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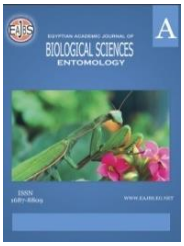

In Vitro Evaluation of Some Therapeutic Applications of Wasp Venom and Mastoparan

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ABSTRACT

An innovative and exciting development in complementary therapies is the utilization of insects as a practical and valuable way to obtain natural products. It has been discovered that a number of insect constituents serve various purposes. This research sought to evaluate the *in vitro* antioxidant, anti-inflammatory, anticancer and anti-Alzheimer impact of wasp venom extract and mastoparan. The present result revealed that wasp venom was much more effective than mastoparan in all the experiments done. Its antioxidant impact with IC_{50}=9.16±0.2 µg/ml., while, mastoparan showed antioxidant with IC_{50}=13.72±0.25 µg/ml. Furthermore, anti-inflammatory impacts for wasp venom and mastoparan = 14.67±0.32 and 32.32±0.75 µg/ml respectively. Besides, anti-Alzheimer outcomes of mastoparan with IC_{50}=12.41±0.2 µg/ml while, wasp venom showed better inhibition of the enzyme with IC_{50}=9.75±0.2 µg/ml} Lastly, wasp venom showed an anticancer towards Hep-G2 cells with IC_{50}=165.85±0.8 µg/ml, while mastoparan showed a promising antitumor impact with IC_{50}= 189.59±0.6 µg/ml and both of them have minimal cytotoxicity versus Vero cells. Wasp venom may be helpful as an auxiliary for liver anticancer and anti-Alzheimer medications as well as an antioxidant and an anti-inflammatory agent for the treatment of disorders linked to inflammation and cellular oxidative stress. To confirm this impact in animal models and determine whether these extracts could be safe and useful for individuals, more *in vivo* experimental research is necessary.

INTRODUCTION

The majority of present utilization anti-cancer and anti-Alzheimer medicines have non-selective targeting and significant adverse effects, which limit their therapeutic potential despite remarkable advancements in chemotherapy (Deng *et al.,* 2016; Abu Gazia

and El-Magd, 2018). Finding innovative anticancer drugs that selectively target cancer cells and have a strong inhibitory impact is therefore becoming increasingly important (El-Magd *et al.,* 2019; Magdy *et al.,* 2020 and Magdy *et al.,* 2022). Targeted therapy has been looking for safe, natural medications to prevent the negative effects of anticancer therapies in recent years (Awad *et al.,* 2020). Significant anti-inflammatory, antioxidant, anti-Alzheimer and anticancer potential can be found in extracts made from natural products and medicinal plants (Attia *et al.,* 2020). Recent numerous bioactive components isolated from insects have been proven to have anticancer properties, (Amer *et al,*. 2021 and Magdy *et al.,* 2022). Although their significance has not been thoroughly tested, insects and their larvae may be a useful source of numerous bioactive chemicals (Yan *et al.,* 2015 and Amer et al 2021). According to earlier research, certain insect extracts and products exhibit antibacterial, antioxidant, and antitumor qualities (Guo *et al.,* 2017 and Di Mattia *et al.,* 2019). Insects are a staple food for at least two billion people worldwide due to their high protein and vitamin content, along with their therapeutic benefits. Nutrition with high antioxidants could reduce tumors, and Alzheimer's disorders brought on by oxidative damage (El-Demerdash *et al.,* 2021).

The family Vespidae includes insects known as oriental hornets (*Vespa orientalis*), which are found in the Middle East, Southwest Asia, Southern Europe, and Northeast Africa. They are colonial in nature. Wasp venom was used to treat gastritis and (colds) in folk treatments, despite the fact that their stings may be quite unpleasant for humans and trigger allergies (Ken *et al.,* 2005). Strong antibacterial activity against a wide range of microorganisms was demonstrated by the venom isolated from *V. orientalis* (Jalaei *et al.,* 2018), as well as anticancer activity (Mukund and Manjunath, 2017). The cytotoxic and neurotoxic effects of insect venom on normal cells are one of the main disadvantages of employing them as an anticancer treatment. An effective substitute might be to use insect pupae and larvae (Saidemberg *et al.,* 2010). Chemically, wasp venom contains different biologically active molecules including, proteins, volatile compounds, phospholipase A2, mastoparan, and decoralin (Abd El-Wahed *et al.,* 2021).

