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Research Article

Protection of histomorphology of vital organs by methanolic seed extract of *Nigella sativa* against cadmium-induced tissue injuries in rats

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Abstract

Background: Chemical-induced organ injuries have been on a fast rise for decades and these injuries have become common causes of mortality and morbidity in the society. Edible plant materials with medicinal properties have been used for treating various diseases for many centuries in folk medicine. Recently, the role of food or medicinal plants in human health has received considerable attention. Traditional uses of *N. sativa* seed range from soothing wounds to remedying cough, eczema, diabetes, inflammation of the bronchi and tooth aches; and these point to substantial tissue effects.

Objective: We investigated the protective effects of methanolic seed extract of *Nigella sativa* (MENS) against cadmium-induced histomorphological alterations in heart, kidney and liver tissues of albino rats.

Methods: Twenty five (25) male albino rats, weighing (200 \pm 20g), were randomly grouped into five groups: A, B, C, D, and E. Group B (Negative Control) received intraperitoneal administration of cadmium chloride (CdCl₂, 5mg/kg) only, group C received CdCl₂ and low dose MENS (300mg/kg, oral), group D received CdCl₂ and high dose MENS (600mg/kg, oral), and group E (Positive control) received CdCl₂ and Vitamin C (200mg/kg, oral), for 14 days. Group A (Normal control) received no administration. Heart, kidney and liver were harvested for histopathological analyses.

Results: Cadmium (CdCl₂) induced significant histomorphological changes in the studied organs, and the heart was the most damaged of all the organs studied; however a significantly ameliorative effect by methanolic seed extracts was observed.

Conclusion: Nigella sativa seed extract is potentially tissue-protective against harmful chemical toxins like cadmium.

Keywords: anticardiotoxic, cadmium, *Nigella sativa*, medicinal food, antinephrotoxic, ethnopharmacology, antihepatotoxic

1. INTRODUCTION

Nigella sativa (N. sativa) (Family Ranunculaceae), commonly known as black seed or black cumin or seed of blessing in different languages, is a grassy plant, and has green to blue flowers with small black seeds. A few foods of plant origin and plants species have been thoroughly evaluated for their medicinal properties. The medicinal properties of Nigella sativa has been reported to be due to its composition of stable and volatile oils which contain good amounts of unsaturated fatty acids, arachidonic acid and eicosenoic acids in little amount. Traditional uses of N. sativa seed range from soothing wounds to remedying cough, eczema, diabetes, inflammation of the bronchi and tooth aches. One of the active ingredients of N. sativa is thymoquinone, a flavanoid with proven anti-inflammatory, anti-cancerous, anti-bacterial along with anthelmintic properties.

rapid increase in developments, environmental pollutants, heavy metals (e.g. cadmium and lead inclusive), now top the list of toxicants which pose serious risk or threat to human health and wellbeing.

Cadmium is of a great use especially in the utilization of its conductor properties and thus found to be of great importance in the manufacture of batteries and reactors. However, this element is found to be very toxic, even in very little amount and could cause abnormalities in man after exposure.⁵ Cigarette smoking is considered to be the most significant source of human cadmium exposure. When ingested, cadmium accumulates in the body with age and has an extremely long biological half-life.⁶⁻⁸

Although cases of cadmium-induced nephropathy have been described in chronic exposures, recent studies suggest that

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cadmium at acute or low environmental exposures in industrialized countries can also cause subtle renal effects leading to a modest increase in the urinary excretion of low weight micro proteins. Exposure to cadmium could produce serious adverse effects in humans and one of the ways through which this happens is through induction of oxidative stress. Cadmium chloride (CdCl₂), recognized as an experimental toxin, is known to be highly cardiotoxic, 11,12 and may cause severe liver damage. 12

Despite all studies performed to date, therapy choices for cardiac, liver and kidney injuries are very few. Foods or plants with medicinal value have proven to be foremost or firstline therapy choice in the treatment of diseases in most of the developing countries, and they provide important sources of most of the world's pharmaceutical; thus they have served a valuable starting material in drug discovery or drug development.¹³ To date, no study has holistically evaluated the protective effect of black seed extracts against cadmium-induced histomorphological alterations of heart, kidney and liver in rat.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh samples of *Nigella sativa* seeds were obtained from local market in Enugu, Nigeria. The plant material was authenticated by a consultant taxonomist at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, and a voucher specimen was deposited at the herbarium with reference number UNH No 662 for future reference.

