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ETHANOL EXTRACT OF GOTU LEAVES (CENTELLA ASIATICA (L.) URBAN) INCREASES PDGF LEVELS BUT DOES NOT CHANGE TNF-A AND MALONDIALDEHYDE IN WISTAR RATS IN A CARRAGEENIN-INDUCED ACUTE INFLAMMATORY MODEL

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ABSTRACT
Conventional anti-inflammatory therapies with NSAIDs and glucocorticoids are limited by gastrointestinal, renal, and metabolic side effects, thus encouraging the search for plant-based alternatives. Gotu kola leaves (Centella asiatica (L.) Urban) are rich in triterpenoids and flavonoids which are reported to have anti-inflammatory and wound-healing activities. This study aimed to analyze the effect of gotu kola leaf ethanol extract on tumor necrosis factor-alpha (TNF-α), platelet-derived growth factor (PDGF), malondialdehyde (MDA), and leg edema volume in male Wistar rats with carrageenan-induced acute inflammation model. Thirty rats were randomized into five groups (n=6): normal, negative control (carrageenan), positive control (carrageenan + sodium diclofenac), and two extract treatment groups of 2 g/kgBB and 4 g/kgBB orally. Edema was measured with a plethysmometer in the first minute and every hour for 4 hours; Serum TNF-α, PDGF, and MDA levels were measured by ELISA. Data were analyzed with one-way ANOVA and LSD follow-up test (α=0.05). Screening for positive phytochemicals of flavonoids, alkaloids, saponins, tannins, phenols, glycosides, and steroids/triterpenoids. PDGF levels differed significantly between groups (p=0.001), highest in the 4 g/kgBB group (2.14±0.12 ng/mL) and lowest in the negative control (1.66±0.10 ng/mL). The volume of edema was significantly different at the beginning and 1st hour (p=0.001) but not at the 2nd to 4th hour. The levels of TNF-α (p=0.423) and MDA (p=0.072) were not significantly different. Ethanol extract of Centella asiatica leaves, especially at a dose of 4 g/kgBB, increased PDGF and decreased early-phase edema, showing modulation potential towards tissue repair, but had no significant effect on TNF-α and MDA in this acute model.

Keywords: acute inflammation; carrageenan; PDGF; centella asiatica; malondialdehyde; TNF-α

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INTRODUCTION

Inflammation is the body's innate defense mechanism against both infectious and non-infectious injuries, characterized by heat, rub, tumor, dolor, and functio laesa. Based on the passage of time, inflammation is divided into acute, subacute, and chronic; acute inflammation occurs immediately after injury and lasts several days, while failure of resolution can progress to chronic inflammation accompanied by tissue damage and fibrosis (Hannoodee & Nasuruddin, 2022; Pahwa et al., 2022). Since inflammation underlies most acute and chronic diseases, its control remains an important therapeutic goal (Hannoodee & Nasuruddin, 2022). Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine that is primarily produced by activated macrophages and monocytes in acute inflammation; overproduction contributes to the tissue-damaging effects (Xiao et al., 2025). Platelet-derived growth factor (PDGF) is a potent mitogen and chemoattractant secreted by platelets and macrophages, playing a role in encouraging fibroblast proliferation, angiogenesis, and

granulation tissue formation in wound healing (Irma et al., 2025). Oxidative stress accompanies acute carrageenan-induced inflammation; malondialdehyde (MDA), the end product of lipid peroxidation, is widely used as a biomarker of membrane oxidative damage (Mas-Bargues et al., 2021; Mohideen et al., 2023).

Current anti-inflammatory therapies rely on non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids. NSAIDs inhibit arachidonic acid metabolism, but are often associated with gastrointestinal bleeding, nephrotoxicity, and increased cardiovascular risk, while corticosteroids are at risk of glucose intolerance, hypertension, osteoporosis, and immune suppression (Ong et al., 2007; Rhen & Cidlowski, 2005). Therefore, WHO encourages the use of herbal medicines that are proven to be safe and efficacious (World Health Organization, 2002).

Gotu kola leaf (Centella asiatica (L.) Urban) is a tropical medicinal plant rich in triterpenoid glycosides (asiaticosides, madecassosides, asiatic and madecassic acids), flavonoids (kaempferol, quercetin), and phytosterols, and is empirically used to treat inflammation and wounds (Biswas et al., 2021). Previous research by (Biswas et al., 2021; Nafiisah et al., 2021; Mardiyah, 2022) showed that gotu kola counteracts oxidative stress due to heavy metals accelerates wound healing, lowers serum TNF-α in traumatic brain injury models, and in cream preparations inhibits leg edema dose-dependently. However, integrated data on the effect of gotu kola ethanol extract on TNF-α, PDGF, MDA, and edema volume simultaneously in a single acute inflammatory model are limited. This study aimed to analyze the effect of gotu kola leaf ethanol extract on these four parameters in male Wistar rats with carrageenin-induced acute inflammation.

