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The Protective Effects of Vernonia Amygdalina, Ocimum Gratissimum and Talinum Triangulare on Cardiac Toxicity in Dichlorvos-Treated Wistar Rats

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Abstract

This research assessed the efficacy of Vernonia amygdalina, Ocimum gratissimum, and Talinum triangulare extracts in ameliorating dichlorvos (DDVP)-induced cardiotoxicity. Fifty adult male Wistar rats (272 g) were distributed into ten groups (n=5), including negative and positive controls (saline and 8.0 mg/kg DDVP, respectively), and treatment groups co-administered DDVP with either plant extracts (200 or 400 mg/kg) or vitamin C (20 or 40 mg/kg) for 28 days. DDVP intoxication significantly increased ($p < 0.05$) cardiac injury biomarkers (LDH and CK). However, all three plant extracts significantly attenuated this rise, demonstrating a dose-dependent protective effect. Histological analysis of cardiac tissue confirmed the biochemical evidence of cardioprotection. These findings indicate that the tested botanicals hold promise as therapeutic agents against pesticide-induced myocardial damage

KEYWORDS: DDVP, cardiac toxicity, *Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare*, LDH, Cardioprotective.

Introduction

Organophosphate pesticides, like dichlorvos (2,2-dichlorovinyl dimethyl phosphate), are widely used in agriculture and domestic pest control due to their effectiveness and affordability (Sankhla et al., 2024). However, their toxicological effects on non-target organisms, including humans and animals, remain a major concern. Dichlorvos exerts its toxicity primarily through the irreversible

inhibition of acetylcholinesterase, leading to excessive accumulation of acetylcholine at synapses and neuromuscular junctions (Žnidaršič et al., 2023; Jiang et al., 2024). Beyond neurotoxicity, chronic exposure has been associated with oxidative stress, lipid peroxidation, and cardiovascular complications including arrhythmias, myocardial injury, and impaired cardiac function (Salem et al., 2023). These deleterious effects necessitate the search for natural protective agents that can mitigate pesticide-induced cardiotoxicity.

Medicinal plants remain a rich source of bioactive compounds with therapeutic potential against oxidative and inflammatory damage (Narayanankutty et al., 2024). Vernonia amygdalina, commonly known as bitter leaf, is widely consumed in Africa both as food and medicine. It is rich in phytochemicals such as flavonoids, alkaloids, saponins, and terpenoids that have been reported to exhibit antioxidant, anti-inflammatory, and cardioprotective effects (Pol et al.,2024). Similarly, Ocimum gratissimum (scent leaf), a culinary and medicinal herb, contains essential oils, phenolic compounds, and other bioactives that demonstrate free radical scavenging, antihypertensive, and cardioprotective properties (Zahran et al., 2020; Ezeorba et al., 2024). Talinum triangulare (waterleaf), another widely consumed vegetable, is abundant in vitamins, minerals, and polyphenols that have been linked to antioxidant, anti-lipid peroxidation, and cytoprotective activities (Barman et al., 2024; Oladele et al., 2024)

The combined use of these plants may provide protection against xenobiotic-induced toxicity by enhancing endogenous antioxidant defenses, improving lipid metabolism, and preserving cellular integrity. Previous studies have shown that dietary antioxidants from medicinal plants can ameliorate pesticide-induced oxidative stress and organ damage in experimental animals (Sajad et al., 2024; Taychaworaditsakul, et al., 2024). However, there is limited information on the cardioprotective potential of *Vernonia amygdalina*, *Ocimum gratissimum*, and *Talinum triangulare* against cardiotoxicity resulting from dichlorvos exposure.

Therefore, this study aims to investigate the protective potentials of *Vernonia amygdalina*, *Ocimum gratissimum,* and *Talinum triangulare* on cardiac toxicity in dichlorvos-treated Wistar rats. The findings will provide insight into the possible use of these plants as dietary or therapeutic interventions for pesticide-induced cardiovascular damage.

