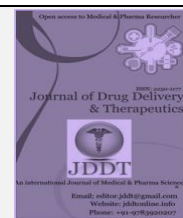
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Short Communication

## *Nigella sativa* seed extract protects against cadmium-induced cardiotoxicity in rats

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### ABSTRACT

**Objective:** The aim of this study is to investigate the protective effects of methanolic extract of *Nigella sativa* (MENS) (Black seed) against cardiotoxicity of cadmium in albino rats.

**Methods:** Twenty five (25) male albino rats, weighing (150-170g), were randomly grouped into five groups: A-E. Group B (Negative Control) received intraperitoneal administration of cadmium chloride (CdCl<sub>2</sub>, 5mg/kg) only, group C received CdCl<sub>2</sub> and low dose MENS (300mg/kg, oral), group D received CdCl<sub>2</sub> and high dose MENS (600mg/kg, oral), group E (Positive control) received CdCl<sub>2</sub> and Vitamin C (200mg/kg, oral), for 7 days. No treatment was administered to group A (Normal control). Cardiac injury was assessed by measuring serum levels of Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) and Creatine kinase (CK-MB) using standard methods. The heart was harvested for histopathological examination.

**Results:** CdCl<sub>2</sub> induced significant cardiotoxicity with marked elevation in the levels of biochemical markers of cardiac functions ( $p < 0.05$  or  $p < 0.01$ ); these were however attenuated by MENS. Histopathological examination of the heart sections supported the biochemical findings.

**Conclusion:** *Nigella sativa* seed extract is potentially cardioprotective against harmful chemical toxins such as cadmium.

**Keywords:** Cadmium, CdCl<sub>2</sub>, *Nigella sativa*, Medicinal food, cardiotoxicity

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### INTRODUCTION

Ingestion or exposure to chemicals (toxins) poses a serious health risk. Early intervention against cellular or biochemical changes induced by such events is vital to help prevent organ damage. Cadmium chloride (CdCl<sub>2</sub>), recognized as an experimental toxin, is known to be highly cardiotoxic. Cadmium toxicity poses adverse effects on target organs such as the kidney, lungs, liver, testes, bones and the cardiovascular system.<sup>1</sup>

Despite all studies performed to date, therapy choices for heart injuries are very few. Foods or plants with medicinal value have proven to be most useful 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 for drug development.<sup>2</sup> Recently, beneficial effects of medicinal plants in human health have received considerable attention. These beneficial effects have been partly attributed to the phytochemical compounds which possess antioxidation. We evaluated the protective effects of methanolic extract of *Nigella sativa* seed against cadmium-induced cardiotoxicity in rats.

### MATERIALS AND METHODS

#### Collection of plant material

Fresh samples of *Nigella sativa* seeds were obtained from local market in Enugu, Nigeria. It was authenticated by a consultant taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, and a voucher specimen was deposited at the herbarium with reference number UNH 662 for future reference.

#### Preparation of methanolic extracts of *Nigella sativa* (MENS)

*Nigella sativa* seeds were dried and shaded from sun light, then powdered with a grinder. The extracts were prepared using Babaei *et al.*<sup>3</sup> method with minor modifications. Five hundred gram (500g) of *N. sativa* powder was macerated with 2 litres of analytical grade 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 by a shaker. At the end of the extraction, the extract was filtered through a Whatman filter (Whatman Clifton, NJ, USA). Finally, using a waterbath 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.

### Phytochemical analysis of *Nigella sativa*

Preliminary phytochemical screening of *N. sativa* was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Procedures outlined by Trease and Evans<sup>4</sup> were employed for the analyses.

### Chemical reagents and drugs

The chemicals used in the study were of analytical grade and include absolute methanol, for plant extraction, and Cadmium chloride salt for induction of cardiotoxicity, and were purchased from Ogbete main market, Enugu. Drug used includes vitamin C (Alpha Pharmaceuticals, Enugu, Nigeria). AST assay kit was purchased from Randox Laboratory (UK); CKMB ELISA kit was from Elabscience (Texas, USA); and LDH ELISA kit from MyBioscience (San Diego, USA).