2.1.1 Preparation of Methanolic extracts of Nigella sativa (MENS)

Nigella sativa seeds were dried and shaded from sun light, then powdered with a grinder. Extraction was done using Babaei et al.14 method with minor modifications. Five hundred gram (500g) of N.sativa powder was macerated with 2 litres of absolute (100%) methanol (as methanolic extract) for seventy two (72) hours. The mixture was stirred in an Erlenmeyer flask for twenty four (24) hours using a laboratory shaker. At the end of the extraction, the extract was filtered through a Whatman filter (Whatman Clifton, NJ, USA). Finally, using a water bath set at 30°C, the solvent evaporated, and 4g of dried methanolic extracts was obtained. This was reconstituted in distilled water, used to prepare the required concentration, and stored at 4°C until when needed for use.

2.1.2. Acute toxicity test (LD₅₀)

The median lethal dose (LD_{50}) of methanolic extracts of *N.sativa* (MENS) was performed on mice and Lorke procedure of LD_{50} determination was used.¹⁵

2.1.3 Phytochemical analysis of Nigella sativa seeds

Preliminary phytochemical screening of N.sativa was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria. Procedures outlined by Trease and Evans¹⁶ were employed for the analyses.

2.2 Drug and Chemicals used

The chemicals used in the study include analytical grade of absolute methanol, for plant extraction, and Cadmium chloride salt for induction of selected organs toxicity. The chemicals were purchased from Ogbete main market, Enugu.

Drug used includes vitamin C (Alpha Pharmaceuticals, Enugu, Nigeria).

2.2.1 Preparation of vitamin C solution

Stock concentrations (20mg/ml) of vitamin C were prepared and used for the research.

2.2.2 Preparation of cadmium chloride solution

Eighty milligram (80mg) of cadmium salt (CdCl₂) was dissolved in distilled water and made up to 100ml in a measuring cylinder to give a stock concentration of 0.8mg/ml.

2.3 Induction of selected organs toxicity

Sub-acute cardiac, kidney and liver injuries were induced in each animal by intraperitoneal injection with cadmium chloride solution (5mg/kg), daily for 14 days.

2.4 Animals and maintenance

Twenty five (25) adult albino rats, weighing (200±20g), were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria. The animals were housed in standard condition and properly fed with commercial rat pellets and water *ad libitum*. The animals were kept under observation for 14 days before the onset of the experiment for acclimatization. The experimental protocol was approved by the institution animal ethics committee of the University of Nigeria Teaching Hospital (UNTH/CSA. 452/VOL. 19).

2.5 Experimental Design

The twenty five (25) male rats were grouped into (A-E) and received the following treatments daily and within 2 hours.

Group A: (normal Control): No treatment was administered to this group.

Group B: (Negative Control): received CdCl₂ (5mg/kg, i.p) only for 14 days.

Group C: received $CdCl_2$ and low dose (300mg/kg, oral) of methanolic extract of *N. sativa* MENS for 14 days.

Group D: received CdCl₂ and high dose (600mg/kg, oral) of methanolic extract of *N. sativa* (MENS) (600mg/kg, oral) for 14 days.

Group E (Positive control): received $CdCl_2$ and Vitamin C (200mg/kg, oral) for 14 days.

2.6 Sample collection

After 14 days, the animals were sacrificed via cervical dislocation under chloroform anesthesia. The heart, kidney and liver were harvested for histopathological analysis.

2.7 Histopathological analysis

The excised heart, kidney and liver tissues were processed using the paraffin wax embedding technique, sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] staining procedure. The histological sections were examined using an Olympus Might microscope.

2.7.1 Histopathological image analysis

Interpretation was done following standard guidelines as described by Gurcan *et al.*,¹⁸.