Materials and Method

Plant Materials and Preparation of Extracts

Fresh leaves of Vernonia amygdalina (V.A), Ocimum gratissimum (O.G), and Talinum triangulare (T.T) were procured from Amassoma in Bayelsa State, Nigeria. The plant species were taxonomically identified by Prof. Kola Ajibesin (Department of Pharmacognosy, Niger Delta University). The leaves were destemmed, washed with distilled water, and air-dried at room temperature with periodic turning to ensure uniform drying and prevent mold. Drying times varied by species, with T. triangulare requiring 33 days and V. amygdalina and O. gratissimum drying within 13 days. The desiccated leaves were milled into a fine powder. Aqueous extraction was conducted by steeping 250 g of each powder in 1 L of distilled water for 72 hours. The macerates were filtered and the filtrates were concentrated via rotary evaporation. The resulting crude extracts were freeze-dried (lyophilized) and stored at 4°C for subsequent analysis.

Experimental Animals

Fifty mature male Wistar rats (mean weight: 272 g) were sourced from the University of Port Harcourt's animal breeding unit. Prior to experimentation, the rats underwent a 14-day acclimatization period in well-ventilated cages at the Niger Delta University's Department of Pharmacology. The animals were fed a standard laboratory diet and had continuous access to clean drinking water. All protocols were conducted in strict compliance with relevant animal ethics regulations.

Chemicals and Reagents

The organophosphate compound dichlorvos (DDVP) were obtained in its technical grade from a local supplier in Bayelsa State, Nigeria. The product, branded as Sniper, is specified to contain 100 g/L of the active ingredient 2,2-dichlorovinyl dimethyl phosphate with 100% purity. For biochemical analyses, Randox™ (Crumlin, UK) assay kits were used to determine the enzymatic activity of lactate dehydrogenase (LDH) and creatine kinase (CK

Experimental Procedure

Fifty adult-male Wistar rats were randomly allocated into 10 experimental groups (n=5 per group) and subjected to the following treatments for 28 days:

1. Group 1 (Normal Control): Administered 1 mL/kg body weight of normal saline.
2. **Group 2 (Positive Control):** Received 8.0 mg/kg body weight of dichlorvos (DDVP) alone.
3. **Group 3:** Treated with 8.0 mg/kg DDVP + 200 mg/kg *Vernonia amygdalina* extract.
4. **Group 4:** Treated with 8.0 mg/kg DDVP + 400 mg/kg *Vernonia amygdalina* extract.
5. **Group 5:** Treated with 8.0 mg/kg DDVP + 200 mg/kg *Ocimum gratissimum* extract.
6. **Group 6:** Treated with 8.0 mg/kg DDVP + 400 mg/kg *Ocimum gratissimum* extract.
7. **Group 7:** Treated with 8.0 mg/kg DDVP + 200 mg/kg *Talinum triangulare* extract.
8. **Group 8:** Treated with 8.0 mg/kg DDVP + 400 mg/kg *Talinum triangulare* extract.
9. **Group 9** (Standard Control 1): Received 8.0 mg/kg DDVP + 20 mg/kg Vitamin C.
10. **Group 10** (Standard Control 2): Received 8.0 mg/kg DDVP + 40 mg/kg Vitamin C.

All treatments were administered orally, with dosages determined based on prior LD_{50} studies of the respective plant extracts.

Blood samples were collected from the submandibular vein on days 0, 1, 7, 14, 21 and 28 for biochemical analysis. On day 28, all rats were euthanized under isoflurane anesthesia and heart tissue samples were excised for histological examination.

Ethical Approval

This was approved by and monitored by The Research Ethical Committee of the College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State.

The Research Ethical Committee of the College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, approved all experimental protocols, ensuring compliance with institutional ethical standards.

Biochemical Assays:

Cardiac function biomarkers were quantified using Randox™ biochemical assay kits, following the manufacturer's protocols.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA), with post-hoc comparisons performed using Tukey's test (SPSS Version 20, IBM, USA). Results were expressed as mean ± standard error of the mean (mean ± SEM. A probability value of $p < 0.05$ was considered statistically significant.

