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Anti-inflammatory and Nociceptive Properties of the Root Aqueous Extract of *Vernonia amygdalina* and *Ocimum graticimum* Using Animal Models

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Abstract

Vernonia amygdalina is a medicinal plant used in folk medicine for the treatment of several diseases. *Ocimum graticimum* plant roots act as sedatives for children. *Ocimum* tea is an infused form of the herb used in treating fever and diaphoresis. The study aims to evaluate the anti-inflammatory and analgesic properties of the root extracts of biherbal using Swiss mice and Wistar rats. The plant samples were collected, air-dried, ground, and processed into a root biherbal extract. Adult Swiss mice and Wistar rats were obtained to assess anti-inflammatory and analgesic effects using animal models. The results obtained showed a significant reduction in edema at 100 and 200 mg/kg of the extract. The analgesic effects at 100 and 200 mg/kg had a significant reduction in acetic acid and formalin-induced pain models. All effects were statistically significant ($p < 0.05$). The biherbal extract exhibits significant anti-inflammatory and analgesic effects, with further research needed to identify bioactive compounds and explore its modern medical potential.

Keywords: Anti-inflammatory; Antinociceptive; *Vernonia amygdalina*; *Ocimum graticimum*; Animal Models.

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction to prevent the spread of an injurious agent and to remove the necrosed cells and tissues. Inflammation may be caused by bacteria, viruses, fungi, parasites, antigen-antibody reaction. Mechanical trauma, organic and inorganic poisons, and foreign bodies. Inflammation involves 2 basic processes: the early inflammatory response, later followed by healing. Signs of inflammation include redness, swelling, heat, pain, and loss of function. There are two types of inflammation (Kumari *et al*., 2017). An acute transient phase characterized by local vasodilation and permeability. increased capillary (Alara *et al*., 2017). A subacute phase, characterized by infiltration of leukocytes and phagocytic cells (Ebong *et al*., 2008). A chronic proliferative phase, in which tissue degeneration and fibrosis occur. Methods for testing acute and sub-acute inflammation are: Acute and Chronic. Acute inflammation is of short duration and represents the early body reaction, resolves quickly, and is usually followed by healing. Chronic inflammation is defined as a prolonged process in which tissue destruction and inflammation occur at the same time.

Medicinal plants have demonstrated their significance as a source of compounds with therapeutic potential and continue to be a vital resource for discovering new drug candidates (Atanasov *et al*., 2015). The WHO characterizes herbal medicine as substances or preparations derived from plants that provide therapeutic or other health benefits for humans, containing either raw or processed components from one or more plant sources. In certain traditions, materials of animal or inorganic origin may also be included.

Vernonia amygdalina (bitter leaf) belongs to the family Asteraceae, which has a characteristic bitter taste. It is a small shrub that can grow up to three meters and is native to tropical Africa. The plant is also well distributed in Asia and more commonly found near drainage lines and in natural forests (Alara *et al*., 2017). The leaves of the plant are used to make soup and stew in tropical Africa, and are mainly used ethno-botanically to treat various ailments (Luo *et al*., 2017). *V. amygdalina* is a medicinal plant used in folk medicine for the

treatment of several diseases. The bitter taste of V. amygdalina can be reduced by boiling or soaking in water (Ebong *et al.,* 2008). Several bioactive alkaloid saponins and tannins are present in the plant leaves. These bioactive components made out of *V. amygdalina* leaf act as an antimicrobial agent in brewing industries (Kadiri and Olawoye, 2016). Several ailments, such as fever, kidney problems, hiccups, and stomach discomfort, can be treated ethno-medically using extracts of V. amygdalina leaves and roots. *V. amygdalina* leaf and root extracts are used as purgative, antifungal, and anti-malaria agents (Mohammed and Sharif, 2007). In the pharmaceutical industry*, V. amygdalina* has acquired special relevance recently due to its possession of antitumorigenic properties (Usunohun and Okolie 2016). Several studies conducted on different parts of *V. amygdalina* indicated that it contains different bioactive components such as alkaloids, flavonoids, tannins, saponins, triterpenoids, and many more (Luo *et al*., 2017). These bioactive components are responsible for their different pharmacological properties, such as antimalarial, antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antithrombotic, laxative, anticancer, anthelmintic, and hypoglycemic, among others (Alara *et al*., 2017).