METHOD

This laboratory's experimental research uses a post-test only randomized controlled group design. The research was carried out at the Phytopharmacy Laboratory, Integrated Laboratory, and Animal House, Faculty of Medicine, Andalas University, Padang, for ± 60 days (April-June 2024). Fresh gotu kola herb was obtained from plantations in Medan City and was determined as *Centella asiatica* (L.) Urban var. asiatica (Apiaceae family). Dried simplicia are pollinated, sifted (mesh 40), then 250 g are extracted by percolation using 70% ethanol. Percolate is concentrated with a rotary evaporator and evaporated in a water bath (≈ 40 °C) until a viscous extract with constant weight is obtained; Yields are recorded. Qualitative screening was performed for alkaloids (Mayer, Bouchardat, Dragendorff reagents), flavonoids (Mg–HCl–amyl alcohol), tannins ($FeCl_3$ 1%), saponins (foam test), and steroids/triterpenoids (Liebermann–Burchard). The total flavonoid content was determined by the aluminum chlorimetric method against the quercetin raw (expressed as QE/g extract), and the total phenolic content by the Folin–Ciocalteu method against the raw gallic acid (expressed as GAE/g extract), using the UV-Vis spectrophotometer (Shimadzu UV-1700).

Used 30 male Wistar rats (Rattus norvegicus) aged 2.5–3 months with a weight of 150–220 g. Animals were acclimatized for two weeks with feed and drink ad libitum, and fasted 18–24 hours before treatment by remaining given drinks. Treatment followed the principles of 3R and 5F (Percie du Sert et al., 2020). Ethical feasibility was obtained from the Health Research Ethics Commission, Faculty of Medicine, Methodist University of Indonesia, Medan. The sample size was calculated using Federer's formula $[(n-1)(t-1) \geq 15]$, resulting in a minimum of five rats per group; six were used in anticipation of death. Rats were randomized into five groups (n=6): (i) normal (no induction/treatment); (ii) negative control (carrageenan only); (iii) positive control (carrageenan + diclofenac sodium per orally); (iv) treatment I (carrageenan + extract 2 g/kgBB, p.o.); and (v) treatment II (carrageenan + extract 4 g/kgBB, p.o.). Acute inflammation is induced by subplantar injection of 1% (0.1 mL) of carrageenan on the sole of the hind foot, and treatment is administered once a day. The volume of the soles of the hind legs was measured with a mercury plethysmometer before induction and every hour for 4 hours after induction. The percentage of edema inhibition was calculated relative to the comparison group as an index of anti-inflammatory activity. At the end of

the observation, the animal is sacrificed and blood is drawn through a cardiac puncture. The serum was separated by cold centrifugation and checked for TNF-α, PDGF, and MDA levels using a commercial ELISA kit (quantitative sandwich ELISA for PDGF), with absorbance read on an ELISA reader. The data is presented as an average ± standard deviation. Normality and homogeneity are tested; Normally-distributed and homogeneous data were analyzed with one-way ANOVA followed by LSD tests, while if not eligible, the Kruskal–Wallis test was used. The analysis was performed with SPSS, and $p < 0.05$ was considered meaningful

RESULT

Phytochemical screening

Determination confirmed the sample as *Centella asiatica* (L.) Urban var. asiatica (Apiaceae). Qualitative screening of ethanol extracts was positive for flavonoids, alkaloids, saponins, tannins, phenols, glycosides, and steroids/triterpenoids (Table 1), according to the profile of compounds that support antioxidant and antiinflammatory activity. Triterpenoid saponins and gotu kola flavonoids have been reported to inhibit NF-κB activation and suppress proinflammatory mediators, while stimulating fibroblast proliferation and growth factor production (Witkowska et al., 2024; Xiao et al., 2025).

PDGF rate

Serum PDGF levels differed significantly between the five groups (ANOVA, p=0.001; Table 2). The negative control showed the lowest average (1.66±0.10 ng/mL), while the 4 g/kgBB extract group showed the highest average (2.14±0.12 ng/mL), exceeding the normal and positive control groups. Follow-up LSD tests found significant differences between negative controls with treatment I (p=0.011) and treatment II (p<0.001), between positive controls with treatment II (p=0.010), and between treatments I and II (p=0.025). This pattern indicates a dose-dependent increase in PDGF. Since PDGF is a mitogen and chemoattractant that promotes fibroblast proliferation, angiogenesis, and granulation tissue formation, these findings are consistent with the pro-reparative effects of gotu kola extract during acute inflammation (Irma et al., 2025). Gotu kola triterpenoid compounds (asiaticoside, asiatic acid and madrasik) are reported to stimulate fibroblast proliferation and growth factor production and accelerate wound healing in animal models, thus providing a mechanistic basis for the observed increase in PDGF (Witkowska et al., 2024; He et al., 2023). This increase in PDGF is the main finding of the study and places the potential of gotu kola more on modulating tissue repair than on suppression of inflammation alone.

