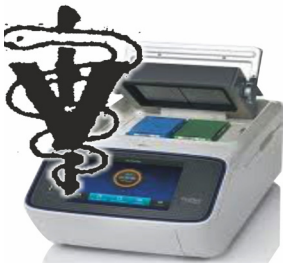
Journal of Veterinary and Biomedical Sciences
Vol. 8(1). PP. 134-144, July, 2026
ISSN:2659-0743

DOI: 10.36108/jvbs/6202.80.0111



Evaluation of Anti-inflammatory and Analgesic Effects of Aqueous Buds Extract of Syzygium aromaticum (L.) Merr. and L.M. Perry. in Rats

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Abstract

The synthetic drugs in use for the treatment of inflammation and pains often come with side effects and high costs; this has led to increased interest in exploring natural remedies from the plants. Syzygium aromaticum is one of the widely used medicinal plants for its pain-relieving and anti-inflammatory properties and the plant was extracted in distilled water using cool maceration method. Acute toxicity test was performed to determine the safety dose of Syzygium aromaticum extract Three different models: Acetic acid-induced writhing test, Hot plate reaction test, and Tail flick test were used to evaluate the analgesic effects in which doses of 100, 200 and 400 mg/kg of the aqueous extract of the plant were administered orally to three groups of mice for each model, while anti-inflammatory effect was evaluated using egg albumin-induced paw edema method in rats with 100, 200 and 400 mg/kg were also administered orally to three groups of rats. Indomethacin and distilled water were used in both analgesic and anti-inflammatory evaluations as positive and negative controls respectively. The three models were used for analgesic action in order to evaluate the effects of the plant on both deep and peripheral pains. The results showed that the aqueous extract of the plant significantly ($p<0.05$) reduced inflammation and pain in dose-dependent manner when compared with control group. The extract also increased the pain reaction time in rats, indicating both central and peripheral analgesic actions, and these results confirm the traditional use of the plant in the treatment of pain and inflammation-related health problems.

Keywords: Analgesic, anti-inflammation, Syzygium aromaticum, Rats, Mice.

Introduction

Synthetic drugs used as anti-inflammatory agents such as NSAIDs, in most cases come with some adverse effects (Vane and Botting, 1998.) while some are very costly especially for the rural dwellers, which therefore compel them to the use of natural products especially medicinal plants. Over the years, it has been shown that medicinal plants produce a wide range of secondary metabolites that have been reported to exhibit both anti-inflammatory and analgesic activities, these metabolites include: flavonoids (Middleton et al., 2000), alkaloids (Kumar et al., 2010, Oladele et al,. 2018), terpenoids (Liu, 1995), tannins (Edeoga et al., 2005), saponins and glycosides (Harborne, 1998) among others. Syzygium aromaticum also known as clove is of the family Myrtaceae. It has been prized for centuries as both an aromatic and a medicinal plant, mostly due to its essential oil content, with eugenol being the dominant bioactive compound (Chaieb et al., 2007). Syzygium aromaticum is a chemically rich plant, with its pharmacological importance strongly linked to diverse chemical constituents. These chemical constituents can be broadly divided into two categories: the volatile or essential oil fraction, which is predominantly concentrated in the unopened flower buds, and the non-volatile phenolic and polyphenolic compounds which are present in both volatile-poor and solvent-based extracts. The volatile fraction is the most researched due to its aromatic and pharmacological properties and the major compound present is eugenol (4-allyl-2-methoxyphenol), a phenylpropanoid that has analgesic, local anesthetic, anti-inflammatory, and antioxidant effects, and that typically accounts for 50–90% of the bud essential oil, depending on origin and extraction method (Chaieb et al., 2007; Cortés-Rojas et al., 2014). Syzygium aromaticum has a wide variety of application in its use as a medicinal tool with effects on pain, inflammation, infection, and digestion. Some of these applications include; analgesic, anti-inflammatory, antimicrobial, digestive aid used to alleviate bloating, flatulence etc. (Chaieb et al., 2007; Cortés-Rojas et al., 2014), dental and oral care as it is incorporated into zinc oxide–eugenol cement, root canal sealants, and periodontal dressings (Jirovetz et al., 2006). The antioxidant effects protect cells against oxidative stress implicated in cancer, diabetes, and cardiovascular diseases (Harborne, 1998; Gülçin et al., 2012).

