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RESEARCH ARTICLE
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Effect of *Ocimum sanctum* Extract in Controlling Life Stages of *Callosobruchus chinensis* L.: An In-silico Approach

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ABSTRACT

In silico studies based on the targeted effect of bioactive phytochemicals allows the preparation of a more precise and less time-consuming experimental setup. *Ocimum sanctum* is an aromatic plant species whose secondary metabolites and essential oils are known for their therapeutic effects against a large number of human health disorders. It is also known to possess insecticidal properties. The targeted inhibition of biologically significant enzymes within insect bodies using plant-derived phytochemicals from *O. sanctum* presents a promising approach for controlling the life cycle of target insects. Assessment of in-silico interactions between these phytochemicals and key enzymes such as glutathione S-transferase and alpha-amylase reveals their efficacy in biologically controlling pest species *Callosobruchus chinensis*. The results of the *in silico* analysis are further corroborated with direct toxicity tests using methanolic extracts of *O. sanctum* targeted at specific stages of the life cycle of the target pest. Based on the result obtained from the present study 6.16% concentration of *O. sanctum* is found to be LC_{50} for adult stage and 3.9% concentration of *O. sanctum* is found to be LC_{50} for egg stage of *C. chinensis*. The results affirm that *O. sanctum* extracts can be used in the biocontrol of *C. chinensis*, a stored grain pest.

Keywords: Biocontrol, *Callosobruchus chinensis, in silico, Ocimum sanctum,* Phytochemicals

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INTRODUCTION

Food security poses a significant global challenge, particularly given the world's ever-growing population and the concurrent global pandemic. Any instance of food loss can be considered disastrous in the context of food security. Infestation by insect pests contributes to both quantitative and qualitative losses in stored food products, thereby affecting their nutritional value (Stathers et al., 2020). *Callosobruchus chinensis* is a significant stored grain pest primarily affecting legumes, particularly in tropical regions of Asia (Olajire et al., 2011). The life cycle of the pest begins with the insects laying their eggs on the surface of legume pods. When the eggs hatch, the larvae bore inside the pods, where they complete their life cycle. As they develop, the larvae consume the grains within the pods, causing significant damage. The mature larvae eventually emerge as adults, completing the cycle and further contributing to grain destruction (Varma and Anadi, 2010). The infestation of *C. chinensis* renders the entire affected produce valueless, leading to substantial economic losses for farmers. To mitigate the damage caused by these insects, farmers employ various control measures, including traditional methods and chemical pesticides. However, in the current context, the indiscriminate use of pesticides has rendered these practices either ineffective or hazardous to human health and the environment (Mahmood et al., 2016; Lee and Choi, 2020). This situation has led to the search for more environmentally friendly alternatives to the chemical pesticides. A study by Kalpna et al. (2022) on the management of *Callosobruchus* sp. also emphasizes on the implementation of modern techniques like freezing and heating, use of resistant varieties, botanical extracts, transgenic approach, radiation treatments etc. for the control of this major stored grain pest.

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Ocimum sanctum is a perennial herb with a wide range of pharmacological properties (Mahajan et al., 2013). It has been used in both Ayurveda and Traditional Chinese Medicine for centauries for its anticancer, anti-inflammatory, analgesic, immunomodulatory, gastro protective, antimicrobial, antioxidant and antidiabetic properties (Pattanayak et al., 2010; Asala et al. 2022; Farooq et al. 2022). The plant also finds uses as an effective larvicide (Anees, 2008) as well as pesticide (Žabka et al., 2021). In this study, *O. sanctum* leaves are investigated as a potential agent for controlling *C. chinensis*. The study mainly focuses on two stages of the pest's life cycle i.e. adult and egg stage, as the larval and pupal stages are completed within the seed grain and topical application method cannot be effective in controlling those two stages.

