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Fullerene C_{60} Nanoparticles Potentiate the Antioxidant Defense System of Brain and Liver by Increment of Catalase Activity in Normal Rats

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Abstract

Background: It has been demonstrated that fullerene C_{60} nanoparticles and their derivatives showed the antioxidant properties in a wide range of the *in vitro* and *in vivo* studies.
Objectives: Hence, we examined the effects of oral administration of fullerene C_{60} nanoparticles for eight weeks on antioxidant capacity of brain and liver through assessment of catalase activity in normal rats.
Methods: The experiment was performed in two groups of Wistar rats (each group, n=6); untreated and treated normal animals. Treated rats received orally fullerene C_{60} nanoparticles via oral gavage at dose of 1 mg/kg/day. Blood glucose and body weight of rats were measured during the study. At termination of the study, catalase activity of brain and liver was determined using the method of Aebi.
Results: Treatment with fullerene C_{60} did not change blood glucose of treated rats compared to untreated animals. The rats of both groups showed similarly progressive weight gain during the study. Fullerene administration significantly increased catalase activity in the brains of treated rats (0.34±0.10 U/mg protein) compared to untreated animals (0.12±0.03 U/mg protein), ($p<0.05$). Fullerene C_{60} also significantly increased the mean value of catalase activity in the livers of treated rats (6.14±0.76 U/mg protein) compared to untreated animals (2.07±1.43 U/mg protein) ($p<0.05$).
Conclusion: Fullerene C_{60} nanoparticles potentiate the antioxidant capacity of brain and liver through enhancement of catalase activity. Hence, fullerene C_{60} can be used for prevention of damage to brain and liver against ROS accumulation and oxidative stress in various pathological situations.

Keywords: Fullerene, Nanomaterial, Antioxidant capacity, Catalase, Oxidative stress.

Introduction

Reactive oxygen species (ROS) accumulation and oxidative stress in the tissues has been demonstrated that plays a crucial role in pathophysiology of various chronic diseases as well as aging.[1,2] In the physiological situations, ROS are often produced in the tissues and to preserve a redox balance are eliminated by the antioxidant systems.[3,4] It is believed that to balance between oxidation and anti-oxidation, the endogenous antioxidant systems are not sufficient and therefore, the exogenous antioxidants are the constant demand to prevent oxidative stress in the tissues.[5] Nevertheless, change in the antioxidant capacity of the tissues is the main reason of ROS accumulation and oxidative stress, so that antioxidant therapy might diminish these lesions.[4,6,7] Therefore, application of many natural or synthetic materials with the anti-ROS properties determines the attractiveness of antioxidants for pharmacotherapy. For example, using several antioxidants prevented progression of chronic neurodegenerative diseases such as Parkinson's disease (PD) disease and Alzheimer's disease (AD) as well as delayed aging process.[5,8]

Spherical fullerenes (buckminsterfullerene or fullerene C_{60}) are nanomaterials made of pure carbon atoms in a cage-like shape.[8] Several beneficial effects of these nanoparticles and their derivatives have been

demonstrated in biological systems.[9] The antioxidant function of fullerene C_{60} and its derivatives have been shown in a wide range of the *in vitro* and *in vivo* studies.[5,8,10,11] These nanoparticles behave as an excellent antioxidant through passing the cell membrane and localizing preferentially in the mitochondria.[8] Tokuyama et al., reported that fullerene C_{60} derivatives protected the cells against photo-induced cytotoxicity as well as photo-induced DNA damage.[12] It has been reported that oral administration of fullerene C_{60} prolonged the lifespan in an experimental model of CCl_4 intoxication in rats.[9] Promising findings are shown fullerene C_{60} and its derivatives among the new nanoparticles are useful in skin care and cosmetics that make eager many manufacturers to use them in cosmetics and topical preparations.[11] Application of fullerene compounds also developed neuroprotection and improved the axonal loss and disability in a mouse model of progressive multiple sclerosis, which might be useful in treatment of neurodegenerative diseases.[13] Moreover, the neuroprotective functions of fullerene C_{60} have been reported in ischemic stroke. Administration of fullerene during ischemic stroke decreased the injuries of cerebral ischemia by inhibition of oxidative damage and inflammatory response.[14-16]

Objectives

As regards numerous studies have demonstrated that fullerene C_{60} nanomaterials behave as a powerful scavenger of ROS and an excellent antioxidant in the preclinical *in vitro* and *in vivo* studies, we examined the effects of oral repeated administration of fullerene C_{60} nanoparticles on the antioxidant capacity of brain and liver by assessment of catalase activity in normal rats.