TNF- α Levels

Serum TNF-α levels did not differ significantly between groups (p=0.423; Table 3), with an average ranging from 219.10±30.02 pg/mL (positive control) to 249.01±33.38 pg/mL (normal). The absence of a marked decrease in TNF-α in the treatment group compared to the negative control suggests that, at the single acute dose and time point used, the extract has not suppressed these cytokines in a measured manner. It should be noted that the average TNF-α normal group was actually higher than that of negative controls; this unpredictable pattern may be related to the temporal dynamics of TNF-α that peaks early (about 1–3 hours post-induction) so that at the time of terminal blood collection the levels have decreased, in addition to inter-individual variability and limited sample size. Theoretically, triterpenoids and gotu kola flavonoids can inhibit NF-κB activation and suppress TNF-α (Nafiisah et al., 2021). Therefore these meaningless results do not negate the extract's anti-inflammatory potential, but rather signal the need for dose modifications, scheduling sampling according to cytokine peaks, or the addition of tissue markers to follow-up studies.

MDA rate

Serum MDA levels did not differ significantly between groups (p=0.072; Table 4), with an average of 1.08±0.22 nmol/mL (negative control) to 1.50±0.15 nmol/mL (normal). As with TNF-α, the normal group showed the highest average; This confirms that serum oxidative markers in this acute

model are influenced by biological variability and sampling time, so that more consistent antioxidant effects of extracts reported in chronic or metal-induced oxidative stress conditions have not been detected in this acute design (Biswas et al., 2021). Measurements of MDA in local tissues, not just serums, can provide a more sensitive picture in future studies.

Volume of Foot Edema

The volume of leg edema differed significantly between groups at the initial and 1st hours post-induction (both p=0.001), but not at the 2nd hour (p=0.503), 3rd (p=0.312), or 4th (p=0.180) (Table 5). At the 1st hour, follow-up tests showed significant differences between negative control and positive control (p=0.032) and between positive control and treatment II (p=0.009). The differences that arise in the initial phase disappear in the advanced phase in line with the concept that early edema is mainly mediated by histamine and serotonin which are more easily inhibited by the bioactive compounds of the inhibitor, while the advanced phase involves the recruitment of neutrophil-macrophages and more complex mediators so that the inhibitory effect of the extract becomes less pronounced (Mardiyah, 2022). It should be recognized that the volume of initial edema between groups was already different, meaning that the treatment group was even lower than the normal group before induction so the interpretation of edema data needs to be cautious. This limitation confirms the importance of uniformity of basal volume and reporting of edema as a change (delta) or percentage of inhibition to the basal value of each animal in subsequent studies.

DISCUSSION

This study demonstrated that ethanol extract of Centella asiatica significantly increased serum PDGF levels and reduced paw edema during the early phase of carrageenan-induced acute inflammation, while no significant changes were observed in TNF-α and MDA levels. These findings suggest that the biological activity of C. asiatica in acute inflammation is more closely associated with tissue repair and regeneration than with suppression of systemic inflammatory cytokines or oxidative stress biomarkers.

The significant increase in PDGF observed in the extract-treated groups, particularly at the dose of 4 g/kgBW, indicates that C. asiatica may accelerate the transition from the inflammatory phase to the proliferative phase of tissue repair. PDGF is one of the earliest growth factors released following tissue injury and plays a central role in fibroblast proliferation, extracellular matrix synthesis, angiogenesis, and granulation tissue formation. The higher PDGF concentration found in this study is biologically plausible considering that C. asiatica contains asiaticoside, madecassoside, asiatic acid, and flavonoids, compounds known to stimulate fibroblast migration and collagen synthesis. Previous studies have demonstrated that asiaticoside enhances wound healing by promoting collagen deposition and angiogenesis, thereby accelerating tissue regeneration (Witkowska et al., 2024; He et al., 2023). Therefore, the elevation of PDGF observed in this study supports the hypothesis that the primary pharmacological action of C. asiatica may involve enhancement of regenerative signaling rather than direct inhibition of inflammatory mediators.

