***Original Research Article***

***Parinari curatellifolia, biosafety and pharmacological efficacy against biological end points of pneumonia***

**Abstract**

*Parinari curatellifolia (P. curatellifolia*) of the *Chrysobalanaceae* family is a widely utilized medicinal plant in Zimbabwe. The major traditional applications include the management of key biological end points of pneumonia, a critical global health concern particularly in the sub-Saharan Africa and south Asia regions where it contributes to over 14% of under-five deaths. Pneumonia is characterized by inflammatory biomarkers such as elevated procalcitonin and C-reactive protein. Given the limitations and challenges associated with current paediatric pneumonia treatments, there is an urgent need for safe, effective, and affordable alternatives capable of modulating the disease’s biological endpoints. This study investigated the phytoconstituent profile and the anti-inflammatory and antibacterial activities of *P. curatellifolia*. Various wet chemical techniques were employed to screen for the pharmacologically active secondary metabolites; the anti-inflammatory activity was determined through the egg albumin assay using diclofenac as a standard. The antibacterial effectivity was assessed through the agar well diffusion method against *S.* *aureus* and *K. pneumoniae*  and the acute oral toxicity evaluation was done using an amended OECD technical guideline 423 (the limit test). The phytochemical screening revealed the presence of diverse biomedically relevant secondary metabolites. Biosafety assessment in Sprague Dawley rats confirmed the absence of acute oral toxicity effects at a fixed dose of 4000 mg/kg body weight. The lyophilised hydroethanolic bark extract also exhibited significant anti-inflammatory activity, comparable to diclofenac. These. The antibacterial tests confirmed antibacterial inhibition of selected microbes. It was therefore concluded that the numerous bioactive secondary metabolites present in *P. curatellifolia* bark possess anti-inflammatory, antibacterial and potentially antioxidant properties and are toxicologically safe. This study validates the use of *Parinari curatellifolia* as a potential complementary treatment in the management of key biological endpoints of pneumonia in traditional Zimbabwean medicinal practices.

**Key words**: *Parinari curatellifolia*, anti-inflammatory, pneumonia, secondary metabolites

# Introduction

## *Parinari curatellifolia*

*Parinari curatellifolia (P. curatellifolia*), is known in varying regions of Zimbabwe as *muchakata, mubuni, mobola, muisha and umkhuna* [1]. Previously classified under the Rosaceae order *P. curatellifolia* belongs to the *Chrysobalancaceae* family [2,3]. It is a medium to large evergreen tree that can grow up to 20m tall, with a bare stem and a dense, roundish mushroom-shaped crown [3]. The trees’ small, white fragrant flowers are borne in large panicles, and the fruit is fleshy and yellow when ripe containing a single seed [4,5]. This species is widespread throughout Southern Africa and is an indigenous fruit species that serves as a food source for many rural communities, and an important medicinal plant [6,7]. Traditionally, it has been used to treat a variety of ailments mostly pneumonia as well as fever, bacterial infections and inflammatory disorders [7,8,9]. The bark and leaf extracts can also be used for tanning, and bark produces a pink-brown dye which is used in basket work [10].

  

Figure 1: Images of P curatellifolia plant, aerial parts, foliage and fruit

## Antibacterial activity of Secondary metabolites

Resistance to conventional antimicrobial drugs has been on the rise and this has necessitated the need to discover more effective therapies. Antibiotic-resistant bacterial infections account for more than a million deaths worldwide every year, with the number expected to rise over the next decades unless new antibiotics can be developed [11]. Plants are a promising source of antibiotics due to their diverse array of bioactive secondary metabolites. Secondary metabolites are diverse chemical structures that play a role in the adaptation of plants to their environment, for example in defense against predators and pests but are not essential for their life and are broadly classified in three groups: phenolics, terpenes and steroids, and alkaloids [12].

