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Fullerene C_{60} nanoparticles potentiate the antioxidant defense system of the brain and liver by increasing catalase activity in normal rats

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Abstract

Background: Previous studies have shown that fullerene C_{60} nanoparticles and their derivatives possess antioxidant properties, both *in vitro* and *in vivo*.

Objectives: The aim of this study was to investigate the effects of orally administered C_{60} fullerene nanoparticles on the antioxidant capacity of the brain and liver of normal rats, especially on catalase activity.

Methods: Two groups of Wistar rats (n = 6) were studied for 8 weeks: a group that received no treatment and a group that received C_{60} fullerene nanoparticles orally at a dose of 1 mg/kg/day. Blood glucose and body weight were monitored throughout the study. At the end of the study, catalase activity in the brain and liver was assessed using the Aebi method.

Results: Blood glucose levels remained unchanged in treated rats compared to untreated rats. Results showed similar progressive weight gain in both groups. Fullerene C_{60} injection, on the other hand, significantly boosted catalase activity in treated rats' brains (0.340.10 U/mg protein) compared to untreated rats (0.120.03 U/mg protein) (p 0.05). Furthermore, fullerene C_{60} boosted mean catalase activity in treated rats' livers (6.140.76 U/mg protein) compared to untreated rats (2.071.43 U/mg protein) ($p<0.05$).

Conclusion: The findings suggest that fullerene C_{60} nanoparticles may enhance the antioxidant capacity of the brain and liver through the enhancement of catalase activity. This may have implications for the prevention of ROS accumulation and oxidative stress in various pathological situations.

Keywords: Fullerenes, Nanostructures, Antioxidants, Catalase, Oxidative stress.

Introduction

Reactive oxygen species (ROS) accumulation and oxidative stress play a crucial role in the pathophysiology of various chronic diseases and aging.[1,2] Although ROS are created in tissues under physiological circumstances, antioxidant mechanisms remove them to maintain redox equilibrium.[3,4] However, endogenous antioxidant systems may not be sufficient to balance oxidation and anti-oxidation, leading to ROS accumulation and oxidative stress.[5] Antioxidant therapy can diminish these lesions.[4,6,7] Therefore, pharmacotherapy that utilizes natural or synthetic materials with anti-ROS properties is attractive. Antioxidants have been shown to prevent the progression of chronic neurodegenerative diseases, such as Parkinson's and Alzheimer's disease, and to delay the aging process.[5,8]

Fullerene C_{60} is a spherical nanomaterial consisting of pure carbon atoms in a cage-like shape.[8] This nanoparticle and its derivatives have exhibited several beneficial effects in biological systems.[9] Fullerene C_{60} and its derivatives have demonstrated antioxidant functions in both in vitro and in vivo studies.[5,8,10,11] They behave as excellent antioxidants by passing through the cell membrane and

localizing preferentially in the mitochondria.[8] Tokuyama et al. reported that fullerene C_{60} derivatives protected cells against photo-induced cytotoxicity and photo-induced DNA damage.[12] Oral administration of fullerene C_{60} has been shown to prolong lifespan in rats with CCl4 intoxication.[9] Fullerene C60 and its derivatives show promise for use in cosmetics and topical preparations due to their antioxidant properties.[11] Fullerene compounds also exhibit neuroprotection and improve axonal loss and disability in a mouse model of progressive multiple sclerosis, potentially making them useful treatments for neurodegenerative diseases.[13] Furthermore, the neuroprotective functions of fullerene C_{60} have been reported in ischemic stroke,[14–16] as fullerene administration during ischemic stroke inhibits oxidative damage and the inflammatory response, thereby decreasing cerebral ischemia injuries.

Objectives

Numerous preclinical in vitro and in vivo studies have shown that fullerene C_{60} nanomaterials have powerful ROS-scavenging and excellent antioxidant properties. Therefore, the aim of this study was to investigate the effect of repeated oral administration of fullerene C_{60} nanoparticles on the antioxidant capacity of the brain and liver of normal rats, as measured by catalase activity.

Methods

Animals

Male Wistar rats weighing about 220±20 g and aged 8–10 weeks were used for the study. The rats were accommodated in the institutional animal house, where they received ad libitum standard chow and water. The animals were housed under controlled conditions of light exposure (12 h light/dark cycles), temperature (22–24 ˚C), and humidity (40–60%).