### Preparation of vitamin C solution

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

### Preparation of cadmium chloride solution

Eighty milligram (80mg) of cadmium salt (CdCl<sub>2</sub>) was dissolved in distilled water and made up to 100ml in a measuring cylinder to give a stock concentration of 0.8mg/ml.

### Induction of cardiotoxicity

Acute cardiac injury was induced in each animal by intraperitoneal injection with cadmium chloride solution (5mg/kg), daily for 7 days.

### Animals

A total of twenty five (25) adult albino rats, weighing (150-170g), were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria. The animals were housed under standard condition. The animals were kept under observation for about 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. 524/VOL. 19).

### Experimental Design

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

Group B: (Negative Control): received CdCl<sub>2</sub> (5mg/kg, i.p) only for 7 days.

Group C: received CdCl<sub>2</sub> and low dose MENS (300mg/kg, oral) for 7 days.

Group D: received CdCl<sub>2</sub> and high dose MENS (600mg/kg, oral) for 7 days.

Group E (Positive control): received CdCl<sub>2</sub> and Vitamin C (200mg/kg, oral) for 7 days.

### Sample collection

After 7 days, 6 ml of blood was collected from the axillary vein under chloroform anesthesia. The blood was dispensed into a plain container, allowed to clot at room temperature, spun at 5,000 r.p.m for 5 min; serum was separated and cryopreserved for analysis of cardiac biomarkers (CK-MB, LDH and AST). The heart was harvested for histopathological studies.

### Biochemical analysis

The serum activities of the creatine kinase isoenzyme, CK-MB and LDH were determined using enzyme immunoassay method as described by Tietz (5). AST was determined using colorimetric method.<sup>5</sup>

### Histopathological analysis

The excised heart and kidney 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.<sup>6</sup> The histological sections were examined using an Olympus™ light microscope.

### Statistical analysis

Data analysis was done using GraphPad prism version 7.0 (GraphPad, San Diego, CA, USA). The results of the biochemical assays were reported as mean±SEM (standard error of mean). One way analysis of variance (ANOVA), followed by the Tukey post hoc analysis, was used to test for the level of significance (p<0.05).

## RESULTS

### 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.

### Biochemical results

The functionality of the heart was established by estimating the serum level of CK-MB (U/L), LDH (U/L) and AST (U/L). A statistically significant (P<0.05) elevated levels of CK-MB (U/L), LDH (U/L) and AST (U/L), were seen in CdCl<sub>2</sub>-treated group B (negative control) when compared with group A (normal control) and group E (positive control). However, co-administration of CdCl<sub>2</sub> with high and low doses of methanolic extracts of *Nigella sativa* (MENS) separately, restored the level of these parameters to near normal when compared with CdCl<sub>2</sub>-treated group (negative control). Furthermore, we observed the extracts had a dose-dependent protection, with the low dose MENS showing better cardioprotection than high dose MENS (Table 1).