The perennial plant *O. gratissimum* (scent leaf) is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiatae, and it is the most abundant of the genus *Ocimum*. In the southern part of Nigeria, the plant is called "effinrin-nia" by the Yoruba-speaking tribe, "Nchonwu" in Igbo, while in the northern part of Nigeria, the Hausas call it "Daidoya". These perennial plants are woody at the base. Several studies have been performed that lend credence to the herbalist's use of this plant for treating diarrhoea and other gastrointestinal infections (Usunohun and Okolie 2016). In folk medicine, *O. gratissimum* is extensively used throughout West Africa as a febrifuge, anti- malarial, and anti-convulsant. The crushed leaf juice is used in the treatment of convulsions, stomach pain, and catarrh. Oil from the leaves has been found to possess antiseptic, antibacterial, and antifungal activities (Luo *et al*., 2017). In the coastal area of Nigeria, the plant is used in the treatment of epilepsy, high fever, and diarrhoea. While in the savannah areas, decoctions of the leaves are used to treat mental illness. *Ocimum gratissimum* is used by the Igbos of southern Nigeria in the management of the baby's cord. It is believed to keep the baby's cord and wound surface sterile. It is used in the treatment of fungal infections, fever, cold, and catarrh. Clinical trials of creams formulated against dermatological disease have yielded favorable results. Several tonics are produced from these herbs and used in treating skin infections, bronchitis, and conjunctivitis. The plant roots act as sedatives for children (Kadiri and Olawoye, 2016). *Ocimum* tea is an infused form of the herb used in treating fever and diaphoresis.

MATERIALS AND METHODS

Collection of plant materials

The roots of *Vernonia amygdalina and Ocimum graticimum* were meticulously collected in June from the University of Benin, located in Edo State, Nigeria. The plant was identified and authenticated by Prof. O. Timothy, a plant Taxonomist in the Department of Plant Biology and Biotechnology University of Benin, Benin City.

Preparation of plant materials

The roots of *Vernonia amygdalina and Ocimum graticimum* were sectioned into various sizes and air dried at room temperature. Subsequently, they were dried in a hot air oven and then pulverized with a grinding machine to obtain a powdered form. A total of 593 grams of the pulverized root powder was weighed and transferred into a beaker; six (6) liters of distilled water were added. The mixture was agitated using a magnetic stirrer and left to stand for three days, with shaking occurring every four hours. Following the three days, extraction was performed using the maceration process with a glass jar, and the pulverized root extract was concentrated using a water bath. Finally, the concentrated extract was transferred into a small amber glass bottle for storage.

Experimental Animal

Adult Swiss mice and Wistar rats, of both sexes, weighing between 20 and 30 grams and 150 and 180 grams, respectively, were sourced from the Animal House at the Department of Pharmacology, Faculty of Pharmacy, University of Benin, located in Benin City. The animals were acclimatized for a period of seven days. The animals were kept in properly ventilated plastic cages under controlled laboratory conditions (12-hour light/dark cycle, 23 ± 2°C) and provided with a standard diet of Bendel Grower Pellet Chew. Food and water were made available at all times. All animal-related procedures followed the established ethical guidelines.

Experimental Design

The animals were transferred to the laboratory 24 hours before the experiment began, and they were randomly allocated to five groups (n=5), including controls and treated groups, according to the study protocol. This grouping was part of the experimental design for the analgesic study. Pain was induced using chemicals method, and analgesic effects were measured by observing the number of writhes. Inflammatory studies typically involved the induction of inflammation with chemical agents, and inflammation was assessed by measuring paw swelling. The animals were given a standard diet, and all procedures involving them complied with the ethical guidelines, with ethical number

issued (LS21106) by the ethical committee of Life Sciences.