The experiment begins with an *in-silico* study that allows a precise and time efficient preliminary understanding of the effect of the plant phytochemicals on the target pest. Two crucial enzymes of the pest were focused on. Glutathione S transferase, this enzyme plays a crucial role in facilitating the metabolic detoxification of foreign compounds in insects, making it a significant target for investigation (Eaton and Bammler, 1999). Inhibiting the Glutathione S-transferase enzyme would disrupt the pathway that enables the insect to detoxify a variety of external toxins. Alpha amylase on the other hand is a digestive enzyme that catalyzes the first step in the conversion of starch to maltose (Da Lage, 2018) thus playing an important role in the digestive physiology of the insect. Blocking this enzyme would lead to severe metabolic disruptions in the insect's digestive system. The targeted deactivation of the two enzymes using in-silico methods demonstrates the potential of the test extract as a biocontrol agent against the pest species. To validate the findings from the in-silico analyses, direct toxicity assays were conducted using methanolic extracts derived from *O. sanctum* leaves. Integrating the results from both methodologies was undertaken to authenticate the effectiveness of *O. sanctum* leaf extracts in the biological control of the stored grain pest *C. chinensis*.

MATERIALS AND METHODS

In silico Analysis

Preparation of Receptors and Ligands

The protein structures of the targeted enzymes, Glutathione S-transferase (1pn9) and Alpha-amylase (1viw), were retrieved from the Research Collaborators for Structural Bioinformatics Protein Data Bank (RCBS PDB). This repository provides three-dimensional structural data of large biological molecules, encompassing proteins and nucleic acids (Parasuraman, 2012). Ligands were selected based on available literature, and a total of 23 ligands were chosen for the study. The structures of these ligands were acquired from the ZINC database, which serves as a repository of commercially available compounds specifically designed for virtual screening (Irwin and Shoichet, 2005).

Molecular Docking

Molecular docking analyses were conducted employing Molegro Virtual Docker (MVD 2010.4.0) for Windows, a software tool utilized for identifying potential binding sites within receptor structures and predicting the primary binding orientations within the three-dimensional structure (Thomsen and Christensen, 2006). The receptor and ligand structures were imported into the software interface, and the docking wizard was executed. Docking scores generated by the software were tabulated, and the most favorable interactions were subsequently chosen for further examination.

Visualization

The visualization of receptor-ligand interactions was conducted utilizing Discovery Studio Visualizer 2021 Client software. The three-dimensional representation of receptor-ligand interactions derived from the molecular docking experiments facilitated the precise localization of ligand-binding sites within the target enzyme. Additionally, two-dimensional visualization enabled the examination of ligand interactions with specific amino acids of the target enzymes (Biovia, 2021).

Direct Toxicity Test

Collection and Rearing of Insects

The *C. chinensis* stock culture was cultured on *Vigna radiata* seeds and maintained under controlled conditions of room temperature (18-32°C) and relative humidity (70±5%). The insects were housed in plastic containers (12×8cm) covered with muslin cloth to facilitate proper ventilation and containment.

Preparation of Plant Extract

Ocimum sanctum leaves were collected, washed, and air-dried at ambient temperature. Subsequently, the dried leaves were pulverized mechanically using a grinder to obtain a fine powder. Methanolic extraction was carried out using a Soxhlet apparatus, followed by concentration of the extract using a rotary evaporator. The resulting crude extract was stored in hermetically sealed glass vials. Preliminary phytochemical analysis was conducted to identify the presence of alkaloids, glycosides, terpenoids, saponins, flavonoids, phytosterols, phenolic compounds, etc., in accordance with the methodology outlined by Kokate (1994).

Toxicity Test

The insects were anesthetized and subsequently exposed to escalating concentrations of the plant extract (2, 4, 6, 8, 10, and 12%) dissolved in acetone as the solvent, as per the method described by Odebiyi and Sofowora (1978). A volume of 20µL of each solution was applied to the dorsal surface of individual insects using a micropipette. Following treatment, the insects were maintained under identical culture conditions. Three sets of replicates, along with a control group treated with acetone alone, were established. Insect mortalities were monitored daily and recorded at 24-, 48-, and 72-hours post-treatment (HAT), following the protocol outlined by Jatsch and Ruther (2021).

