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ANTI-INFLAMMATORY ACTIVITY OF ETHANOL EXTRACT FROM *GNETUM MONTANUM* LIANAS IN EXPERIMENTAL MODELS

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Summary

Anti-Inflammatory Activity of Ethanol Extract from *Gnetum montanum* Lianas in Experimental Models

The anti-inflammatory activity of ethanolic extract of *Gnetum montanum* Markgr lianas (GME) harvested in the North of Vietnam was investigated in experimental models: carrageenan-induced peritonitis in rats, heat-induced hemolysis and hypotonicity-induced hemolysis in human red blood cells (HRBC), LPS-induced nitric oxide (NO) production in RAW 264.7 cells. GME at doses of 87.5, 170, and 350 mg/kg significantly reduced leukocyte count, myeloperoxidase activity, and total protein in carrageenan-induced peritoneal exudates when compared with the control group. HBRC stabilization models revealed that GME inhibited hemolysis in both the heat-induced model and hypotonicity-induced model with the IC_{50} of 50.5 µg/mL and 22.3 µg/mL, respectively. GME also inhibited NO production in stimulated RAW 264.7 cells with the IC_{50} of 12.6 µg/mL. The obtained results have provided scientific evidence for the use of GM to treat inflammatory diseases according to folk experience.

Keywords: *Gnetum montanum, Anti-inflammation, Peritonitis, Hemolysis, NO production.*

1. Introduction

Gnetum montanum (GM) is a type of vine that grows wild in the mountainous areas of Vietnam. In Vietnamese traditional medicine the roots and lianas of the plant have been used to treat rheumatism, bone pain, menstrual disorders, and snakebites…[1]. Some remedies use leaves, lianas, and roots of GM to treat low back pain, gout, rheumatoid arthritis, and chronic bronchitis [2]. Although there has been widespread application of GM in the treatment of diseases related to inflammation and gout, so far the use of this medicinal herb is based mainly on experience. Regarding biological activity, recent research showed that the crude extract had anti-inflammatory effects in experimental models [3], suggesting the need for further comprehensive studies on the anti-inflammatory effects and mechanisms of this plant. Studies evaluating the *in vivo* and *in vitro* anti-inflammatory activity of GM will provide scientific evidence, and support the progress of research and development of this valuable medicinal herb.

2. Materials and methods

2.1. Plant material and extract preparation

Lianas of *Gnetum montanum* Markgr were collected in Mường Nhé, Điện Biên province in April 2022. The plant was identified by Prof. Dr. Tran The Bach, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (IEBR-VAST). A voucher specimen (DL2) was deposited at IEBR-VAST. The lianas were cleaned, sliced, dried to a constant weight using a hot air oven at 50°C and ground in a blender to powder. The powder (3 x 2000 g) then was thrice extracted with 95% aq. ethanol by reflux for 3 h. The extracts were combined and evaporated at 50°C under low pressure to obtain residue (1008 g, 21.5% loss on drying). The yield of the extract was 13.2% relative to the dried lianas powder. The total extract was stored at −20°C until use.

2.2. Animals

Wistar albino male rats (10 weeks old, weighing 150-180 g) were purchased from the Centre of Experimental Animals, Vietnam Military Medical University. The rats were acclimatized for at least 7 days to adapt to their environment before any experimental manipulation. They were provided with free access to a standard rodent pellet diet and tap water.

2.3. Cells

RAW 264.7 macrophages were obtained from American Type Cell Culture (ATCC; Rockville, MD) and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37°C in an incubator containing 5% CO2.

