Evaluation of Anti-inflammatory and Analgesic Effects of Aqueous Extract of Syzygium aromaticum (L.) Merr. and L.M. Perry. Using Four Different Models

Oladele G.M[1]., Isimah P.E[1]., Tetteh M.B[1]. and Oladele G.O[2].

[1]Department of Veterinary Pharmacology and Toxicology, University of Abuja, Nigeria

[2]Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria

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ABSTRACT

The synthetic drugs in use for the treatment of inflammation and pains often comes with side effects and high costs, this has led to the 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. Three different models; Acetic acid-induced writhing test, Hot plate reaction test, and Tail flick test were used to evaluate the analgesic effects where 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 in which 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 confirms the traditional used 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 comes with some adverse effect (Vane and Botting 1998.) while some are very costly especially for the rural dwellers, which therefore prompts 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 is 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,

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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 includes 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, mixes clove oil with honey or ghee which is used for the relieve of earaches, sore throats, and inflamed swellings (Sofowora, 1993). This study evaluates the anti-inflammatory and analgesic effects of the crude aqueous extract of this plant using different models and the three models were used for analgesic action 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 1315ml of distilled water was added to the beaker. The mixture was macerated for 72 hrs 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 are 25 adult male and female albino Wistar rats weighing between 90 g and 130 g and 25 albino male and female mice weighing between 40g to 90 g. the rats and mice were obtained from the animal house at the Faculty of Veterinary Medicine, University of Abuja, where they were housed in metal cages and maintained 12 h light and 12 h dark 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 foe easy identification.

Experimental procedures

Administration of aqueous extract of Syzygium aromaticum to the albino Wistar rats

Fresh 10% (w/v) solution of the extract was prepared with distilled water to produce appropriate dosages required for each group. The various groups were given different doses of the extract per os using oral canula. Group A was given 100 mg/kg body weight of the extract, Group B was given 200 mg/kg body weight of the extract while Group C was given 400 mg/kg body weight of the extract, Group D was the negative control group and they were given 3 ml/kg of distilled water, Group E was the positive control and they were given 10 mg/kg of Indomethacin. All these administrations were done orally for 28 days and weight of the rats were taken every week for four weeks.

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Evaluation of Anti-inflammatory Activity

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 inhibition of paw edema was calculated in relation to the control group using the formula: (Vc - Vt)/Vc × 100. Where, Vc is the Mean percentage increase in paw volume in control rats and Vt is Percentage increase in paw volume in each treated rat (Abatan and Adeagbo, 1986; Oladele et al,. 2009).

Evaluation of Analgesic Activity

The analgesic activity of the aqueous extract of Syzygium aromaticum was assessed in mice using three standard methods such as follows;

Acetic Acid-Induced Writhing Test

To induce pain in the experimental animals, an intraperitoneal administration of 0.6% (v/v) acetic acid solution prepared in normal saw line, at a dosage of 10 ml per kilogram of body weight was carried out. 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.

Hot Plate 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.

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 $p < 0.05$.

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Results

Anti-inflammatory study

Table 1**:** The mean ± SEM values of circumference of the rat paws administered with crude aqueous extract of Syzygium aromaticum, indomethacin and distilled water.

Time/Min	0	30	60	90	120	150	180
Dist	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
Indo	2.45±0.67	2.85±0.31	2.95±0.12	2.55±0.52	2.55±0.57	2.75±0.31	2.60±0.57
SAE 100mg/kg	2.35±0.57	2.70±1.20	2.90±0.82	2.45±0.47	2.40±0.61	2.45±0.47	2.55±0.34
SAE 200mg/kg	2.40±1.02	2.70±0.27	2.90±0.77	2.75±0.31	2.65±0.85	2.60±0.23	2.65±0.38
SAE 400mg/kg	2.25±0.91	2.60±0.57	2.90±0.87	2.60±0.27	2.40±0.84	2.55±0.23	2.30±0.81

SAE is Syzygium aromaticum extract, Dist is distilled water at 3ml/kg and Indo is indomethacin at 10mg/kg

The study shows that the aqueous extract of Syzygium aromaticum (SAE) effectively reduced inflammation in the paws of rats in a dose-dependent manner. The 400 mg/kg extract group exhibited the most significant anti-inflammatory effect, comparable to those produced by the standard drug Indomethacin.

Table 2: The mean ± SEM values of the percentage inhibition of increase in paw volume of the rats administered with crude aqueous extract of Syzygium aromaticum, indomethacin and distilled water.