Mastoparans is a class of wasp linear cationic α-helical peptides, or they are short peptides, usually tetra-decapeptides with an amidated C-terminal, that were first shown to stimulate mast cell degranulation and histamine release. Several species of social and solitary wasps have venom that contains this category of peptides (Dos Santos Cabrera *et al.,* 2019& Abd El-Wahed *et al.,* 2021). Numerous investigations have exhibited the diverse physiological implications of mastoparans, which encompass activation of protein G (Higashijima *et al.,* 1988), activation of phospholipase A2, C, and D, release of serotonin and insulin, as well as antimicrobial, hemolytic, and anticancer properties. Mastoparans have been utilized in plants to stimulate an increase in intracellular Ca^{+2} that controls cell-to-cell communication (De Santana. *et al.,* 2022)

This study aimed to assess and compare some *in vitro* biomedical impacts of venom of *V. orientalis* and mastoparan as a preliminary step for other future investigations.

MATERIALS AND METHODS

Samples (*Vespa orientalis* & Mastoparan):

Throughout the summer months (July to September 2023), samples of the oriental hornet (*Vespa orientalis*) were collected from hornet traps positioned amidst honeybee colonies at the Department of Honey Bee Research, Institute of Plant Protection, Ministry of Agriculture. (Egypt's Giza). In order to catch *V. orientalis* while it was consuming workers and honey bees, traps were set up (Amer *et al.,* 2021). The taxonomic key was used to classify *V. orientalis* L. in accordance with (Smith-Pardo *et al.,* 2020). Then Using

the electrical shock method, one gram of venom was collected from the *V. orientalis* and then preserved at -15.0°C in glass vials (Amer *et al.*, 2021; Amin *et al.*, 2022).

Mastoparan was brought from (Sigma-Aldrich, Germany) company. It kept at -20°C. Its molecular weight is 1479 daltons, and its chemical formula is $C_{70}H_{13}1N_{19}O_{15}$.

Preparation and Extraction:

To remove unwanted detritus, the collected venom was mixed with 95 mL of distilled water or pure ethyl alcohol. The combination was then centrifuged at 8000 rpm/20 min/4 °C. The supernatant and mastoparans were extracted as 70% ethanol and kept in the freezer until further use (Hasaballah, *et al.*, 2019).

Screening for Antioxidant Effect:

To evaluate the investigated samples of mastoparan and wasp venom (*in vitro* antioxidant impact, ethanol solution that contained 0.1 mM DPPH (2,2-Diphenyl-1-picrylhydrazyl) was used. DPPH solution was divided into three milliliters (1 ml/ each to perform the test three times) and combined with different levels of samples (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml) which dissolved in ethanol content. After giving the mixture a good shake, it was let to stand at room temperature for half an hour. The absorbance at 530 nm was obtained using spectrophotometry (Jenway 6305, USA) (Sherif *et al.*, 2023).

Screening for Anti-Inflammatory Effect:

The following assay was used to evaluate the tested samples' in vitro anti-inflammatory effects. Samples were immersed in distilled water. Duplicate pairs (per dose) of the hypotonic solution (5 ml) containing graded doses of the samples (100, 200, 400, 600, 800, and 1000 g/ml) were placed inside the centrifuge tubes. Duplicate pairs (5 ml) of an isotonic solution containing graduated quantities of the standard drug (Ibuprofen) (100 - 1000 µg/ml) were added to the centrifuge tubes. The control tubes contained five milliliters of indomethacin 200 mg/ml and the vehicle was distilled water. 0.1 ml of the erythrocyte (Red blood cells) suspension was added to each tube and mixed thoroughly. Prior to being centrifuged for three minutes at 1300 g, the solutions were incubated for one hour at 37 degrees Celsius. Using a (Jenway 6305, USA) spectrophotometer, the absorbance (OD) of the supernatant's hemoglobin content was determined at 560 nm (Chioma *et al.*, 2012).