2.4. Chemicals

Carrageenan (Sigma Aldrich-Pcode: 1001606651); diclofenac (Secondary standard, National Institute of Drug Quality Control, C0619047.05); indomethacin (Secondary standard, National Institute of Drug Quality Control, 0203094); prednisolone (*Prednisolon 5 mg*, Nahapharm, VD-16472-12); heparin (*Heparin 50000UI/mL*, Rotexmedica, VN-15617-

12); total protein kit (Erba Diagnostics, lot 2001050); RPMI 1640, fetal bovine serum (FBS), penicillin and streptomycin (Thermo Scientific). N^G-Methyl-L-arginine acetate (L-NMMA), LPS (*Escherichia coli* 0111.B4), 3-(4,5-di methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sulfanilamide, phosphoric acid, naphthyl ethylenediamine dihydrochloride, dimethyl sulphoxide (DMSO), $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$, NaCl, KH_2PO_4 (Sigma Aldrich). All other chemicals and reagents were of analytical grade.

2.5. Experimental design

2.5.1. Carrageenan-induced rat peritonitis model:

Rats were randomly assigned into 6 groups (10 rats per group) each receiving vehicle (normal/control), prednisolone 5 mg/kg p.o. (reference), and 87.5 - 17 5 - 350 mg/kg p.o. dose of the GME, respectively. Saline (1 mL/ 100 g for the normal group) or carrageenan 1% in 0.9% saline (1 mL/100 g for the other group) was injected into the peritoneal cavity 1 hour after test drug administration. Then, after 4 hours, euthanasias were conducted, and peritoneal cavities were washed with 10 mL of heparinized (20 IU/mL) saline. The peritoneal exudate is collected. Leukocyte count, myeloperoxidase (MPO) activity, and total protein concentration in the exudates are considered measures of inflammation [4].

Leukocyte counts were performed using an automated cell counter (Autohematology analyzer URIT-3000 VET Plus). Total protein assay was performed using the quantitative kit (Erba, Mannheim) following the manufacturer's instructions.

MPO activity assay was assessed according to Fertz (2008) [5] with some modifications. Briefly, 0.1 mL of exudate was homogenized in phosphate buffer (50 mmol/L, pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) using ultrasonic homogenizers. After removing cell debris by centrifugation (15.000 rpm, 4°C, 30 min), homogenates (30 µL) were mixed with 290 µL of 0.5 mmol/L *o*-dianisidine solution containing 0.0005% H_2O_2 in a 96-well plate. The absorbance at 460 nm was monitored every 1 min for 5 min. The MPO activity was determined as the change in absorbance *versus* time. Volume enzyme activities (A) were calculated according to the following equation: $A(mU/\mu L)=(\Delta E\times V)/(t\times\varepsilon\times d\times v)$, where ΔE, $E_{5\,min} - E_{0\,min}$ at 460 nm; V, final volume (µL); t, time (min); ε, molar absorption coefficient ($mM^{-1}\,cm^{-1}$); d, light path (cm); and v, sample volume (µL).

2.5.2. Heat-induced hemolysis in the HRBC model:

The heat-induced hemolysis of erythrocytes was carried out as described by Ranasinghe et al. (2012) [6] with some modifications. Blood from healthy volunteers was freshly collected and transferred to heparinized centrifuge tubes. The tubes were centrifuged at 2500 rpm for 5 min, and the resultant erythrocyte pellet was washed three times with an equal volume of NaCl 0.9%. Finally, the erythrocyte pellet was suspended in NaCl 0.9 % to make a 20% suspension of HRBC. Various concentrations of GME at 200, 100, 50, 25, and 12.5 µg/mL were prepared. The test mixture consisted of 20 µL of the sample, 30 µL 10 mM sodium phosphate buffer pH 7.4, and 150 µL of the 20% HRBC suspension in test tubes. Indomethacin (100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) was used as a positive control. DMSO was used as a control. The final concentration of DMSO (0.1%) in the control group was the same as in all other groups. The reaction mixture was incubated at 55°C for 20 min. After heating, the reaction mixtures were cooled to room temperature and centrifuged at 5000 rpm for 5 min. 100 µL of supernatant were collected. The hemoglobin content of the supernatant solution was measured indirectly through its absorbance (OD) at 540 nm using a microplate reader. The blank was prepared as above, except that the reaction mixture was incubated at 4°C. The percentage inhibition of hemolysis (I%) was calculated using the following formula: $I\,(\%) = \{[(OD_{control}-OD_{blank})-(OD_{sample}-OD_{blank})]/(OD_{control}-OD_{blank})\}\times 100\%$

