Evaluation of Anti-inflammatory and Analgesic Effects of *Allophylusspicatus* Leaf Extract inMice

Original Research Article

ABSTRACT

This study examined the analgesic and anti-inflammatory activity of an ethanolic extract of *Allophylusspicatus* leaves in albino mice, taking into account the socioeconomic impacts of pain and inflammation as well as the need to search for effective antipain and anti-inflammatory drugs with minimal side effects from traditional medicinal plants. The acetic acid writhing test and the hot plate method were used to assess the peripheral and central analgesic activity, while carrageenan-induced paw oedema and egg white-induced hind paw oedema were used to assess the anti-inflammatory activity. *A. spicatus*extract significantly ($p<0.05$) reduced discomfort in the acetic acid writhing test when 1% acetic acid (10ml/kg) was used to induce abdominal contractions.Similarly, in comparison to the control, the extract demonstrated noteworthy ($p<0.05$) analgesic effects in the hot plate method. Mice given different doses of the extract as well as those given indomethacin showed a substantial increase in the latency to pain response after 30 minutes when compared to the control group. In every time period tested, animals given 500 mg/kg of the extract showed the strongest analgesic effects. Gradual dosages of *A. spicatus*leaf extract and 10 mg/kg of standard Diclofenac sodium were administered after 0.1% carrageenan induced oedema, and after 120 and 180 minutes, respectively, the paw edema was significantly ($p<0.05$) reduced in control as equated.In a similar vein, graded dosages of *A. spicatus* leaf extract and conventional Diclofenac sodium dosed at 10mg/kg valuably decreases level of paw edema in the egg white-induced hind paw edema test as compared to the control. According to the study, ethanolicextract from *A. spicatus* leaves can effectively treat both centrally and peripherally caused pain. These findings validate reports of the plant's traditional usage in Nigerian communities for the treatment of inflammatory and analgesic ailments.

KEYWORDS: Pain, *Allophylusspicatus*, oedema, inflammation

1. INTRODUCTION

One of the most frequent concerns brought to a doctor is pain. Pain is defined as an unpleasant sensory-emotional experience connected to existing or potential tissue damage by the International Association for the Study of Pain (IASP) [1]. To put it simply, pain is an unpleasant sensation that is frequently brought on by strong or harmful stimuli, like stubbing a

toe, burning a finger, or applying alcohol to a cut [2]. However, as a defensive reaction involving immune cells, blood vessels, and chemical mediators, inflammation is a part of the complicated biological response of bodily tissues to harmful stimuli, such as infections, damaged cells, or irritants [3].As an all-encompassing reaction, inflammation is classified as an innate immunity mechanism, in contrast to adaptive immunity, which is thought to be pathogen-specific [4-5].

Any medication in the class used to provide analgesia, or pain relief, is referred to as an analgesic or painkiller. The central and peripheral nerve systems are affected in different ways by analgesic medications. Paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs) like salicylates, and opioid medications like morphine and oxycodone are examples of analgesics [6]. Due to their proven effectiveness in lowering pain and inflammation, non-steroidal anti-inflammatory medicines (NSAIDs) are among the most often prescribed drugs worldwide [7].

The primary mechanism of action of nonsteroidal anti-inflammatory drugs (NSAIDs) involves inhibiting the pro-inflammatory enzyme cyclooxygenase (COX). COX enzymes, comprising two isozymes, COX-1 and COX-2, convert arachidonic acid into prostaglandins, which are involved in the intricate process of inflammation and also cause pain [8]. Hence, inhibiting prostaglandin synthesis leads to a reduction in inflammation in inflamed tissues. Conventional nonselective NSAIDs that nonspecifically inhibit COX-1 are effective in reducing pain and inflammation, but have a considerable risk of serious gastrointestinal adverse events with long-term use [9].In order to address this issue, certain COX-2 isoenzyme inhibitors were created. But serious worries about COX-2 drugs' possible cardiovascular toxicity have lately surfaced, which has resulted in limitations on usage and the removal of some COX-2 inhibitors from the market [10-11]. Adverse musculoskeletal, ophthalmologic, cardiovascular, and endocrine disorders have also

been demonstrated to be brought on by long-term use of steroidal anti-inflammatory medicines [12].