Screening for Anti-Alzheimer Effect:

The samples were exposed to 0.2 U/mL of butyrylcholinesterase enzyme obtained from Sigma Aldrich (enzyme marker for Alzheimer's), which caused the reactions. The rate of increase in absorbance was measured spectrophotometrically using (Jenway 6305, USA) running at 405 nm. The first sixty seconds of the progress curves were utilized to calculate the enzyme activity, with an extinction coefficient of 14.2 mM/cm-1. The butyrylcholinesterase enzyme could be examined for inhibition by adding 0-0.375 M DMMB to the reaction mixture (final volume: 1.2 mL). the reaction mixture contained 1.25% (v/v) of methanol and showed no effect on the enzyme's activity (Sezgin *et al.*, 2013).

Screening for Anti-Tumor Effect:

The cytotoxic effects of the samples were examined on HepG-2 (Human hepatocellular carcinoma cells) obtained from Sigma Aldrich. Following 24 hours of adhesion till confluence, samples ranging in concentration from 500 to 15.63 µg/mL were added, and the cells were subsequently cultured on RBMI growth media for another 24 hours at 37 °C. Following the addition of the new medium, 100 µL of MTT solution (5 mg/mL) was added and left to sit for 4 hours at 37 °C. Using a microplate reader (BioTeck, Agilent Technologies, USA), absorbance was measured at 570 nm. A digital camera and an inverted microscope (CKX42; Leica Micosystems, Germany) were used to take the images. as (Ahmed *et al.*, 2022).

***In Vitro* Cytotoxicity Effect:**

Vero cells (kidney epithelial cells extracted from an African green monkey) obtained from Sigma Aldrich were seeded with 1×10^5 cells and 100 µl of DMEM growth media in 96-well plates. After being seeded for 24 hours, confluent cell monolayers were transferred using a multichannel pipette into 96-well flat-bottomed microtiter plates (Falcon, Jersey, NJ, USA). Subsequently, different concentrations of the samples were added to fresh DMEM medium, and the inspected specimen was diluted twice. The microtiter plates were placed in a humid incubator with 5% CO_2 and incubated for 48 hours at 37°C. After staining with crystal violet, absorbance was measured at 570 nm. The pictures were acquired by a digital camera coupled with an inverted microscope (CKX42; Leica Micosystems, Germany) (Al-Salahi *et al.*, 2015).

Statistical Analysis:

The data were evaluated using GraphPad Prism (version 5.0, San Francisco, CA, USA), and were presented as means with deviations of means (SD) (Sehim *et al.*, 2023).

RESULTS

Antioxidant Action:

Applying DPPH test for wasp venom revealed an effective antioxidant impact with IC_{50}=9.16±0.2 µg/ml. While mastoparan showed good antioxidants with IC_{50}=13.72±0.25 µg/ml. Ascorbic acid was applied as a standard for antioxidant measurement with IC_{50}=3.36±0.4 µg/ml as illustrated in (Fig. 1).

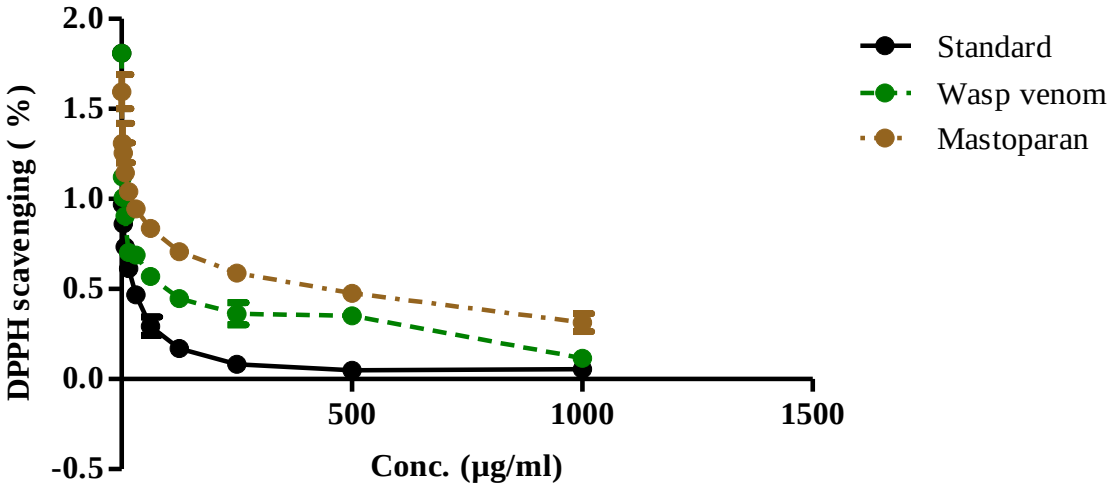

Fig. 1: Anti-oxidant action of wasp venom and mastoparan (results are represented as means ± S.D).

