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Research Article

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In Vivo Evaluation of Anti-Inflammatory Activity of *Bridelia Micrantha* (Hochst.) Baill. Stem Bark and *Ganoderma Applanatum* (Pers.) Pat. Extracts

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Abstract

Ethnopharmacological Relevance

Inflammation is a natural defense response to injury or infection; however, persistent inflammation is associated with the development of chronic diseases. In traditional medicine, numerous plant and fungal species are used for the management of inflammatory conditions, although many of these remedies lack sufficient scientific validation. Bridelia micrantha and Ganoderma applanatum are traditionally used in various communities for the treatment of pain, swelling, and inflammation-related disorders.

Aim of the Study

This study aimed to evaluate the anti-inflammatory activity of Bridelia micrantha stem bark and Ganoderma applanatum extracts using an in vivo experimental model.

Materials and Methods

The anti-inflammatory activity of the extracts was evaluated in Swiss albino mice using the formalin-induced paw edema model. B. micrantha extract was administered at doses of 25, 50, and 100 mg/kg, while G. applanatum extract was tested at doses of 50, 100, and 200 mg/kg. Paw edema was measured at 60, 120, 180, and 240 minutes following formalin injection. Diclofenac sodium was used as the reference drug.

Results

All doses of B. micrantha significantly reduced paw edema compared to the negative control ($p < 0.05$), with anti-inflammatory effects becoming more pronounced from the second to the fourth hour. The 50 mg/kg dose exhibited the highest activity, surpassing diclofenac at 180 minutes. G. applanatum extracts significantly inhibited paw edema at 60 and 180 minutes across all tested doses ($p < 0.05$), while no significant effects were observed at 120 and 240 minutes ($p > 0.05$). A non-linear dose-response relationship was observed, with the 50 mg/kg dose at 180 minutes showing greater efficacy than diclofenac, whereas the 100 mg/kg dose demonstrated inconsistent activity.

Conclusions

Both Bridelia micrantha and Ganoderma applanatum exhibited significant anti-inflammatory activity, with optimal effects observed at specific doses rather than in a dose-dependent manner. These findings support their traditional use in the management of inflammatory conditions and suggest that the observed activity may be attributed to bioactive constituents such as flavonoids, tannins, and terpenoids. Further studies are warranted to elucidate their pharmacokinetics and underlying mechanisms of action.

Keywords: Bridelia Micrantha, Ganoderma Applanatum, Anti-Inflammatory, Swiss Albino Mice, Paw Edema, Formalin

Abbreviations

ANOVA - Analysis of Variance
B. micrantha - Bridelia Micrantha
COX - Cyclooxygenase
G. applanatum - Ganoderma Applanatum
IL - Interleukin
kg - Kilogram
LOX - Lipoxygenase
mg/kg - Milligrams Per Kilogram Body Weight
min - Minutes
NF-κB - Nuclear Factor Kappa B
NSAIDs - Non-Steroidal Anti-Inflammatory Drugs
p - Probability Value
ROS - Reactive Oxygen Species
SEM - Standard Error of the Mean
TNF-α - Tumor Necrosis Factor Alpha

1. Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants [1]. While it serves as a protective mechanism aimed at removing injurious stimuli and initiating the healing process. Chronic inflammation has been implicated in the pathogenesis of various diseases, including rheumatoid arthritis, cardiovascular disorders, diabetes, and cancer [2]. Conventional anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are commonly used to manage inflammatory conditions. However, their long-term use is often associated with adverse effects, including gastrointestinal disturbances, renal toxicity, and immunosuppression. This has spurred a growing interest in exploring plant-derived compounds with anti-inflammatory potential, which may offer safer alternatives with fewer side effects [3].

The inflammatory response is primarily mediated by the release of signaling molecules such as cytokines, chemokines, prostaglandins, and reactive oxygen species (ROS) [4]. These mediators are produced by activated immune cells, including macrophages and neutrophils, and contribute to vasodilation, increased vascular permeability, and leukocyte infiltration. Natural compounds with antioxidant and enzyme-inhibitory properties can modulate these pathways by suppressing the activity of key inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), or by downregulating pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6 [5].

