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Anti-inflammatory activity of *Urtica dioica* root and *Ganoderma lucidum* in Swiss albino mice

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ABSTRACT

Background: Inflammatory response, a beneficial body's response to injury and infection, can have various detrimental effects, ranging from pain, edema, and fever. There are several modern remedies to these ailments, which include the use of Nonsteroidal anti-inflammatory agents (NSAIDs) drugs as well as steroids. These treatments are associated with various adverse effects such as gastrointestinal irritation, liver and kidney toxicity, cardiovascular risks, and immunosuppression. Other methods of treatment in East Africa include the use of plant remedies such as *Urtica dioica* root and *Ganoderma lucidum*, though their efficacy has not been scientifically evaluated. **Objective:** This study aimed to assess the anti-inflammatory effects of methanol extracts of *Urtica dioica* root and *Ganoderma lucidum* using an animal model. **Materials and methods:** Formalin-induced hind paw edema in mice was used as the model of acute inflammation. The animals were administered with 12.5, 25 & 50 mg doses of the two plants 30 minutes prior to injection of 5% formalin solution in the left hind paw. Paw diameter was taken before injection of formalin and every hour after for three hours using digital Vernier callipers. The difference between the initial reading and subsequent readings was quantified as edema formed in the hind paw. Qualitative phytochemical analyses were carried out using standard methods. The data obtained was expressed as means and their standard errors, then analysed using one-way Analysis of Variance (ANOVA) and Tukey as the post hoc test. A value of $p < 0.05$ was considered significant. **Results:** The two extracts significantly reduced ($p < 0.05$) the paw edema diameter. Phytochemical analyses identified secondary metabolites that can be associated with anti-inflammatory effects. **Conclusion:** These findings suggest that *Urtica dioica* root and *Ganoderma lucidum* may possess compounds with anti-inflammatory effects, hence supporting their folklore use for inflammation management.

Keywords: Anti-inflammatory, *Urtica dioica*, *Ganoderma lucidum*, Mice.

INTRODUCTION

Remedies from natural sources, such as plants and fungal extracts, play an important part in the management of a wide range of ailments, with about 80% of the population in developing countries depending on them for their primary benefits [1]. The major motivating factors are the belief that they are easily available, effective, cheaper, and have fewer side effects than modern synthetic drugs [2]. In recent years, natural products derived from plants and fungi have garnered attention due to their potential therapeutic benefits, minimal side effects, and wide availability [3]. Plants such as *Withania somnifera* contain withanolides, which have been shown to have anti-inflammatory and analgesic properties. Similarly, among other plants with known medicinal properties, *Urtica dioica* (Urticaceae) (stinging nettle in English, *thabai/hatha* in Kikuyu, *sambakhalu* in Luhya) is a herbaceous flowering plant that is 50 to 300 cm long, and its leaves, roots, and flowers are used as medicines [4]. The leaves are also used as vegetables and in tea [5]. *Ganoderma lucidum* (Reishi mushroom) in English, *nduru* in Kikuyu), commonly used in traditional Chinese medicine, has been valued for centuries for promoting longevity and overall wellness. Reishi contains bioactive compounds such as polysaccharides, triterpenoids, and peptidoglycans, notable for their diverse biological activities, including anti-inflammatory and analgesic effects. *U. dioica* roots have been used as a folklore medicine for pain and inflammation, while *G. lucidum* for its immune-modulating and anti-inflammatory properties. They are used as natural alternatives in managing inflammation and pain, supporting their use alongside other traditional remedies like *W. somnifera* for treating chronic inflammatory conditions [6]. The effects of Urtica include anti-inflammatory and antioxidant properties, making it useful in managing conditions like arthritis and protecting against oxidative stress. Urtica also acts as a natural diuretic, antioxidant, allergy relief, blood sugar regulator, among other uses. Ganoderma lucidum exhibited an antioxidant effect. Compounds isolated from *Ganoderma lucidum* and Urtica dioica include tannins, flavonoids, terpenoids, and alkaloids [7]. The root contains bioactive compounds such as sterols, flavonoids, and lignans, which have demonstrated significant anti-inflammatory and antinociceptive effects [4]. Despite extensive use of the two plant parts as a folklore remedy for inflammation and pain, there is scarcity of data on their efficacy.

Hence this study aimed evaluated the ant inflammatory effect using animal model and phytochemical profile.