Anti-Inflammatory Action:

The outcome of hemolysis Inhibition percentage in (Fig. 2) showed the effective anti-inflammatory action for both wasp venom and mastoparan, where the level of protection elevated upon increasing level of specimens. Hemolysis inhibition percentage for wasp venom and mastoparan = 14.67±0.32 and 32.32±0.75 µg/ml respectively. While the hemolysis inhibition percentage for the standard was 1.6±0.30 µg/ml.

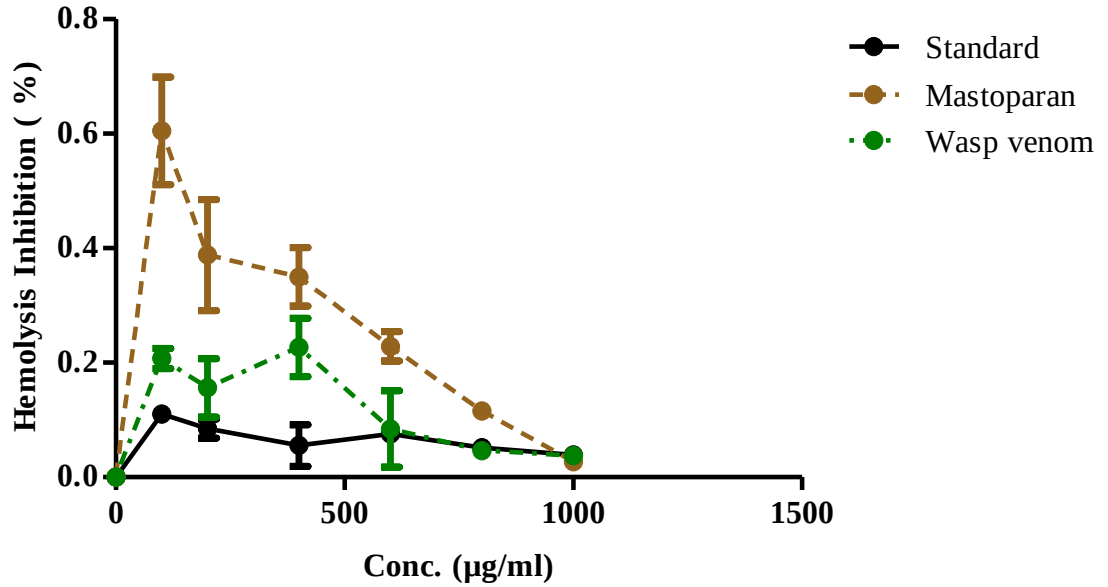

Fig. 2: Anti-inflammatory action of wasp venom and mastoparan (results are represented as means ± S.D)**.**

Anti-Alzheimer Action:

Butyrylcholinesterase (BChE) inhibition test was used to examine anti-Alzheimer action for wasp venom and mastoparan. Where, both materials gave a good inhibition of the enzyme, as mastoparan showed inhibition with IC_{50}=12.41±0.2 µg/ml. While wasp venom showed better inhibition of the enzyme with IC_{50}=9.75±0.2 µg/ml where the standard IC_{50}=4.01±0.1 µg/ml as illustrated in (Fig. 3).