Time/Min	30	60	90	120	150	180
Dist	15.56±2.03	31.11±2.32	8.89±1.25	8.89±1.54	8.89±2.21	13.33±1.76
Indo	4.95±0.37	34.39±3.76*	54.55±4.73*	54.55±2.76*	-37.68±3.87	54.00±4.64*
SAE 100mg/kg	4.24±0.34	24.78±2.17	52.08±3.57*	56.04±4.78*	52.08±4.76*	36.16±3.87*
SAE 200mg/kg	19.67±3.70	33.04±4.12*	64.00± 3.42*	7.21±1.23	6.30±0.67	21.83±3.41
SAE 400mg/kg	0.06±0.02	7.20±1.24	5.03±5.79	24.97±2.43	4.94±4.32	52.97±4.78*

*Asterisk values indicates significant difference at $p<0.05$ between the treated and the control groups.

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SAE is Syzygium aromaticum extract, Indo is indomethacin at 10 mg/kg and Dist is distilled water at 3ml/kg. % IPV is the percentage increase in paw volume while % IIPV is the percentage inhibition of increase in paw volume of the rats.

The results shows that Syzygium aromaticum extract (SAE) significantly inhibited paw edema at specific doses and time points. The 100 mg/kg dose of SAE produced a steady inhibition of inflammation throughout the observation period, while the 400 mg/kg dose exhibited a delayed but strong inhibitory effect, which became comparable to that of Indomethacin at 180 minutes. These findings suggest that Syzygium aromaticum extract contains bioactive compounds capable of reducing inflammation, although the response varies with dosage, indicating a dose-dependent pattern of activity.

Analgesic study

Table 3: The mean ± SEM writhing movement of the mice administered with crude aqueous extract of Syzygium aromaticum, indomethacin and distilled water as control.

Treatment	No. of writhing movement/20minutes
Dist water (3 ml/kg)	67.00±7.18
Indo (5 mg/kg)	24.50±4.25*
SAE (100 mg/k)	54.00±8.32
SAE (200 mg/kg)	47.00±4.76*
SAE (400 mg/kg)	33.50±3.68*

*Asterisk values indicates significant difference at $p<0.05$ between the treated and the control groups.

SAE is Syzygium aromaticum extract, Dist is distilled water, Indo is indomethacin.

The study showed that Syzygium aromaticum extract (SAE) significantly reduced writhing movements in rats in a dose-dependent manner. Both the 200 mg/kg and 400 mg/kg doses produced statistically significant reductions in pain responses ($p<0.05$). Notably, the 400 mg/kg dose was almost as effective as Indomethacin, suggesting a strong analgesic potential for the extract.

Analgesic study using reaction time on hotplate

Table 4: The mean ± SEM values of the reaction time of the mice administered with crude aqueous extract of Syzygium aromaticum, indomethacin and distilled water as control on hot plate maintained at 55^0C.

Treatment	Reaction Time in seconds
Dist water (3 ml/kg)	22.01±2.18
Indo (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*

*Asterisk values indicates significant difference at $p<0.05$ between the treated and the control groups.

SAE is Syzygium aromaticum extract, Dist is distilled water, Indo is indomethacin.

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The results indicated that Syzygium aromaticum extract (SAE) significantly 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 produced significant effects ($p<0.05$), with the 400 mg/kg dose showing the strongest response, although it was still slightly less effective than Indomethacin.

Analgesic effects using tail flick model

Table 5: The mean ± SEM values of the Tail flick (reaction time) of the mice administered with crude aqueous extract of Syzygium aromaticum, indomethacin and distilled water as control in water maintained at 55^0C.

Group	Tail Flick in seconds
Dist 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*

29.70±0.68*

*Asterisk values indicates significant difference at $p<0.05$ between the treated and the control groups.

SAE is Syzygium aromaticum extract, Dist is distilled water, Indo is indomethacin.

The study revealed that Syzygium aromaticum extract (SAE) 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.

DISCUSSION

The findings of this study clearly demonstrated that the aqueous extract of Syzygium aromaticum possesses significant anti-inflammatory and analgesic activities in experimental models using Wistar rats. Some of the models employed in assessing the efficacy of these plant extracts, include; carrageenan-induced paw edema in rats, formalin-induced pain in mice, and hot plate or 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 effectively interferes 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 downregulates 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 acts 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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