Bridelia micrantha (Hochst.) Baill. is widely used in African traditional medicine for the management of inflammatory conditions, including pain, wounds, swelling, arthritis, and fever. Ethnobotanical surveys conducted in Kenya, Nigeria, and Zimbabwe document the use of stem bark decoctions to relieve joint pain, treat wounds, and reduce inflammatory swellings, indicating its longstanding role in the management of inflammation-related conditions [6].

Ganoderma applanatum (Pers.) Pat., commonly known as the artist's conk, has a history of use in traditional Asian medicine for managing chronic inflammatory disorders such as arthritis, musculoskeletal pain, and inflammation-associated fatigue. Ethnomedicinal records from Chinese and Japanese traditional practices report its use in powdered or decoction forms to alleviate chronic pain and inflammatory conditions, similar to other medicinal species of the genus *Ganoderma* [4-7].

Phytochemical studies have shown that B. *micrantha* contains flavonoids, tannins, alkaloids, saponins, terpenoids, and phenolic compounds, while G. *applanatum* is rich in triterpenoids, phenolics, and polysaccharides. These classes of compounds are widely associated with anti-inflammatory, antioxidant, and immunomodulatory activities, providing a scientific basis for their traditional use and supporting further experimental investigation [8].

Given the global burden of inflammatory diseases and the need for safer therapeutic agents, it is imperative to evaluate the pharmacological potential of medicinal plants such as *B. micrantha* and *G. applanatum.* This study aimed to investigate the anti-inflammatory activity of B. micrantha stem bark and G. applanatum extract using in vivo models, and to provide scientific evidence supporting its traditional use in the treatment of inflammatory conditions.

2. Materials and Methods

2.1. Collection and Preparation of Plant Materials

The bark of *B. micrantha* verified according to World Flora Online was collected from its natural habitat in Manyatta Constituency, Embu County. The samples were transported to the laboratory

in polythene bags. Species identification was confirmed by a taxonomist at the Kenyatta University Herbarium, where a voucher specimen KMB001/2018 was also deposited. *G. applanatum* was collected in Kitale, Trans Nzoia County, from an acacia tree at a riverbank. Botanical identification of the mushroom was done in the herbarium at the National Museum of Kenya, Nairobi, where a voucher specimen EAHNMK 265 was deposited. The samples were thoroughly washed with running tap water, cut into small pieces, and shade-dried for seven days. The dried material was then ground into a fine powder and stored in an airtight polythene bag for further analysis.

2.2. Extraction

100 g of the powdered sample was soaked in 500 mL of methanol overnight. The mixture was then subjected to ultrasonic extraction at 60 °C for two hours using an ultrasonic bath to enhance compound recovery. Following sonication, the extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator to yield the crude extract.

2.3. Experimental Animals

Adult Swiss albino mice of both sexes were equitably distributed and used for both assays. The animals were randomly assigned into groups of six and housed in cages under controlled conditions of 20-25°C with a 12-hour light/dark cycle. They were given a seven-day acclimatization period before the experiments began. Standard mouse pellets (Unga Feeds, Kenya Ltd) and tap water were provided throughout the experimental period. All animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Ethics Committee of Kenyatta University, approval number KU-ACUC/04/2018 [9].

2.4. Anti-Inflammatory Assay

The anti-inflammatory activity was evaluated using the formalin-induced hind paw edema model in albino mice, with 5.0 % formalin solution serving as the phlogistic agent, as described by [10]. Albino mice of either sex were randomly assigned to nine groups of five animals each. Group 1 received 0.85 mg/mL of normal saline and served as the negative control. Group 2 received diclofenac sodium (15 mg/kg) as the positive control, administered 30 minutes before formalin injection. Groups 3 to 9 were administered varying doses of the test extract via intraperitoneal injection.

Inflammation was induced by injecting 50 µL of 5 % formalin into the subplantar region of the left hind paw. Paw diameter was measured before formalin injection using a digital caliper and subsequently at one-hour intervals for a total of four hours. The increase in paw diameter was calculated as the difference between post- and pre-injection measurements.