2.5.3. Hypotonicity-induced hemolysis in the HRBC model:

The hypotonicity-induced hemolysis of erythrocytes was carried out as described by Ranasinghe et al. (2012) [6] with some modifications. The suspension of HRBC 20 % in NaCl 0.9% (with the preparation process described in section 2.5.2) was used. Various concentrations of GME at 62.5, 31.25, 15.625, 7.8125, and 1.9513 µg/mL) were prepared. The test mixture consisted of 20 µL of the sample, 40 µL NaCl 0.9%, 30 µL of the 20% HRBC

suspension, and 110 µL distilled water in test tubes. Sodium diclofenac (500, 250, 125, 62.5, 3125, and 1.625 µg/mL) was used as a positive control. DMSO was used as a control. The concentration of DMSO in the control group was the same as in all other groups. The reaction mixture was incubated at 37°C for 30 min. The reaction mixtures, then, were cooled to room temperature and centrifuged at 5000 rpm for 5 min. 100 µL of supernatant were collected. The hemoglobin content of the supernatant solution was measured indirectly through its absorbance (OD) at 540 nm using a microplate reader. The blank was prepared as above, except that the reaction mixture consisted of 20 µL of the sample, 150 µL NaCl 0.9%, and 30 µL of the 20% HRBC suspension. The percentage inhibition of hemolysis (I%) was calculated using the following formula: $I\ (\%) = [(OD_{control} - OD_{sample})/ (OD_{control} - OD_{blank})] \times 100\%$

2.5.4. LPS-induced NO production in RAW 264.7 cells model:

The RAW 264.7 cells were seeded in 96-well culture plates with $2x10^5$ cells/well and incubated for 24 h at 37°C in an incubator containing 5% CO_2. The cells were pretreated with various concentrations (0.8, 4, 20, and 100 µg/mL) of GME or of L-NMMA (positive control) for 2 h. DMSO was used as a control. The concentration of DMSO in the control group was the same as in all other groups. The cells were stimulated with or without LPS (1 µg/mL) at 37°C for 24 h. After a 24 h incubation at 37°C, 100 µL of supernatant was collected for the NO assay. The remaining cells were used for assessing cell viability using the MTT assay.

The amount of NO in the culture medium was measured by the Griess reaction. Briefly, 100 µL of the supernatant above were mixed with 100 µL of Griess reagent (equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid, and 0.1% (w/v) naphthyl ethylenediamine dihydrochloride). The reaction mix was incubated at room temperature for 10 min, and the absorbance at 540 nm was measured using a microplate reader. The concentration of NO in the samples was calculated according to a standard curve of sodium nitrite. Fresh culture medium was used as a blank in all experiments.

Cell viability was assessed by MTT assay. After collecting the supernatant for NO assay, the remaining cells in each well were incubated with 100 µL medium containing MTT (500 µg/mL) for 4 h at 37°C. Viable cells with active metabolism convert MTT into a purple-colored formazan product. The medium was discarded, and the formazan was dissolved with 100 µL DMSO by shaking for 5 min. The absorbance of each well at 540 nm, measured using a microplate reader, was considered directly proportional to the number of viable cells [7],[8].

2.6. Statistical analyses

Data were presented as the means ±standard error of the mean (S.E.M). Statistical significance was determined by one-way analysis of variance ANOVA followed by LSD's or Dunnett T3 multiple comparison test to determine treatment differences. With non-normal distribution data, results are presented as the median (min-max) of at least three independent experiments, statistical significance was determined by the Kruskal-Wallis test followed by the Mann-Whitney U test to determine treatment differences. Differences were considered statistically significant when p-values were <0.05. For *in vitro* experiments, concentration providing 50% inhibition (IC_{50}) was calculated by non-linear regression using GraphPad Prism Software.

