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Effectiveness of ethanol extract of Morinda citrifolia L. as an anti-inflammatory: A preclinical study

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Abstract: The inflammatory response is triggered by infections or tissue damage, often requiring effective anti-inflammatory treatments. *Morinda citrifolia L*. (noni fruit) contains bioactive compounds such as flavonoids, steroids, and triterpenoids with potential anti-inflammatory properties. The aims of this research to evaluate the anti-inflammatory effects and optimal dosing of ethanol extract of noni fruit in reducing paw edema and improving histopathological conditions in male rats induced with Complete Freund's Adjuvant (CFA). This experimental study involved 30 male rats divided into six groups: normal control, negative control (CFA-only), positive control (triamcinolone 0.72 mg), and three treatment groups receiving noni fruit extract (150, 300, 600 mg/200 g BW). Edema volume was measured using a plethysmometer, and histopathological analysis of joint tissues was conducted. Noni fruit extract significantly reduced edema volume, with 600 mg/200 g BW being the most effective dose. However, histopathological analysis revealed persistent inflammatory cell infiltration and synovial hyperplasia in treated groups, indicating incomplete tissue repair compared to triamcinolone. Ethanol extract of noni fruit demonstrates significant anti-inflammatory effects, particularly at higher doses, but is less effective than triamcinolone in resolving tissue inflammation.

Keywords: Morinda citrifolia L, anti-inflammatory, histopathology

INTRODUCTION

The body's inflammatory response is a critical defense mechanism triggered by infection or tissue damage. This response is characterized by heat, redness, swelling, discomfort, and functional impairment. Common causes of inflammation include microorganisms, mechanical injury, chemical exposure, and physical effects. The ultimate goal of inflammation is to attract plasma proteins and phagocytes to the site of injury or invasion, isolate or neutralize invaders, clear debris, and prepare tissues for healing processes. However, persistent or chronic inflammation can result in tissue damage and disease progression.[1,2,3,4,5,6]

To address inflammation, anti-inflammatory drugs are widely used and are generally classified into two groups: steroidal anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs). SAIDs, such as corticosteroids, block the release of prostaglandins, reducing inflammation at its source. NSAIDs, including ibuprofen, aspirin, and naproxen, inhibit cyclooxygenase (COX), an enzyme responsible for prostaglandin synthesis. While effective, both types of drugs carry risks: SAIDs may cause immunosuppression, osteoporosis, and diabetes, while NSAIDs are associated with gastrointestinal ulcers, anemia, and renal complications.[7,8,9] These limitations necessitate the exploration of alternative, safer anti-inflammatory agents.

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Morinda citrifolia L., commonly known as noni, is a traditional medicinal plant widely recognized for its potential therapeutic properties, including anti-inflammatory effects. Noni fruit contains bioactive compounds such as flavonoids, iridoids, and damnacanthal, which inhibit pro-inflammatory enzymes like COX-2 and cytokines such as TNF-α and IL-6. Previous studies have demonstrated that noni extract can suppress the NF-κB pathway, reduce prostaglandin and nitric oxide production, and mitigate inflammation, making it a promising alternative for managing inflammatory conditions such as arthritis.[10,11]

Despite these promising findings, significant gaps remain in understanding the specific mechanisms and optimal dosing of noni fruit extract for anti-inflammatory purposes. For instance, while earlier studies have shown the efficacy of noni in reducing cytokine expression and NF-κB activity, its direct impact on histopathological changes and its comparative effectiveness against standard treatments like corticosteroids remain underexplored.[12,13] Additionally, while noni's therapeutic potential has been supported by biochemical and cellular studies, there is limited evidence on its effectiveness in preclinical animal models.

Freund's Complete Adjuvant (CFA), commonly used to induce inflammation in experimental settings, provides a robust model for studying anti-inflammatory agents. CFA induces localized inflammation, allowing researchers to assess both macroscopic changes, such as edema volume, and microscopic alterations, such as inflammatory cell infiltration in joint tissues.[14] Male *Rattus norvegicus* are often employed in such studies due to their stable hormonal profile and suitability for surgical and sampling procedures, further ensuring reliable results.[15,16] In this context, this study aims to address the research gap by evaluating the effectiveness of ethanol extract of *Morinda citrifolia* L. in reducing inflammation and improving histopathological outcomes in CFA-induced male rats. By comparing the effects of noni extract to those of standard treatments, this study seeks to provide critical insights into its potential as a safer, alternative anti-inflammatory agent.