2.5. Data Analysis

Data were expressed as mean ± standard error of the mean (SEM) and analysed using one-way ANOVA, followed by Scheffé's post hoc test. A p-value < 0.05 was considered statistically significant [11].

2.6. Phytochemical Analysis

The presence of flavonoids was tested as per guidelines [12]. A few drops of sodium hydroxide solution was added to 1 mL of the extract. An intense yellow color that disappears on the addition of dilute acid indicates the presence of flavonoids. To confirm the presence of terpenoids, 0.8 g of the extract was transferred to a test tube, and 10 mL of ethanol was added. The sample was thoroughly shaken and then filtered. To 5 mL of the filtrate, 2 mL of chloroform was added, followed by 3 mL of sulphuric acid. The formation of reddish-brown colour was an indication of the presence of terpenoids in the extract. This was followed by the tannins' test, where 10 mL of bromine (prepared by decanting the liquid bromine into a bottle with distilled water in the fume hood) was used. After the airspace above the water was covered with red bromine vapor, both bottles were capped. The mixture was gently swirled and added to 0.5g of the extract.

3. Results and Discussion

Phytochemical	*Bridelia micrantha bark*	*Ganoderma applanatum*
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Terpenoids	+	+

(+) Present

Table 1: Phytochemicals Present in B. Micrantha Bark and G. Applanatum Extract

The presence of key phytochemicals such as alkaloids, tannins, flavonoids, and terpenoids, shown in Table 1 in both *B. micrantha* bark and *G. applanatum,* suggests a strong potential for anti-inflammatory activity. These bioactive compounds are well-documented for their ability to modulate inflammatory responses. Flavonoids and terpenoids, for instance, are known to inhibit pro-inflammatory mediators like prostaglandins and cytokines, thereby reducing inflammation [13]. Tannins exhibit tightening properties

that help in tissue contraction and wound healing, while alkaloids are reported to interfere with the inflammatory signalling pathways. The presence of these phytochemicals in both species supports their traditional use in managing inflammatory conditions and provides a biochemical basis for their observed anti-inflammatory effects in experimental models [5].

This study evaluated the anti-inflammatory activity of *B. micrantha* bark and *G. applanatum* extract in Swiss albino mice using the formalin-induced paw edema model, a well-established method for assessing acute inflammation. The extract for *B. micrantha* was tested at three different doses: 25 mg/kg, 50 mg/kg, and 100 mg/kg, as shown in Table 2. The findings provide evidence that the extract significantly reduced paw edema at all doses compared to the negative control (normal saline), indicating that *B. micrantha* possesses dose-dependent anti-inflammatory properties.

Treatment	Time in Minutes				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
25 mg/kg	0	0.97 ± 0.05	0.81 ± 0.06a	0.70 ± 0.06	0.95 ± 0.06a
50 mg/kg	0	1.29 ± 0.09a	0.73 ± 0.06a	0.35 ± 0.09a	0.98 ± 0.09a
100 mg/kg	0	1.04 ± 0.03a	0.72 ± 0.05a	0.47 ± 0.04a	1.01 ± 0.09

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the superscript are considered to have no significant difference relative to normal saline.

Table 2: Effect of B. Micrantha Extract on Formalin-Induced Paw Edema in Mice

The initial observation revealed that all doses exhibited minimal edema reduction in the first hour post-administration, as shown in Figure 1, with error bars indicating variability. This suggests that the active constituents of the extract may require more time to be absorbed and exert their biological effects. Such a delayed onset is common in phytotherapeutic agents, which often rely on natural compound metabolism, gastrointestinal absorption, and systemic circulation before achieving peak efficacy. This lag phase may also reflect the pharmacokinetics of the extract, whereby plasma concentrations of active metabolites were still sub-therapeutic within the first hour.

By the second, third, and fourth hours, a notable reduction in paw edema was observed in all treatment groups, with the 50 mg/kg dose showing the most pronounced effect. Interestingly, this dose outperformed even the standard drug, diclofenac sodium, particularly at the 180-minute mark. Diclofenac is a potent non-steroidal anti-inflammatory drug (NSAID) known for its cyclooxygenase (COX) inhibitory activity, which reduces the synthesis of prostaglandins responsible for pain and inflammation [13]. The superior performance of the 50 mg/kg dose suggests that *B. micrantha* may act through both COX-dependent and COX-independent pathways, possibly involving histamine, serotonin, and bradykinin antagonism or inhibition of nitric oxide synthase and cytokine expression.