Results

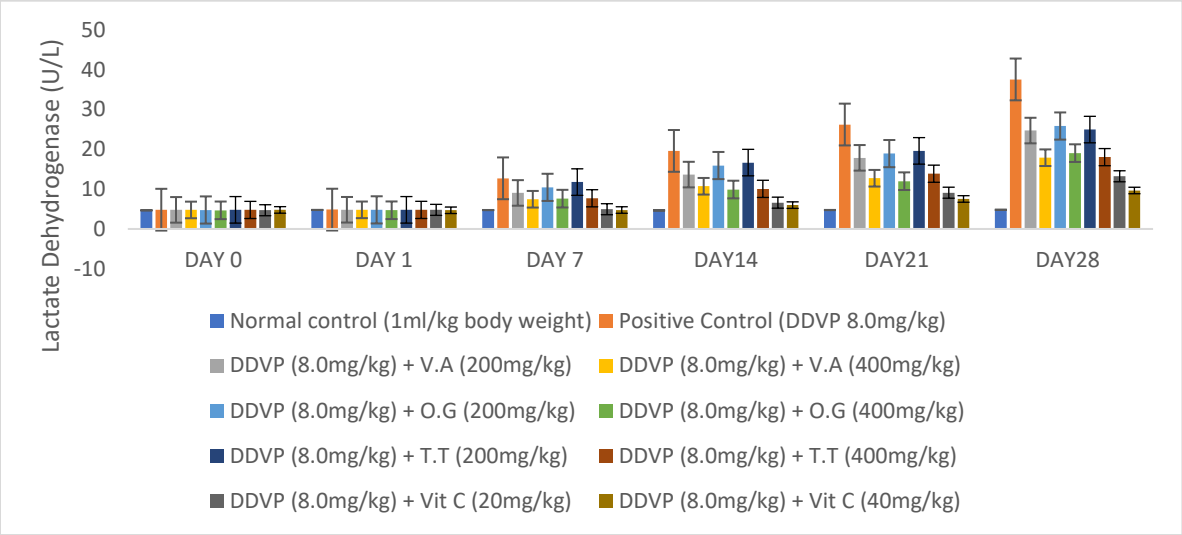


Fig. 1 LDH activity following 28-day DDVP exposure (8.0 mg/kg) and treatment with plant extracts or Vitamin C. Data are mean ± SEM. $p < 0.05$ vs. DDVP-only group.