MATERIALS AND METHODS

Collection and treatment of plant materials

Roots of the *Urtica dioica* were collected from their natural habitat between January and September 2018, from the Manyatta constituency, Embu County, in the afternoon, and transported in a polythene bag to the laboratory. *G. lucidum* was collected in September from Kitale, Trans Nzoia County, at around 4.00 pm from an acacia tree at a river bank. Botanical identification of the mushroom was done in the herbarium at the National Museum of Kenya, Nairobi, and a voucher specimen was deposited. While *Urtica dioica* was identified by a taxonomist in the Kenyatta University herbarium, and voucher specimen was deposited at the university herbarium. The samples were washed thoroughly with running tap water, cut into small pieces, and shade dried for 7 days before grinding them into fine powder. The ground powder was stored in an air-tight polythene bag for further use. A total of 100 g of each ground plant sample was weighed and soaked overnight in 500 mL of methanol. The mixture was then subjected to ultrasonic extraction at 60°C for two hours using an ultrasonic bath to enhance the extraction process. After sonication, the extract was filtered through Whatman No. 1 filter paper. The resulting clear filtrate was concentrated under reduced pressure using a rotary evaporator to obtain the crude extract.

Chemicals and reagents

The analytical-grade chemicals and reagents utilized in the study included diclofenac sodium ($C_{14}H_{10}C_{12}NNaO_2$), formalin (CH_2O), sodium chloride (NaCl), deionized water (H_2O), and methanol.

Experimental animals

The experiments were conducted per the ethical guidelines outlined by Wolfensohn for the care and use of laboratory animals, ensuring that all procedures complied with established standards for humane treatment and welfare [8]. Proper housing, feeding, and handling protocols were followed throughout the study to minimize stress and discomfort to the animals. All interventions were performed to reduce pain and distress, and the experimental design incorporated principles of the 3Rs: Replacement, Reduction, and Refinement to uphold the highest standards of animal welfare. Ethical approval was obtained from the Institutional Ethics Committee (Ref. No. IAEC/I56/38350/2016).

Anti-inflammatory activity assay

The anti-inflammatory activity was induced using the formalin-induced hind paw edema in Swiss albino mice, where 5.0% formalin solution as the phlogistic agent [9]. Albino mice of either sex, distributed equally, were divided into nine groups of five each. Inflammation was induced by injecting 50 µl of 5 % formalin solution into the subplantar region of the left hind paw of the animal. The paw diameter before injection of formalin was measured using a digital caliper. One hour after the formalin injection, the paw diameter was measured. This was repeated for hours. The difference between the final diameter and the initial was recorded [10].

Phytochemical analysis

The screening of phytochemicals present in *Ganoderma lucidum* and *Urtica dioica* was carried out using a method described by [11].

Data analysis

The data were presented as means with standard errors of the mean, then analysed using one-way ANOVA, followed by *Scheffe's* post *hoc* test. A student's t-test was applied to compare the activity between the two extracts. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Injury and tissue damage are linked to inflammation and pain. Formalin-induced edema test involves a biphasic response [12]. The first phase is neurogenic pain, which is as a result of the direct effect of formalin. The second phase is caused by an inflammatory reaction brought by histamine, prostaglandin, serotonin, cytokines, and bradykinin, such as nitric oxide [13]. This study used formalin-induced paw edema in mice to determine the anti-inflammatory activity of *G. lucidum* and *U. dioica* roots in experimental mice. The anti-inflammatory effect of *G. lucidum* appeared to be more potent in reducing inflammation than *U. dioica*. The dose of 50 mg showed a significant effect ($p < 0.05$) on formalin-induced paw edema at times 60, 120, 180, and 240 minutes, as shown in Table 2.

Other doses had a significant effect at different times (Table 2; Figure 2). In a study done in South India by Sheena and coworkers on anti-inflammatory activity using *G. lucidum,* the mice were treated with 500 and 1000 mg/kg BW of both ethyl acetate and methanol extract of *G. lucidum*, methanol extract of dose 1000 mg/kg exhibited a reduction in paw edema compared to ethyl acetate extract. This might be due to the solubility difference of methanol and ethyl acetate, which might affect the availability and concentration of bioactive compounds [14]. In comparison, our study used a much lower dose of *G. lucidum* (50 mg/kg BW), yet we observed a noticeable anti-inflammatory effect. This lower dose of the extract produced a similar reduction in paw edema, which could suggest that our extract may be more potent or that the mechanism of action of *G. lucidum* in reducing inflammation is dose-dependent and potentially more efficient at lower concentrations, as shown in Figure 1.

Flavonoids have been shown to hinder inflammation by inhibiting lipoxygenase and cyclooxygenase pathways, which play a critical role in the inflammatory response [15]. Alkaloids, one of the largest groups of secondary metabolites in plants, exhibit a broad spectrum of pharmacological effects, including anti-inflammatory activity [16]. These phytochemicals are likely contributors to the observed anti-inflammatory activity in our study. All three doses of *U. dioica* roots exhibited a reduction in paw edema (Table 3 and Figure 3). The 12.5 and 25 mg/kg doses showed a significant effect ($p < 0.05$) as shown in table 3, relative to normal saline at 60, 120, 180, and 240 minutes, while the dose of 50 mg/kg showed a significant effect ($p < 0.05$) at 60 and 180 minutes. Nevertheless, the dose of 50 mg/kg failed to reduce the edema at 120 and 240 minutes.