Evaluation of Additional Parameters

The seeds were treated with varying concentrations of plant extract, and batches of 5g of treated seeds were allocated to individual mating chambers. Each chamber was then populated with a newly emerged pair of insects to facilitate mating. Three replicates, along with a control group and a positive control, were maintained for each treatment condition. To assess the impact of the plant extract on oviposition, adult insects were removed from the chambers after 8 days, and the number of eggs laid was quantified, following the protocol outlined by Zafar et al. (2018). To evaluate the effect on the emergence of adult insects, the mating chambers containing the eggs were left undisturbed for 15-20 days. Then the % of adult emergence is calculated using following formula:

$$\%\ \text{of adult emergence} = \frac{\text{Number of adults emerged}}{\text{Number of eggs laid}} \times 100$$

Data Analysis

Probit analysis was conducted utilizing the Henry simplified table, following the methodology outlined in Kalita (2016). The preparation of tables, graphs, and measurement of standard deviation (SD) were performed using Microsoft Excel, 2016.

RESULTS AND DISCUSSION

To evaluate the efficacy of *O. sanctum* for the control of different life stages of *C. chinensis*, the present investigation has been carried out in two stages. First, *in-silico* technique was used to analyze the effect of bioactive phytochemicals present in *O. sanctum* on the target insect which was then followed by direct toxicity tests based on different parameters to obtain a comprehensive result. For the computational analysis, two enzymes from the insect were chosen: Glutathione S Transferase and Alpha amylase. The impact of the chosen phytochemicals on these enzymes was subsequently examined through structure-based virtual screening and molecular docking methodologies. The docking software provides a re-rank score on the basis of the energy of receptor-ligand interactions. The negative re-rank scores thus obtained signify stable interactions between the selected molecules. The results when tabulated showed that all 23 selected ligands show interactions with the target receptors. Among the selected ligands Luteolin with a minimum re-rank score of -77.87±5.40 shows highest level of interaction with Alpha amylase (Fig. 1). On the other hand, Escolin with a minimum re-rank score of -78.00±12.46 shows the highest level of interaction with Glutathione S Transferase (Fig. 2). 2D and 3D pictorial data also show the significant interactions between the molecules. The interactions between the ligands and the amino acids of target enzymes, including Van der Waals forces, hydrogen bonds, C-H bonds, and non-covalent bonds such as Pi-Alkyl, Pi-pi T shaped, and Pi-Sulfur bonds, were visualized using computer-generated data. (Fig. 3). Various researchers have employed an in-silico approach to investigate the capacity of plant extracts in inhibiting enzyme activity. Inhibition of alpha amylase activity by an allergenic protein Ric C1 & Ric C3 from *Ricinus communis* was reported by Nascimento et al. (2011). Ghaffar et al. (2020) also revealed the inhibitory effect of quercetin, paraoxon and tetraethyl pyrophosphate on enzyme CYP450 isoenzyme of *Tribolium casteneum*. Another study by Gupta et al. (2023) on insecticidal and detoxification activity of *Acorus calamus* and *Lavandula angustifoli* revealed that these plant extracts have the potential to inhibit Glutathione S Transferase activity of *C. chinensis*. The findings from the in-silico analysis indicate that the chosen ligands possess the ability to interact with the target enzymes, thereby disrupting their normal biological functions. This suggests that the phytochemicals derived from *O. sanctum* hold promise as potential insecticides against the target pest *C. chinensis*.

To corroborate the findings from the in-silico analysis, direct toxicity assessments were conducted in a laboratory setting. These assessments involved the use of methanolic extract derived from *O. sanctum*. The tests were performed against both adults and eggs of *Callosobruchus chinensis*. The results of direct mortality test show a dose-dependent response with a notable value of significance over the control at all used concentrations (Fig. 4 and 5). Probit analysis provides an LC50 value of 6.16% and 3.9% against adult and egg stages respectively (Table 1 and 2). Similar work by Ekeh et al. (2013) reveals 83% mortality of *Callosobruchus sp*. when treated with *O. gratissmum*. Dose dependent adult mortality was reported by Bindu et al. (2005) Murasing et al. (2018) also reports that the LC_{50} value for petroleum ether extract of *Leucas lavandufolia* was found to be 2.3% at 72 HAT. Bincy et al. (2023) revealed that the chemical constituent estragole derived from *O. Basilicum* shows 100% mortality of *C. chinensis*. Pipariya et al. (2022) also found the essential Oil *Mentha arvensis* show insecticidal activity against *C. chinensis*.