Phenolics are found in leaves, stems, roots, flowers, and fruits and have demonstrated antimicrobial properties. Some of the most extensively studied compounds are eugenol, carvacrol, thymol, resveratrol, cinnamaldehyde, and pyrogallol [13]. The ability of phenols and phenolic acids to link with and disable some bacterial enzymes essential for bacterial cell wall synthesis could play a positive role in the treatment of infections caused by resistant bacteria and their use as adjunct therapies to existing antibiotics could have a synergistic effect [14]. Polyphenols can be classified as phenolic acids, flavonoids, lignans, stilbenes and tannins based on the number of aromatic rings and the structural elements, but can also be classified by their source of origin, natural distribution and biological activity [14]. Plant polyphenols’ antibacterial pathways include interaction with proteins and bacterial cell walls, alteration of the cytoplasmic functions and of membrane permeability, inhibition of energy metabolism and DNA damage or inhibition of nucleic acids synthesis with mechanisms of inhibition differing, depending on the specific properties given by functional groups and aromatic rings as well as the bacterial species [15,14]. Terpenes are linear or cyclic hydrocarbons, whereas terpenoids are oxygen-containing terpene analogues found in all living organisms. Steroids are a subclass of terpenoids that are biosynthesized from terpene precursors[16]. The multitarget impact of terpenes and their derivatives make them potent antimicrobial agents against multidrug-resistant organisms, particularly bacteria and fungi [17]. Due to their multiple target sites and methods of action, no microbial resistance has yet been observed to terpenes [17]. Examples of terpenes include monoterpenes, sesquiterpenes, diterpenes, triterpenes and terpenoids. Terpenes have antimicrobial activities against both the antibiotic-susceptible and antibiotic-resistant bacteria, mainly via their abilities to promote cell rupture and inhibition of protein and DNA synthesis [17]. In a study by Gallucci et al.,[18] carvacrol, carvone, eugenol, geraniol, and thymol were among the terpenes that demonstrated antibacterial action by causing structural and functional changes to the inner or outer cytoplasmic membrane, interacting with membrane proteins, and their effects on intracellular targets. Alkaloids are found in bacteria, fungi, plants and animals but flowering plants, particularly angiosperms, are the main source [19]. Structurally, alkaloids are extremely diverse and have served as scaffolds for important antibacterial drugs such as metronidazole and the quinolones due to their wide antibacterial spectrum on common clinical strains, including drug-resistant bacteria [20,21]. The antibacterial mechanisms of natural alkaloids include disruption of the bacterial cell membrane, disruption of the DNA function and inhibition protein synthesis [21]. Examples of alkaloids include purines, aporphine, isoquinoline and piperedine alkaloids.

The use of indigenous plants in the treatment of bacterial infections is prevalent in Southern Africa with a reliance on plant species such as Acacia, Aloe vera and Cassia abbreviate. These are used in forms such infusions, decoctions or topical ointments to fight bacterial infection such as pneumonia, tuberculosis and skin infections. P. curatellifolia has been used traditionally to treat ailments such as bacterial pneumonia but its bioactivity and safety profiles are not well characterized. Hence, the purpose of this study is to investigate the bioactivity and biosafety of hydro-ethanolic lyophilized P. curatellifolia bark extract in order to validate its use in the treatment of bacterial pneumonia.

## Anti-inflammatory activity secondary metabolites

Inflammation can be defined as the nonspecific immune response against physical, chemical, or even biological attack to the body resulting in physiological changes that provide the indistinguishable redness characteristics, heat in the affected area due to an increase in blood flow and vascular permeability, pain, swelling, and movement difficulty or loss of function [22]. Inflammation in pneumonia occurs as an immune response to infection in the lungs that can help fight the infection but can also cause lung injury when prolonged [23]. Harmful stimulus triggers the release of proinflammatory proteins, known as cytokines and in cases where the stimulus persists, such as in the cases of drug-resistant bacteria, inflammation becomes chronic. Nonrevolving inflammation is a major driver of disease resulting in tissue damage and necrosis [24]. While current treatment includes the use of non-steroidal anti-inflammatories (NSAIDS), concerns have been raised over their safety and tolerability highlighting the need to develop new and effective NSAID formulations that are efficacious in management of inflammation and pain [25]. Phytochemicals, such as phenols, polyphenols, tannins, flavonoids, and more recently thiosulfinates and polysulfides have been found to play an important role as anti-inflammatories, antioxidants and immunosuppressants in humans [22,26]. Studies has shown they inhibit pro-inflammatory enzymes, for example phenolic compounds inhibit enzymes such as cyclooxygenase (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS), and modulate cellular signaling, by scavenging free radicals to reduce oxidative stress and regulate inflammatory pathways on the body [26].