3. Results

3.1. Effect of the GME on carrageenan-induced peritonitis in rats

3.1.1. Effect of the GME on leukocyte count in peritoneal exudate:

As shown in Table 1, white blood cell (WBC) count, MPO activity, and protein levels in the peritoneal exudate in the model group were significantly higher than the physiological control group. Prednisolone significantly reduced all these parameters compared to the model group. Pretreatment of animals with GME 175 mg/kg was able to reduce the migration of leukocytes to the peritoneal exudate with the decreasing of total WBC, granulocytes (Gran) count, and MPO activity as well as total protein when compared with the model group. At the dose of 87.5 mg/ kg, GME significantly reduced WBC, Gran, and MPO activity. The effects of the dose 350 mg/kg were only significantly exhibited in parameters of WBC and Gran when compared with the model group. In addition, GME at three doses did not significantly affect the number of lymphocytes and monocytes in carrageenan-induced peritoneal exudate of rats (data not shown).

Table 1. Effect of the GME on carrageenan-induced peritoneal exudate

Group	WBC (G/L)	Gran (G/L)	MPO activity (μU/mL)	Total protein (g/L)
Normal	3.3±0.3	2.8±0.3	0.029±0.011	1.43±0.11
Control	10.2±1.4$^{###}$	9.4±1.3$^{##}$	0.065±0.023$^{###}$	5.49±0.37$^{###}$
Prednisolone 5 mg/ kg	4.5±0.9***	3.4±0.6***	0.021±0.007***	3.50±0.29**
GME 87.5 mg/ kg	5.4±0.5**	4.7±0.5***	0.039±0.014**	5.00±0.37
GME 175 mg/ kg	4.3±0.8**	3.5±0.7***	0.025±0.009***	3.61±0.46**
GME 350 mg/ kg	6.2±0.6**	5.8±0.6**	0.058±0.020	5.20±0.26

*($^{###}$: $p < 0.001$ compared to normal; $^{##}$: $p < 0.01$ compared to normal; **: $p < 0.01$ compared to control; ***: $p < 0.001$ compared to control).*

3.2. Effect of the GME on hemolysis of HRBC induced by heat

All the tested concentrations of GME at 200, 100, 50, 25, and 12.5 μg/mL were able to protect HBRC from heat-induced lysis with the inhibition percentage of hemolysis, respectively 85, 73, 52, 26, and 11%. It can be seen in Fig. 1 that in the same manner as positive control indomethacin, GME caused a concentration-dependent stabilization of the red blood cell membranes. The IC_{50} value of GME on heat-induced hemolysis was found to be 50.5 (44.8 - 57.0) μg/mL, compared with indomethacin 13.2 (10.6 - 16.3) μg/mL.

3.3. Effect of the GME on hemolysis of HRBC induced by hypotonicity

In the model of hemolysis induced by hypotonicity, GME exhibited potent membrane protection of HBRCs. At the GME concentrations of 62.5, 31.2, 15.6, 7.8, and 1.9 μg/mL, the inhibition percentage of hemolysis was, respectively 80, 64, 36, 16, and 7%. It can be seen in Fig. 2 that in the same manner with positive control diclofenac, GME displayed a concentration-dependent effect. The IC_{50} value of GME on hypotonicity-induced hemolysis was found to be 22.3 (18.8 - 26.4) μg/mL, compared with diclofenac 95.1 (77.3 - 117.0) μg/mL. Thus, the potent of GME on red blood cell membrane stabilization was even higher than the positive control diclofenac.

