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Impacts of fullerene C_{60} and virgin olive oil on cadmium-induced genotoxicity in rats

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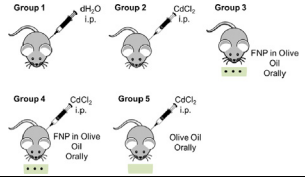
HIGHLIGHTS

- Fullerene C_{60} induces both genotoxicity and anti-genotoxicity in cadmium chloride exposed rats.
- Strong anti-genotoxicity of virgin olive oil is reported.
- Establishment of optimal settings for fullerene nanoparticles C_{60} and virgin olive oil biomedical applications is required.

GRAPHICAL ABSTRACT

Experimental design and genotoxic and anti-genotoxic effects of fullerene C_{60} and virgin olive oil against $CdCl_2$ toxicity in rats.



Group		Group		Compound	Genotoxicity	Anti-genotoxicity
2	vs.	1	::::::::::>	$CdCl_2$	++++	-
3	vs.	1	::::::::::>	FNP	+++	-
3	vs.	2	::::::::::>	$CdCl_2$ and FNP	+++	-
4	vs.	2	::::::::::>	FNP+VOO	++	++++
5	vs.	2	::::::::::>	VOO	++	+++
5	vs.	3	::::::::::>	VOO	++	+++
5	vs.	4	::::::::::>	FNP	+++	+++++

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ABSTRACT

Currently, cadmium is considered to be one of the major environmental pollutants. Environmentally, cadmium is released in various forms e.g. oxide, chloride and sulphide. The aim of the present study was to examine the genotoxic impact of fullerene nanoparticles C_{60} (C_{60}) and virgin olive oil (VOO) on cadmium chloride ($CdCl_2$)-induced genotoxicity in rats. To evaluate these effects on DNA damage and chromosomal frequency, 25 albino rats were randomly assigned to 5 groups ($n = 5$ per group): Group 1 served as a control; Group 2 received a single intraperitoneal dose of $CdCl_2$ (3.5 mg/kg); Group 3 animals were treated with C_{60} (4 mg/kg, orally) every other day for 20 days; Group 4 received a single intraperitoneal dose of $CdCl_2$ (3.5 mg/kg) and an oral dose of C_{60} (4 mg/kg); and Group 5 received a single intraperitoneal dose of $CdCl_2$ (3.5 mg/kg) and oral doses of VOO every other day for 20 consecutive days. Genotoxic and anti-genotoxic effects of C_{60} and VOO were evaluated in the liver, kidney and bone marrow using molecular and cytogenetic assays. As expected, $CdCl_2$ and C_{60} administration was associated with band number alterations in both liver and kidney; however, C_{60} pretreatment recovered to approximately basal number. Surprisingly, C_{60} and VOO significantly attenuated the genotoxic effects caused by $CdCl_2$ in livers and kidneys. In bone marrow, in addition to a reduction in the chromosomal number, several chromosomal aberrations were caused by $CdCl_2$. These chromosomal alterations were also reversed by C_{60} and VOO. In conclusion, molecular and cytogenetic studies showed that C_{60} and VOO exhibit anti-genotoxic agents against $CdCl_2$-induced genotoxicity in rats. Further studies are needed to investigate the optimal conditions for potential biomedical applications of these anti-genotoxic agents.

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1. Introduction

Cadmium is often used in industrial purposes. It is generally accepted that cadmium exerts severe toxicity to biological systems, including mammals, and it is classified by the International Agency for Research on Cancer (IARC, 1993) as a group I carcinogen. Cadmium toxicity can occur as a result of exposure via inhalation of polluted air and ingestion of contaminated food or water (Szczygłowska et al., 2014). At the organ level, cadmium may lead to liver, lung, and testis failure in acute cases (Kasuya et al., 1992). Furthermore, cadmium is well-described as an immunotoxic and genotoxic metal (Desforges et al., 2016; Giri et al., 2016; Rocha et al., 2014). The genotoxic effect includes DNA damage and chromosomal aberrations (Skipper et al., 2016).

Recently, we and others have shown numerous hepatoprotective effects of fullerene C_{60} (C_{60}) against cyclophosphamide-induced acute toxicity (Elshater et al., 2018) and oxidative stress (Afanasieva et al., 2015; Foley et al., 2002; Prylutska et al., 2009, 2012). By contrast, some reports show that C_{60} itself has some genotoxic effects on mouse and human cell lines (Sayes et al., 2005; Shierley Chu et al., 2006). Specifically, it has been shown that C_{60} induces DNA strand breaks and oxidative DNA damages in a concentration-dependent manner (Al-Subiai et al., 2012; Dhawan et al., 2006; Jacobsen et al., 2008). Today, it is clear that C_{60}-induced genotoxicity in in vitro and in vivo systems strongly depends on the size of the aggregates, the concentration tested, the cell type, as well as and the duration of exposure (Sayers et al., 2016).

Virgin olive oil (VOO, a typical solvent for C_{60}) can reduce the rate of oxidation of DNA (Machowetz et al., 2007) due to the antioxidant potential of its polyphenols. Furthermore, VOO has hepato- and nephroprotective effects against hydrogen peroxide-induced oxidative damage (Gill et al., 2005). Based on these reports, the present study was undertaken to investigate the genotoxic and protective effects of C_{60} and VOO in rats induced by cadmium chloride ($CdCl_2$). We report that C_{60} and VOO are potent anti-genotoxic agents against cadmium-induced genotoxicity in rats.