A study was done in Iran by Hajhashemi on *U. dioica* leaf using carrageenan-induced edema using mice. Doses of 100, 200, and 400mg/kg body weight (BW) were used. Extract dose at 400mg/kg BW significantly reduced the inflammation ($p < 0.05$) [17].

In contrast, in our study, the anti-inflammatory activity was investigated using *U. dioica* root extract at much lower doses of 12.5 mg/kg BW and 25 mg/kg BW. Despite the lower doses, we observed significant reductions in inflammation, suggesting that the root extract may be more potent, or that its active components are more effective at lower doses compared to the leaf extract

Nonsteroidal Anti-inflammatory Drugs (NSAIDs), including diclofenac sodium, are commonly used to manage inflammation by inhibiting the synthesis of prostaglandins, which are key mediators of the inflammatory response [18]. However, this inhibition can sometimes exacerbate the inflammatory condition in the long term [19]. In the study, edema was induced in mice 30 minutes before the administration of diclofenac sodium. Following this, various doses of methanol extracts were administered to evaluate their anti-inflammatory effects.

The observed reduction in inflammation was attributed to the active metabolites present in the methanol extract. Among these metabolites, flavonoids were identified as shown in Table 1 as a significant contributor.

Flavonoids are known for their potent anti-inflammatory properties, primarily by inhibiting the metabolism of arachidonic acid. Arachidonic acid, when released from cell membranes, undergoes enzymatic conversion through the COX and LOX pathways, resulting in the production of pro-inflammatory mediators like prostaglandins and leukotrienes [20]. By blocking these pathways, flavonoids effectively reduce the synthesis of these inflammatory compounds, thereby exerting their anti-inflammatory effects.

Table 1: Phytochemicals present in *Urtica dioica* root and *Ganoderma lucidum* extract

Phytochemical	*Urtica dioica* root	*Ganoderma lucidum*
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Terpenoids	+	+

(+) Present (–) Absent

Table 2: Effect of phytochemical-rich fractions of *Ganoderma lucidum* extract on formalin-induced paw edema in mice

Treatment	Time (min)			
	60	120	180	240
Vehicle	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0.94 ± 0.06	0.56 ± 0.09	0.47 ± 0.03	0.94 ± 0.09
12.5 mg	0.92 ± 0.07	0.94 ± 0.09	0.79 ± 0.08*	0.88 ± 0.05*
25 mg	0.86 ± 0.03	0.64 ± 0.06*	0.51 ± 0.08*	0.65 ± 0.07*
50 mg	0.73 ± 0.07*	0.44 ± 0.09*	0.28 ± 0.08*	0.40 ± 0.09*

Mean change in the paw edema diameter in mm ± SEM (*p < 0.05) relative to vehicle (normal saline).

Table 3: Effect of *Urtica dioica* root extract on formalin-induced paw edema in mice

Treatment	Time (min)			
	60	120	180	240
Vehicle	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
12.5 mg	1.06 ± 0.06*	0.68 ± 0.01*	0.62 ± 0.02*	0.88 ± 0.05*
25 mg	1.01 ± 0.05*	0.59 ± 0.06*	0.50 ± 0.06*	0.82 ± 0.07*
50 mg	1.09 ± 0.02*	0.77 ± 0.03	0.46 ± 0.03*	1.03 ± 0.06

Mean change in the paw edema diameter (mm ± SEM) (*p < 0.05) against the vehicle. Dose 50 mg/kg reduced the inflammation better than diclofenac sodium.