3. RESULTS

3.1 Acute toxicity studies result.

Median lethal dose (LD_{50}) value of the extract was 2400 mg/kg which indicates that MENS is safe and is not toxic to mice, at the doses used in the experiment (Table 1).

3.2 Phytochemical results.

The result of the preliminary phytochemical analysis of *Nigella sativa* revealed abundant presence of alkaloids and flavonoids (+++); moderate presence of tannins and phenols (++). However glycosides, saponins and steroids were absent (table 2).

3.3 Histopathological results

In Figure 1, the heart of normal control rats appeared functionally and structurally normal. The cardiac fibres showed a well conserved morphology (1A). The heart of CdCl₂-treated group (negative control) showed abnormal changes; there was evidence of fibrosis and mild infiltration by inflammatory cells. Fibres appear wavy showing signs of significant degeneration (1B). However, the cardiac fibres of test group rats (low dose MENS at 300mg/kg) appeared normal with very mild infiltration by inflammatory cells (1C). While in the other test group rats (high dose MENS at 600mg/kg), the myocardial fibres appear wavy; some fibres are necrotic, with presence of leucocyte infiltration (1D). Furthermore, photomicrograph of heart section from CdCl₂ + Vitamin C (200mg/kg), showed normal appearance of cardiac fibres (1E).

In Figure 2, the kidney of normal control rats appeared functionally and structurally normal. The glomeruli and tubule showed a well conserved morphology (1A). The kidney of CdCl2-treated group (negative control) showed abnormal changes; there was severe tubular degeneration; the glomeruli are all enlarged (1B). However, the kidney tubules of test group rats (low dose MENS at 300mg/kg) showed mild signs of autolytic degeneration and erosions; most glomeruli were normal while some were enlarged (1C). While in the other test group rats (high dose MENS at 600mg/kg), the kidney tubules showed moderate signs of autolytic degeneration, mild infiltration by inflammatory cells; and the glomeruli were enlarged and hyper cellular (1D). Furthermore, photomicrograph of kidney section from CdCl₂ + Vitamin C (200mg/kg), showed normal appearance of tubules; glomeruli also appear normal with a few eroded Bowman's capsule (1E).

In Figure 3, the liver of normal control rats appeared functionally and structurally normal. The hepatocytes showed a well conserved morphology (1A). The Liver of CCl_4 -treated group (negative control) showed abnormal changes; there was extensive vacuolation of the hepatocytes with mild infiltration by inflammatory cells (1B). However, the liver of test group rats (low dose MENS at 300mg/kg) showed mild pericentral vacuolation of hepatocytes with mild infiltration (1C). In the other test group rats (high dose MENS at 600mg/kg), the liver showed evidence of fatty degeneration (giving a foamy appearance) and vacuolation of some hepatocytes. Furthermore, photomicrograph of liver section from $CdCl_2$ + Vitamin C (200mg/kg), showed normal appearance of hepatocytes (1E).

Table 1: The median lethal dose (LD₅₀) of methanolic extracts of *N.sativa* (MENS)

| Phase | Dose | Death | Observation |
|-------|------|-------|----------------------------|
| 1 | 10 | 0/3 | Nil |
| | 100 | 0/3 | Nil |
| | 1000 | 0/3 | Nil |
| 2 | 1200 | 0/4 | Calm and no death occurred |
| | 2500 | 2/4 | Died within 48hours |
| | 3500 | 2/4 | Died within 48hours |
| | 5000 | 4/4 | Died within 24hours |

Two doses, 1200 mg/kg and 5000 mg.kg were used to calculate the LD_{50} of the plant extract.