The reduction of paw edema during the first hour after carrageenan injection further supports the anti-inflammatory activity of the extract. Carrageenan-induced inflammation is characterized by a biphasic response. The early phase (0–2 hours) is mediated predominantly by histamine, serotonin, and bradykinin release, whereas the late phase (3–6 hours) involves prostaglandins, cyclooxygenase products, nitric oxide, and leukocyte infiltration. The extract significantly reduced edema only during the early inflammatory phase, suggesting that its active constituents may interfere primarily with vasoactive mediator release rather than with prostaglandin-mediated inflammatory pathways. Similar findings have been reported by Mardiyah (2022), who demonstrated dose-dependent inhibition of carrageenan-induced paw edema by C. asiatica cream during the initial inflammatory period. The absence of significant inhibition during the later phase may indicate that a single oral

administration was insufficient to maintain effective concentrations of bioactive compounds throughout the inflammatory process.

Contrary to expectations, serum TNF-α levels were not significantly different among experimental groups. Although numerous studies have shown that triterpenoids from C. asiatica suppress NF-κB activation and reduce TNF-α production, the discrepancy observed in the present study may be explained by the temporal kinetics of cytokine release. TNF-α is rapidly produced within one to three hours following carrageenan administration and declines thereafter. Since blood sampling was performed only at the end of the observation period, transient alterations in TNF-α concentration may have been missed. Furthermore, the relatively small sample size (n = 6 per group) may have limited statistical power to detect modest biological differences. Nafiisah et al. (2021) reported significant reductions in TNF-α following repeated administration of C. asiatica extract in a traumatic brain injury model, suggesting that longer treatment duration or repeated dosing may be required before systemic cytokine suppression becomes apparent.

Similarly, no significant difference was observed in serum MDA concentrations among treatment groups. Although C. asiatica possesses well-established antioxidant properties through its flavonoid and phenolic constituents, oxidative stress biomarkers may not have reached sufficiently elevated levels in this short-term acute inflammatory model. Previous investigations demonstrating reductions in MDA generally involved chronic oxidative stress models, including heavy metal toxicity or prolonged inflammatory conditions (Biswas et al., 2021). Therefore, the absence of significant changes in serum MDA in the present study should not be interpreted as evidence of absent antioxidant activity but rather as a consequence of the timing of measurement and the relatively mild oxidative challenge. Future studies incorporating tissue MDA, superoxide dismutase (SOD), catalase, glutathione peroxidase, and other oxidative biomarkers would provide a more comprehensive evaluation of antioxidant mechanisms.

The phytochemical screening confirmed the presence of flavonoids, alkaloids, saponins, tannins, phenols, glycosides, and triterpenoids in the ethanol extract. These compounds likely exert synergistic biological effects. Flavonoids inhibit cyclooxygenase and lipoxygenase pathways while scavenging reactive oxygen species, whereas triterpenoid saponins such as asiaticoside and madecassoside stimulate fibroblast proliferation and extracellular matrix remodeling. Consequently, the observed increase in PDGF together with early edema inhibition reflects a coordinated pharmacological response involving both anti-inflammatory and tissue regenerative mechanisms.

Several limitations should be acknowledged. First, biomarker measurements were obtained from serum at a single terminal time point, which may not accurately reflect the dynamic changes in inflammatory mediators. Second, baseline paw volume differed among experimental groups, potentially influencing interpretation of edema inhibition. Third, the relatively limited sample size reduced the statistical power to detect differences in TNF-α and MDA. Finally, molecular signaling pathways such as NF-κB, COX-2, inducible nitric oxide synthase (iNOS), VEGF, transforming growth factor-β (TGF-β), and collagen expression were not evaluated. Future investigations should include serial biomarker measurements, histopathological analysis, immunohistochemistry, and gene or protein expression studies to better elucidate the mechanisms through which C. asiatica modulates inflammation and tissue repair.

Overall, the present findings indicate that the ethanol extract of Centella asiatica primarily enhances tissue repair by increasing PDGF production while providing modest anti-inflammatory effects during the early phase of acute inflammation. These results strengthen the potential of C. asiatica as a natural therapeutic candidate for conditions requiring both inflammation control and accelerated tissue regeneration, although further mechanistic and clinical studies remain necessary before translational application.

CONCLUSION

Ethanol extract of gotu kola leaves (Centella asiatica (L.) Urban) contains flavonoids, alkaloids, saponins, tannins, phenols, glycosides, and steroids/triterpenoids. In the carrageenan-induced acute inflammatory model, the extract, especially at a dose of 4 g/kgBB, significantly increased PDGF levels and decreased the volume of edema in the early phase (1st hour), but had no significant effect on TNF-α and MDA. These results show a modulating effect towards tissue repair, and support follow-up research with higher doses, longer observation periods, uniformity of basal values, and immunohistochemistry examinations.

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