Fullerene nanoparticles

For this study, fullerene C_{60} was purchased from Sigma-Aldrich (USA). The purity of the compound was >99%. Fullerene C_{60} was dissolved in sesame oil to a dose of 1 mg/kg/day and administered orally via gavage for eight weeks, according to previous studies.[9,17]

Experimental Design and Grouping

Twelve rats were randomly assigned to untreated and treated groups (n=6 each). Treated rats received fullerene C_{60} nanoparticles orally at a dose of 1 mg/kg/day for eight weeks via oral gavage. Untreated rats received the same volume of sesame oil without fullerene C_{60} nanoparticles orally for eight weeks. The blood glucose and body weight of rats were measured throughout the study.

Tissue preparation

At the end of the study, brains and livers were quickly removed under deep anesthesia. Brain and liver tissues were homogenized in ice-cold phosphate-buffered saline (PBS), and the homogenates were centrifuged at 14,000 g and 4°C for 15 min. The supernatants were then used to determine the protein concentration and catalase activity of the brain and liver tissues.

Determination of protein levels

Protein levels of the brains and livers were quantified using the Bradford method,[18] and bovine serum albumin (BSA; Sigma, Germany) was used as a standard for protein concentration calculation.

Determination of catalase activity of brain and liver

The Aebi method was used to determine the activity of catalase in the brain and liver tissue homogenates.[19] First, the homogenate was incubated in a reaction mixture containing 0.1 ml of homogenate and 0.85 ml of potassium phosphate buffer (50 mM and pH 7.0) at room temperature for 10 min. Following that, 0.05 ml of H_2O_2 (30 mM, produced in 50 mM potassium phosphate buffer, pH 7.0) was added to start the reaction. A spectrophotometer set to 240 nm recorded the reduction in absorbance for 3 minutes. The specific activity of catalase in the brain and liver tissues was calculated as U/mg protein, where one unit (U) of catalase was defined as 1 nmol H_2O_2 decomposed per minute.

Statistical analysis

All statistical analyses were performed with SPSS (version 21.0, SPSS Inc, Chicago, IL, USA). The t-test was used to analyze the data between two groups (treated and untreated animals). All data were expressed as mean±SD. A “P-value” less than 0.05 was considered significant.

Ethical considerations

All experimental protocols used in the current study were approved by the Institutional Animal Ethics Committee of the University of Baqiyatallah Medical Sciences (Tehran, Iran). The ethical code for present study is IR.BMSU.REC.1397.029.

Results

Effect of fullerene C_{60} on blood glucose

Figure 1 represents the changes in blood glucose levels in the normal and treatment groups during the study. The blood glucose level in normal animals was 6.19±0.64 mmol/l at the beginning of the test, which remained unchanged on days 30 (6.46±0.56 mmol/l) and 60 (6.73±0.55 mmol/l). In the fullerene-treated normal rats, the blood glucose level was 6.66±0.39 mmol/l at the beginning of the test and did not change significantly during the test on days 30 (6.80±0.31 mmol/l) and 60 (6.95±0.25 mmol/l). Furthermore, there were no significant differences in the blood glucose levels of the two groups at different times during the test.

Effect of fullerene C_{60} on body weight

Figure 2 shows that the mean body weight of the normal and treatment groups was 225±22 g and 205±5 g at the beginning of the test, respectively. Both groups exhibited similar progressive weight gains during the study. The body weight of untreated animals on days 30 and 60 was 265±19 g and 295±23 g, respectively. Similarly, the body weight of fullerene-treated animals on days 30 and 60 was 264±22 g and 298±29 g, respectively. There were no significant differences in the body weight values of the two groups over time.

Effect of fullerene C_{60} on catalase activity of brain

Figure 3 displays the catalase activity in brain tissues at the end of the experiment. The mean catalase activity in untreated rats was 0.12±0.03 U/mg protein, while fullerene treatment significantly increased this value in treated rats to 0.34±0.07 U/mg protein ($p<0.05$).

Effect of fullerene C_{60} on catalase activity of liver

Similarly, Figure 4 depicts the catalase activity level in liver tissues at the end of the experiment. The mean catalase activity in untreated rats was 2.07±1.43 U/mg protein, while treatment with fullerene nanoparticles significantly increased the catalase activity in treated rats to 6.14±0.76 U/mg protein ($p<0.05$).