Table 1: Mortality of different life stages of *C. chinensis* when treated with different concentrations of selected plant extract

Sl. No.	Stage of life cycle	Plant Extracts	Total no. of Dead Insects					
			Doses					
			2%	4%	6%	8%	10%	12%
1	Adult	*Ocimum sanctum*	3.33±1.15	6±1	8±2	11.33±0.57	13.66±0.57	16.66±1.15
		Control	0±0	0±0	0±0	0.33±0.57	0±0	0.33±0.57
2	Egg	*Ocimum sanctum*	7.33±0.57	10±1	12.66±0.57	12.66±2.51	16.66±0.57	18.66±0.57
		Control	0±0	0±0	0±0	0.33±0.57	0±0	0.33±0.57

Table 2: Data of Probit analysis for LC_{50} determination (adult and egg stage)

Sl. No	Stage of life cycle	Plant extract	Regression Equation	R-square value	Significance F value in regression	LC_{50} (% Conc.)
1	Adult	*Ocimum sanctum*	y=2.41x+3.08	0.9428	0.001249135	6.16
2	Egg	*Ocimum sanctum*	y=2x+3.8	0.8258	0.012114341	3.9

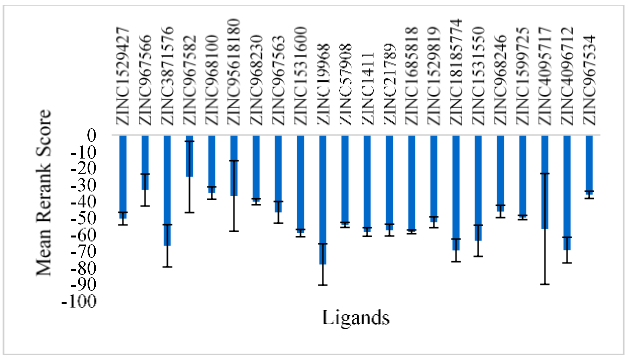

Fig. 1: Graph showing rerank scores of ligands of *Ocimum sanctum* against Alpha amylase.

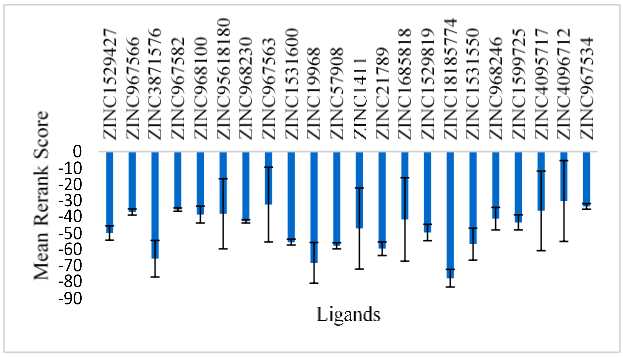

Fig. 2: Graph showing rerank scores of ligands of *Ocimum sanctum* against Glutathione S Transferase.