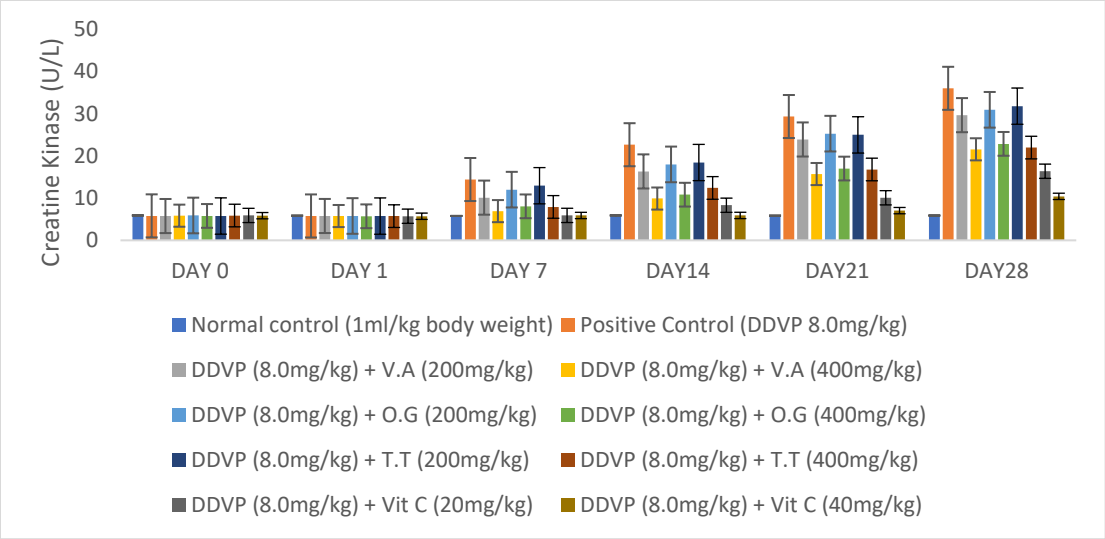
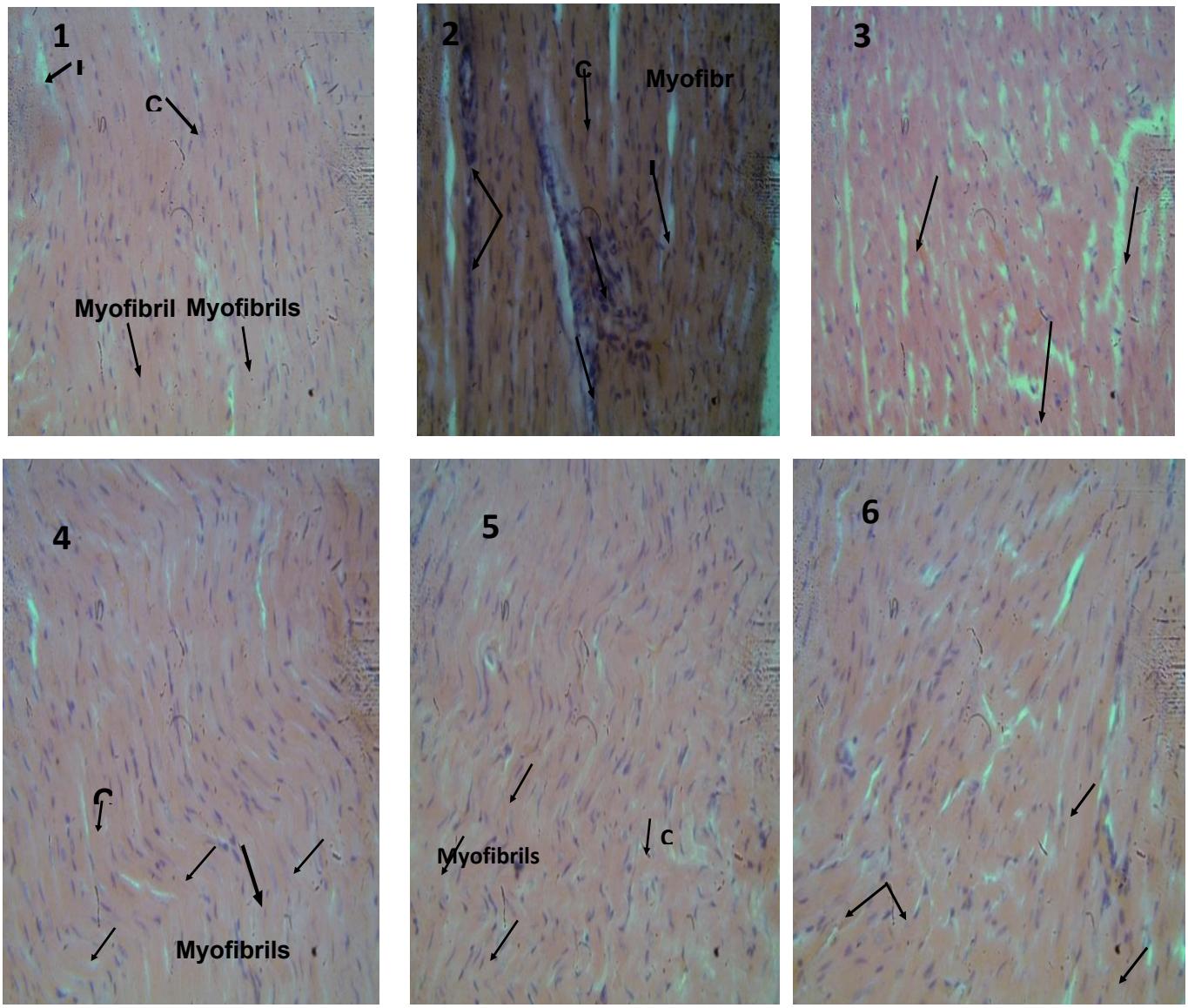


Fig. 2. CK activity following 28-day DDVP exposure (8.0 mg/kg) and treatment with plant extracts or Vitamin C. Data are mean ± SEM. $p < 0.05$ vs. DDVP-only group.