With the aim to expand the range of orally administered antibacterial agents, this study was carried out to confirm the bioactivity of lyophilized hydro-ethanolic extracts of *P. curatellifolia* bark against markers of pneumonia progression such as inflammation. This study also is intended to determine the biosafety in animal models.

# Materials and methods

## Materials, equipment and facilities

All chemicals, associated reagents, equipment and facilities for the *in-vivo* laboratory animal toxicity investigations and the bioactivity assays were obtained from the University of Zimbabwe, Faculty of Medicine and Health Sciences laboratories.

## Animal use approval

Prior to the investigations, animal use and research ethics approvals were obtained from the Joint Parirenyatwa Research Ethics Committee (JREC) which is the local research Institutional Review board for the University of Zimbabwe.

## Parinari curatellifolia plant material collection and preparation

Plant material was collected from Spitzkop, 24 km West to the City of Harare (17.7861° S, 30.8705° E). In observing the rules for Zimbabwe's Sustainable Harvesting of Traditional Medicinal Plants, the material was collected from 5 different plants. The plant material was authenticated as *P. curatellifolia* by the National Herbarium and Botanical Garden in Harare, Zimbabwe. The bark was thoroughly washed using clean water to remove debris and other contaminants, shade dried at room temperature to constant weight for three weeks, and then pulverized using mortar and pestle. The pulverized material was ground into a fine powder using a coffee grinder (Hamilton Beach Coffee Grinder Model- 80410).

The phyto-extraction was done by adding 100g plant powder into 1000ml of 70% (v/v) hydro-ethanolic mixture in a 2-litre sterile amber bottle and macerated for 5 days, with 3 minutes physical shaking twice a day. A muslin cloth was used to obtain a filtrate from the solution, which was further clarified by filtration using Whatman filter paper number 1. The filtrate was then evaporated under vacuum and low pressure (Rotavapor® R-300, Buchi, Switzerland), followed by lyophilization (Lyovapor l-200, Buchi, Switzerland) under 140Pa pressure and -50 °C. The lyophilized extract was stored in an airtight sample bottle

### **2.4 Phytochemical Screening of Parinari curatellifolia**

5g of the lyophilized hydro-ethanolic extracts of *P. curatellifolia* were dissolved in 50ml of distilled water in a 100ml round bottom flask and subjected to various phyto-screening techniques to confirm the presence or absence of relevant phytoconstituents of pharmacological interest. The following qualitative tests were conducted on the extract liquor using studies by Gwaze et al [27].

*Table 1: Phytochemical screening tests done on P. curatellifolia*

|  |  |
| --- | --- |
|  Detection for alkaloids by the Iodine test |  To 3ml of the lyophilized extract solution, a few drops of iodine solution were slowly added along the sides of the test tube |
|  The presence of alkaloids was then identified by the appearance of a blue colour, which disappears on boiling and reappears on cooling |
|  Detection of tannins by the Braymer’s test |  To 1ml lyophilised extract solution, 3 drops of a 10% Ferric chloride solution were added |
| The presence of tannins was confirmed by conversion of the solution to a blue-green colour |
| Detection of Glycosides by the Keller-Killani test | To 5Ml of the lyophilised solution, 5mL glacial acetic acid was added and a few drops of 5% ferric chloride were added followed by a few drops of concentrated sulphuric acid along the side of test tube |
|  The presence of glycosides was confirmed by the emergence of a blue coloured solution in mixture acetic acid layer |
| Detection of Phenolic compounds by the Gelatin test  | 2ml the lyophilised extract solution was added to 5ml of a 1% gelatin solution and 5 drops of a 10% NaCl were further added |
| Phenolics were identified by the appearance of a white precipitate |
|  Detection of saponins by the simplified foam test | 2ml of the extract was added to 20ml distilled water. The mixture was shaken in a graduated cylinder for 15 minutes |
| The presence of saponins would be confirmed by the formation of form with a head height of at least 1cm3 |
| Detection of flavonoids by the Ammonia test | 5ml dilute ammonia solution was added to 5ml of the lyophilised solution followed by a few drops of concentrated sulphuric acid |
| The emergence of a yellow colour indicates the presence of flavonoids |

## 2.5 Anti-inflammatory activity of *Parinari curatellifolia using* the egg albumin denaturation test

The anti-inflammatory activity of *P. curatellifolia* was assessed using the egg albumin denaturation assay. The method is based on the idea that compounds with anti-inflammatory properties have the ability to stabilize proteins and prevent their denaturation. Hence, the principle of the test is to measure the extent to which a substance prevents the denaturation of egg albumin. The assay was carried out following the method described by Madhuranga & Samarakoon [28].