MATERIAL AND METHOD

This study employed an experimental design to assess the impact and correlation between the independent and dependent variables. The dependent variables were the reduction in inflammation, edema volume, and histopathological changes in joint tissues, while the independent variable was the ethanol extract of *Morinda citrifolia* L. The study was conducted between November 1, 2023, and February 12, 2024, at the Pharmacology Laboratory of the Faculty of Pharmacy, University of North Sumatra, and the Anatomical Pathology Laboratory at Royal Prima Hospital Medan. Ethical approval for the use of experimental animals was obtained from the Prima Indonesia University Health Research Ethics Committee (No. 061/KEPK/UNPRI/IX/2023).

Tools and Materials

The study utilized a variety of laboratory tools and reagents essential for conducting the experimental procedures. Ethanol extracts of *Morinda citrifolia* L. were prepared through maceration followed by concentration using ethanol and silica gel chromatography for compound separation. Thirty male *Rattus norvegicus* (aged 2–3 months and weighing 150–200 g) were used as test animals, with Complete Freund's Adjuvant (CFA) employed to induce inflammation. During the treatment phase, paw edema volume was measured using a plethysmometer to evaluate the anti-inflammatory activity. Histopathological analysis of the joint tissues was performed following paraformaldehyde fixation, tissue dehydration, paraffin embedding, and Hematoxylin-Eosin (HE) staining for microscopic evaluation. Standard reagents such as PBS-azide solution, nitric acid, and hematoxylin-eosin dyes were used throughout the experimental procedures.

Preparation of Ethanol Extract of Noni Fruit

Fresh noni fruits were washed, seeds and pulp removed, and the remaining material oven-dried. The dried material was ground into powder. Maceration was performed using 96% ethanol for 24 hours, followed by filtration and concentration with a rotary evaporator to obtain a thick ethanol extract.[17,18]

CFA-Induced Inflammation in Test Animals

Male rats were subplantarly injected with 0.1 mL CFA into the left hind paw to induce inflammation. After 16 days, the paw edema volume was measured using a plethysmometer. Edema measurements were performed on day 0 (before CFA injection) and on days 17, 20, 23, 26, 29, and 31 post-injection.[19]

Study Groups and Treatments

The 30 test animals were acclimatized for two weeks and randomly divided into six groups (n=5 per group):

1. **Normal control:** No treatment.
2. **Negative control:** CFA injection only.
3. **Positive control:** CFA injection followed by triamcinolone (0.72 mg intramuscularly) on day 17.
4. **Dose I:** CFA injection followed by 150 mg/200 g BW noni fruit extract daily from day 17 to day 31.
5. **Dose II:** CFA injection followed by 300 mg/200 g BW noni fruit extract daily from day 17 to day 31.
6. **Dose III:** CFA injection followed by 600 mg/200 g BW noni fruit extract daily from day 17 to day 31.

Anti-inflammatory Activity Test

The anti-inflammatory effect was evaluated by measuring the reduction in paw edema volume using a plethysmometer. The decrease in paw edema was interpreted as an indicator of anti-inflammatory activity.[20,21]

Histopathological Analysis

On day 32, after 15 days of treatment, joint tissues were collected from the left hind paws of the rats. The procedure involved:

1. **Dissection:** Rats were euthanized by cervical dislocation, and the left hind paw joint was excised.
2. **Tissue Processing:** The joint tissues were washed with cold 0.9% NaCl and immersed in PBS-azide solution (pH 7.4) and paraformaldehyde (PFA).
3. **Staining:** Tissue samples were processed using the Hematoxylin-Eosin (HE) staining method, involving fixation, decalcification, dehydration, infiltration, embedding in paraffin, sectioning, and staining. The slides were analyzed microscopically to evaluate inflammatory cell infiltration and synovial hyperplasia.[22,23]

Data Analysis

Data were analyzed using Shapiro-Wilk test was applied to assess data normality. The paired sample *t*-test was used to evaluate the effectiveness of different doses of noni fruit extract. A post-hoc LSD test determined the most effective dose for reducing edema.[24,25]

RESULTS AND DISCUSSION

Phytochemical Test Results

Based on the results of phytochemical tests, the content in the ethanol extract of noni fruit (Morinda citrifolia L) contains steroids and triterpenoids, saponins, flavonoids and tannins.