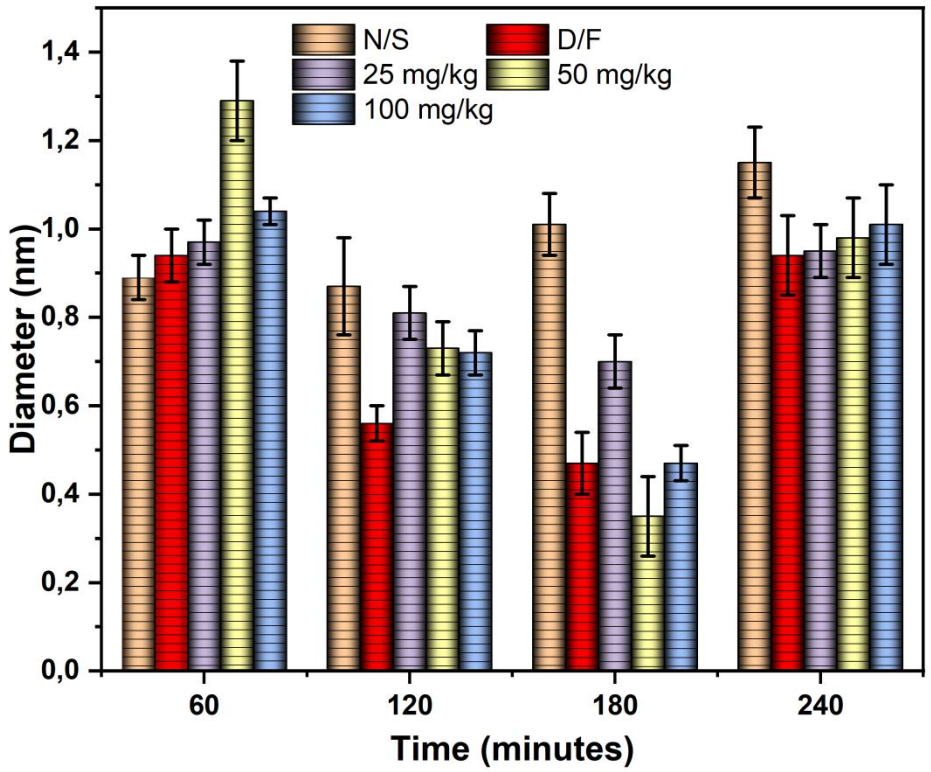

Figure 1: Anti-Inflammatory Effects of Bridelia Micrantha Stem Bark

The results also revealed a non-linear dose-response relationship. Although 100 mg/kg is a higher dose, it did not produce consistently greater anti-inflammatory activity than the 50 mg/kg dose. While it did show significant activity at 60, 120, and 180 minutes ($p < 0.05$), its effect diminished by the 240-minute time point, where no significant reduction in paw edema was observed compared to the control. This decline in efficacy could be attributed to several factors, including a shorter half-life of the active compounds, the induction of metabolic enzymes leading to faster degradation, or possible receptor desensitization at high concentrations.

Moreover, phytochemical extracts often contain a complex mixture of constituents, some with synergistic effects and others that may antagonize the activity of key anti-inflammatory metabolites. At higher doses, inhibitory interactions may occur, reducing the overall pharmacological response. Another possibility is that high concentrations may lead to the up-regulation of certain enzymes that counteract the anti-inflammatory effect, such as prostaglandin synthetase, or cause cellular feedback mechanisms that attenuate the response. This is consistent with the observation that excessive concentrations of enzyme inhibitors or metabolites can, paradoxically, reduce drug activity.

Conversely, the 25 mg/kg dose, although effective at certain time points, demonstrated inconsistent activity. It did not produce statistically significant results at 60 and 180 minutes ($p > 0.05$), indicating that this dose may be below the minimum effective concentration required for sustained anti-inflammatory action. This could be due to inadequate levels of active metabolites in systemic circulation, which fail to reach the pharmacological threshold needed to inhibit inflammatory mediators. The fluctuating activity of the 25 mg/kg dose underscores the importance of optimizing dosage for phytotherapeutic interventions.