**Table 1: Statistical Comparison of cardiac biomarkers of treated groups with negative controls**

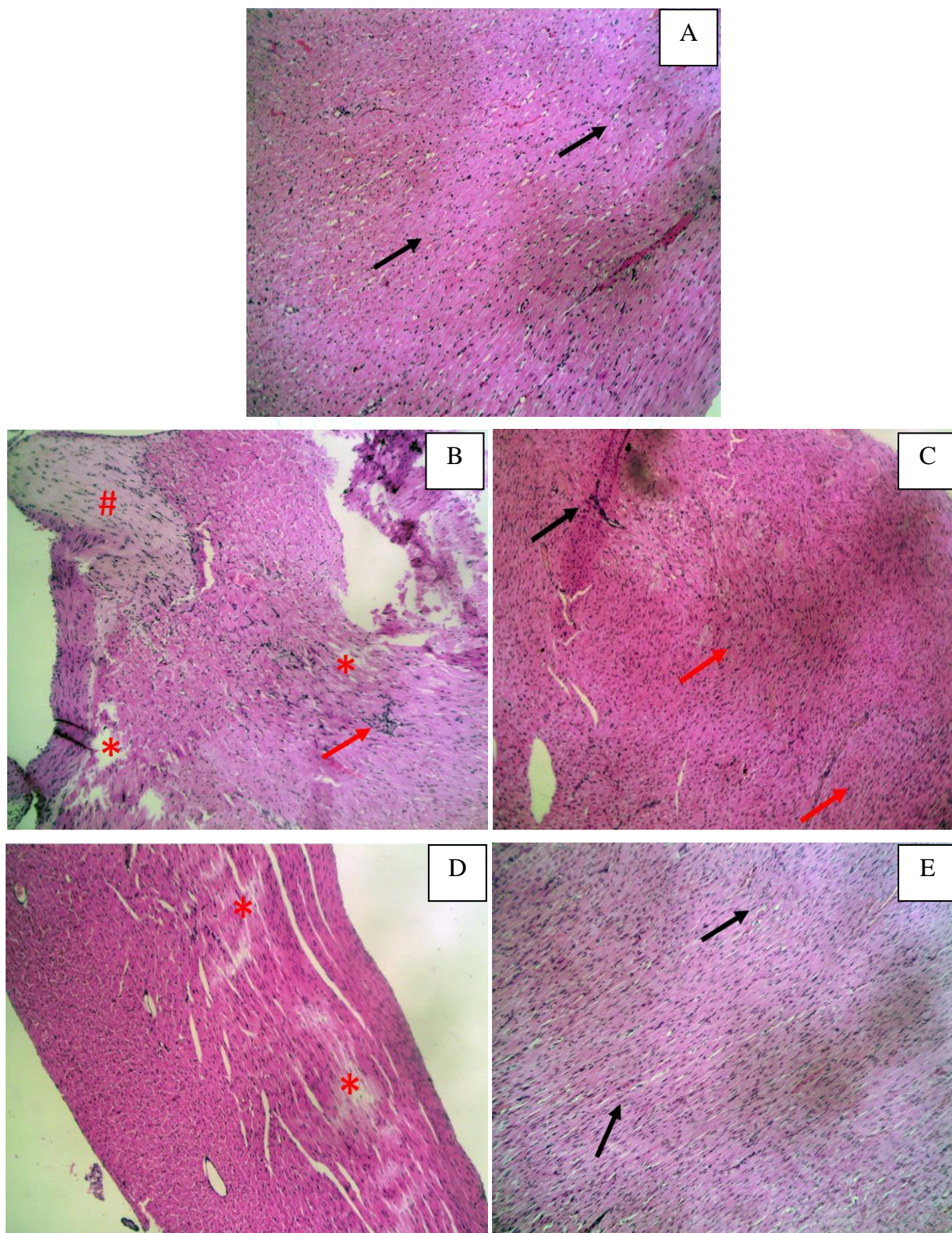
Groups	CK-MB (U/L)	LDH (U/L)	AST (U/L)
<b>A: Normal Control</b>	193.75 ± 11.09**	195.03 ± 14.72**	20.51 ± 3.69*
<b>B: CdCl<sub>2</sub> Alone</b>	255.08 ± 20.82	254.75 ± 13.05	40.75 ± 10.92
<b>C: CdCl<sub>2</sub> + MENS (300mg/kg)</b>	201.81 ± 13.77*	200.75 ± 15.32**	21.13 ± 4.33*
<b>D: CdCl<sub>2</sub> + MENS (600mg/kg)</b>	235.55 ± 20.62	227.75 ± 22.75	34.09 ± 5.24
<b>E: CdCl<sub>2</sub> + Vit. C (200mg/kg)</b>	200.12 ± 18.26*	199.51 ± 15.42**	20.88 ± 4.37*

Values given as Mean ± SD. \*\*p<0.01 or \*p<0.05 is significant when CdCl<sub>2</sub> alone (negative control) is compared with all other groups.