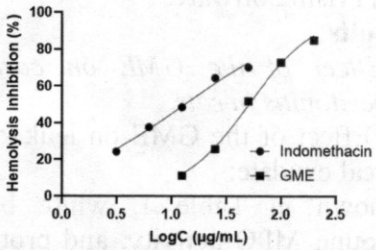


Fig. 1. Effect of the GME on hemolysis of HRBC induced by heat

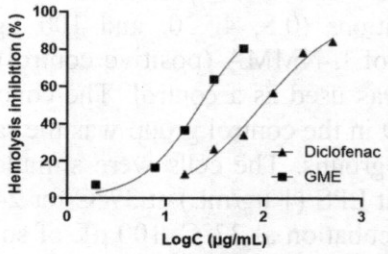
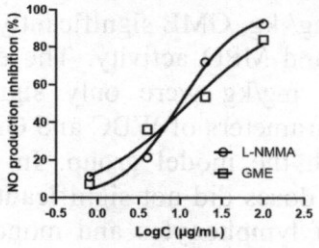


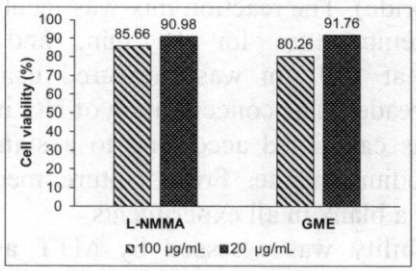
Fig. 2. Effect of the GME on hemolysis of HRBC induced by hypotonicity

3.4. Effect of the GME on cell viability and NO production of LPS-induced RAW 264.7 macrophages

As shown in Fig. 3A, GME inhibited NO production in a dose-dependent manner. The IC_{50} value of GME on NO release was found to be 12.6 μg/mL, compared with L-NMMA 9.9 μg/mL. The effect of GME on the viability of activated RAW 264.7 cells was studied using an MTT assay. No toxic effects were observed in the presence of GME at concentrations up to 100 μg/mL after 24 h LPS treatment.



A



B

Fig. 3. Effect of the GME on NO production (A) and cell viability (B) of LPS-induced RAW 264.7 macrophages

4. Discussion

4.1. Influence of GME on carrageenan-induced peritonitis in rats

Carrageenan is a sulfated polysaccharide extracted from *Chondrus crispus*, a marine alga, used for decades in research for its potential to induce inflammation in different animal models. The injection of carrageenan triggers an acute inflammation. Carrageenan-induced peritonitis promotes the selective migration of neutrophils and does not stimulate the recruitment of macrophages, lymphocytes, or mast cells [9]. It was obvious that in the model group, carrageenan caused peritonitis with the rising of WBC count, Gran count, MPO (a marker of neutrophil activation) activity, and protein levels in the peritoneal fluid. And positive control prednisolone, a steroidal anti-inflammation drug that decreased inflammation via suppression of the migration of polymorphonuclear leukocytes and reversing increased capillary permeability, exhibited strong activities by reducing all the indicators of WBC, Gran, MPO, and protein levels. Three experimental doses of GME (designed based on folk medicinal use experience [2]) showed anti-inflammatory activity with different potency. The dose that shows the best activity was 175 mg/kg. At that dose, GME significantly reduced all the parameters: WBC, Gran, MPO activity, and total protein, similar to the potency of prednisolone 5mg/kg. The lower dose of 87.5 mg/ kg of GME reduced WBC, Gran, and MPO activity slightly less. Interestingly, the largest dose 350 mg/kg only significantly exhibited anti-inflammatory potency in parameters of WBC and Gran. Dose-dependent anti-inflammation property of GME needs to be further investigated. This may relate to the issues of molecular mechanism and constituents of the extract.