The peak anti-inflammatory activity observed at the third hour across the effective doses may reflect the extract's peak plasma concentration. In drug development, understanding the time to peak effect (Tmax) is crucial in establishing dosing schedules [14]. The delayed but robust response of the 50 mg/kg dose indicates it may have a favourable pharmacokinetic profile with sufficient bioavailability, supporting its potential for further development.

Comparatively, previous studies in Nigeria also demonstrated the anti-inflammatory efficacy of *B. micrantha* using various models, including histamine-induced, formaldehyde-induced, and carrageenan-induced paw edema in rats. These studies revealed that higher doses; 200 mg/kg and 400 mg/kg, were effective in reducing inflammation, with the 400 mg/kg dose having prolonged activity lasting up to 24 hours in the formaldehyde-induced model. Such findings corroborate the current study and support the broader pharmacological potential of *B. micrantha* across both acute and chronic inflammation models [6]. The duration of action in the formaldehyde model implies that some of the extract's constituents may act on delayed-pwassehase mediators such as prostaglandins,

leukotrienes, or cytokines, which are more involved in chronic inflammatory responses [14].

Phytochemical analysis of *B. micrantha* has shown that it contains numerous secondary metabolites, including tannins, flavonoids, alkaloids, and terpenoids. These compounds have been widely reported in the literature for their anti-inflammatory activity. Flavonoids, for instance, are known to inhibit arachidonic acid metabolism by blocking COX and lipoxygenase pathways. Tannins exhibit astringent properties that may reduce capillary permeability and tissue swelling, while alkaloids and terpenoids can modulate immune responses and inhibit pro-inflammatory cytokines such as TNF-α and IL-1β [13].

Given the results of the current study, it is plausible that the anti-inflammatory effect of *B. micrantha* bark extract is due to a combination of these phytochemicals working synergistically. However, without fractionation and compound isolation, it is difficult to determine which specific constituents are responsible for the observed effects. Future research should focus on bioassay-guided fractionation to identify and characterize the most active anti-inflammatory agents within the extract. Additionally, studies on the extract's mechanism of action at the molecular level, including its impact on inflammatory signalling pathways such as NF-κB and MAPK, would provide more detailed insight into its pharmacodynamics [5].

From a translational point of view, the extract's effectiveness, particularly at 50 mg/kg, indicates its potential utility as a natural anti-inflammatory remedy. However, before any clinical application, it is essential to establish the extract's safety profile through acute and chronic toxicity studies, determine its pharmacokinetic parameters, and ensure batch-to-batch consistency in its phytochemical composition. Standardization of the extract would also be crucial to ensure reproducibility and efficacy in different populations.

The data in Table 3 illustrate the anti-inflammatory effects of different treatments over 240 minutes using the formalin-induced paw edema model. The doses 50, 100 and 200 mg/kg of *G. applanatum* extract exhibited a significant effect ($p < 0.05$) on induced edema on mice at 60 and 180 minutes. The normal saline group, serving as the negative control, shows a continuous increase in paw edema from 0.89 ± 0.05 mm at 60 minutes to 1.15 ± 0.08 mm at 240 minutes, indicating sustained inflammation. In contrast, diclofenac, the standard anti-inflammatory drug, significantly reduces paw swelling, reaching a low of 0.47 ± 0.07 mm at 180 minutes. Notably, treatment with G. applanatum extract, particularly at doses of 50 mg/kg and 200 mg/kg, shows a marked reduction in inflammation. At 180 minutes, paw thickness decreases to 0.42 ± 0.14 mm and 0.44 ± 0.07 mm, respectively, closely aligning with the effect of diclofenac. This suggests that *G. applanatum* exhibits potent, dose-dependent anti-inflammatory activity, likely due to its rich phytochemical composition, including flavonoids, alkaloids, tannins, and terpenoids. These findings support the potential therapeutic application of *G. applanatum* in managing acute inflammation.