 $LD_{50} = \sqrt{A \times B}$

A = Maximum dose with 0% mortality (1200mg/kg)

B = Minimum dosed with 100% mortality (5000mg/kg)

 LD_{50} of methanolic extract of *N.sativa* seed = $\sqrt{1200}$ X 5000 = 2449.48mg/kg

 LD_{50} of methanolic extract of *N.sativa* seed ≈ 2400 mg/kg

Table 2: Preliminary qualitative phytochemical results of methanol extract of N.sativa (MENS)

| Test | Result |
|-----------|--------|
| Alkaloid | +++ |
| Flavonoid | +++ |
| Tannins | ++ |
| Glycoside | - |
| Phenol | ++ |
| Saponin | - |
| Terpenoid | - |
| Steroid | - |

Key: +++ = present (in abundance); ++ = present (in moderate amount); - = absent

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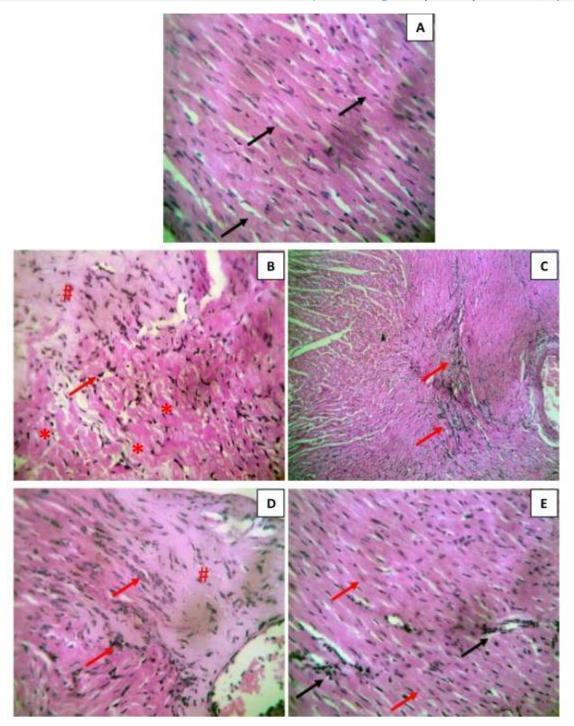


Figure 1: Photomicrograph of heart section. (A) Cardiac fibres (black arrows) appear normal with no degenerative changes. (B) Evidence of fibrosis (#) and mild infiltration by inflammatory cells (arrows). Fibres appear significantly wavy and damaged (*). (C) A section of the cardiac fibres appear necrotic and inflamed with infiltration by inflammatory cells (arrows).. (D) Evidence of fibrosis (#) and mild infiltration by inflammatory cells (arrows). (E) Cardiac fibres (red arrow) appear normal with very mild infiltration by inflammatory cells (black arrows) [Stain: H and E; ×400].

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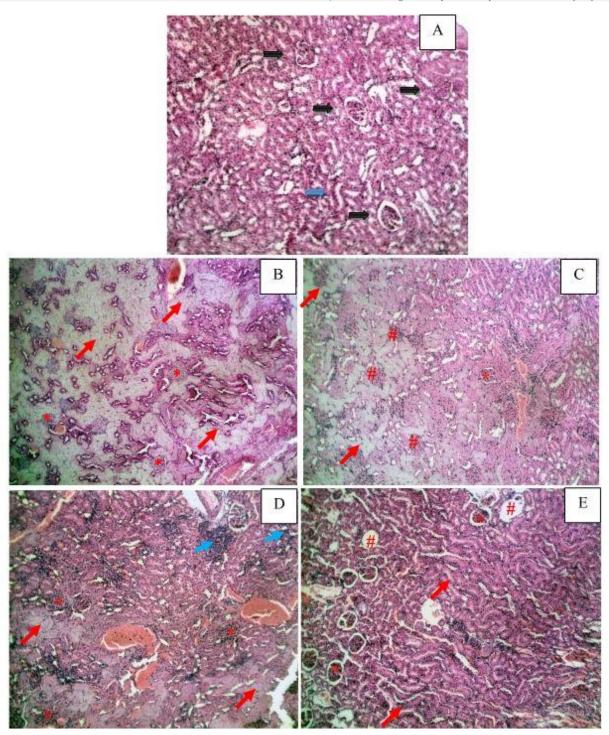


Figure 2: Photomicrograph of kidney section. (A) There is normal appearance of glomeruli (black arrows) and renal tubule (blue arrow). (B) There is severe tubular degeneration (red arrows); the glomeruli are all enlarged (*). (C) Most glomeruli are enlarged and hypercellular (#); few are still normal (*). (D) The tubules show signs of autolytic degeneration (red arrows); the glomeruli are enlarged (*) and there is infiltration by inflammatory cells (blue arrows). (E) Most tubules appear normal (red arrows); glomeruli also appear normal (*) with a few eroded Bowman's capsule (#). [Stain: H and E; ×100].