# 2.5.1 Preparation of the Egg Albumin Solution

Albumin was separated from the yolk of a fresh hen’s egg. The albumin was then diluted with sterile phosphate-buffered saline (PBS, pH7,4) to achieve a final concentration of 2% (v/v).

# 2.5.2 Preparation of test samples

A stock solution of *P. curatellifolia* extract was prepared at a concentration of 200mg/ml. Serial dilutions were done to obtain working concentrations of 100mg/ml, 50mg/ml, 20mg/ml and 10mg/ml. Diclofenac sodium, a standard non-steroidal anti-inflammatory drug was used as a positive control and prepared similarly at concentrations of 5mg/ml and distilled water served as the negative control.

**2.5.3 Assay procedure**

A reaction mixture of 2ml of the test sample, 2.8ml of PBS and 0.2ml of 1% egg albumin solution was prepared in test tubes. The tubes were incubated at 37oC for 15 minutes then heated in a water bath at 70oC for 10 minutes in a water bath. After cooling, the absorbance of the resulting solution was measured using a spectrophotometer (UV spectrophotometer, Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments) at 660nm.

**2.5.4 Calculation of the Percentage Inhibition**

To calculate the percentage inhibition of protein denaturation, the following formula was used:

Equation 1

 

## Anti-bacterial evaluation of *Parinari curatellifolia*

To evaluate the antibacterial activity of *P. curatellifolia* bark extract, the zone diameter of inhibition (ZDI) was carried out using the agar diffusion plate method [29]. In petri dishes containing nutrient agar, 0,2ml of the inoculum was spread. Wells were made using a sterilized borer, then filled with varying concentrations of the extract and incubated at 37oC for 24 hours.

## Minimum inhibitory concentration

To determine the Minimum Inhibitory Concentrations (MIC), the broth macro dilution method was used [30]. We prepared a loopful of each organism with normal saline using 0.5 McFarland standards. About 0.1 ml of the different concentrations of the different extracts was aseptically introduced into test tubes containing 2 ml of Nutrient broth then 0.1 ml of each of the inoculums was also introduced into the different test tubes. The test tubes were then incubated 37°C for 24 h and were observed for turbidity.

## Acute oral toxicity evaluation of *Parinari curatellifolia*

The limit test procedure outlined in the OECD guideline 423 (Acute Oral Toxicity - Acute Toxic Class Method) was used to evaluate the acute oral toxicity of Parinari curatellifolia extracts. The aim of the study was to determine the potential acute toxic effects of the extracts following oral administration.

### **Experimental Animals**

Healthy, adult female Sprague-Dawley rats weighing 200-300 g were used. The rats were sourced from the University of Zimbabwe Animal House and were acclimatized to the laboratory conditions for a week before the experiment. They were kept in standard cages at standard room temperature and pressure conditions with free access to food and water.

**2.4.2. Preparation of Test Substance**

The lyophilized hydro-ethanolic extract of P. curatellifolia was dissolved in distilled water to obtain a stock solution of 2000 mg/ml.

**2.4.3. Limit Test Procedure**

A single dose of 4000 mg/kg body weight was selected as the limit dose, as per OECD guideline 423. Three animals were randomly selected and orally administered the test substance using a gastric gavage needle. The animals were observed continuously for the first 4 hours after dosing, with special attention to signs of toxicity such as tremors, convulsions, salivation, diarrhea, lethargy, and changes in respiration then daily for fourteen days. The body weight of each animal was recorded before dosing and at regular intervals throughout the 14-day observation period. The animals were monitored for clinical signs of toxicity and mortality throughout the study period. At the end of the 14-day observation period, all surviving animals were euthanized by chloroform inhalation and a gross necropsy was performed. Any macroscopic abnormalities in the organs were recorded.