**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 CdCl<sub>2</sub>-treated group (negative control) showed abnormal changes; there was evidence of fibrosis and mild infiltration by inflammatory cells (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 other test group rats (high dose MENS at 600mg/kg) showed sections that are fairly damaged (1D). Furthermore, photomicrograph of heart section from Vitamin C group (200mg/kg), showed normal appearance of cardiac fibres (1E).



**Figure 1: Photomicrograph of heart section.** (A) Cardiac fibres (arrows) appear normal with no degenerative changes. (B) Evidence of fibrosis (#) and mild infiltration by inflammatory cells (arrows). Some fibres are also damaged (\*). (C) Cardiac fibres (red arrow) appear normal with very mild infiltration by inflammatory cells (black arrows). (D) Some sections of the cardiac fibres are damaged (\*). (E) Cardiac fibres appear normal (arrows). [Stain: H and E;  $\times 100$ ].

## DISCUSSION

Accumulation of toxic substances such as drugs, heavy metals, poisons etc can cause significant damage to the heart muscles. 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 impairs antioxidant defense system, leading to oxidative stress in target organs and tissues.<sup>7</sup>

The morbidity and mortality rate associated with the acute myocardial infarction can be evaluated using biochemical markers of cardiac function in assessing heart diseases. Generally, Cardiac marker enzymes such as CK-MB, AST and LDH are very low in serum. They are intracellularly located in the cells of the heart. Disruption of these enzymes via the cell membrane makes them to leak out into plasma and increase tremendously, which give out information of the extent of myocardial injury due to oxidative stress and lipid peroxidation.<sup>8</sup>

Thus, in this study, the functionality of the heart was established by estimating the serum level of CK-MB (U/L), LDH (U/L) and AST (U/L). Treatment with CdCl<sub>2</sub> in group B showed a statistically significant (P<0.05) elevated levels of CK-MB (U/L), LDH (U/L) and AST (U/L) when compared with group A (normal control) and group E (positive control). This observation supports the general knowledge of the cardiotoxic effect of cadmium. We observed a dose dependent protection by the seed extract; and this once against supports the world wide claim that *N.sativa* is a potent medicinal plant.

Over the years, scientists have been researching on food and plant products that possess protective effects against cardiotoxicity. It was discovered that diets rich in natural antioxidants (especially flavonoids) have potent effects against oxidative stress caused by cardiotoxic substances.<sup>9</sup> Thymoquinone, an active ingredient of *Nigella sativa* (black seed), is a constituent of flavonoids. Flavonoids are natural antioxidants that occur in fruits, vegetables flowers and seeds which are very important in human diet.<sup>9</sup> Flavonoids have high antioxidant effects and consequently anti-free radical effects. They have the ability to reduce free radical formation and to scavenge free radicals from blood, thereby preventing cell injury.<sup>9</sup> The result of the preliminary phytochemical analysis of *Nigella sativa* (black seed) revealed abundant presence of alkaloids and flavonoids (+++); moderate presence of tannins and phenols (++) . The findings we observed could be as a result of the singular or combined actions of one or more phytochemical constituents present in the *Nigella sativa*.

According to Kooti *et al.*<sup>10</sup>, long term oral administration of *N. sativa* may decrease vascular contractile responsiveness

and some cardiovascular complications in diabetes. Observation from the biochemical and histological results show that consumption of *N. sativa* may serve as a healthy diet and as well be an effective intervention in improving cardiac injuries. This study and other previous reports suggest a protective role of dietary *N. sativa* intake against cardiotoxicity.

## CONCLUSION

The findings of this work show that cadmium induced cardiotoxicity in albino rats. The administration of methanolic extract of *Nigella sativa* (MENS) (Black seed) ameliorated the effects in the test groups, although in a dose-dependent manner. Thus, the result suggests that methanolic extract of MENS has anti-cardiotoxic properties and could therefore offer significant protection against cardiac injuries.

## COMPETING INTERESTS STATEMENT

The authors declare no conflicts of interest.

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