4.2. Membrane stabilizing effect of GME on HRBC hemolysis models

Lysosomal enzymes are important mediators of acute and chronic inflammatory response. The release of lysosomal enzymes into the cytoplasm stimulates inflammatory mediators like reactive oxygen radicals, prostaglandins, etc. For that inducement, the stabilization of the lysosomal membrane prevents the release of lysosomal constituents and has a vital role in limiting the inflammatory response [7]. The erythrocyte membrane is like the lysosomal membrane so we could screen the anti-inflammatory activity by the HRBC hemolysis models. In this study, GME exhibited HRBC stabilization by inhibiting both heat-induced and hypotonic-induced hemolysis. This indicates that GME possesses biological membrane stabilization properties, protecting against stress-induced destruction of the lysosomal membrane.

4.3. Inhibitory effect of GME on LPS-induced NO production in RAW 264.7 cell lines

Macrophages play a critical role in the initiation, maintenance, and resolution of inflammation. These cells actively participate in the inflammatory responses by releasing proinflammatory cytokines and mediators such as free radicals and NO as well as proinflammatory cytokines. Thus, agents that decrease NO production in macrophages may have a potential effect of anti-inflammation, antisepsis, and anti-carcinogenesis. The results demonstrate that GME strongly inhibited LPS-induced NO production without notable cytotoxicity in RAW264.7 macrophages. The IC_{50} value of GME 12.6 µg/mL may be equivalent to the IC_{50} value of L-NMMA 9.9 µg/mL. This result is in line with a previous paper by Le Thi Hong Nhung (2022) [10] in which ethanol extracts of *G. montanum* collected in Quang Tri and Vinh Phuc province significantly inhibited NO production of LPS-induced RAW264.7 macrophages with the IC_{50} value of 32.3 µg/mL and 21.3 µg/mL, respectively.

5. Conclusions

In conclusion, the total ethanol extract of *Gnetum montanum* lianas expressed anti-inflammatory activity in both *in vivo* and *in vitro* models. The extract significantly inhibited inflammation in rats with the effects of reducing leukocyte immigration, peroxidase activity, and total protein in peritoneal exudate. *In vitro*, results showed that the total ethanol extract of *G. montanum* possessed anti-inflammatory activity through lysosome membrane stabilization and decrease of proinflammatory mediator production (NO) in LPS-induced macrophages. These results contribute to understanding the anti-inflammatory effect and mechanisms of the plant as well as provide pharmacological validation to the traditional use of *G. montanum* in the treatment of inflammatory disorders.

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References

1. Võ Văn Chi (2021), *Từ điển cây thuốc Việt Nam*, NXB Y học, Hà Nội, 377-378. **2.** Đỗ Tất Lợi (2004), *Cây thuốc và vị thuốc Việt Nam*, Y học, Hà Nội, 662. **3.** Nguyễn Thị Ánh Nguyệt, Nguyễn Tiến Chung (2023), Tác dụng chống viêm, giảm đau của dây gắm (*Gnetum montanum* Markgr.) trên động vật thực nghiệm, *Y dược Cổ truyền Việt Nam*, 1, 44-51. **4.** Sousa-Neto B. P., Cunha F. V. M., Nunes D. B., Gomes B. S., Amorim L. V., Lopes E. M., Gutierrez S.J. C., Almeida F. R. C., Arcanjo D. D. R., Souza M. F.V., Oliveira F. A. (2022), Anti-inflammatory and antioxidant effects of the indole-derived *N*-salicyloyltryptamine on peritonitis and joint disability induced by carrageenan in rodents, *Evidence-Based Complementary and Alternative Medicine*, 5524107. **5.** Fietz S., Bondzio A., Moschos A., Hertsch B., Einspanier R. (2008), Measurement of equine myeloperoxidase (MPO) activity in synovial fluid by a modified MPO assay and evaluation of joint diseases - An initial case study, *Research in Veterinary Science*. 84(3), 347-353. **6.** Ranasinghe P., Ranasinghe P., Abeysekera W. P., Premakumara G. A., Perera Y. S., Gurugama P., Gunatilake S. B. (2012), *In vitro* erythrocyte membrane stabilization properties of *Carica papaya* L. leaf extracts, *Pharmacognosy Research*, 4(4), 196-202. **7.** Nguyen T. H., Nachtergael A., Nguyen T. M., Cornet V., Duez P., Muller M., Ly Huong D. T., Kestemont P. (2020), Anti-inflammatory properties of the ethanol extract from *Clerodendrum cyrtophyllum* Turcz based on *in vitro* and *in vivo* studies, *Journal of Ethnopharmacology*, 254, 112739. **8.** Oh Y. J., Jin S. E., Shin H. K., Ha H. (2023), Daeshiho-tang attenuates inflammatory response and oxidative stress in LPS-stimulated macrophages by regulating TLR4/MyD88, NF-κB, MAPK, and Nrf2/HO-1 pathways, *Scitific Reports*, 13: 18891. **9.** Barth C. R., Funchal G. A., Luft C., de Oliveira J. R., Porto B. N., Donadio M. V. (2016), Carrageenan-induced inflammation promotes ROS generation and neutrophil extracellular trap formation in a mouse model of peritonitis. *European Journal of Immunology*, 46(4), 964-970. **10.** Lê Thị Hồng Nhung, Nguyễn Tiến Chung (2022), Khảo sát hoạt tính kháng viêm và ức chế enzyme xanthine oxydase của một số loài thuộc chi dây gắm (*Gnetum*), *Khoa học và Công nghệ*. 58(3), 141-143.

