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Anti-inflammatory Potential of *Tithonia diversifolia* Leaves in Carrageenan-Induced Edema Model in Mice (*Mus Musculus*)

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ABSTRACT

Introduction*: Edema is a common sign of inflammation characterized by increased tissue volume. Tithonia diversifolia leaves have traditionally been used as anti-inflammatory agents because they contain active compounds such as flavonoids, alkaloids, steroids, terpenoids, tannins, sesquiterpenoids, phenolic acids, and saponins.* ***Aims****: Determine the anti-inflammatory effects of ethanol extract of Tithonia diversifolia leaves on rats (Mus musculus) induced by carrageenan.* ***Method****: Extraction was carried out using maceration. The experimental design was pre- and post-test with five groups: healthy controls, positive controls (sodium diclofenac), and three treatment groups receiving 12.5 mg/30 gBW, 25 mg/30 gBW, and 37.5 mg/30 gBW of Tithonia diversifolia leaf extract. Edema was induced with carrageenan and measured using a plethysmometer at certain intervals.* ***Result****: The results showed that a dose of 37.5 mg/30 gBW significantly decreased edema volume, comparable to the diclofenac group (p>0.05).* ***Conclusion****: The ethanol extract of paitan leaves at this dose can function as an effective anti-inflammatory agent.*

KEYWORDS*: Anti-inflammation, Tithonia diversifolia, carrageenan, edema, sodium diclofenac.*

INTRODUCTION

Edema is one of the standard clinical manifestations of an inflammatory response. It is characterized by fluid accumulation in the interstitial tissues, leading to swelling. The inflammatory process that triggers edema involves the release of inflammatory mediators such as histamine, prostaglandins, and cytokines, which increase vascular permeability and allow fluid and protein to escape from capillaries into surrounding tissues. These manifestations are not only uncomfortable but may also indicate the presence of a more serious disorder or disease, such as infection, trauma, or allergic reaction

(Oematan, Manoppo and Runtunuwu, 2013; Chagas-Paula *et al.*, 2015).

In modern medicine, anti-inflammatory agents such as diclofenac sodium are often used to treat edema. However, long-term use of these synthetic drugs can cause side effects such as gastrointestinal disorders, hepatotoxicity, and nephrotoxicity. Therefore, research into natural ingredients with anti-inflammatory potential has grown rapidly as a safer alternative (Budiastuti *et al.*, 2021; Yuda *et al.*, 2022).

One promising source of natural ingredients is paitan leaves (*Tithonia diversifolia*), a plant traditionally used to treat various health conditions. Paitan leaves contain active compounds such as flavonoids, alkaloids, steroids, tannins, terpenoids, and saponins, which have anti-inflammatory, antioxidant, and antimicrobial activities. This pharmacological potential makes paitan an attractive candidate for further development as a natural anti-inflammatory agent (Olayinka, Raiyemo and Etereje, 2015; Sasmita *et al.*, 2017).

This study aims to evaluate the effectiveness of ethanol extract from paitan leaves in reducing edema volume induced using carrageenan in mice (*Mus musculus*). A plethysmometer to measure edema volume, this study is expected to provide scientific data supporting using paitan leaves as an anti-inflammatory.

METHODS

Material

The material used in this research are aluminum foil, aquadest, paitan leaves (*Tithonia diversifolia* (Hemsley) A. Gray), 95% ethanol, animal test mice (*Mus musculus*), carrageenan, chloroform, HCl 2 N, NaCl 0.9%, Na-CMC 0.5%, diclofenac sodium tablets 50 mg, magnesium*, Mayer*, *Wagner*, and *Dragendrof* reagents**.**

Plant Material

The samples used in this research were paitan leaves (*Tithonia diversifolia* (Hemsley) A. Gray) taken at the Makassar, South Sulawesi.

Extraction of *T. diversifolia*

Five hundred grams of powdered leaves were macerated using 1.000 mL of 95% ethanol. Three separate 24-hour extractions with periodic stirring generated filtrate and residue, which were then separated by filtering. The filtrate obtained was evaporated using a rotary evaporator to produce a thick extract and then freeze-dried to get a dry extract (Indrisari *et al.*, 2023).

Qualitative screening

The screening of the main groups of chemical constituents (alkaloid, flavonoid, saponin, tannin, terpenoid, and steroid) of the extract was carried out qualitatively.