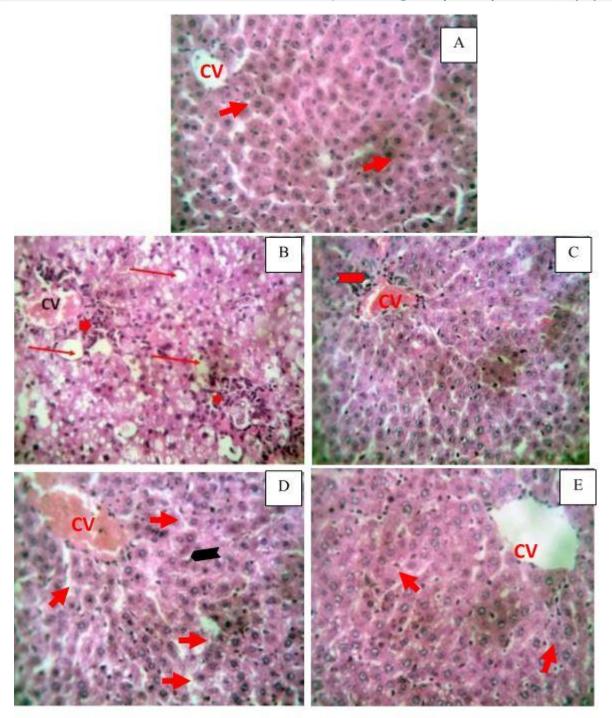


Figure 3: Photomicrograph of liver section. (A) Hepatocytes (arrows) are normal with no signs of degenerative lesions/changes. (B) There is extensive vacuolation of the hepatocytes (red arrows) with mild infiltration by inflammatory cells (arrow head). (C) Hepatocytes are normal; there is mild infiltration of inflammatory cells (arrow head) around the central vein. (D) Hepatocytes appear normal with mild vacuolations (arrow). (E) Liver section appears normal. Hepatocytes (arrow) and central vein appear normal. CV- central vein [Stain: H and E; ×400].

4. DISCUSSION

The heart is a muscular organ; made up of cardiac muscles which constantly pump blood through the blood vessels of circulatory system in human body and animals; while the kidneys are very effective in the excretion of metabolic wastes. Widney disease is both a cause and a consequence of cardiovascular disease, hence the term cardiorenal syndrome. Cardiorenal syndrome is a medical term for disorders involving both the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute

or chronic dysfunction in the other organ. 20 The liver is the main metabolic organ in the body especially in lipometabolism and glycometabolism. 21

Accumulation of toxic substances such as drugs, heavy metals, poisons etc can cause significant damages to the histomorphology of vital organs such as the heart muscles, kidney and liver tissues. Exposure to cadmium could produce serious adverse effects in humans and one of the ways through which this happens is through induction of oxidative stress; whereby it induces early hyperproduction

of Reactive Oxygen Species (ROS) that impair antioxidant defense system leading to oxidative stress in target organs and tissues. 22