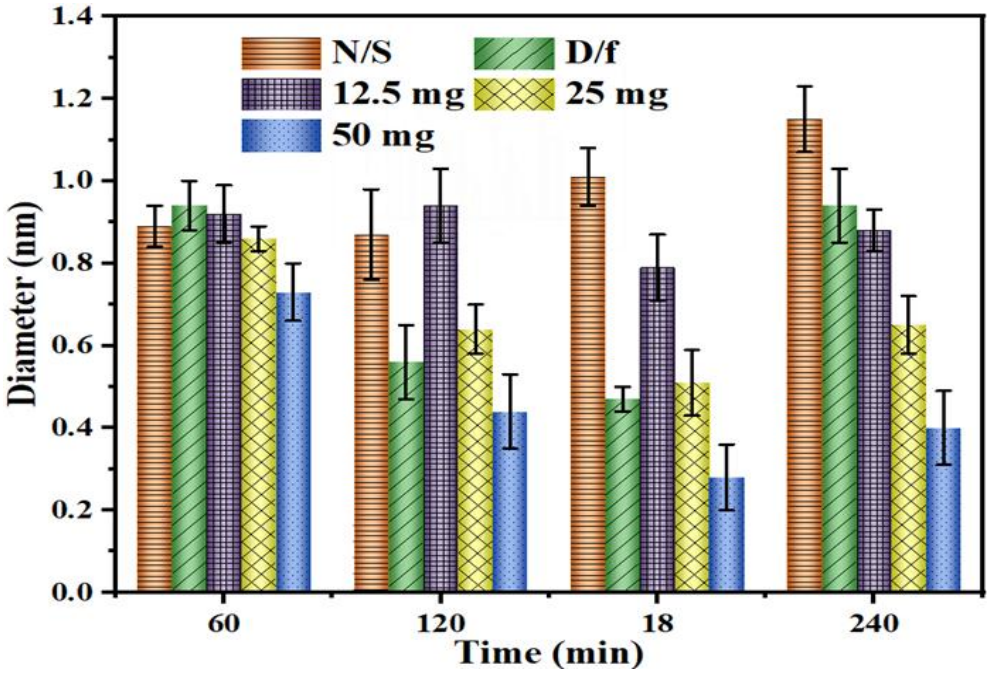

Figure 1: Effect of *Ganoderma. lucidum* extract on formalin-induced paw edema in mice

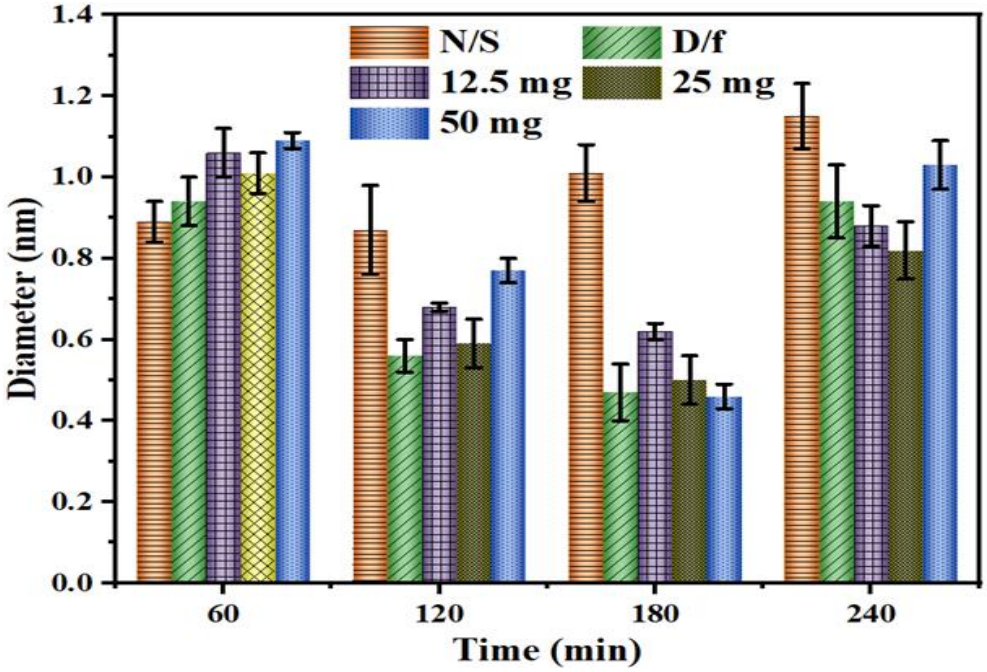

Figure 2: Effect of *Urtica dioica* root extract on formalin-induced paw edema in mice

CONCLUSION

The present study demonstrates that both *U. dioica* root and *G. lucidum* exhibit significant antinociceptive and anti-inflammatory activities in Swiss albino mice, validating their traditional use for pain and inflammation management. Through the formalin-induced pain model, both plant extracts showed a reduction in pain responses, with *U. dioica* root displaying comparable efficacy to the standard analgesic drug, diclofenac sodium. Furthermore, both extracts effectively reduced inflammation in the carrageenan-induced paw edema model, with *U. dioica* root showing a more pronounced anti-inflammatory effect at lower doses. The bioactive compounds present in both plants, such as flavonoids, triterpenoids, and polysaccharides, likely contribute to these therapeutic effects. These findings underscore the potential of *U. dioica* root and *G. lucidum* as natural alternatives to conventional anti-inflammatory and analgesic drugs, offering a promising approach for the management of inflammatory conditions and pain, without the adverse side effects commonly associated with synthetic medications.

Overall, this study provides valuable evidence supporting the therapeutic value of these plant species and lays the foundation for further exploration into their mechanisms of action, safety profiles, and potential clinical applications in the treatment of pain and inflammation.

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Conflict of interest

The authors declared no conflict of interest.

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