Anti-inflammatory activity

For this study, five groups of four Wistar rats of 7 weeks old (180 - 250 g) each were used. The extract at 50, 100, and 200 mg/kg doses was administered orally to the test groups. Hundred (100) mg/kg of Aspirin was orally administered to the positive control, while 0.5 mg/kg of distilled water was administered to the negative control group. After 30 minutes, 0.1 g of Carrageenan with 10 mL of normal saline and 0.1 mL of Formalin solution were injected into the subplantar tissue of the left hind paw and the right hind paw of the animal, respectively. The thickness of the paw was measured using a Vernier caliper at the following time intervals: 30 minutes, 1 hour, 2 hours, and 4 hours.

Analgesic activity on the Mouse Writhe Test

Acetic acid induced pain

Five groups of randomly selected Swiss albino mice, each consisting of three mice, were used in this study. The mice were administered 0.1 mL of acetic acid intraperitoneally. After 30 minutes, the test groups received oral extracts at doses of 50, 100, and 200 mg/kg, while the negative control group received no treatment, but 0.5 mg/kg of Distilled water was administered. Aspirin was administered to the positive control group at a dose of 100 mg/kg. Writhing behavior was observed and counted for 30 minutes, after 5 minutes of waiting.

Formalin-induced pain

Five groups of randomly selected Swiss albino mice, each consisting of three mice, were used in this study. The test groups received oral extracts at doses of 50, 100, and 200 mg/kg, while the positive control group received the standard drug, aspirin (100 mg/kg). The negative control group received just 0.5 mg/kg of water, no treatment. After 30 minutes, all the mice were injected with 0.1 mL of formalin solution. Following a 5-minute waiting period, a 20-minute observation period was initiated to count the number of writhes in each mouse as a result of the induced pain.

Statistical analysis

The data were represented in tables as Mean ± SEM (standard error of the mean) for the different groups. The data was processed with GraphPad Prism. At a 0.05 (95%) significance level was considered.

RESULTS AND DISCUSSION

This study highlights the anti-inflammatory and analgesic effects of biherbal root extract, particularly at specific doses, compared to aspirin in various experimental models (McGeoch *et al*., 2008). The carrageenan-induced paw edema model (Table 1) demonstrated that the 100 mg/kg dose of biherbal root extract exhibited significant edema reduction at multiple time points, with results comparable to aspirin (200 mg/kg). This suggests that biherbal has potent anti-inflammatory properties, capable of reducing both acute and persistent inflammation. However, the 50 mg/kg dose showed moderate efficacy, indicating a dose-dependent effect. These findings align with previous research that has indicated the potential of biherbal extracts in managing inflammatory conditions. Inflammation represents the immune system's reaction to harmful triggers, including pathogens, damaged tissues, toxic substances, or radiation (Medzhitov, 2008; Chavda *et al*., 2024). Its role is to eliminate harmful factors and kickstart the healing process. Thus, inflammation serves as a crucial defense mechanism for maintaining health. Typically, during acute inflammation, various cellular and molecular processes collaborate to mitigate potential injury or infection. This process aids in restoring tissue homeostasis and resolving acute inflammation. However, if acute inflammation is not controlled, it can progress to chronic inflammation, leading to several chronic inflammatory diseases. Carrageenan-induced paw edema, first described by Winter *et al.* (1962) and Zammel *et al.* (2020), serves as a well-established model for studying acute inflammation.

Table 1. Effect of biherbal root extract on Carrageenan acid-induced paw edema.