In this study, the LD₅₀ value of *N.sativa* seed extract was established at 2400mg/kg which indicates that the methanolic extract of N. sativa (MENS) was safe and is not toxic to mice, at the doses used in the experiment (table 1). The preliminary phytochemical analysis of *N.sativa* revealed abundant presence of alkaloids (+++) and flavonoids (+++); moderate presence of tannins (++) and phenols (++). However glycosides (-), terpenoids (-) saponins (-) and steroids (-) were absent (table 2). In the histopathological analyses, cadmium significantly induced histomorphological changes in the heart fibres, kidney and liver tissues. However, treatment with low and high dose methanolic seed extracts of Nigella sativa (MENS) separately, revealed a marked ameliorative effect, thereby protecting the histomorphological architecture of the vital organs studied. Although this present study was not aimed to evaluating the mechanism through which the seed extract showed ameliorative effects; we however observed that the extract, particularly the low dose, acted in similar way as the standard drug (Vitamin C), a known antioxidant, which was used (Figures 1-3). Interestingly, we did not observe dosedependent protection by the seed extract; and these observations once against support the world wide claim that N.sativa is a potent medicinal plant. These findings could be as a result of the singular or combined actions of one or more of these bioactive phytochemical constituents present in the Nigella sativa. Thymoquinone, an active ingredient of Nigella sativa (black seed), is a constituent of flavonoids.4

Several other studies have reported the ameliorative effectsand potential pharmacotherapeutic effects - of crude extracts of N.sativa and thymoquinone separately. N.sativa has been reported to offer protections against cadiotoxic drug such as cyclosporine A; and against toxic heavy metals such as lead (Pb) and cadmium (Cd).23.24 Crude extracts of the seeds of N. sativa have been evaluated for hepatoprotective activity in Wistar rats against various hepatotoxicants, which are widely known for their ability to induce hepatotoxicity in experimental animals.^{25,26} N. sativa has not only been studied for its protective effects against tissue injuries, it has also been evaluated for antimicrobial activities. Different crude extracts of N. sativa exhibited antimicrobial efficacy against different bacterial strains which comprised either gram negative or gram positive bacteria; and these have been reported.^{27,28}

Thymoquinone (TQ) is chemically known as 2-methyl-5-isopropyl-1, 4-benzoquinone.⁴ Nemmar *et al.*²⁹ reported that TQ showed strong anti-inflammatory effects against diesel exhaust particles-induced cardiopulmonary injury in mice. Due to potent anti-oxidant and free radical scavenging action, TQ has been shown to normalize the adverse effect of various environmental toxins or xenobiotics causing oxidative damage and organ dysfunctions leading to pathogenesis of various diseases.³⁰ Alkaloids and Phenols, found to be significantly present in the methanol extracts, also act as natural antioxidants, scavenge free radicals and inhibit their production, stimulate the synthesis of antioxidant enzymes thereby prevent oxidative stress.³¹

CONCLUSION

The findings of this work show that cadmium induced histomorphological alterations in heart, kidneys and liver in the experimental rats; however, methanolic seed extract of *Nigella sativa* ameliorated the effects in the test groups,

although not in a dose-dependent manner. Thus, the result suggests that methanolic seed extract of *Nigella sativa* (MENS) has anti-cardiotoxic, antinephrotoxic and hepatoprotective properties and could therefore offer significant amelioration against cadmium- induced organ injuries.

LIST OF ABBREVIATIONS

MENS = Methanolic Extract of Nigella sativa

ROS = Reactive Oxygen Species

CdCl₂ = Cadmium Chloride

Cd = Cadmium

Pb = Lead

TQ = Thymoquinone

H and E = Haematoxylin and Eosin

ANOVA = Analysis Of Variance

SEM = Standard Error of Mean

AUTHORS' CONTRIBUTION

I.K.U and E..C.O- Conceptualized and designed the experiment, performed the experiment, performed the histopathological image analysis, and prepared the manuscript

C.J.O, J.O.O, A.J.O, T.J.W and C.A.U- Assisted in literature search, and copy-edited the manuscript

C.J.O, K.D.O, A.C.I and C.M.N- Assisted in literature search

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol was approved by the institution animal ethics committee of the University of Nigeria Teaching Hospital (UNTH/CSA. 452/VOL. 19), Nigeria. Experiments were conducted according to the Guidelines on the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA).

HUMAN AND ANIMAL RIGHTS

No humans were used in this research. The procedures involving the use of animals were in accordance with the standards set forth in the eighth edition of "Guide for the Care and Use of Laboratory Animals" (grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory animals_prepub.pdf published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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This study did not receive any fund from any grant agency or organization

COMPETING INTERESTS STATEMENT

The authors declare no conflicts of interest.

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Declared none.

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