Histological examination (Heart Photomicrographs)



Histopathological Analysis of Cardiac Tissue Following Dichlorvos Exposure and Treatment

Plate 1 (Negative Control): The myocardium exhibits a normal histological architecture at 400x magnification (H&E). Striated cardiac myocytes (CM) are arranged in characteristic spiral bundles, with clearly defined interstitium (IS) and myofibrils. **Diagnosis:** Normal myocardial tissue. **Plate 2 (Positive Control - 8.0 mg/kg DDVP):** Marked histopathological alterations are evident. The cardiac tissue displays focal areas of inflammatory cell infiltration (arrows) and significant disruption of the interstitial matrix. **Diagnosis:** Acute inflammation and structural disruption of the myocardium. **Plate 3 (DDVP + *V. amygdalina* [400 mg/kg]):** The tissue architecture shows notable improvement compared to the positive control. A mild dilatation of the interstitium is present, but inflammatory cell activity is substantially reduced (arrows). **Diagnosis:** Near-normal tissue with mild interstitial dilation. The extract demonstrates a strong protective effect, significantly attenuating DDVP-induced cardiotoxicity. **Plate 4 (DDVP**

+ *O. gratissimum* [400 mg/kg]): The myocardium displays a wavy appearance. Cardiac myocytes (CM) and interstitium (IS) are visible, though mild lymphocytic activity is observed (arrows). **Diagnosis:** Mild, persistent lymphocytic infiltration. **Plate 5 (DDVP + *T. triangulare* [400 mg/kg]):** The myocardial structure appears largely normal with a wavy pattern. Cardiac myocytes (CM) are well-integrated within the interstitium (IS), and a significant reduction in lymphocytic activity is noted (arrows). **Diagnosis:** Marked decrease in inflammatory lymphocytic activity, indicating a therapeutic effect. **Plate 6 (DDVP + Vitamin C [40 mg/kg]):** The section reveals minimal edema and minor mononuclear cell activity. The arrangement of cardiac myocytes (CM) within the interstitium (IS) is largely preserved (arrows). **Diagnosis:** Minimal lymphocytic aggregation, confirming the reference antioxidant's protective efficacy.

Discussion

The cardioprotective properties of *Vernonia amygdalina* (V.A.), *Ocimum gratissimum* (O.G.), *Talinum triangulare* (T.T.) were assessed against dichlorvos (DDVP)-induced cardiac injury in male Wistar rats, with a focus on serum lactate dehydrogenase (LDH) and creatine kinase (CK) activities as biomarkers of cardiac injury.

Findings from this study revealed that DDVP exposure significantly elevated serum LDH and CK activities when compared with the untreated control group. This elevation suggests that DDVP exerts cardiotoxic effects, likely through oxidative stress and membrane destabilization, leading to leakage of these enzymes into the circulation (Saka et al., 2024). Both LDH and CK are well-established markers of myocardial damage; their increased activities indicate compromised cardiac cell membrane integrity and enzyme efflux into the serum (Thirumalasetty, 2024); Netala, et al., 2025). Organophosphates such as DDVP are known to induce oxidative stress by generating reactive oxygen species (ROS), impairing antioxidant defense mechanisms, and disrupting mitochondrial function, thereby predisposing cardiac tissues to injury (Farkhondeh et al., 2020; Zhang et al., 2025).