Groups	Doses (mg/kg)	Paw edema (mm)				
		0 minutes	60 minutes	120 minutes	240 minutes	480 minutes
Negative Control	0. 5 ml/kg	5.75 ± 0.35^{a}	5.79 ± 0.57^{a}	5.82 ± 0.49^{a}	5.90 ± 0.49^{a}	6.15 ± 0.52^{a}
Aspirin	100	5.28 ± 0.22^{a}	4.21 ± 0.31^{b}	4.13 ± 0.37^{b}	4.03 ± 0.32^{b}	3.97 ± 0.38^{b}
Biherbal	50	5.77 ± 0.48^{a}	4.41 ± 0.38^{a}	4.25 ± 0.48^{b}	4.11 ± 0.45^{a}	4.01 ± 0.38^{b}
Biherbal	100	5.82 ± 0.40^{a}	3.52 ± 0.48^{a}	3.47 ± 0.34^{b}	3.40 ± 0.34^{b}	3.36 ± 0.39^{b}
Biherbal	200	5.41 ± 0.39^{a}	4.11 ± 0.21^{b}	3.91 ± 0.25^{b}	3.83 ± 0.24^{a}	3.77 ± 0.35^{b}

Values are means ± SEM; n=3 for each group; $p<0.05$. Dw — Distilled water.

This inflammatory response causes increased vascular permeability and cell infiltration, resulting in paw swelling due to fluid leakage and leukocyte accumulation in the affected tissue (Focho *et al*., 2009). Additionally,

the inflammatory response involves the generation of reactive oxygen species (ROS), the release of inflammatory mediators, and changes in blood parameters. Excessive ROS production can damage cells by affecting proteins, lipids, and nucleic acids (Barth *et al*., 2016). It is well recognized that reactive oxygen species can trigger inflammation by promoting the release of pro-inflammatory cytokines and factors that lead to tissue damage. The inflammatory cascade encompasses a variety of receptors and pathways, including Toll-like receptors, which are often targeted in anti-inflammatory therapies. Acute inflammation is prevalent and linked to dangerous conditions like enteritis, gastritis, and bronchitis, all classified as "-itis" diseases in humans. In the formalin-induced paw edema model (Table 2), significant reductions in edema were observed at 60, 120, and 240 minutes for all treatment groups, including aspirin and the different doses of biherbal extract (Bussmann, 2011). Notably, the 200 mg/kg dose of the extract showed results comparable to aspirin, reinforcing the anti-inflammatory activity of the extract. The lack of significant effects at 60 minutes for the 50 and 100 mg/kg doses might suggest that the anti-inflammatory effects of biherbal take time to develop, or that the lower doses are less effective in the acute phase of inflammation. The formalin-induced nociceptive response produces a biphasic pain pattern, useful for evaluating the analgesic effectiveness and mechanisms of drugs (Erami *et al*., 2017). Centrally acting analgesics can inhibit both phases of formalin-induced pain, while peripherally acting medications primarily affect only the second phase. Formalin causes localized inflammation through tissue irritation and promotes a biphasic inflammatory response, activating both acute and chronic inflammatory pathways (Hunskaar and Hole, 1987). These agents provide a reliable means to evaluate anti-inflammatory activity. A similar study by Kiran *et al.* (2012) investigated the anti-inflammatory properties of herbal extracts using carrageenan-induced paw edema. Their findings supported the efficacy of various plant extracts in reducing inflammation, paralleling the results obtained with biherbal.

Table 2. Effect of biherbal root extract on Formalin-induced paw edema.

Groups	Doses (mg/kg)	Paw edema (mm)				
		0 minutes	60 minutes	120 minutes	240 minutes	480 minutes
Negative control	0.5 ml/kg	4.99 ± 0.51^{a}	5.03 ± 0.54^{a}	5.15 ± 0.51^{a}	5.32 ± 0.55^{a}	5.85 ± 0.57^{a}
Aspirin	100	5.07 ± 0.47^{a}	4.99 ± 0.32^{b}	4.80 ± 0.84^{b}	4.75 ± 0.41^{b}	4.61 ± 0.73^{b}
Biherbal	50	4.96 ± 0.46^{a}	4.87 ± 0.42^{a}	4.84 ± 0.62^{b}	4.73 ± 0.74^{b}	4.67 ± 0.54^{b}
Biherbal	100	4.99 ± 0.71^{a}	4.84 ± 0.71^{a}	4.80 ± 0.73^{b}	4.73 ± 0.49^{b}	4.62 ± 0.26^{b}
Biherbal	200	4.97 ± 0.70^{a}	4.88 ± 0.70^{b}	4.83 ± 0.42^{b}	4.61 ± 0.47^{b}	4.50 ± 0.41^{b}

Values are means ± SEM; n=3 for each group; p- value < 0.05.