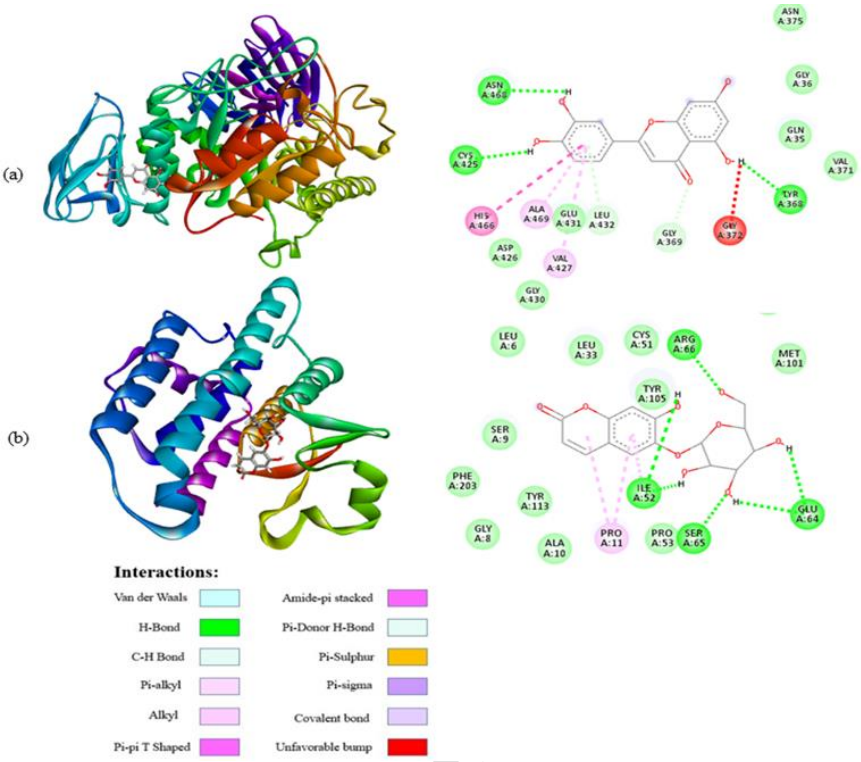

Fig. 3: 3D & 2D visualization of ligands interaction with the Glutathione S Transferase at potential ligand binding cavity: (a) Luteolin with alpha amylase, (b) Escolin with Glutathione S Transferase.

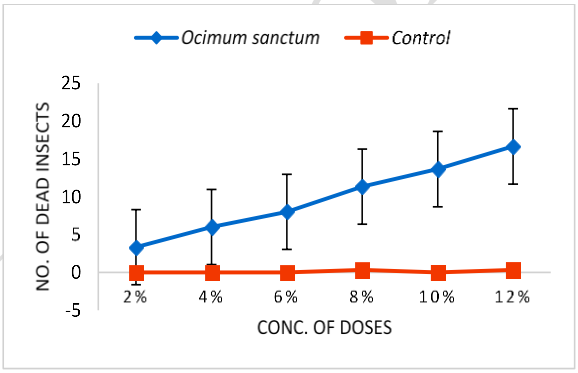

Fig. 4: Graph showing mortality of adult *C. chinensis* when treated with test extract.

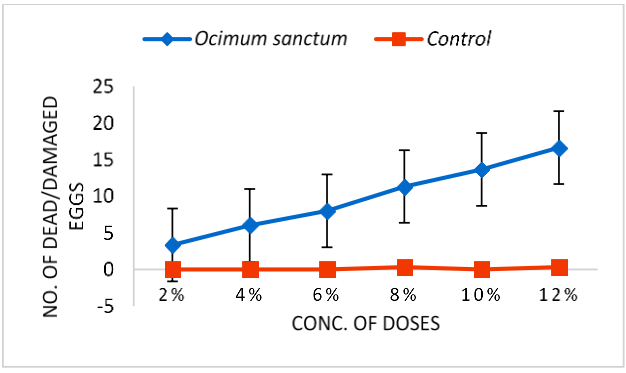

Fig 5: Graph showing mortality of eggs of *C. chinensis* when treated with test extract.

Further, evaluations were conducted on additional parameters including the oviposition potential and the emergence of adults from deposited eggs. The results show that treatment with test extracts shows significant effect in controlling oviposition as well as emergence of insects in the test group as indicated in the graph (Fig. 6; 7).

The results showed an overall oviposition deterrence at 23.88±8.62 which is significant over the control (66.94±1.8) (Table 3). The current results align with those reported by Abd el-Salam (2010). His works show 100% oviposition deterrence when treated with *O. basilicum*. Singh and Chaudhary (2011) also reported that

Table 3: Effect of *O. sanctum* in controlling different parameters of life cycle of *C. chinensis* when treated with different concentrations of selected plant extract

S. No.	Parameters	Plant extracts	Doses					
			2%	4%	6%	8%	10%	12%
1	Oviposition	*Ocimum sanctum*	29±9.16	34±4.58	31.33±4.04	19.66±2.08	14.33±4.04	15±3
		Control	69±1	67±5	67.33±4.72	66.66±5.50	66±7	65.66±6.11
2	Emergence of adult	*Ocimum sanctum*	23.33±4.04	17.66±5.13	17±2.64	14.33±2.08	7.66±2.30	7.33±2.08
		Control	52.33±3.05	58.66±3.51	54.33±3.51	47.33±3.21	45±2.64	53±5.56