Table 1. Phytochemical screening of ethanol extract of noni fruit (*Morinda citrifolia L*)

Secondary Metabolite Compounds	Reagents	Result
Alkaloid	Bouchardart	-
	Maeyer	-
	Dragendroff	-
	Wagner	-
Terpenoids/steroids	Salkowsky	-
	Lieberman-Burchad	+
Saponins	Aquadest + 96% Alcohol	+
Flavonoids	$Mg_{(s)}$+$HCl_{(p)}$	-
	$FeCL_3$ 5%	+
	NaoH 10%	-
	H2SO4	-
Tannin	$FeCL_3$ 1%	+
Glycosides	Mollish	-

Based on the results of phytochemical tests, the content in the ethanol extract of noni fruit (Morinda citrifolia L) contains steroids and triterpenoids, saponins, flavonoids and tannins that function as anti-inflammatory. The anti-inflammatory properties of flavonoids are due to their ability to inhibit cyclooxygenase and lipoxygenase, as well as the concentration of local leukocytes in line with the research of Zaky et al.[26] Found in noni fruit and has anti-inflammatory and anti-allergic effects, noni can also dilate blood vessels that are vasocontracted and improve blood circulation. Scopololetin is a molecule with medical potential, scientists think it can bind to serotonin, an important compound in humans. The scopoletin content in noni fruit can reduce prostaglandin levels by inhibiting cyclooxygenase (COX) and 5-lipoxygenase activity against arachidonic acid. Terpenoids are isometric hydrocarbon molecules found in various lipids or essential oils. These lipids play an important role in organic synthesis and cell repair in the body.[27]

The phytochemical screening of ethanol extract of *Morinda citrifolia* L. revealed the presence of terpenoids, steroids, saponins, flavonoids, and tannins, which are well-documented for their anti-inflammatory properties. Terpenoids and steroids, identified through the Lieberman-Burchard test, inhibit key inflammatory pathways, including cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing the synthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes. Saponins, detected using Aquadest and 96% alcohol, contribute to anti-inflammatory activity by stabilizing cell membranes and reducing vascular permeability, which minimizes edema formation. Flavonoids, confirmed through the $FeCl_3$ 5% test, exert both anti-inflammatory and antioxidant effects by inhibiting COX and LOX enzymes while protecting tissues from oxidative damage caused by inflammation. Similarly, tannins, identified using the $FeCl_3$ 1% test, exhibit astringent properties that reduce tissue swelling and inflammatory exudates while downregulating cytokine production, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). The absence of alkaloids and glycosides suggests that the anti-inflammatory effects of the extract are primarily mediated by the detected compounds. Collectively, these bioactive constituents explain the extract's efficacy in reducing paw edema and histopathological markers of inflammation, particularly in the high-dose treatment group. This phytochemical composition highlights *Morinda citrifolia* L. as a promising natural anti-inflammatory agent with potential therapeutic applications for managing inflammatory conditions.

Thin Layer Chromatography Test (KLT)

Based on the results of Thin Layer Chromatography (KLT) test, the content in the ethanol extract of noni fruit (*Morinda citrifolia L*) is scopoletin with an Rf value of 0.51. The Thin Layer Chromatography (TLC) analysis of the ethanol extract of *Morinda citrifolia* L. confirmed the presence of scopoletin, a coumarin derivative,