**2.4.4. Data Analysis**

Data recorded included the number of dead animals, the presence and severity of clinical signs, changes in body weight and macroscopic pathological findings. If no mortality or significant toxicity was observed at the limit dose of 4000 mg/kg, the extract was considered to have low acute oral toxicity. If mortality or significant toxicity was observed, further studies, such as dose-response studies, would be required to determine the LD50 (median lethal dose). The results were documented and reported in accordance with the OECD guideline 423.

# Results and discussion

## Phytochemical screening

The phytochemical analysis confirmed the presence of primary and secondary metabolites. Of these, phenols, terpenes and alkaloids are of great biomedical relevance with regards to inflammation caused by bacterial agents (Table 2). These results correlate with studies by Fatokun Olakunle et al.,[30] who identified saponins, tannins, alkaloids, flavonoids and glycosides, among other compounds in *P. curatellifolia*. The results also highlighted differences in the phytochemical composition of hydroethanolic and distilled water extracts with glycosides and saponins being absent in the distilled water extract. Of these phytochemicals; phenols, terpenes and alkaloids are of great biomedical relevance with regards to inflammation caused by bacterial agents. This provides evidence for the many traditional applications of the plant, notably in the treatment of pneumonia. The presence of bioactive compounds that have biomedical relevance suggests that the plant can be of pharmaceutical relevance. The plant can be studied and compounds isolated to make novel therapies particularly for bacterial infections. This can be of great importance as global efforts are being made to combat antimicrobial resistance to therapies. The results of this study therefore highlight the need to conduct further research on the specific bioactive compounds in the plant and their potential therapeutic applications.

*Table 2: results of the phytochemical analysis*

|  |  |  |
| --- | --- | --- |
| Test | Presence in hydroethanolic extract | Presence in distilled water extract |
| Alkaloids | ++ | + |
| Tannins | +++ | + |
| Glycosides | + | - |
| Flavonoids | ++ | + |
| Phenolics | + | + |
| Terpenoids | + | + |
| Saponins | ++ | - |
| Phytosterols | + | + |

## Anti-inflammatory tests

The anti-inflammatory activity *of P. curatellifolia* was assayed using the egg albumin test. The egg albumin test is a widely accepted invitro method for assaying anti-inflammatory potential. The results of the study highlighted the potential of P. curatellifolia hydroethanolic extracts in the inhibition of inflammation which is an important aspect to many diseases. The results showed significant inhibition of protein denaturation by the extract. This indicated P. curatellifolia potential anti-inflammatory activity. The inhibition became comparable to the least dose of the positive control Diclofenac (250 µg/ml) used in this study at approximately around 750µg/ml with activity expected to increase further by using bioactive fractions and isolated compounds**.** This anti-inflammatory activity can be attributed to the presence of phytochemicals such as terpenoids, flavonoids and phenolics in the extract as they have been reported to possess anti-inflammatory properties. Further studies may need to be conducted to understand the mechanisms of inhibition of inflammation by the extracts. This may include identifying the specific phytochemicals responsible and their molecular targets. These results also provide a basis for further in vivo and clinical research on the anti-inflammatory activity of *P. curatellifolia.*

*Table 3: results of the anti-inflammatory assay*

|  |  |
| --- | --- |
| **Concentration**  **(µg/ml)** |  **% Inhibition**  |
| ***P. curatellifolia*** **hydroethanolic extract**  | **Diclofenac (standard)**  |
| 250  | 16± 0.08  | 39±0.84  |
| 500  | 27±0.67  | 65±1.78  |
| 1000  | 76±0.28  | 302±3.40  |
| 2000  | 265±1.76  | 760.12± 4.20  |
| 4000  | 386±1.34  | Not tested  |
| 6000  | 488±3.56  | Not tested  |
| 8000  | 716±3.54  | Not tested  |