It is consequently impossible to overstate the need for treatment medicines that are both effective and seldom cause side effects. Analgesic and anti-inflammatory effects, as well as antioxidant qualities, have been reported for secondary metabolites present in medicinal plants [13-19]. Researchers from all over the world have been drawn to this area in an effort to find plant-based antioxidant and anti-inflammatory medicines [20-23].According to Ahmad et al. [24], Furhatun-Noor et al. [20], Orororo et al. [25], and other studies, natural products are safe, effective, economical, biocompatible, and offer suitable options in the treatment of illnesses with negligible or no adverse effects.

A significant genus in the Sapindaceae family is *Allophylus*. There are over 255 recognized species in the genus *Allophylus*, many of which are known to have beneficial properties that could lead to the creation of novel herbal-based medications and products [26]. The majority of these species are native to Asia, although several are also found in Africa (Nigeria, Ghana, and the Ivory Coast), South America, and other tropical and warm regions. People utilize the leaves and roots of *A.spicatus* to treat wounds, cuts, stomach gas, ulcers, and bone fractures [27-28].There is little evidence that the leaves of *A.spicatus* have analgesic or anti-inflammatory properties, despite the fact that species of *Allophylus* have been shown to have antidiabetic, antihypertensive, antimalarial, anticancer, analgesic, and anti-inflammatory potentials [15, 29-30]. For these reasons, this study was conducted to examine the analgesic and anti-inflammatory properties *of A. spicatus* leaf extract (ASE) in albino mice.

2. MATERIALS AND METHODS

2.1 Chemicals

Analytical grade regents and chemicals were used in this study. Sodium dihydrogen phosphate and Disodium hydrogen phosphate used were products of GuanghuaSci-tech Co. Ltd, Shantou, Guangdong, China)**,** while Sodium chloridewasobtainedfromLobaChemie Mumbai, India.

2.2 Plant Material

In the Abraka community of the Ethiope East LGA of Delta State, Nigeria, fresh leaves of *A.spicatus* were collected from a widely growing habitat. The leaves were processed and taken for verification at Delta State University's Department of Pharmacy and Traditional Medicine in Abraka with voucher number DEL/PTM022/044. The plant leaves were then taken to the Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Delta State University, Abraka, for the extraction procedure.

2.3 Preparation of the Plant Extract

The Adebayo et al. [30] method was used for the plant's crude extraction: fresh leaves of *A.spicatus* were collected, cleaned, and air-dried at room temperature. The leaves were then cut into pieces and ground using a blending machine. A 400g plant powder was measured and soaked in 400ml of ethanol in an airtight conical flask with daily shaking at room temperature for 72 hours. The filtrate was collected into airtight bottles, and the ethanol was extracted under reduced pressure using a rotary evaporator at low room temperature to obtain a viscous extract, which was kept in the refrigerator at 4°C until utilized.

2.4 Animals

The study employed adult albino mice weighing between 20 and 30 grams; the mice were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Basic Medical Science, Delta State University, Abraka. Prior to the start of the experiment, the animals were given a two-week period to acclimate, and they were fed a standard laboratory diet (growers mash) and unrestricted access to water.

2.5 Experimental Design

The mice were divided into six groups as shown in the Table below:

Table 1: Experimental Design

Groups	Description	Treatment
A	Positive Control group	Normal Untreated rats
B	Standard Drug	10 mg/kg of standard drug
C	Low does plant extract	Received *ASE 100mg/kg*
D	Medium dose plant extract	Received *ASE 250mg/kg*
E	High dose plant extract	Received *ASE 500mg/kg*

2.6 METHODS

2.6.1 TESTS FOR PAIN EVALUATION

2.6.1.1 WRITHING IN MICE VIA INDUCTION OF ACETIC ACID

This was completed using Raja et al. [31]'s methodology. Thirty mice were split into five groups of six animals each at random (n = 6). Normal saline (10 ml/kg p.o.) was given to group 1, indomethacin (10 mg/kg body weight) was given to group 2, and *A.spicatus* extract (10, 250, and 500 mg/kg p.o.) was given to groups 3, 4, and 5. The animals were given 10 milliliters per kilogram of body weight of 0.6% v/v acetic acid in water one hour after the medication and vehicle administration to induce pain perception. For a 10-minute observation period, the number of times each animal's abdomen writhed was counted five minutes following the acetic acid injection.:Reduction of writhing was calculated in terms of percentage using:

Mean no. of writhes (control) – Mean no. of writhes (Test) / Mean no. of writhes (control) X 100= Reduction (%). In comparison to control, a reduction in the total amount of writhes is suggestive of an analgesic effect.