with an Rf value of 0.51, indicating its significant contribution to the extract's pharmacological properties. Scopoletin is a well-documented bioactive compound with potent anti-inflammatory, antioxidant, and immunomodulatory effects. It exerts anti-inflammatory activity by inhibiting pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and suppressing the activation of nuclear factor kappa B (NF-κB), a transcription factor that regulates inflammatory gene expression. Furthermore, scopoletin inhibits the cyclooxygenase-2 (COX-2) enzyme, thereby reducing prostaglandin synthesis, which is responsible for pain, swelling, and redness during inflammation. Its antioxidant properties complement these effects by scavenging reactive oxygen species (ROS) and reducing oxidative stress, which helps protect tissues from damage and preserves cellular integrity. Additionally, scopoletin's immunomodulatory activity balances immune cell responses, such as macrophages and lymphocytes, preventing excessive inflammatory reactions that could lead to tissue damage. The identification of scopoletin in the extract underscores its potential as a key contributor to the anti-inflammatory effects observed in this study, particularly in reducing paw edema and improving histopathological parameters. This finding supports the therapeutic value of *Morinda citrifolia* L. as a natural source of anti-inflammatory agents and highlights scopoletin's synergistic interaction with other bioactive compounds present in the extract.

Effect of Mouse Leg Volume with Pletismometer

Measurement of rat paw volume with a pletismometer was carried out on day 0, namely before CFA induction and then after CFA induction on day 17, 20, 23, 26, 29 and day 31, with the results in the table 2.

Table 2. Measurement of Mouse Paw Volume Using a Plethysmometer (µL)

Group	Day-0	Day-17	Day-20	Day-23	Day-26	Day-29	Day-31
Normal Control	4.42 ± 0.26	4.40 ± 0.21	4.38 ± 0.24	4.26 ± 0.19	4.29 ± 0.20	4.28 ± 0.22	4.30 ± 0.21
Negative Control	4.22 ± 0.58	9.03 ± 1.12	8.94 ± 1.12	8.78 ± 1.10	8.70 ± 1.09	8.56 ± 1.07	8.40 ± 1.09
Positive Control (Triamcinolone)	3.65 ± 0.25	7.34 ± 0.82	6.93 ± 0.61	6.21 ± 0.44	5.46 ± 0.55	4.98 ± 0.53	4.39 ± 0.46
Dose I (150 mg/200 g BW)	3.71 ± 0.19	7.73 ± 0.79	7.58 ± 0.74	7.30 ± 0.71	7.01 ± 0.66	6.76 ± 0.61	6.28 ± 0.58
Dose II (300 mg/200 g BW)	4.06 ± 0.40	7.30 ± 0.78	6.93 ± 0.73	6.54 ± 0.72	6.22 ± 0.67	5.64 ± 0.61	5.09 ± 0.57
Dose III (600 mg/200 g BW)	3.35 ± 0.23	8.11 ± 1.07	7.56 ± 1.01	6.86 ± 0.92	6.42 ± 0.86	5.59 ± 0.76	4.95 ± 0.69

The results presented in Table 2 demonstrate the progressive reduction in paw volume across different treatment groups, reflecting the anti-inflammatory effects of *Morinda citrifolia* L. extract. In the normal control group, the paw volume remained consistent throughout the study, indicating the absence of inflammation and serving as a baseline. Conversely, the negative control group, which was induced with CFA but received no treatment, exhibited a significant increase in paw volume from Day-17 to Day-31, confirming persistent inflammation caused by the CFA induction. The positive control group treated with triamcinolone showed the most substantial reduction in paw volume, with values decreasing from 7.34 µL on Day-17 to 4.39 µL on Day-31, demonstrating the potent anti-inflammatory and immunosuppressive effects of triamcinolone.

The lowest average decrease in the volume of rat's feet after CFA induction from day-17 to day-31 was the negative control group, from 9.03 µL to 8.40 µL, while the highest average decrease in the volume of rat's feet after CFA induction