*Figure 2: Anti-inflammatory assay of P. curatellifolia*

## Antibacterial assay

The antibacterial assay of the hydroethanolic plant extracts (table 4) exhibited activities against *S.* *aureus* and *K. pneumoniae*. The significant inhibition zone diameters against the tested bacterial strains indicated the potential of the plant in being harnessed for antibacterial therapies. The results indicated a broad spectrum of activity with the extract inhibiting both Gram-negative and Gram-positive bacteria. The differences in the different zone diameter of inhibition reflected the varying degree of sensitivity of the bacteria to the crude extracts and the isolates. The *Klebsiella* mutans was highly sensitive to the hydroethanolic stem bark extract and *Staphylococcus aureus* was moderately sensitive (Table 4). The result of the antibacterial assay of the plant extracts is comparable to Erythromycin the standard antibacterial agent used as the positive control in this work. The results also showed a high bactericidal activity of the hydroethanolic extract at relatively low concentrations of the extract (Table 5). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts of *P. curatellifolia* on the test organisms are of great significance, since these multidrug resistant organisms are of great epidemiological threat. The low MIC value confirms the high antibacterial activity of the extracts at different concentrations. These results suggest that the extracts of *P. curatellifolia* can be further enhanced for the treatment of bacterial infections.

*Table 4: Antibacterial Activity of Hydroethanolic Extracts of P. Curatellifolia Against the Test Organisms*

|  |  |  |
| --- | --- | --- |
| Test organism | concentration (mg/ml) | Zone diameter of inhibition (mm) |
|  | Extract | Control |
| Staphylococcus aureus | 250 | 21 + 0.70 | 32+ 0.50 |
| 100 | 11.5 + 3.40 | 23 + 0.00 |
| 50 | 10 + 0.60 | 18 + 1.20 |
|  |
| Klebsiella pneumoniae | 250 | 26 + 0.20 | 16 + 2.82 |
| 100 | 22 + 2.20 | 12 + 0.70 |
| 50 | 17 + 0.8 | 10 + 0.00 |

## Acute oral toxicity evaluation

*Table 6: Acute toxicity results from the amended limit test*

|  |  |
| --- | --- |
| Observed parameter |  Day of observation |
|  | **1** | **4** | **7** | **10** | **14** | **Control** |
| Food intake | Normal | Normal | Normal | Normal | Normal | Normal |
| Water intake | Normal | Normal | Normal | Normal | Normal | Normal |
| Death | Alive | Alive | Alive | Alive | Alive | Alive |
| Breathing | Normal | Normal | Normal | Normal | Normal | Normal |
| Diarrhoea | Not observed | Not observed | Not observed | Not observed | Not observed | Not observed |
| Urination | Normal | Normal | Normal | Normal | Normal | Normal |
| Skin colour | Normal | Normal | Normal | Normal | Normal | Normal |
| Drowsiness | Not observed | Not observed | Not observed | Not observed | Not observed | Not observed |
| Erection of Fur | Not observed | Not observed | Not observed | Not observed | Not observed | Not observed |

Results and interpretation were done by a qualified veterinary expert. Our findings indicated that the extract at doses up to 4000 mg/kg body weight imparted neither visible signs of toxicity nor mortality in rats, suggesting that *P. curatellifolia* is of low oral toxicity. No animals were withdrawn from the study for any reason during the observation period. According to the Hodge and Steiner toxicity classification, this classifies the extract as biologically safe for oral use. This therefore implies that the use of high extract concentrations to achieve the desired bioactivity effects will not pose any toxicity in *P. curatellifolia* based treatments.

## Rats weights observations

In oral toxicity assays, observation of body weight are a simple and sensitive reflection of toxicity after exposure of study animals to materials. Weight loss or gain of animals is normally an indicative marker of stress, failure to feed or a response to observed or underlying adverse health conditions. In the present study, the lyophilised extracts did not signiﬁcantly affect normal body weight growth during the study period suggesting that the extract did not alter rat growth at the concentration investigated.

*Figure 3: observed rats weights over the toxicity evaluation period*

# Conclusions

The lyophilised hydro-ethanolic extracts of *P. Curatellifolia* bark were shown to possess considerable antibacterial and anti-inflammatory activities. The observed activities were attributable to the presence of secondary metabolites including tannins, phenolic compounds, and phytosterols. These contribute to the underlying mechanisms behind the plant’s proven effects and therapeutic activities in bacterial infections. *P. curatellifolia* was nontoxic at 4000mg/kg. Our biosafety and bioactivity studies therefore authenticate the use of *P. curatellifolia* as a potential antibacterial and anti-inflammatory remedy in traditional medicine in the management of common end points of pneumonia.

# Disclaimer (Artificial intelligence)

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

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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