The acetic acid-induced writhing model (Table 3) tested the analgesic potential of biherbal extract, with the medium (100 mg/kg) and high (200 mg/kg) doses significantly reducing writhing responses compared to the negative control (Ghlichloo and Gerriets, 2023). These results suggest that biherbal exhibits notable analgesic activity at higher doses. The lower dose (100 mg/kg) did not significantly alleviate pain, highlighting a dose-dependent relationship. The effectiveness of aspirin in this model further supports the validity of the experiment and the ability to compare the analgesic properties of the extract. In the formalin test (Table 4), which evaluates both acute and persistent pain responses, the 50 and 100 mg/kg doses of biherbal and aspirin demonstrated significant reductions in writhing behavior during the first 20 minutes. The highest dose (200 mg/kg) showed pain inhibition comparable to the 100 mg/kg dose, suggesting that both lower and higher doses may be similarly effective in alleviating pain in the acute phase (Githingi *et al*., 2012a; Githingi *et al*., 2012b).

Table 3. Effect of biherbal root extract on acetic acid-induced writhing.

Groups	Doses (mg/kg)	Number of Writhe	% Inhibition
Negative control	0.5 ml/kg	22.0 ± 0.51^{a}	0.00
Aspirin	100	7.00 ± 0.20^{b}	68.2
Biherbal	50	15.00 ± 0.89^{a}	31.8
Biherbal	100	2.73 ± 0.23^{c}	87.6
Biherbal	200	1.65 ± 0.17^{c}	92.5

Values are means ± SEM; n=3 for each group; p<0.05.

Table 4. Effect of biherbal root extract on Formalin-induced pain (writhing).

Groups	Doses (mg/kg)	Means ± SEM	% inhibition
Negative control	0.5 ml/kg	31.0 ± 0.70^{a}	0
Aspirin	100	16.30 ± 0.41^{b}	47.4
Biherbal	50	19.00 ± 0.64^{b}	38.7
Biherbal	100	11.00 ± 0.69^{c}	64.5
Biherbal	200	8.41 ± 0.58^{c}	72.9

Values are means ± SEM; n=3 for each group; $p<0.05$.

These results indicate that biherbal extract may act through mechanisms similar to those of aspirin in reducing pain, though the specific pathways require further investigation. This study has also shown that biherbal root extract is effective in reducing pain, as evidenced in the reduction of writhing frequency in the test groups treated with the extract (Ssegawa *et al*., 2007). Acetic acid and formalin are commonly used pain-inducing agents in experimental models to study analgesic effects. Acetic acid induces pain by triggering a localized inflammatory reaction, which leads to the release of pain mediators that activate nociceptors. Historically, acetic acid has been the most commonly used irritant. The animals react with a characteristic stretching behavior, which is called writhing. Writhing is defined as a stretch, tension to one side, extension of hind legs, or contraction of the abdomen so that the abdomen of the mice touches the floor, or turning of the trunk (twist). Analgesic activity of the test compound is inferred from a decrease in the frequency of writhings. The writhing test is often criticized for its lack of specificity, as non-analgesic drugs have been shown to effectively reduce writhing behavior; however, it remains an important experimental model of pain because of its unique sensitivity to weak analgesics (Idu *et al*., 2010; Iwu, 2014; Stafford *et al*., 2008). Thus, the pain induced by acetic acid is relatively mild and brief enough in duration to be ethically acceptable. The test has also been criticized for its relatively high proportion of nonresponders (Treede *et al*., 2015), although this proportion can be reduced by using higher doses of acetic acid. The mechanisms of pain are mediated through various chemical agents, including prostaglandins and bradykinin, which sensitize nociceptors and enhance pain perception.

CONCLUSION

Overall, biherba*l* root extract shows promise as a natural remedy for managing inflammation and pain, hence the need for further investigation for therapeutic applications.

Competing interests: No competing interest

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