2.6.1.2 HOT PLATE TEST

For this test, Williamson et al.'s [32] methodology was used. The mice were initially subjected to a nociceptive pain stimuli created by electrical heat. Animals with reduced nociception were removed from the experiment if they remained on the hot plate for longer than 15 seconds. Five groups of six mice each were randomly selected from among the mice (n = 6). Groups 2, 3, 4, and 5 were given the usual medication indomethacin (10 mg/kg body weight) and extract at doses of 100, 250, and 500 mg/kg body weight, respectively. The normal control is the group 1 treated with (10 ml/kg). After thirty minutes of treatment, each pretreatment animal was housed separately by mounting on hot plate through the medium of a beaker that was kept in a consistent 54 $\pm 1^0$C temperature. The latency to pain response was determined by timing the animals' paw licking or leaping sessions. The control group's latency response was measured at 30 seconds, and it was repeated after intervals of three consecutive 30minutes' different administrations.

2.7 EVALUATION OF ANTI-INFLAMMATORY ACTIVITIES

2.7.1 INDUCTION OF PAW OEDEMA THROUGH CARRAGEENAN

Thirty adult rats were used in this experiment; each group consisted of six animals (n = 5). Groups 2, 3, 4, and 5 were given oral diclofenac sodium (10 mg/kg) as a positive control, and

100 mg/kg, 250 mg/kg, and 500 mg/kg body weight of plant extract, respectively, as the negative control. Group 1 was given normal saline (10 ml/kg). All animals in each group had 0.1 mL of freshly made, sterile, saline carrageenan suspension (1% w/v in 0.9% normal saline) injected into the subplantar surface of their left hind paw thirty minutes after the medication was administered. A vernier caliper was used to assess the size of the hind paw (oedema) starting from origin (0) hour and at one-hour progressive 5 consecutive timingdue to carraggenanadministered [34]. The following formula was used to determine the percentage of inhibition of oedema: average rise in control paw size - average rise in treated paw size/average rise in control paw size X 100.

2.7.2 EGG WHITE INDUCED HIND PAW EDEMA.

The rats were divided into five groups: Group A received 0.1% eggwhite induction, while Group B received 10 mg/kg of diclofenac sodium-treated rats and 0.1% eggwhite induction), Group C received ASE 100 mg/kg and 0.1% eggwhite induction, Group D received ASE 250 mg/kg and 0.1% eggwhite induction, and Group E received ASE 500 mg/kg and 0.1% eggwhite induction. One hour before the research began, all of the medications and the vehicle were administered.A volume of 0.1 milliliters of freshly extracted egg white was introduced into the rat's left hindpaw'ssubplantar tissue. A plethysmometer was used to measure the injected paws' volumes at 0, 60, 120, 180, and 240 minutes. The study examined the inhibitory effects of the medicines and compared the percentage rise in paw oedema between the treatment and control groups [15].

2.8 STATISTICAL CALCULATION

Findings was subjected to analysis of variance of one-way (ANOVA) and evaluated as Mean ± Standard Error of Mean (SEM). Using the Graph Pad software, control group was equated to other groups' outcome, expressing$P< 0.05$ being deemed significant in biostatistics.

3. RESULTS

3.1 ANALGESIC STUDIES

3.1.1 ACETIC ACID WRITHING RESPONSE IN MICE

Outcome of the induced of acetic acid writhing test of the ethanolic extract of *A. spicatus* are displayed on Table 2. The outcome displays a value ($p<0.05$) deduction of the frequency of writhes by mice control group comparison.

Abdominal contractionswere highly raised after 0.1% Acetic acid administered writhing effect (licking of abdomen), however, the given measured doses of*A. spicatus* extract andIndomethacin (10 mg/kg) as standard significantly lower the writhing effect in 60 mins, 90 mins and 180 mins respectively, with outcome showing that the extract could be analgesic at increased dose level.