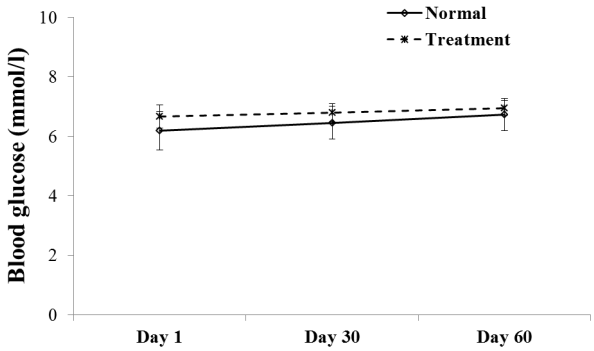

Figure 1. The representative changes of blood glucose (mmol/l) during the study in normal and treatment groups. There were no significant differences in the blood glucose levels of two groups during the test at the different times. All values are presented as mean±SD (n=6).

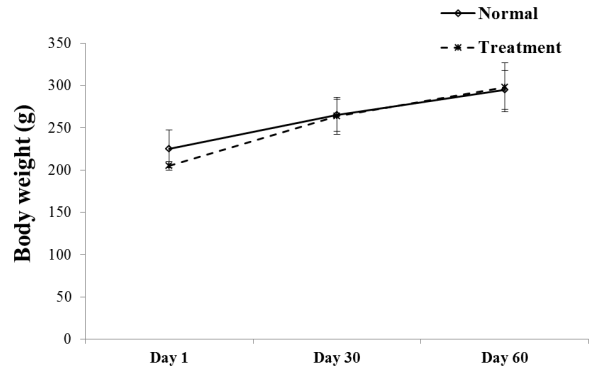

Figure 2. The representative changes of body weight (g) during the study in normal and treatment groups. There were no significant differences in the values of body weight of two groups during the test at the different times. All values are presented as mean±SD (n=6).

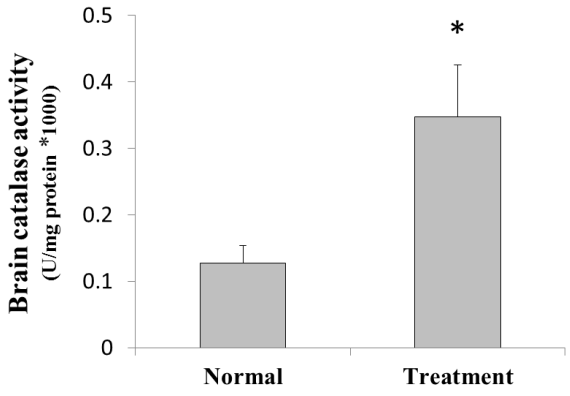

Figure 3. The effects of fullerene nanoparticles on catalase activity of brain (U/mg protein × 1000) in treated rats (treatment group) at termination of the study. All values are presented as mean±SD (n=6). *($p<0.05$) as significant difference compared with normal group

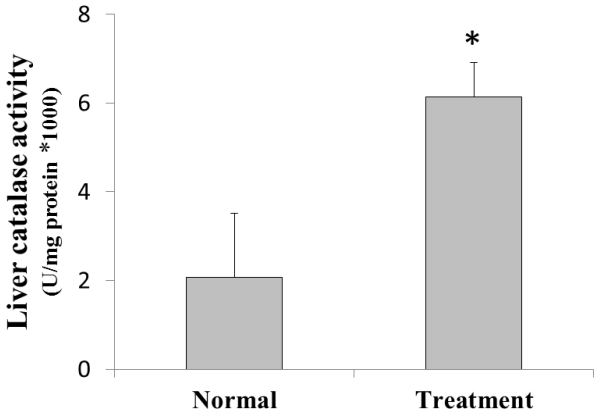

Figure 4. The effects of fullerene nanoparticles on catalase activity of liver (U/mg protein × 1000) in treated normal rats (treatment group) at termination of the study. All values are presented as mean±SD (n=6). * ($p<0.05$) as significant difference compared to normal group

Discussion

Previous researches have revealed that fullerene C_{60} nanoparticles have high antioxidant capabilities in biological settings.[5,8,15] Given that tissue degenerative diseases and aging are linked to the accumulation of oxygen free radicals and the development of tissue oxidative stress,[1,2] this study sought to explore the impact of fullerene C_{60} nanoparticles on the antioxidant capacity of the brain and liver in rats under normal conditions by assessing catalase activity. The findings of the study reveal that administering fullerene C_{60} nanoparticles increased catalase activity in both the brain and liver tissues, enhancing their resistance to oxidative stress.