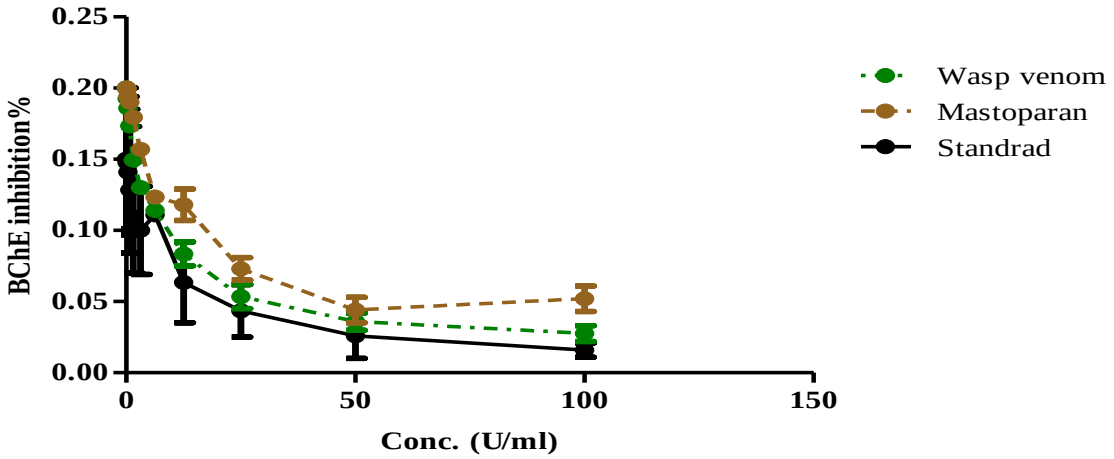

Fig. 3: Anti-Alzheimer action of wasp venom and mastoparan (results are represented as means ± S.D)**.**

Anti-Tumor Action:

The impact of antitumor was examined for wasp venom and mastoparan versus Hep-G2 cells. Examined Mastoparan showed a promising antitumor impact with IC_{50}= 189.59±0.6 µg/ml. While wasp venom showed a better anticancer towards Hep-G2 cells with IC_{50}=165.85±0.8 µg/ml as shown in (Fig. 4).

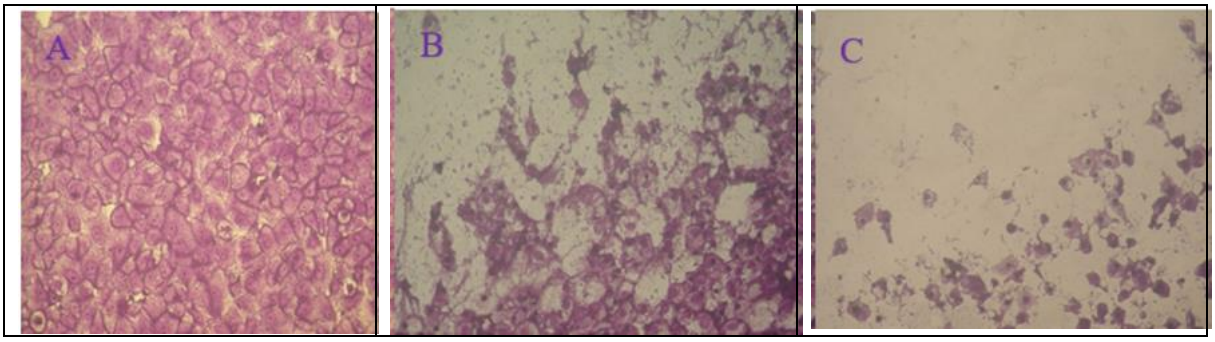

Fig. 4: Anti-tumor effect of wasp venom and mastoparan versus Hep-G2 cells (A) untreated cells; (B) Treated cells with wasp venom and (C)Treated cells with mastoparan (results are represented as means ± S.D)**.**

Cytotoxicity Assay:

Cytotoxic impact tested for wasp venom and mastoparan versus Vero cells. Where mastoparan showed a minimal cytotoxic impact with CC_{50}= 454.79±0.8 µg/ml. While wasp venom showed minimal cytotoxicity towards Vero cells with CC_{50}=439.85±0.7 µg/ml as shown in (Fig. 5).