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ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF THE ETHANOL EXTRACT FROM *CLERODENDRUM CYRTOPHYLLUM* TURCZ IN MOUSE AND RAT MODELS

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Summary

Anti-Nociceptive and Anti-Inflammatory Effects of the Ethanol Extract from *Clerodendrum cyrtophyllum* Turcz in Mouse and Rat Models

The present study aimed to evaluate the anti-nociceptive and anti-inflammatory properties of the ethanol extract from *Clerodendrum cyrtophyllum* leaves (EE) using animal models. The analgesic activities were evaluated using two animal models: the hot-plate test and the acetic acid-induced writhing test. In the acetic acid-induced writhing test, the number of acetic acid-induced writhes was significantly decreased in the presence of EE at doses of 0.54, 1.08, and 2.16 g/kg, demonstrating strong peripheral antinociceptive activity. In the hot plate test, the hot plate pain threshold was significantly increased after 30, 60, and 90 min in the presence of EE at doses of 0.54 and 1.08 g/kg. The inhibitory effects of EE on the two animal models suggest that EE possesses peripheral and central analgesic activities. To evaluate the anti-inflammatory effect, we used two models: carrageenan-induced paw edema and cotton pellet-induced granuloma test. EE at doses of 0.32 g/kg and 0.64 g/kg remarkably inhibited edema of the paw induced by carrageenin in rats. These results indicate that EE exerts anti-inflammatory activity, especially in the acute phase of inflammation. EE at the dose of 0.64 g/kg effectively inhibited cotton pellet granuloma formation, suggesting that EE also displays anti-inflammatory activity in the chronic phase of inflammation. Taken together, the results consistently demonstrate that the raw ethanol extract of *C. cyrtophyllum* leaves presents potent anti-nociceptive and anti-inflammatory activities and may be useful for the treatment of various inflammatory diseases.

Keywords: *Anti-nociceptive, Anti-inflammatory, Hot-plate test, Acetic acid induced writhing test, Carrageenan-induced paw edema, Cotton pellet induced granuloma test, Clerodendrum cyrtophyllum.*

1. Background

Inflammation is a common physiopathological symptom of many diseases including cancer, cardiovascular disease, diabetes, obesity, osteoporosis, rheumatoid arthritis, inflammatory bowel disease, and asthma. There are generally two types of inflammation: acute and chronic inflammation. Chronic inflammatory diseases are the most significant cause of death in the world. The World Health Organization (WHO) ranks chronic diseases as the greatest threat to human health [1].

Clerodendrum cyrtophyllum, a plant belonging to the Verbenaceae family, is widely