from day-17 to day-31 was the positive control group, from 7.34 µL to 4.39 µL. The results of the average decrease in the volume of rat feet after CFA induction from day-17 to day-31 given dose therapy I, II and III, were most effective in dose group III, namely from 8.11 µL to 4.95 µL. This shows that the administration of triamcinolone is more effective in reducing the volume of udema than the administration of ethanol extract of noni fruit. Blocking the phospholipase A2 enzyme in the phospholipid layer of cell membranes, triamcinolone has anti-inflammatory and immunosuppressant effects. This action prevents the formation of arachidonic acid by blocking the breakdown of leukocyte lysosomal membranes. Reduces the expression of cyclooxygenase (COX) and lipoxygenase (LOX), which in turn inhibits the production of prostaglandins and leukotrienes. Corticosteroids manifest anti-inflammatory effects through inhibiting the migration of macrophages and leukocytes to the affected site by restoring dilation and permeability of blood vessels. This action leads to reduced edema, erythema and pruritus. An important anti-inflammatory mechanism is mediated by the inhibition of nuclear factor kappa-B (NF-kappa-B), which leads to decreased expression of interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), and COX-2.[28]

These findings highlight the anti-inflammatory potential of *Morinda citrifolia* L. extract, particularly at higher doses, in mitigating CFA-induced inflammation. However, the extract's efficacy remains lower than that of triamcinolone, indicating that while the extract provides substantial anti-inflammatory effects, it may serve better as a complementary or adjunctive therapy rather than a standalone treatment. The dose-dependent reduction in paw volume underscores the importance of optimizing dosing strategies for maximizing therapeutic outcomes.

Effectiveness of Ethanol Extract of Noni Fruit (*Morinda citrifoilia L*)

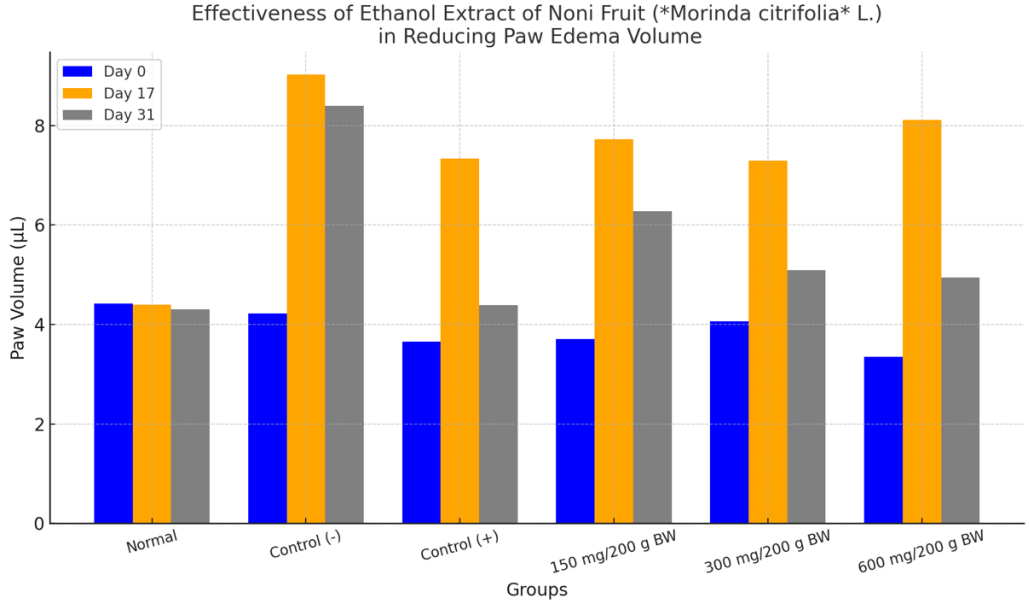

Figure 1. Effectiveness of Ethanol Extract of Noni Fruit (Morinda citrifoilia L) in Reducing the Volume of Udem in Mouse Legs

The graph demonstrates the effectiveness of ethanol extract of *Morinda citrifolia* L. and triamcinolone in reducing paw edema in CFA-induced mice, measured at different time points (Day 0, Day 17, and Day 31). The normal control group maintained consistent paw volumes throughout the study, indicating no inflammatory response. In contrast, the negative control group showed a marked increase in paw volume following CFA induction, with minimal reduction by Day 31, reflecting persistent inflammation in the absence of treatment. The positive control group, treated with triamcinolone, exhibited a significant reduction in paw volume

from Day 17 to Day 31, demonstrating its potent anti-inflammatory effects. Among the groups treated with noni fruit extract, a dose-dependent reduction in paw volume was observed. The group receiving the highest dose (600 mg/200 g BW) showed the greatest efficacy, with paw volumes approaching those of the positive control group by Day 31. Lower doses (150 mg/200 g BW and 300 mg/200 g BW) were less effective but still reduced paw edema compared to the negative control. These findings highlight the potential anti-inflammatory effects of *Morinda citrifolia* L. extract, particularly at higher doses, while emphasizing that its efficacy is lower than that of triamcinolone.