Table 2: *E*ffect of *A. spicatus* extract on Acetic acid induced writhing response in albino mice

Groups	0min	30min	60min	90min	120min
Control	28.00±1.68^{a}	27.00±2.64^{a}	23.50±1.44^{a}	20.25±2.25^{a}	22.00±4.49^{a}
Standard **Indomethacin 10 mg/kg**	25.75±3.75^{b}	20.75±1.43^{b}	15.25±.1.85^{b}	11.75±0.47^{b}	7.75±0.47^{b}
ASE 100mg/kg	25.75±2.80^{b}	20.75±1.65^{b}	17.00±1.08^{c}	11.75±1.31^{b}	9.25±1.49^{c}
ASE 250mg/kg	22.00±2.94^{c}	19.50±.6.40^{c}	12.00±1.08^{d}	9.25±0.85^{c}	6.00±1.08^{d}
ASE 500mg/kg	25.50±2.75^{b}	23.00±1.47^{d}	18.00±2.85^{c}	10.00±0.40^{d}	8.00±1.47^{e}

Results are shown as Mean ± SD (n=6). outcome on the same column with different superscripts distinct significantly ($p>0.05$). GROUP A = mice administered only induced with 0.1% Acetic acid, GROUP B = 10 mg/kg of Indomethacin treated rats and induced with 0.1% Acetic acid. GROUP C = Received *ASE 100mg/kg* and induced with 0.1% Acetic acid, GROUP D = Received *ASE 250mg/kg* and induced with 0.1% Acetic acid, GROUP E = Received *ASE 500mg/kg* and induced with 0.1% Acetic acid

3.1.2 HOT PLATE TEST

The result of the hot plate induced pain analysingethanolic extract of *A. spicatus* as seen in Table 3.

Latency to pain response was significantly increased after 30 minutes in mice administered various doses of the extract and indomethacin compared to the control. The highest analgesic effects was observed in mice administered 500mg/kg of the extract in all the time frames examined,

Table 3: The Impact of*A.spicatus* extract on Hot plate targeted pain in albino mice

Groups	0min	30min	60min	90min	120min
Control	30.00 ± 2.12^{a}	33.75 ± 0.75^{a}	35.75 ± 1.62^{a}	32.50 ± 2.02^{a}	27.50 ± 2.02^{a}
Standard **Indomethacin 10 mg/kg**	24.00 ± 2.41^{b}	54.50 ± 2.02^{b}	73.00 ± 3.29^{b}	91.50 ± 2.90^{b}	89.50 ± 3.92^{b}
ASE 100mg/kg	30.25 ± 4.92^{a}	50.25 ± 3.98^{c}	68.75 ± 5.46^{c}	74.25 ± 6.42^{c}	77.25 ± 1.93^{c}
ASE 250mg/kg	33.25 ± 2.28^{a}	48.75 ± 6.60^{d}	72.00 ± 3.55^{b}	90.00 ± 2.48^{b}	118.00 ± 5.95^{d}
ASE 500mg/kg	25.50 ± 5.63^{b}	62.75 ± 4.80^{e}	101.25 ± 8.64^{d}	145.25 ± 14.39^{d}	205.00 ± 7.66^{e}

results are shown as Mean ± SD (n=6). Values on the same column with different superscripts differ significantly ($p>0.05$). GROUP A = hot plate induced pain in normal rats, GROUP B = 10 mg/kg of Indomethacin treated rats in respect of hot plate induced pain. GROUP C = Received *ASE 100mg/kg* coupled with hot plate induced pain, GROUP D = Received *ASE 250mg/kg* and induced pain by hot plate, GROUP E = Received *ASE 500mg/kg* and hot plate induced pain.

3.2 ANTI-INFLAMMATORY STUDIES

3.2.1 CARRAGEENAN INDUCED HIND PAW OEDEMA

The effect of *A. spicatus* extract on carrageenan induced oedema in Albino mice is shown in Table 4. Following 0.1% carrageenan induced oedema, treatment of graded doses of the *ASE* extract and 10 mg/kg of Diclofenac sodium as standard significantly reduced the paw edema later at 120 min and 180 mins respectively compared to the control.