Alkaloid

The ethanol extract was added with 1 ml of 2 N HCl, then heated for 2-3 minutes, cooled, and filtered. The filtrate was divided into three parts and put into test tubes, each added with *Mayer*, *Wagner*, and *Dragendrof* reagents. Identifying alkaloids in the sample is positive if it produces a white precipitate after adding *Mayer* reagent, brown precipitate on *Wagner* reagent, and orange precipitate on *Dragendrof* reagent (Indrisari *et al.*, 2023).

Flavonoid

The Shinoda test was performed to assess the presence of the flavonoid. In reaction tubes, 1 mL of extract was dropped with concentrated hydrochloric acid, and a bit of magnesium was added. After adding reagents, green to black hues were thought to indicate the presence of flavonoids (Indrisari *et al.*, 2023).

Saponin

Foaming formed after shaking the extract in 2 mL of water indicates the presence of saponin (Indrisari *et al.*, 2023).

Tannin

The previously boiled and filtered 0.1 g of extract received a few drops of 0.1% of ferric chloride. Then, a blue-black or green-black coloring appeared (Indrisari *et al.*, 2023).

Terpenoid/Steroid

Salkowski tests were used to determine the presence of terpenoids. One milliliter of concentrated sulfuric acid was added to 0.1 g of the extract and 0.4 mL of chloroform. Then, it was observed for reddish brown coloration (Indrisari *et al.*, 2023).

Ethical Clearences

This research requested approval from the STIFA Makassar Health Research Ethics Commission No. 217/EC.1.1.B/VIII/KEPK/ 2023.

Experimental Animals

The test animals used in the study were 15 healthy mice (*Mus musculus*) weighing 20-30 grams. Divided into five groups, each consisting of 3 heads. Before treatment, mice were adapted for ±14 days by giving standard feed.

Preparation of Carrageenan 1% b/v

Carrageenan weighed as much as 0.1 g plus 5 ml NaCl solution, then homogenized, and the volume reached 10 ml (Rahman, Wati and Sukmawati, 2018).

Preparation of Diclofenac Sodium Suspension

Diclofenac sodium was weighed at as much as 5.138 mg, which was then put into a mortar and 1% Na-CMC was added. CMC colloidal solution little by little while being crushed until homogeneous and then put

Table 1. Phytochemical screening results of paitan leaf extracts

Phytochemical compound	Result
Saponin	+
Tannin	+
Alkaloid	+
Flavonoid	+
Steroid/triterpenoid	+

into a volumetric flask, and the volume was sufficient to 10 mL (Rahman, Wati and Sukmawati, 2018).

Treatment of Test Animals

Before being treated, mice were fed for ±8 hours. Mice were divided into five groups; group I (control) was given Na-CMC, group II (positive control) was given diclofenac sodium suspension, and groups III, IV, and V were given extracts at a dose of 12.5 mg/30 gBW, 25 mg/30 gBW, and 37.5 mg/30 gBW. Each group of test animals was induced with carrageenan. The mice's paw volume was measured using a *Pletysmometer* before, after, and after treatment at hours 1, 2, 3, 4, and 5 (Mansouri T. M., 2015).

Data Analysis

Data obtained from the size of the edema volume of the mice's feet at each time in all groups were tabulated. The average volume of inflammatory feet was calculated, and the average decrease in edema of the mice's feet was calculated by comparing it to the baseline volume before the carrageenan injection. Furthermore, the results obtained from the calculation were analyzed statistically, and then the *Kruskal Wallis* and *Mann-Whitney* tests were continued to see differences between treatment groups.

RESULTS AND DISCUSSION

The results of the phytochemical screening of ethanol extract of paitan leaves can be seen below to table 1. The sample preparation results showed that the extract yield obtained was 1.92%. The compounds contained in paitan leaf extract are saponins, tannins, alkaloids, flavonoids, steroids, and terpenoids. Flavonoids are phenolic compounds that have great potential as an anti-inflammatory. Flavonoids have anti-inflammatory activity by inhibiting enzymes such as aldose reductase, xanthine oxidase phosphodiesterase Ca^{2+} ATPase, lipooxygenase, and cyclooxygenase (Budiastuti et al., 2021; Chagas-Paula et al., 2015). All groups were induced with 1% b/v carrageenan intraplantar and showed increased edema. The measurement of edema volume was carried out using a *pletismometer* based on Archimedes' law, namely, objects inserted into a liquid will exert an upward force or pressure equal to the volume moved. The mice's legs were dipped into the *pletismometer*, and leg volume was increased.