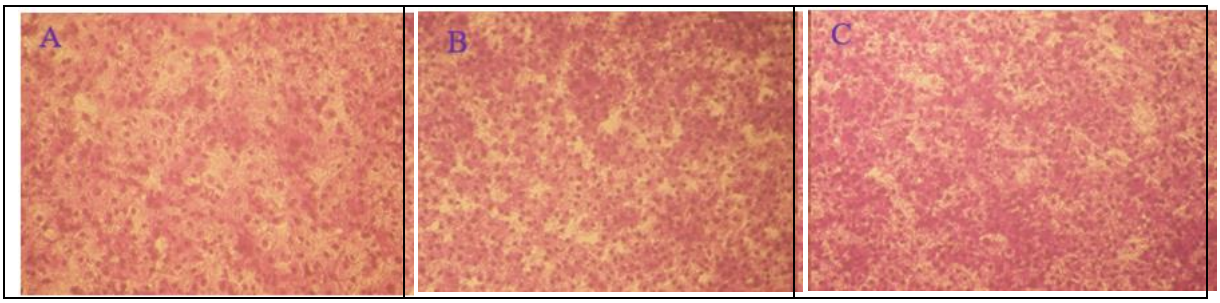

Fig. 5: Cytotoxicity of wasp venom and mastoparan versus Vero cells (A) untreated cells; (B) Treated cells with wasp venom and (C)Treated cells with mastoparan (results are represented as means ± S.D)**.**

DISCUSSION

Investigating the functions of bioactive substances of insects is becoming more and more popular, particularly those with antitumor and antioxidant qualities (Saidemberg *et al.*, 2010). Only the anticancer potential of Vespa sp. venom and its bioactive peptides have been studied in the past (Leite *et al.*, 2015). Insect larvae and pupae, aside from their venom, show promise as antioxidants and anticancer agents with minimal damage to normal cells. An antioxidant effect for the aqueous pupal extract of *V. affinis* was recorded by (Dutta *et al.*, 2016). Additionally, *M. domestica*'s larval hemolymph exhibited cytotoxic and antioxidant qualities towards MCF7, but no cytotoxicity against normal Vero cells (El-Garawani *et al.*, 2020).

The venom of *V. orientalis* has been shown to exhibit cytotoxic effects in earlier research, which have been linked to the bioactive peptide mastoparan, which destabilizes membranes and subsequently causes cell lysis. Moreover, mastoparan activates G-protein, which sets off apoptosis and permeability of mitochondria. Nonetheless, scant information exists regarding the biological impacts of mastopran and larval extracts from *V. orientalis*, (Habib *et al.*, 2020).

In the present work *V. orientalis* venom showed a promising antioxidant, and anti-inflammatory which was higher than the impact of mastopran levels. Besides, the capacity

of a strong anticancer medication to destroy cancer cells while having little effect on healthy cells is one of its key characteristics. The majority of cancer-related deaths could be caused by metastases and the ensuing multiorgan failure. It's interesting to see that Hep-G2 was suppressed by V. orientalis larval extracts with minimal effects on Vero cells.

(El-Garawani *et al.,* 2020) found that the hemolymph of *M. domestica* larvae had reduced levels of MDA, the lipoperoxidation marker, and greater levels of oxidative enzymes, all of which had an antioxidant impact. Insect larval extracts from blowflies and beetles have also been shown in a number of other investigations to have antioxidant properties. It is noteworthy that the aqueous extract's enhanced anticancer capability was linked to a higher antioxidant status. This association may be explained by the defense components present in larvae. Accordingly, antioxidants can cause cancer cells to undergo apoptosis and prevent carcinogenesis and development. Larval preparations of *V. orientalis* have been shown to have a beneficial effect against cancer and disorders dependent on oxidative stress, as evidenced by their inhibition of excessive Reactive oxygen species generation and stimulation of antioxidant enzymes (Elgazar *et al.,* 2018).

Accelerated impairment of cognition and impairment of essential processes are hallmarks of Alzheimer's disease (AD), a debilitating and ultimately deadly neurodegenerative illness (Scheltens *et al.,* 2021). More than 50 million people are impacted by AD, which is the fifth greatest reason for mortality globally. AD places a significant economic and psychological burden on communities and medical systems (Hu *et al.,* 2022). The present study reported the role of both *V. orientalis* venom and mastopran as active compounds showing *in vitro* anti-Alzheimer impact where *V. orientalis* extract has a higher value than mastopran. Collectively, the current research investigation proved the multipurpose capabilities of both mastopran and *V. orientalis* venom as a first step towards investigating fresh compounds from sources found in nature that may be used in animal experiments to validate laboratory findings.

Declarations:

Ethical Approval: This research paper was approved by the research ethics committee from the Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2024/1/3).

Competing interests: The authors have no competing interests to declare that are relevant to the content of this article.

Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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