Table 4: The effect of*A. spicatus* extract on carrageenan induced oedema in Albino mice

Groups	0min	60min	120min	180min
Control	2.27±0.05[a]	3.37±.07[a]	3.58±0.06[a]	3.58±0.06[a]
Standard **diclofenac 10 mg/kg**	2.28±0.04[a]	2.77±.05[b]	2.63±0.13[b]	2.63±0.13[b]
ASE 100mg/kg	2.28±0.03[a]	2.98±.13[c]	2.78±0.09[c]	2.78±0.09[c]
ASE 250mg/kg	2.30±0.04[a]	2.87±.05[d]	2.68±0.05[b]	2.58±0.05[d]
ASE 500mg/kg	2.23±0.02[a]	2.78±.05[b]	2.48±0.03[d]	2.30±0.04[e]

Outcome shown as Mean ± SD (n=6). Values on the same column with different superscripts differ significantly (p>0.05). Group A = Untreated rats but induced with 0.1% carrageenan, Group B = 10 mg/kg of diclofenac sodium administered rats and subjected to 0.1% carrageenan. Group C = Received *ASE 100mg/kg* and induced with 0.1% carrageenan, Group D = Received *ASE 250mg/kg* and induced with 0.1% carrageenan, Group E = Given*ASE 500mg/kg* and induced with 0.1% carrageenan.

3.2.2 EGG WHITE INDUCED HIND PAW EDEMA

The effect of *A. spicatus* extract on Egg white induced hind paw edema in Albino miceis represented in Table 5. The output shows significant increase with the rats paw after 0.1% Egg white induced oedema, however, the administration of graded doses of the extract of *A. spicatus* and 10 mg/kg of Diclofenac sodium as standard significantly reduced the paw edema after 180 min and 240 mins respectively.

Table 5: The effect of*A. spicatus* extract on Egg white induced hind paw edema in Albino mice

Group	Initial	T_0	T_1	T_2	T_3	T_4
Negative control	2.51±0.08[a]	3.21±0.08[a]	3.72±0.13[a]	3.75±0.10[a]	3.52±0.13[a]	3.33±0.14[a]
***Standard* diclofenac 10 mg/kg**	2.51±0.04[a]	3.13±0.09[a]	3.46±0.05[b]	3.40±0.12[b]	3.08±0.07[b]	2.95±0.06[b]
ASE 100mg/kg	2.47±0.08[a]	3.07±0.08[a]	3.82±0.08[c]	3.38±0.19[c]	3.31±0.19[c]	2.75±0.16[c]
ASE 250mg/kg	2.66±0.09[b]	3.21±0.13[a]	3.45±0.08[b]	3.18±0.03[d]	3.13±0.03[b]	3.01±0.10[d]
ASE 500mg/kg	2.61±0.08[b]	3.46±0.19[b]	3.66±0.11[d]	3.11±0.01[d]	3.07±0.03[b]	2.91±0.04[b]

Figuresare shown as Mean ± SD (n=6). Values on the same column with different superscripts differ significantly (p>0.05). Group A = Physiologically Untreated rats but induced with 0.1% eggwhite, Group B = 10 mg/kg of diclofenac sodium administered mice and induced with 0.1% eggwhite. Group C = Given*ASE 100mg/kg* and induced with 0.1% eggwhite, Group D = Given*ASE 250mg/kg* and induced with 0.1% eggwhite, Group E = Administered*ASE 500mg/kg* and induced with 0.1% eggwhite.

4. DISCUSSION

Finding efficient antipain and anti-inflammatory medications with few side effects from traditional medicinal plants makes sense, especially in light of the economic and social impacts of pain and inflammation as well as our understanding of potential herbal medicines from traditionally claimed plants. Therefore, using albino mice, this study examined the analgesic and anti-inflammatory properties of an ethanolic extract of *A.spicatus* leaves. The extracts prevented the mice's writhing reflex in the acetic acid writhing test. Plants are tested for peripheral analgesic efficacy using this method. Acetic acid produced pain because it stimulated pain nerve terminals by releasing endogenous substances such prostaglandins [31].According to Tesfaye et al. [1], the release of prostaglandin E2 (PGE2) causes a chemical reaction at the nerve terminals, which then transmits the pain signal to the brain via the neurological system.

