to mucosa injury [24]. Oxidative stress includes ROS, reactive oxygen intermediates (ROI), and the byproduct of cellular metabolism,

which causes oxidative damage to the tissue. NSAIDs were one of the exogenous factors that caused ROS production [25]. ROS production induced lipid peroxidation on the epithelial cells of the stomach, and pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-1β (IL-1β) resulted in mucosa injury. The noni antioxidant supports the stomach defensive factor by compensating for ROS-induced epithelial damage and protecting further damage from oxidative stress [16, 19, 26]. Recent meta-analysis studies showed 19% selective COX-2 increased upper gastrointestinal side effects but less gastrointestinal toxicity compared with non-selective NSAIDs [27]. This was in accordance with our results, which showed that several samples using noni juice fruit scored 1-2 of the Barthel Manja score (epithelial desquamation) but less than a score when giving diclofenac sodium (score 2-3).

The anti-inflammatory effect in pure noni juice significantly decreased carrageenan-induced paw edema [28]. Yet, this study's 90% concentration of noni juice also significantly reduced paw edema volume**.** The study's results, the comparison between both in terms of lowering paw edema and the histopathological appearance of the stomach was not significantly different. We can conclude that the 100% and 90% concentrations of noni juice have the same potency. However, more investigation is required to establish the optimal noni juice concentration through studies at lower concentrations.

This study only observes the acute effect of noni juice administration on gastric mucosa compared with diclofenac sodium. Noni juice was administrated instead of noni extract in this study. For further research, the author suggested administering noni juice extract instead of juice and observing this treatment's chronic effect on mucosa. This study also has other limitations in showing anti-inflammatory effects; further research is needed to measure pro-inflammatory markers.

CONCLUSION

The administration of noni juice could reduce paw edema as an anti-inflammatory effect. The anti-inflammatory effect between noni juice and diclofenac sodium had the same potential. The safety effect of noni juice was shown in the histopathological future of the stomach, which proved that noni juice could protect against gastric mucosal injury. Mucosa injury caused by noni juice treatment was not worse than diclofenac sodium because of the selective COX-2 and the antioxidant activity of quercetin and scopoletin. Otherwise, COX-2 inhibitors in diclofenac sodium cause mucosal injury due to oxidative stress.

ACKNOWLEDGMENT

None.

AUTHOR CONTRIBUTIONS

All authors developed the ideas, designed the study, conceptualized, collected data, analyzed the data, drafted the manuscript, and revised and edited the manuscript; DA supervised the study. The final manuscript has been read and approved by all authors.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1] Medzhitov R. Inflammation 2010: New adventures of an old flame. Cell. 2010;140:771-6.

[2] Yoshikawa T, Naito Y. The role of neutrophils and inflammation in gastric mucosal injury. Free Radic Res. 2000;33:785-94.

[3] Jisha N, Vysakh A, et al. Anti-inflammatory efficacy of methanolic extract of muntingia calabura l. Leaves in carrageenan induced paw edema model. Pathophysiology. 2019;26:323-30.

[4] Kumar V, Abbas AK, Aster JC. Robbins and cotran pathologic basis of disease. 9th edition. Philadelphia: Saunders; . 2015.

[5] Ou Z, Zhao J, et al. Anti-inflammatory effect and potential mechanism of betulinic acid on λ-carrageenaninduced paw edema in mice. Biomed Pharmacother. 2019;118:109347.

[6] Ferrer MD, Busquets-Cortés C, et al. Cyclooxygenase-2 inhibitors as a therapeutic target in inflammatory diseases. Curr Med Chem. 2019;26:3225-41.

[7] Alfaro R.A., Davis D.D. Diclofenac. In xPharm: The Comprehensive Pharmacology Reference; StatPearls Publishing: Treasure Island, FL, USA. 2022:1-7. Available online: https://www.ncbi.nlm.nih.gov/books/NBK557879/.

[8] Tai FWD, Mc Alindon ME. Non-steroidal anti-inflammatory drugs and the gastrointestinal tract. Clin Med (Lond). 2021;21:131-4.

[9] AU A. An overview of inflammation: Mechanism and consequences. Front Biol China. 2011;6:274-81.