Some of the folkloric use of Syzygium aromaticum in traditional medicinal treatment has been documented and these include the following; In Ayurveda, Syzygium aromaticum oil used for toothaches, gum pain, sore throats, and arthritis. Traditional Chinese Medicine used cloves for abdominal pain and cold-induced inflammation (Parthasarathy et al., 2008; Chaieb et al., 2007). African traditional medicine uses decoctions and poultices of clove in treatment of rheumatism, chest pain, stomachache, and wound inflammations (Edeoga et al., 2005). Middle Eastern and Unani medicine, mixed clove oil with honey or ghee which is used for the relief of earaches, sore throats, and inflamed swellings (Sofowora, 1993). This study evaluated the anti-inflammatory and analgesic effects of the crude aqueous extract of this plant using three different models in order to evaluate the effects of the plant on both deep and peripheral pains.

Materials and methods

Collection and preparation of Syzygium aromaticum

The dried buds of the plant Syzygium aromaticum, were bought from Gwagwalada market, in Gwagwalada Area Council of the Federal Capital Territory Abuja, Nigeria. The buds were identified and authenticated at the Botany Unit of the Department of Biology, University of Abuja. They were properly cleaned and left to dry in the shade at room temperature. The dried-out clove buds were properly blended into fine powder using electricity-powered blender and the resulting powered sample was then stored in an air-tight container for aqueous extraction.

Aqueous extraction of the buds

The powdered plant material (315 gram) was poured into a beaker and 1315 ml of distilled water was added to the beaker. The mixture was

macerated for 72 h with occasional stirring; it was then filtered using a Whatman's No. 1 filter paper and the filtrate was poured into another beaker and placed in a water bath maintained at 45^0C to obtain a thick, coffee brown, sticky paste extract which was then stored in an air-tight bottle and refrigerated.

Experimental animals

The animals used for this study were 25 adult male and female Wistar rats weighing between 90-130 g and 25 male and female mice weighing between 40-90 g. The rats and mice were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Abuja, where they were housed in metal cages and maintained at 12 h light/dark cycle at room temperature and given food and water ad libtum. The rats and mice were both divided into 5 groups of 5 animals per group. Picric acid solution was used to label each animal distinctly for easy identification.

Acute toxicity test

Acute toxicity test was conducted to determine the safety dose of Syzygium aromaticum buds extract in mice; the method of Lorke (1983) was adopted with some modifications. In the first phase of the study, nine (9) rats were randomly allocated to three groups (A-C) of rats consisting of 3 rats per group. The animal groups were given 10, 100, and 1000 mg/kg of the extract respectively. The animals were then observed for signs of toxicity and death within 2 h.

The second phase of the experiment commenced in the absence of mortality of the test rats in the first phase of the study. A new set of nine (9) rats were randomly assigned to three groups (A-C), as previously done in the first phase of the study. The animals were treated with higher doses 1500, 2000, and 3000 mg/kg of the extract, respectively. A 4^{th} group consisting of 3 rats served as normal control in both phases of the experiment and was administered distilled water (10 ml/kg). All treatments were given orally using gastric intubation. The experimental rats were also observed for signs of toxicity and death within 2 h. the LD_{50} of the extract was calculated using the formula (Enegide et al., 2013):

$LD_{50} = \sqrt{(D_0 \times D_{100})}$ Where D_0 = Highest dose that gave no mortality, and D_{100} = Lowest dose that produced mortality.

Evaluation of anti-inflammatory activity with egg albumin-induced rat paw edema

The egg albumin-induced paw edema method (Winter et al. 1962) with little modifications was used to assess the anti-inflammatory activity of the aqueous extract of Syzygium aromaticum. The paw diameter of each rat was measured carefully to obtain a baseline reading and so to induce inflammation, 0.1 ml of fresh egg albumin was injected into the sub-plantar region of the right hind paw of each rat. The injection produced a localized inflammatory response characterized by swelling (edema). After the injection of egg albumin, subsequent measurements were taken at intervals of 30, 60, 90, 120, 150, and 180 minutes using a non-elastic thread and a calibrated ruler. The control groups (both negative and positive) and the extract-treated groups were all assessed under identical conditions. The percentage edema inhibitory activity of the extract was calculated as shown below:

% inhibition of edema = $(C_{t-}C_{O)}$ negative control $-(C_t-C_{o)}$

$(C_{t-}C_O)$ negative control

Where C_O = mean paw size in control group

C_t = mean paw size in treatment group

Evaluation of Analgesic Activities of Syzygium aromaticum buds extract

The analgesic activity of the aqueous extract of Syzygium aromaticum was assessed in mice using three standard methods.