The technique, also known as the abdomen tighter reaction, is very porous and can identify pain relief of substance at dose levels where other techniques, such as the tail-flick test, may not be able to detect them. It is hypothesized that local peritoneal receptors and prostaglandins' rise in

peritoneal fluid contribute to the abdominal constriction reactions. It is possible that the phytochemical contains capacity to reduce the moderate count of abdominal writhes results from its interaction with acetic acid-sensitive visceral receptors [34-35]. The plant extract's peripheral analgesic action might be mediated by cyclo-oxygenase inhibition.This hypothesis aligns with the findings of Williamson et al. [32], who proposed that the acetic acid-induced writhing method is a valuable technique for assessing peripherally-acting analgesic drugs. These proposed mechanisms align with the theoretical framework that states that any substance that reduces the number of writhing will exhibit pain relief by blocking the synthesis and excitement of Prostagladins and by blocking the transmission of pain to the periphery [35].

The current study's findings are consistent with those of Hossain et al. [34] and Furhatun-Noor et al. [20], who looked at the ethanolic leaf extracts of *Phyllanthusacidus* L's and *Cynodondactylon*potential as analgesics and anti-inflammatory agents, respectively. A rise in concentration of phytochemicals that exhibit analgesic activity at the highest dose may be the cause of the extract's increasing analgesic effect with higher doses.

Hot plate testing was the second model employed, and it was in this model that central antinociceptive mechanism and supra-spinal nociception were identified [35]. Upon exposing the mice to a continuously heated plate and monitoring their reaction times (jumping, pulling away, and licking their paws), the central antinociceptive mechanisms of the extract were identified. This was because mice's paws are extremely sensitive to heat at temperatures that do not cause skin damage. The treatment with measured doses of *A.spicatus* extract and 10 mg/kg of standard Indomethacin significantly increased mice's latency time at 60, 90, and 120 minutes, according to the results of the hot plate test. This suggests that the mice's high doses of the drug have a promising analgesic potency. Drugs that work well in this model also have a central

analgesic effect. The hot plate test model is used to assess a drug's effect on central pain. According to Tesfaye et al. [1], pain brought on by the hot plate's thermal stimulus is unique to centrally mediated nociception.According to Tamrat et al. [36] and Demsie et al. [37], the extract's capacity to extend the thermally generated pain reaction time in mice suggests that it has central analgesic effect.

When compared to the control group, which shown a progressive rise in the rats' paw circumference, the inflammation inhibition activity results demonstrated that *A. spicatus* exhibited considerable anti-inflammatory action on carrageenan-activated inflammation. In addition, *A. spicatus* significantly reduced eggwhite-induced inflammation in comparison to the control group, which demonstrated a gradual rise in the rats' paw circumference. According to Adebayo et al. [28], this indicates that the ethanolic extract of A. spicatus has an anti-inflammatory action and could be helpful in the treatment of inflammatory pain.This is most likely caused by the cyclooxygenase (COX) route of arachidonate metabolism, which results in prostaglandins, which affect blood vessels, nerve terminals, and inflammatory cells in different ways. According to Tesfaye et al. (2020), the extracts' anti-inflammatory action was most likely caused by preventing the release of inflammatory mediators including prostaglandins and polypeptide kinins from being synthesized. According to Çadirc et al. [38], the extract may also have anti-proliferative properties against fibroblasts, limit neutrophil and macrophage infiltrations, and impede leucocyte and phagocyte migration into the inflamed area.Previous reports published in scientific journals indicate that plants primarily containing alkaloids, flavonoids, saponins, tannins, phenolic compounds, glycosides, coumarins, and triterpenoid chemical constituents have strong anti-inflammatory properties [1, 39-40].These findings lend support to the anti-inflammatory action of *A. spicatus* extract in the current study

5. CONCLUSION

According to the research, *A. spicatus* leaf extract may have analgesic and anti-inflammatory properties. The findings validate the traditional medical practitioners' folklore usage of *A. spicatus* to treat a variety of aches and inflammatory disorders, including fever, rheumatism, and arthritis. However, more research is required to determine the likely mode of action for the analgesic and anti-inflammatory properties as well as to identify the bioactive components of *A. spicatu*s that are accountable for the effects. It is strongly advised to identify, measure, and isolate the active compound(s) in order to standardize and assess the level of the plant's anti-inflammatory action.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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