Previous researches have revealed that fullerene C_{60} nanoparticles have strong antioxidant properties in biological settings.[5,8,14] Given that ROS play a critical role in the pathogenesis of tissue degeneration in various disorders and the aging process,[1,2] fullerene C_{60} may help reduce tissue damage. The study's results highlight a significant increase in catalase activity in the brains of treated rats following the administration of fullerene C_{60} nanoparticles. Catalase is in charge of detoxifying H2O2 produced by superoxide dismutase,[20] and prior studies have shown that fullerene C_{60} has catalase-like characteristics in vitro.[21] Consequently, fullerene C_{60} enhances the antioxidant capacity of the normal brain by increasing catalase activity and could aid in preventing brain damage induced by ROS accumulation in several neurodegenerative diseases and the aging process. This ability to enhance the brain's antioxidant capacity demonstrates a potential protective effect of fullerene C_{60} application in these conditions.

As previously established, oxidative stress induced by a number of exogenous and endogenous substances plays a significant role in the incidence of hepatotoxicity.[22-24] Therefore, enhancing the liver's antioxidant capacity may be beneficial in preventing liver damage and hepatotoxicity induced by ROS accumulation. The study's findings indicate a significant increase in catalase activity in the livers of treated rats following administration of fullerene C_{60} nanoparticles. In line with these results, it has been demonstrated that fullerene C_{60} possesses catalase and superoxide dismutase mimetic properties in vitro.[8,25] Catalase, in conjunction with superoxide dismutase, safeguards hepatic cells against damage caused by free radicals such as hydroperoxides and lipoperoxides, which are neutralized by catalase.[26] In the absence of catalase activity, hydrogen peroxide converts into hydroxyl radicals through Fenton reactions, inducing oxidative damage to macromolecules like lipids, proteins, and nucleic acids.[1] Furthermore, fullerene C_{60} regulates hydrogen peroxide-dependent signal transduction pathways, resulting in alterations to cellular catalase activity, as hydrogen peroxide can serve as a signaling molecule.[27] Thus, fullerene C_{60} administration might offer hepatoprotective effects against ROS-induced toxicity during various pathophysiological states or conditions of ROS accumulation in the liver by enhancing the organ's antioxidant capacity.

Conclusions

The findings of this study suggest that fullerene C_{60} nanoparticles may offer neuroprotective and hepatoprotective effects against ROS-induced brain and liver toxicity. These protective effects may stem from the potentiation of antioxidant defense systems, as evidenced by the enhanced catalase activity observed in both the brain and liver tissues. Consequently, the administration of fullerene C_{60} nanomaterials is hypothesized to be useful in preventing tissue damage in the brain and liver caused by ROS accumulation.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

Reactive oxygen species: ROS; Parkinson's disease: PD; Alzheimer's disease: AD; Hydrogen peroxide: H_2O_2; Phosphate buffered saline: PBS.

Authors' contributions

All authors have actively participated in the presentation of the idea, search for sources, writing and reviewing the article, and with the final acceptance of this article, accept responsibility for the accuracy of the content presented in it.

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Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval (code: IR.BMSU.REC.1397.029) was obtained.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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Supplementary data: Table illustrates the measured parameters for each individual animal in both groups (**N;** normal rats, **NF;** fullerene treated normal rats)

Animals	Blood glucose (mmol/l)			Body weight (g)			Catalase activity (U/mg protein)	
	Day 1	Day 30	Day 60	Day 1	Day 30	Day 60	Brain *100	Liver *1000
N_1	6.11	6.33	6.55	221	240	256	0.11	0.92
N_2	6.66	6.97	7.27	250	300	320	0.10	1.37
N_3	6.11	6.13	6.16	247	270	300	0.14	0.31
N_4	5.55	5.83	6.11	234	260	280	0.16	3.51
N_5	7.22	7.33	7.44	200	262	311	0.11	2.54
N_6	5.55	6.22	6.88	200	260	305	0.15	3.82
NF_1	6.66	6.94	7.22	200	250	300	0.22	6.23
NF_2	6.38	6.52	6.66	210	300	337	0.47	4.73
NF_3	7.22	7.24	7.27	210	280	317	0.36	6.11
NF_4	6.11	6.41	6.72	210	240	252	0.35	6.11
NF_5	6.66	6.74	6.83	200	24	283	0.35	6.87
NF_6	6.94	6.99	7.05	200	250	304	0.34	6.80