Analgesic study with Syzygium aromaticum buds extract on Acetic Acid-Induced Writhing Test

Pain was induced in the experimental animals with intra-peritoneal administration of 0.6% (v/v) acetic acid solution prepared in normal saline, at a dosage of 10 ml per kilogram of body weight. After the injection, the animals were placed in separate transparent observation chambers and monitored for abdominal constrictions (writhing responses). Each writhing movement, defined as a contraction of the abdominal muscles accompanied by an elongation of the body and extension of the hind limbs, was recorded over a fixed observation period of 20 minutes. The degree of analgesic activity of the plant extract was then determined by comparing the mean number of writhes in the treated groups with those in the control group, with a lower writhing count indicating higher analgesic potential. The percentage pain inhibition of each treatment was determined relative to the negative control.

Analgesic study with Syzygium aromaticum buds extract on Hot Plate Pain Reaction Test

Each mouse was individually placed on a hot plate apparatus maintained at a constant temperature of 55 ± 1°C to evaluate the central analgesic activity of the extract. The hot plate test is a well-established method used to evaluate pain response that is primarily mediated through the central nervous system (Eddy and Leimbach, 1953). The reaction time, that is, the interval between the placement of the animal on the heated surface and the first observable response such as paw licking, jumping, or withdrawal of the hind paw, was recorded in seconds. Positive indication of central analgesic activity was interpreted when there was an increase in reaction latency after administration of extract or standard drug.

Analgesic study with Syzygium aromaticum buds extract on Tail Flick Test

In this method, the distal portion of each mouse's tail was gently positioned and exposed to a focused beam of radiant heat to assess the analgesic potential of the test substance. The reaction time which is minutes after the administration of the extract or standard drug is the interval between the onset of the heat stimulus and the animal's first observable response, such as a quick flick or withdrawal of the tail.

Statistical Analysis

All data were expressed as Mean ± Standard Error of Mean (SEM). Statistical comparisons were carried out using One-way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

Results

Acute toxicity test

There was no manifestation of overt toxic effects or death in any of the experimental mice given various oral doses (10, 100, and 1000 mg/kg) at the 1st phase and 1500, 2000, and 3000 mg/kg at the

2nd phase of aqueous S. aromaticum extract, respectively. The extract did not induce acute toxicity or mortality in mice within the 24 h duration of the test (Table 1).

Table 1. Acute toxicity of the aqueous extract of Syzygium aromaticum in mice

Dose ($mgkg^{-1}$)	Group A	Group B	Group C
Phase I			
10	0/3	0/3	0/3
100	0/3	0/3	0/3
1000	0/3	0/3	0/3
Phase II			
1500	0/3	0/3	0/3
2000	0/3	0/3	0/3
3000	0/3	0/3	0/3

Key: 0/3 = Number of animals which died/number of animals used in the experiment.

Anti-inflammatory study with Syzygium aromaticum buds extract on egg albumin-induced paw edema in rats

Peak inflammatory reaction in rats following egg albumin-induced paw edema occurred at 60 minutes. Treatments did not produce visible anti-inflammatory effect in any of the rat groups before 90 minutes.

At 90 minutes post treatment, indomethacin (10 $mgkg^{-1}$) exerted 13.6% inhibition of inflammation relative to 15.5%, 5.2% and 10.3% inhibition with 100, 200 and 400 mg/kg Syzygium aromaticum bud extract respectively.

At 120 minutes post treatment, indomethacin (10 $mgkg^{-1}$) again had 13.6% inhibition of inflammation compared to 17.2%, 8.6% and 17.2% inhibition with 100, 200 and 400 mg/kg S. aromaticum bud extract respectively.

At 150 minutes post treatment, indomethacin (10 $mgkg^{-1}$) produced 6.8% inhibition of inflammation relative to 15.5%, 10.3% and 12.1% inhibition with 100, 200 and 400 mg/kg S. aromaticum bud extract respectively.

At 180 minutes post treatment, indomethacin (10 $mgkg^{-1}$) induced 11.9% inhibition of inflammation compared to 12.1%, 8.6% and 20.7% inhibition with 100, 200 and 400 mg/kg S. aromaticum bud extract respectively (Table 2).