Edema inducers use carrageenan, which causes cell injury and releases pain mediators to cause inflammation. This is because carrageenan is a hydrochloride

Table 2. Average reduction in edema volume

Group	Initial Volume	Induction Volume	After Treatment (Hour) T1	T2	T3	T4	T5
Normal	0,19±0.010	0,19±0.010	0,19±0.012	0,19±0.012	0,19±0.010	0,19±0.013	0,19±0.012
Na-CMC	0,20±0.016	0,28±0.0012	0,27±0.023	0,26±0.021	0,25±0.025	0,24±0.022	0,24±0.024
Na. Diklofenak	0,20±0.018	0,27±0.014	0,26±0.023	0,24±0.025	0,23±0.026	0,21±0.025	0,20±0.028
Dose I	0,20±0.016	0,27±0.020	0,26±0.026	0,26±0.022	0,25±0.021	0,24±0.018	0,23±0.020
Dose II	0,21±0.017	0,27±0.027	0,26±0.022	0,25±0.020	0,24±0.025	0,24±0.025	0,22±0.026
Dose III	0,20±0.010	0,27±0.023	0,26±0.024	0,24±0.028	0,23±0.026	0,22±0.028	0,21±0.028

compound consisting of esters of potassium, sodium, and magnesium or calcium sulfate with galactose and physiological body so that the inflammatory response occurs quickly and is easy to observe. Carrageenan is neutral, which only causes edema and does not cause necrosis (tissue death) (Mansouri, M.T, et al., 2015).

The test animals used were 18 mice divided into six treatment groups. Each group consisted of 3 mice. Group I is normal, and group II is a negative control given Na-CMC, group III is a positive control given diclofenac sodium, where diclofenac sodium is a class of NSAIDs with anti-inflammatory activity. The mechanism of action is inhibiting cyclooxygenase nonselectively to inhibit the formation of prostaglandins (Budiastuti et al., 2021; Chagas-Paula et al., 2015). Diclofenac nonselectively to inhibit the formation of prostaglandins (Budiastuti et al., 2021; Chagas-Paula et al., 2015). Diclofenac sodium is a derivative of phenylacetic acid. Groups IV, V, and VI were given ethanol extract of paitan leaves with three different copolymers of 3,6 anhydrogalactose (Budiastuti et al., 2021; Yuda et al., 2022). This compound is readily accepted by the doses of 12.5 mg/30 gBW, 25 mg/30 gBW, and 37.5 mg/30 gBW. Edema reduction was observed for 5 hours with a time interval of 1 hour.

Table 2 shows the average results of edema volume reduction observed every 1 hour for 5 hours, which shows a decrease in edema volume, including the Na.CMC group, this may be due to the body's response in repairing tissues. In contrast, the positive control group (diclofenac sodium), extract groups IV, V, and VI at hours 1 to 5 experienced decreased edema. Groups IV, V, and VI showed that from hour 1 to hour 5 there was a decrease in edema volume in the legs of mice.

Data from Table 3 is then calculated for the percent decrease in edema volume after treatment at the 5th hour, and the results can be seen in Table 3 through the following percent decrease formula:

Table 3. Percent reduction in edema volume

Group	Average percent reduction in edema volume (%)				
	T1	T2	T3	T4	T5
Na.CMC	0,23±1,76	6,00±2,52	8,42±1,82	12,04±4,56	14,42±6,06[a]
Na. Diklofenak	3,62±1,74	10,93±2,50	17,02±3,84	21,91±4,58	28,00±6,10[b]
Dose I	3,66±1,72	6,04±2,55	9,70±3,80	12,31±4,54	14,59±5,06[a]
Dose II	2,43±2,00	7,32±2,51	10,98±3,79	13,40±3,60	18,25±4,08[a]
Dose III	4,89±1,80	10,98±2,53	15,87±3,86	19,53±2,46	24,38±6,03[b]

$$\text{Percentage of edema} = \frac{Vo - V1}{Vo}\ x\ 100\%$$

Description:

Vt = Edema volume after time t

Vo = Initial volume of mouse paws

Data on the average percent decrease in edema volume at each hour of treatment can be seen in Table 3, which shows the Na.CMC group at the 5th hour has an average percent decrease in edema of 14.42%, the diclofenac sodium group has an average percent decrease of 28%, while the paitan leaf ethanol extract group doses of 12.5mg / 30 gBW, 25 mg / 30 gBW and 37.5 mg / 30 gBW have an average percent decrease of 14.59%, 18.25%, and 24.38%. This shows that the ethanol extract group dose of 37.5 mg / 30gBW reduces the inflammatory effect more significantly than the dose of extract 12.5mg / 30 gBW and 25 mg/30 gBW.