The effects of the treatments highlight the ability of ethanol extract of *Morinda citrifolia* L. and triamcinolone to modulate the inflammatory response and reduce tissue swelling. The high-dose noni extract (600 mg/200 g BW) showed a notable reduction in edema, suggesting its effectiveness in decreasing vascular permeability and inflammatory mediator production, which are key processes in the progression of inflammation. The bioactive compounds, such as scopoletin, flavonoids, and tannins, likely contribute by inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) pathways, thus reducing prostaglandin and leukotriene synthesis. The improvement in edema reduction over time in the treated groups also indicates a potential cumulative effect of the extract with prolonged administration. However, while the noni extract reduced inflammation significantly, its effects were not as rapid or pronounced as triamcinolone, which acts through more direct mechanisms, such as suppressing cytokine expression and stabilizing lysosomal membranes. This positions the noni extract as a potential complementary therapy in managing inflammation, particularly in cases where corticosteroid use may be contraindicated.

Table 3. Post-Hoc Test Results Using LSD (Least Significant Difference)

Day	Control (-)	Control (+)	Dose 1 (150 mg/200 g BW)	Dose 2 (300 mg/200 g BW)	Dose 3 (600 mg/200 g BW)
Day 0	0.667	0.667	0.829	0.667	0.829
Day 17	0.003	0.003	0.796	0.796	0.606
Day 20	0.002	0.002	0.314	0.137	0.779
Day 23	0.001	0.001	0.000	0.002	0.000
Day 26	0.000	0.000	0.000	0.000	0.000
Day 29	0.000	0.000	0.000	0.000	0.000
Day 31	0.000	0.000	0.000	0.000	0.000

The effectiveness of ethanol extract of noni fruit (Morinda citrifoilia L) which is most effective in reducing the volume of udema in the legs of male rats induced by CFA can be seen using the Post-Hoc test with LSD. Based on the doses used, namely 150 mg/200 gBB, 300 mg/200 gBB and 600 mg/200 gBB of the three doses of extracts have effectiveness as anti-inflammatory. However, the dose of 150 mg/200 gBB has shown effectiveness on day 23 and the dose of 600 mg/200 gBB is the most effective dose in reducing the volume of edema in the legs of male rats induced by CFA. The most effective dose of noni fruit ethanol extract in reducing the volume of udema is in dose group III of 600 mg/200 gBB. Scoploletin, flavonoids, triterpenoids, and steroid chemicals are included in the ethanol extract of noni fruit. Scopolonetin contains anti-inflammatory and anti-allergic effects in addition to dilating narrowed blood vessels and improving blood circulation. The analgesic effect of flavonoid concentrations of noni fruit is due to its ability to block the enzyme cyclooxygenase and its substrates from binding oxygen, thereby causing prostaglandin synthesis.[29]