Table 2: Effects of aqueous Syzygium aromaticum buds extract on rat paw circumference following

egg albumin-induced paw edema

	Mean rat pedal edema circumference ± S.E.M. (cm) post treatment (Percentage oedema inhibition in parentheses)						
Time/Min	0	30	60	90	120	150	180
D.H_2O	2.25±0.03	2.60±0.27	2.95±0.81	2.45±0.37 (-)	2.45±0.57 (-)	2.45±0.27 (-)	2.55±0.55 (-)
Indometh (10 $mgkg^{-1}$)	2.45±0.67	2.85±0.31	2.95±0.12	2.55±0.52* (13.6%)	2.55±0.57* (13.6%)	2.75±0.31* (6.8%)	2.60±0.57* (11.9%)
SAE 100 mg/kg (12.1%)	2.35±0.57	2.70±1.20	2.90±0.82	2.45±0.47 (15.5%)	2.40±0.61** (17.2%)	2.45±0.47* (15.5%)	2.55±0.34*
SAE 200 mg/kg	2.40±1.02	2.70±0.27	2.90±0.77	2.75±0.31* (5.2%)	2.65±0.85* (8.6%)	2.60±0.23* (10.3%)	2.65±0.38 (8.6%)
SAE 400mg/kg	2.25±0.91	2.60±0.57	2.90±0.87	2.60±0.27* (10.3%)	2.40±0.84* (17.2%)	2.55±0.23* (12.1%)	2.30±0.81** (20.7%)

SAE = Syzygium aromaticum extract; D. H_2O = distilled water (10 $mlkg^{-1}$); Indometh = indomethacin (10 $mgkg^{-1}$); n=5; *,** = significantly ($p<0.05$, 0.01) reduced compared to values at 60 min on the same row; column with red colored figures = maximal inflammatory response due to edema induction.

Analgesic study with Syzygium aromaticum buds extract on Acetic Acid-induced Writhing Test

The study showed that Syzygium aromaticum extract (SAE) significantly ($p<0.05$) reduced writhing movements in rats in a dose-dependent manner. All doses (100, 200 and 400 mg/kg) of Syzygium aromaticum extract produced significant ($p<0.05$) reductions in mice pain responses. Indomethacin (5 mg/kg), 100, 200 and 400 mg/kg extract induced pain inhibition of 64.2%, 19.4%, 29.9% and 50% respectively. Similarly, distilled water-treated group had writhing responses of 67±7.18 but indomethacin (5 mg/kg), 100, 200 and 400 mg/kg of S. aromaticum bud extract produced significantly ($p<0.05$) reduced writhing reflexes in the values of 24.50 ± 4.5, 54± 8.32, 47± 4.76 and 33.50± 3.68 respectively (Table 3).

Table 3: Analgesic effects of aqueous extract of Syzygium aromaticum buds on mice pain response in

Acetic acid-induced Writhing test

Treatment	No. of writhing reflexes/20minutes	% Pain inhibition
Distilled water (3 ml/kg)	67.00±7.18	-
Indomethacin (5 mg/kg)	24.50±4.25*	64.2
SAE (100 mg/k)	54.00±8.32*	19.4
SAE (200 mg/kg)	47.00±4.76*	29.9
SAE (400 mg/kg)	33.50±3.68*	50.0

*significant ($p<0.05$) reduction compared to distilled water-treated control; SAE=Syzygium aromaticum buds extract.

Analgesic effects of Syzygium aromaticum buds extract on mice pain reaction time in Hotplate test

The results indicated that Syzygium aromaticum extract significantly ($p<0.05$) increased the reaction time of mice in the hot plate test in a dose-dependent manner. The 200 mg/kg and 400 mg/kg doses of the extract produced significantly ($p<0.05$) delayed pain reaction time, with the 400 mg/kg showing the strongest response, although it was still slightly less effective than Indomethacin (Table 4).

Table 4: Analgesic effects of Syzygium aromaticum buds extract on mice in Hotplate pain test

Treatment	Reaction Time in seconds
Distilled water (3 ml/kg)	22.01±2.18
Indomethacin (5 mg/kg)	57.40±1.25*
SAE (100 mg/kg)	31.35±3.32
SAE (200 mg/kg)	35.17±2.76*
SAE (400 mg/kg)	42.70±0.68*

*=significant ($p<0.05$) increase relative to distilled water-treated control; SAE = Syzygium aromaticum buds extract; n = 5.

Analgesic effects of Syzygium aromaticum buds extract on pain with tail flick model

The pain reaction time elicited with distilled water was 22.01±2.18; indomethacin (5 mg/kg) produced a reaction time of 57.40±1.25 while Syzygium aromaticum buds extract increased the tail flick reaction time in a dose-dependent manner, indicating enhanced pain tolerance. The 400 mg/kg dose produced a significant central analgesic effect that was comparable to Indomethacin, while the lower doses of 100 mg/kg and 200 mg/kg were less effective.