Data from each treatment group were processed statistically. From the results of data processing, the normality value $p<0.05$ was obtained. The test was continued using the *Kruskal-Wallis* test, and the test results showed a value of $p <0.05$, which means there is a significant difference between treatment groups. Further analysis was carried out to compare the two groups using *Mann-Whitney*. The *Mann-Whitney* test results showed results that were not significantly different ($p>0.05$), with a significant value of 0.105. This means that the diclofenac sodium group and dose III (37.5 mg/30 gBW) have the same effect in reducing edema volume.

CONCLUSION

Based on the results of research and data analysis, it can be concluded that ethanol extract of paitan leaves (*Tithonia diversifolia*) at a dose of 37.5 mg/30 gBW has potential as an anti-inflammatory agent that is effective in reducing edema volume in carrageenan-induced mice.

REFERENCES

Budiastuti, B., Sukardiman, S., Primaharinastiti, R., & Nurcholida, R. D. (2021). *Anti-Inflammatory Activity of Cinnamon Bark Oil (Cinnamomum Burmannii (Nees & T. Nees) Blume) From Kerinci Regency of Indonesia*. Proceedings of the 1st International Conference on Education, Humanities, Health and Agriculture (ICEHHA), 3-4 June 2021, Ruteng, Flores, Indonesia.

Chagas-Paula, D. A., Zhang, T., Da Costa, F. B., & Edrada-Ebel, R. (2015). *A Metabolomic Approach to Target Compounds From the Asteraceae Family for Dual COX and LOX Inhibition*. Metabolites, 5(3), 404–430. https://doi.org/10.3390/metabo5030404

Hall, J. E. (2020). *Guyton and Hall Textbook of Medical Physiology* (14th ed.). Philadelphia: Elsevier.

Indrisari, M., Aksa, R., Khairi, N., Bau Agiel, F. M., Harmawati, H., & Muslimin, L. (2023). *Acute Safety Assessment of Ethanol Leaves Extract of Cemba (Acacia Rugata L.) on the Kidney in Male Wistar Rats*. Tropical Journal of Natural Product Research, 7(7), 3360–3363. https://doi.org/10.26538/tjnpr/v7i7.12

Mansouri, M. T., Hemmati, A. A., Naghizadeh, B., Mard, S. A., Rezaie, A., & Ghorbanzadeh, B. (2015). *A Study of the Mechanisms Underlying the Anti-Inflammatory Effect of Ellagic Acid in Carrageenan-Induced Paw Edema in Rats*. Indian Journal of Pharmacology, 47(3), 292–298. https://doi.org/10.4103/0253-7613.157127

Oematan, Y., Manoppo, J. I. C., & Runtunuwu, A. L. (2009). *Peran Inflamasi Dalam Patofisiologi Sepsis dan Syok Septik pada Anak*. Jurnal Biomedik (JBM), 1(3), 166–173. https://doi.org/10.35790/jbm.1.3.2009.831

Olayinka, B. U., Raiyemo, D. A., & Etereje, E. O. (2015). *Phytochemical and Proximate Composition of Tithonia Diversifolia (Hemsl.) A. Gray, a Perennial Broad-Leaved Weed Species Reported Ornamental Plant*. Annals Food Science and Technology, 16(1).

Rahman, S., Wati, A., & Sukmawati, E. (2018). *Efek Antiinflamasi Ekstrak Etanol Daun Kamboja (Plumeria Rubra L.) pada Tikus Putih (Rattus Norvegicus)*. Jurnal Ilmiah As-Syifaa, 10(1), 51–59. https://doi.org/10.33096/jifa.v10i1.329

Sasmita, F. W., Susetyarini, E., Husamah, H., & Pantiwati, Y. (2017). *Efek Ekstrak Daun Kembang Bulan (Tithonia Diversifolia) terhadap Kadar Glukosa Darah Tikus Wistar (Rattus Norvegicus) yang Diinduksi Alloxan*. Biosfera, 34(1), 22. https://doi.org/10.20884/1.mib.2017.34.1.412

Yuda, P. E. S. K., Mahardika, I. M. A., Cahyaningsih, E., Sasadara, M. M. V., Nayaka, N. M. D. M. W., & Dewi, N. L. K. A. A. (2022). *Aktivitas Anti-Inflamasi Minyak Herbal Tradisional dari Bahan Usada Bali pada Mencit Inflamasi yang Diinduksi Karagenan*. JPSCR: Journal of Pharmaceutical Science and Clinical Research, 7(3), 319. https://doi.org/10.20961/jpscr.v7i3.60529