Treatment	Time in Minutes				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
50 mg/kg	0	1.21 ± 0.06a	0.75 ± 0.28	0.42 ± 0.14a	0.95 ±0.20
100 mg/kg	0	1.21 ± 0.07a	0.81 ± 0.05	0.72 ± 0.04a	1.06 ± 0.12
200 mg/kg	0	1.08 ± 0.08a	0.70 ± 0.04	0.44 ± 0.07a	1.08 ± 0.12

Table 3: Effect of Ganoderma Applanatum Extract on Formalin-Induced Paw Edema in Mice

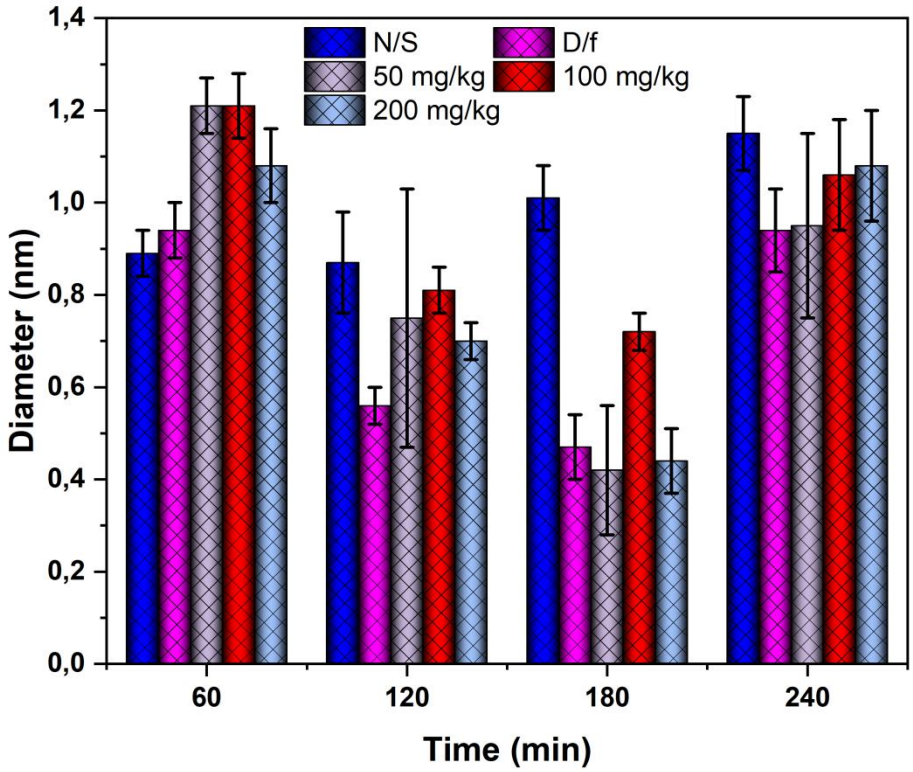

Figure 2: Effect of Ganoderma Applanatum Extract on Formalin-Induced Paw Edema in Mice

Furthermore, the observed decline in activity at higher doses points to the need for precise dose optimization. While herbal remedies are often considered safe, inappropriate dosing can lead to sub-therapeutic effects or even adverse reactions [5]. Thus, the findings of this study emphasize the importance of evidence-based dosing in traditional medicine and the potential for integrating such plant extracts into modern pharmacotherapy.

4. Conclusion

In conclusion, Bridelia micrantha bark extract demonstrated significant anti-inflammatory activity in a dose-dependent manner, with the 50 mg/kg dose showing the highest efficacy, even outperforming diclofenac sodium at specific time points. Ganoderma applanatum extract possesses significant, dose-dependent anti-inflammatory activity comparable to the standard drug diclofenac. The extract, particularly at doses of 50 mg/kg and 200 mg/kg, effectively reduced formalin-induced paw edema in mice, with substantial inhibition observed at 180 minutes. The study highlights the complex interplay between dose, time, and bioactivity in herbal medicine and reinforces the therapeutic potential of B. micrantha and G. applanatum as a source of natural anti-inflammatory compounds. These promising findings warrant further investigation through compound isolation, mechanistic studies, and clinical evaluation to fully exploit its pharmacological potential.

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