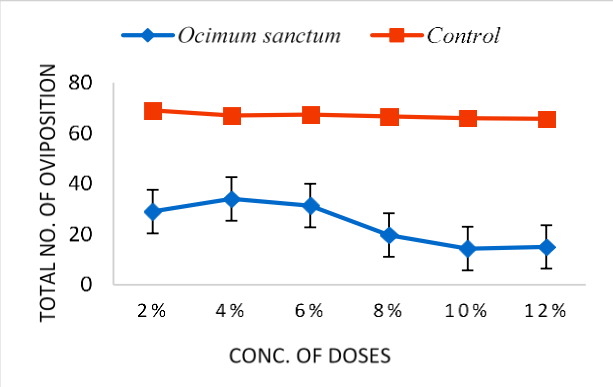

Fig. 6: Graph showing effect of test extract on oviposition of *C. chinensis*.

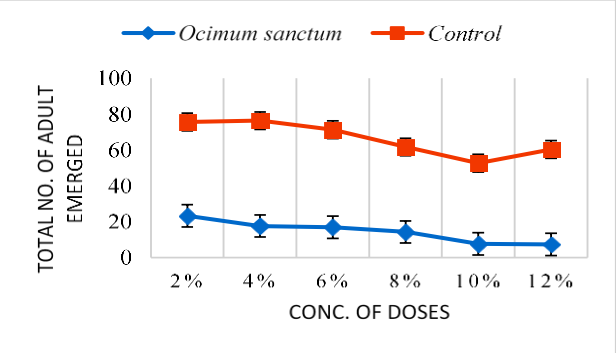

Fig. 7: Graph showing effect of test extract on emergence of adults in *C. chinensis*.

O. sanctum showed 53.77% oviposition deterrence of *C. chinensis* at 1gm/100gm concentration. Another study by Herald and Tayde (2023) revealed significant oviposition deterrent by Castor, Neem and Pungam oil against *C. chinensis*. Rawat (2023) also revealed the oviposition activity of *Prosopis cineraria* extracts against *C. chinensis*. The overall effect on emergence of adult on treated group is 14.55±6.20 and is significant over the control group (51.77±4.92) (Table 3). The present finding is closely similar to the work of several other authors. Reduction in adult emergence when treated with *O. sanctum* was also reported by Kalita (2016). Similar work by Yankanchi and Lendi (2009) reveals 100% F_1 emergence when treated with *Pongamia pinnata*. Another study by Vindhya et al. (2022) also revealed the effectiveness of *O. sanctum*, *Murraya koenigii*, *Pongamia pinnata* and *Syzygium aromaticum* on the emergence of adult *C. chinensis*.

So, it is evident that the test extract not only affects the mortality of the insect species but also effects the oviposition capacity of the adults and emergence of new larvae from the eggs. Thus, from the results of both *in silico* studies and laboratory tests, it can be confirmed that *O. sanctum* leaf extract can be used in the biological control of the pest species *C. chinensis*.

Conclusion

The current study demonstrates the potent insecticidal properties of *Ocimum sanctum* leaf extracts against *Callosobruchus chinensis*, as evidenced by both in-silico analysis and direct toxicity testing. Additionally, the application of these extracts notably impacted the oviposition and emergence of adults within the target species. In silico identification helps in reducing down the potential lead compounds from a diverse range of compound databases to pick prospective matches by using molecular docking techniques, thus helping in optimization of bioactivity of the phytochemicals of the plant. It reduces the uncertainty on the outcomes, resulting in a faster and more efficient development of the pesticide. Thus, *O. sanctum* can be effectively used as an alternative plant resource in the natural control of *C. chinensis*.

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Conflict of Interest

The authors declare there is no conflict of interest.

Ethical Approval: Ethical approval for the work on insect pest is not required.

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Data Availability Statement: The primary data is available with the corresponding author and can be made available on reasonable request.

Author Contributions: This work was carried out in collaboration among all the authors. Author Suraj Chetri was involved in designing the study and involved in in-silico analysis. Author Dipanjan Dey and Lupamudra Borah did the wet lab experiments. Sansaka Sekhar Ghosh and Suraj Chetri analyzed the data, finalized the results and prepared the draft of the manuscript. All authors read and approved the final manuscript.

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