[10] Lee D, Yu JS, et al. Identification of anti-inflammatory compounds from hawaiian noni (morinda citrifolia l.) fruit juice. Molecules. 2020;25:4968.

[11] Jamaludin R, Kim DS, et al. Kinetic study of subcritical water extraction of scopoletin, alizarin, and rutin from morinda citrifolia. Foods. 2021;10:2260.

[12] Jiang Q, Charoensiddhi S, et al. A review on the gastrointestinal protective effects of tropical fruit polyphenols. Crit Rev Food Sci Nutr. 2023;63:7197-223.

[13] Halil S, Cihan G, et al. Investigation of the anti-inflammatory effects of caffeic acid phenethyl ester in a model of λ-carrageenan–induced paw edema in rats. Human and Experimental Toxicology. 2021;40:721-38.

[14] Marta Szandruk-Bender AM-L, Benita Wiatrak, Maciej Danielewski, Stanislaw Dzimira, Danuta Szkudlarek, Lukasz Szczukowski, Piotr Swiatek, Adam Szelag. Novel i, 3,4-oxadiazole derivate of pyrrolo [3,4-*d*]pyridazinone exert anti-inflammatory activitry without acute gastrotoxicity in the carrageenan-induced rat paw edema test. Journal of Inflammation Research. 2021;14:5739-56.

[15] Tanikawa T, Kitamura M, et al. Anti-inflammatory effects of morinda citrifolia extract against lipopolysaccharide-induced inflammation in raw264 cells. Medicines (Basel). 2021;8:43.

[16] Manavalan, Arulmania, et al. Nrf2 activation by morindacitrifolia l. (noni) fruit juices. World Journal of Traditional Chinese Medicine. 2021;7:184-91.

[17] Sabirin I, Yuslianti E. Effect of topical noni (morinda citrifolia l.) leaf extract paste in carrageenan-induced paw edema on wistar rats. Global Medical and Health Communication. 2019;7:116-22.

[18] Zhang X, Zhang B, et al. Fermented noni (morinda citrifolia l.) fruit juice improved oxidative stress and insulin resistance under the synergistic effect of nrf2/are pathway and gut flora in db/db mice and hepg2 cells. Food Funct. 2022;13:8254-73.

[19] Narasimhan KK, Jayakumar D, et al. Morinda citrifolia and its active principle scopoletin mitigate protein aggregation and neuronal apoptosis through augmenting the dj-1/nrf2/are signaling pathway Oxid Med Cell Longev. 2019;2019:2761041.

[20] Mansouri MT, Hemmati AA, et al. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian J Pharmacol. 2015;47:292-8.

[21] Altman R, Bosch B, et al. Advances in nsaid development: Evolution of diclofenac products using pharmaceutical technology. Drugs. 2015;75:859-77.

[22] Sostres C, Gargallo CJ, et al. Adverse effects of non-steroidal anti-inflammatory drugs (nsaids, aspirin and coxibs) on upper gastrointestinal tract. . Best Pract Res Clin Gastroenterol. 2010;24:121-32.

[23] Bruno A, Tacconelli S, Patrignani P. Variability in the response to non-steroidal anti-inflammatory drugs: Mechanisms and perspectives. Basic Clin Pharmacol Toxicol. 2014;114:56-63.

[24] Cryer B, Goldstein JL. Gastrointestinal injury associated with nsaid use : A case study and review of risk factors and preventative strategies. Drug, Heal Patient Saf. 2015:31-41.

[25] Bhattacharyya A, Chattopadhyay R, et al. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev. 2014;94:329-54.

[26] Yeo D, Hwang SJ, et al. The aqueous extract from artemisia capillaris inhibits acute gastric mucosal injury by inhibition of ros and nf-kb. Biomed Pharmacother. 2018;99:681-7.

[27] Curtis E, Fuggle N, et al. Safety of cyclooxygenase-2 inhibitors in osteoarthritis: Outcomes of a systematic review and meta-analysis. . Drugs Aging. 2019;36:25-44.

[28] Yilmazer N, Coskun C, et al. Antioxidant and anti-inflammatory activities of a commercial noni juice revealed by carrageenan-induced paw edema. Pol J Vet Sci. 2016;19:589-95.