Mouse Foot Histopathology

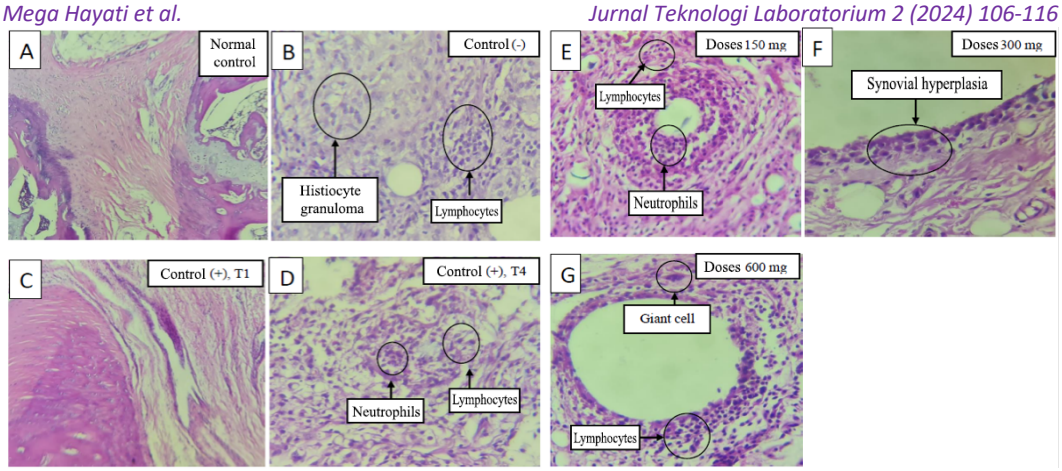

Figure 2. Rat paw histopathology

The results of the histopathological examination of the negative control group can be seen in Figure 2 (B) that there is an infiltration of inflammatory cells in the tissue of the rat's foot joints in the form of inflammatory cell infiltrates of lymphocytes, plasma cells, PMN neutrophils and the distribution and group of histiocytes forming a granuloma structure. The histopathology results of the positive control group (triamcinolone injection) can be seen in Figure 2 (C) and (D) that almost all rats (rats 1,2,3 and 5) show a cleaner picture of the foot joint tissue and are close to a normal picture. The histopathology results of dose groups 1, 2 and 3 (ethanol extract of noni fruit) can be seen in Figure 2 (E), (F) and (G) that there is an infiltration of inflammatory cells in the rat foot joint tissue in the form of infiltrates of lymphocyte inflammatory cells, plasma cells and scattered, histiocyte groups forming a granuloma structure accompanied by the form of many-nucleated datia cells and there is also synovial hyperplasia. The histopathological picture of inflammatory cell infiltrates and thickened synovials in rats given mengkudu fruit extract doses of 150 mg / 200 gBB, 300 mg / 200 gBB and 600 mg / 200 Gbb, this indicates that the ethanol extract of mengkudu fruit (Morinda citrifolia L) cannot improve the histopathology of joint tissue in CFA-induced rat feet.

Inflammation that occurs in rat feet after being induced by CFA, interpret the parameters of histopathological changes in rat foot joint tissue after the administration of ethanol extract of noni fruit as an inflammatory therapy. The results showed that the tissues in the rat's feet experienced inflammation in all groups of rats except the normal control group and the positive control group (triamcinolone injection), it can be seen in Figure 2 (C) and 2 (D) that almost all rats (rats 1, 2, 3 and 5) showed a cleaner picture of the foot joint tissue and approached a normal picture. Only in rat 4 which still shows inflammatory cell infiltration. This is in line with research according to Katzung et al., 2023 triamcinolone inhibits the postpolyphase enzyme, which results in the inhibition of the release of arachidonic acid, which is needed to activate the next enzyme.[30]

CONCLUSION

The ethanol extract of *Morinda citrifolia* L. demonstrates significant anti-inflammatory activity, as evidenced by its ability to reduce paw edema and inflammatory cell infiltration in CFA-induced rats. Among the tested doses, the 600 mg/200 g BW dose exhibited the highest efficacy, with reductions in paw volume and improvements in histopathological parameters approaching those of the positive control (triamcinolone). However, the extract did not achieve complete tissue recovery as observed with triamcinolone, suggesting its anti-inflammatory effects are less potent. The presence of bioactive compounds such as scopoletin, flavonoids, and tannins contributes to the anti-inflammatory effects through mechanisms like COX and LOX inhibition, reduction of cytokine production, and

modulation of immune responses. These findings imply that while *Morinda citrifolia* L. extract shows potential as a natural anti-inflammatory agent, it is best suited as a complementary therapy or for conditions where corticosteroid use is contraindicated. Future research should focus on refining extraction methods, exploring higher doses, and conducting clinical trials to validate its therapeutic application in managing chronic inflammatory conditions.

AUTHORS' CONTRIBUTIONS

Mega Hayati prepared the samples, Adek Amansyah designed the protocol, implemented the protocol, and Fioni wrote the manuscript. Ali Napiah Nasution and Ermi Girsang reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigastion are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

There is no conflict of interest.

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