Table 5: Analgesic effects of Syzygium aromaticum buds extract on pain with tail flick model

Group	Tail Flick in seconds
Distilled water (3 ml/kg)	17.22±0.18
Indo (5 mg/kg)	31.30±1.25*
SAE (100 mg/kg)	19.35±2.32
SAE (200 mg/kg)	21.20±2.12
SAE (400 mg/kg)	29.70±0.68*

*Asterisk values indicates significant ($p<0.05$)

increase compared to distilled water (control); SAE = Syzygium aromaticum extract.

Discussion

Acute toxicity study revealed that Syzygium aromaticum buds extract was tolerated by experimental rats up to a maximum test dose of 3000 mg/kg; there was no visible toxic manifestations or death within 24 hour study period. The findings of this study clearly demonstrated that the aqueous extract of Syzygium aromaticum possessed significant anti-inflammatory and analgesic activities in experimental models used. Some of the models often employed in assessing analgesic efficacy of plant extracts include carrageenan-induced paw edema in rats, formalin-induced pain in mice, hot plate and tail flick tests (Vinegar et al., 1969). These models employed gives us an insight into the possible mechanisms of action, and also evidence of efficacy which supports the integration of medicinal plants into mainstream pharmacology.

The extract showed a dose-dependent inhibition of paw edema in the egg albumin-induced inflammation model and a reduction in pain responses in the acetic acid-induced writhing, hot plate, and tail flick tests. These results confirm the traditional use of cloves as a remedy for pain and inflammation and are in agreement with previous scientific reports.

The anti-inflammatory effect observed may be attributed mainly to the presence of bioactive compounds such as eugenol, eugenyl acetate, and β-caryophyllene, which have been reported to inhibit pro-inflammatory mediators like prostaglandins, histamine, and bradykinin (Hassan et al., 2017). Anti-inflammatory agents, whether synthetic or natural, typically act by blocking the synthesis or activity of these mediators such as; prostaglandins, histamine, cytokines, and leukotrienes (Kumar et al., 2010).

The significant reduction in paw edema at lower doses (100 mg/kg) suggests that the extract might have interfered with the synthesis or action of inflammatory mediators. This agrees with the report of Mittal et al. (2014), who demonstrated that eugenol suppresses cyclooxygenase (COX) enzyme activity and down-regulates tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and nitric oxide production. Therefore, the anti-inflammatory mechanism of Syzygium aromaticum could be through inhibition of COX enzymes and modulation of cytokine release.

Similarly, the analgesic activity observed in the acetic acid-induced writhing, hot plate, and tail flick tests indicates that the extract acted through both peripheral and central mechanisms. The acetic acid-induced writhing test reflects peripheral pain mediated by inflammatory prostaglandins and bradykinin. The marked reduction in writhing at 200 mg/kg and 400 mg/kg doses suggests that the extract suppresses peripheral pain transmission. On the other hand, the prolonged reaction time in the hot plate and tail flick tests shows that Syzygium aromaticum also exerts central analgesic effects, possibly through modulation of central pain receptors or neurotransmitters in the spinal cord and brain (Ali et al., 2019).

The presence of eugenol, a phenolic compound known to possess local anesthetic and analgesic properties, may explain the central activity observed in this study. Eugenol which is the major active compound in clove inhibit prostaglandin synthesis, modulate cytokines, and block pain conduction (Chaieb et al., 2007; Gulcin et al., 2012) and animal studies further validate these analgesic and anti-inflammatory effects (Cortés-Rojas et al., 2014). This compound eugenol has been shown to block voltage-gated sodium and calcium channels, thereby inhibiting neuronal excitability and transmission of pain signals (Chaieb et al., 2007). The dual action observed both central and peripheral make Syzygium aromaticum an interesting candidate for further pharmacological development.

The overall effect of the extract was comparable to the standard drug indomethacin, especially at higher doses. This suggests that the aqueous extract of Syzygium aromaticum could serve as a safer natural alternative or complement to

synthetic drugs, particularly in the management of inflammatory and painful conditions where long-term therapy is required.

However, while the findings are promising, further studies are necessary to isolate and characterize the specific active compounds responsible for these effects and to assess possible toxicity during prolonged administration. The results of this study support the growing body of evidence advocating for the use of Syzygium aromaticum in traditional and modern medicine as an effective natural anti-inflammatory and analgesic agent.

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