2. Material and methods

2.1. Experimental animals

Twenty-five male albino rats (20–25 g body weight) were brought from Animal House Facility (Faculty of Veterinary Medicine, South Valley University, Qena, Egypt). Rats were caged and kept under normal laboratory and nutritional conditions until they each reached a weight of 180–200 g (at which they were approximately 2 months old). Rats were randomly assigned to 5 groups ($n = 5$) as follows (Graphical abstract): i) Group 1 served as a control and received distilled water intraperitoneally (i.p.); ii) Group 2 was injected i.p. with a single dose of $CdCl_2$ (3.5 mg/kg dissolved in 2 ml distilled water); iii) Group 3 was treated orally with C_{60} (4 mg/kg dissolved in 1 ml VOO every other day for 20 days), according to Baati et al. (2012) and Elshater et al. (2018); iv) Group 4 was injected with a single i.p. dose of $CdCl_2$ (3.5 mg/kg) and treated orally with C_{60} (4 mg/kg dissolved in 1 ml VOO every other day for 20 days) and v) Group 5 was injected with a single i.p. dose of $CdCl_2$ (3.5 mg/kg) and treated orally with VOO (1 ml every other day for 20 days). The experimental protocols with rats were carried out in full compliance with the guidelines for animal care and were approved by the Animal Care Committee from the Faculty of Veterinary Medicine, South Valley University, Egypt (Application number: VetEg.0265R-2016-2018).

2.2. Preparation of fullerene C_{60}

Fullerene C_{60} (purity 99.9%, Lydow Group Limited Research Corporation, China)-VOO solution was prepared according to Baati et al. (2012) and Elshater et al. (2018) as follows: 1 g of fullerene C_{60} was dissolved in 200 ml of VOO, this solution was stirred for 15 days at ambient temperature in the dark. The mixture was the centrifuged (5000 rpm) for 1 h. After centrifugation, the supernatant was filtered through 0.25 μm Millipore filters and administered immediately.

2.3. DNA extraction

Rats were euthanized 24 h after the final administration. Liver and kidney tissues were collected immediately, fixed in Carnoy's fixative and stored at −80 °C until analysis. Genomic DNA was extracted from liver and kidney tissues according to the method described by Zietkiewicz et al. (1994).

2.4. PCR reaction

Inter simple sequence repeat (ISSR) analysis was performed using five primers which were synthesized by Eurofins, Germany (Table 1). Amplification reactions for ISSR analysis were used in a volume of 25 μl (1× of green Go Taq®Flexi buffer, 1.5 mM of $MgCl_2$, 200 μM of dNTPs (Promega), 25 pucM of primer, 1 U of Go Taq® Flexi DNA Polymerase (Promega), 25 ng of template DNA and up to 25 μl distilled H_2O). Amplification was carried out in a Gene Amp®PCR System 9700 thermal cycler programmed as follows: i) An initial denaturation at 94 °C for 5 min (1 cycle); ii) Annealing at 94 °C for 45 s, 45 °C for 50 s and 72 °C for 1.5 min (40 cycles) and iii) Extension at 72 °C for 7 min (1 cycle) and 4 °C (infinitive). Amplification was performed according to (Sharaf-Eldeen et al., 2006).

2.5. Gel electrophoresis

PCR products were analyzed using (1.5%) agarose gel electrophoresis and visualized with 10 μg/μl ethidium bromide staining. The size of the fragments was estimated based on a DNA ladder of 100 to 2000 bp (MBI, Fermentas) and DNA molecular weight was used as a standard. Results were visualized on a UV transilluminator and photographed using a Molecular Imager® Gel Doc™ System with Image Lab™ Software, Bio-Rad. Clear and distinct amplification products were scored as (1) for presence and (0) for absence of the bands, and were compared to determine the genetic alterations across the different groups included in the study.

2.6. Chromosome preparations

Mitotic chromosomal spread microscopic slides were prepared using the technique described by Yosida and Amano (1965) with some modifications. Rats were given an i.p. injection of 0.05% colchicine and then euthanized 2.5 h. For chromosomal aberrations analysis, rat femurs were removed and the bone marrow cells were aspirated from both femurs in 5–6 ml warmed 0.56% KCl for 30 min. The extract was centrifuged, the supernatant decanted, and the residual mass was fixed three times in a mixture of methanol–glacial acetic acid (3:1). Slides for chromosome analysis were prepared by dropping the cell suspension onto ethanol cold slides and flaming them slightly. Slides were stained with 10% Giemsa in phosphate buffer (pH 6.8). Approximately 250 metaphase spreads per group were analyzed and the structural chromosomal aberrations per cell counted.

Table 1
ISSR primer sequences used for ISSR-PCR.

Primer	Sequence (5′ → 3′)
ISSR- 1	AGAGAGAGAGAGAGAGYC
ISSR- 2	AGAGAGAGAGAGAGAGYG
ISSR- 3	ACACACACACACACACYG
ISSR- 4	GTGTGTGTGTGTGTGTYG
ISSR- 5	CGCGATAGATAGATAGATA