However, treatment with aqueous leaf extracts of V.A., O.G., T.T., and vitamin C significantly reduced LDH and CK activities in a dose-dependent manner compared with the positive control. This suggests that the extracts possess cardioprotective properties capable of mitigating DDVP-induced myocardial damage. The dose-dependent reduction indicates that higher concentrations of the extracts confer stronger protective effects, possibly due to enhanced bioavailability of phytochemicals and antioxidants.

The cardioprotective effects observed may be attributed to the phytochemical constituents of the plants. *Vernonia amygdalina* is rich in flavonoids, saponins, and phenolic compounds that exhibit antioxidant and membrane-stabilizing effects (Gildas et al., 2024; Oloruntola et al., 2025). Similarly, *Ocimum gratissimum* contains essential oils (eugenol, thymol), polyphenols, and terpenoids with strong free-radical scavenging and anti-inflammatory activities (Chatterjee and Sarkar, 2025; Udi et al., 2025). *Talinum triangulare*, widely consumed as a leafy vegetable, is also rich in vitamins, flavonoids, and minerals that contribute to its antioxidative capacity (Sridhar and Pavithra, 2021). Collectively, these phytochemicals may reduce oxidative damage, prevent lipid peroxidation of cardiac membranes, and preserve enzyme localization within cardiac cells.

Vitamin C, a known antioxidant, also significantly lowered LDH and CK activities. Its efficacy is consistent with its established role as a scavenger of ROS, regenerating other antioxidants and protecting cellular membranes against oxidative insult (Kükürt and Gelen, 2024). Interestingly,

the extracts exhibited comparable protective effects to vitamin C, highlighting their potential as natural alternatives or adjuncts in mitigating pesticide-induced cardiotoxicity.

These results are in agreement with earlier studies where plant extracts ameliorated pesticide-induced biochemical alterations. For instance, *Vernonia amygdalina* was shown to reduce cardiac enzyme activities in doxorubicin-induced rats (Syahputra et al., 2021), while *Ocimum gratissimum* demonstrated cardioprotective effects in streptozotocin-induced diabetes in rats (Ajiboye et al., 2024). Similarly, *Talinum triangulare* has been reported to enhance antioxidant enzyme activities and reduce lipid peroxidation in toxin-exposed models (Inwang et al., 2024). These findings corroborate the current study, suggesting that the protective effects of these plants may involve a combination of antioxidant, anti-inflammatory, and membrane-stabilizing mechanisms.

The histopathological findings indicate that DDVP causes significant inflammatory and structural alterations in the myocardium, but co-treatment with medicinal plants and Vitamin C mitigated these effects to varying degrees. Among the interventions, *V. amygdalina* and *T. triangulare* appeared most effective in preserving myocardial integrity, while *O. gratissimum* and Vitamin C provided moderate protection. These results are consistent with previous studies reporting the cardioprotective and antioxidant roles of medicinal plants in organophosphate-induced toxicity (Saka et al., 2025).

Overall, the dose-dependent reduction in LDH and CK activities following treatment with the extracts and vitamin C underscores their therapeutic relevance in protecting against pesticide-induced cardiac injury. The findings indicate that supplementation with these plant extracts could serve as a natural intervention strategy in populations exposed to organophosphate pesticides, thereby reducing the risk of cardiac damage.

Conclusion

This study showed that dichlorvos (DDVP) exposure causes cardiotoxicity in Wistar rats, marked by elevated LDH and CK levels due to oxidative stress and mitochondrial damage. Pretreatment with *Vernonia amygdalina*, *Ocimum gratissimum*, *Talinum triangulare*, and vitamin C significantly reduced these effects, demonstrating cardioprotective potential. The protective activity may be linked to their phytochemical constituents with antioxidant and anti-inflammatory properties, with efficacy comparable to vitamin C. Histological analysis confirmed that *V. amygdalina* and *T. triangulare* were most effective in preserving heart structure. Overall, these findings suggest that these plant extracts may serve as natural therapeutic or preventive agents against pesticide-induced cardiac injury, though further studies are needed to isolate active compounds and assess clinical relevance.

Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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