Methods

Animals

Adult male Wistar rats, weighting about 220±20 gr (aging 8-10 weeks old), were employed in the current study. The rats acclimatized in the institutional animal house and were given standard chow and water ad libitum. 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

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

Experimental Design and Grouping

Twelve rats were randomly assigned into two untreated and treated groups (each group, n=6). Treated rats received orally fullerene C_{60} nanoparticles via oral gavage at dose of 1 mg/kg/day for eight weeks. Untreated rats received orally sesame oil per day without fullerene C_{60} nanoparticles in the same volume of the treated rats for eight weeks. Blood glucose and body weight of rats were measured during the study.

Tissue preparation

At termination of the study, the tissues (brains and livers) were quickly removed under deep anesthesia for examination of the protein concentration and catalase activity. After homogenization of the brains and livers in ice-cold phosphate buffered saline (PBS), the homogenates were centrifuged at 14000 g at 4 °C for 15 min. Then, the supernatants were used to determine the protein concentration and catalase activity in the brains and livers.

Determination of protein levels

The method of Bradford was used to quantify the protein levels of brains and livers for data calculation.[18] To calculate the protein concentration, bovine serum albumin (BSA; Sigma, Germany) was used as a standard.

Determination of catalase activity of brain and liver

The method of Aebi was used to determine the activity of catalase in the brain and liver tissues homogenates.[19] First, the homogenate was incubated in the reaction mixture that contained 0.1 ml homogenate and 0.85 ml potassium phosphate buffer (50 mM and pH 7.0) at room temperature for 10 min. Then, the reaction was started by adding 0.05 ml H_2O_2 (30 mM prepared in 50 mM potassium phosphate buffer, pH; 7.0). A decrease in the absorbance was recorded by a spectrophotometer at 240

nm for 3 min. Specific activity of catalase in the brain and liver tissues was calculated as U/mg protein. One unit (U) of catalase was defined as 1 nMol H_2O_2 decomposed per min.

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 illustrates the representative changes of blood glucose during the study in normal and treatment groups. Blood glucose of the normal animals was 6.19±0.64 mmol/l at beginning of the test. This value did not significantly change during the test at days 30 (6.46±0.56 mmol/l) and 60 (6.73±0.55 mmol/l). Also, blood glucose of the fullerene-treated normal rats was 6.66±0.39 mmol/l at beginning of the test. This value did not change in treatment group during the test at days 30 (6.80±0.31 mmol/l) and 60 (6.95±0.25 mmol/l). Finally, there were no significant differences in the blood glucose levels of two groups at the different mentioned times during the test.

Effect of fullerene C_{60} on body weight

As shown in Figure-2, the mean value of body weight in normal and treatment groups was 225±22 g and 205±5 g at beginning of the test. The rats of both groups showed similarly progressive weight gain during the study. The body weight of untreated animals at days 30 and 60 was 265±19 g and 295±23 g, respectively. Also, the body weight of fullerene-treated animals at days 30 and 60 was 264±22 g and 298±29 g, respectively. There were no significant differences in the body weight values of two groups at the different mentioned times during the test.

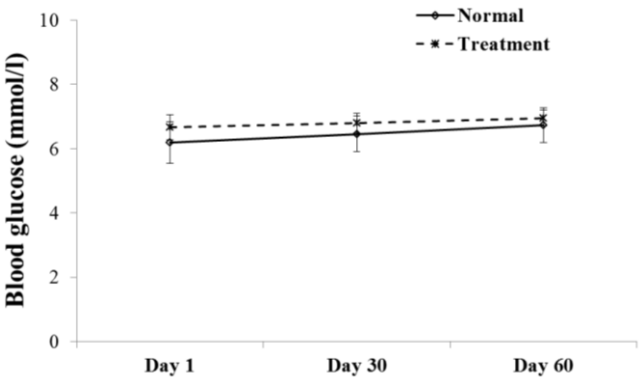

Figure-1. The representative changes of blood glucose (mmol/l) during the study in normal and treatment groups. There were no significant differences in the blood glucose levels of two groups during the test at the different times. All values are presented as mean±SD (n=6).

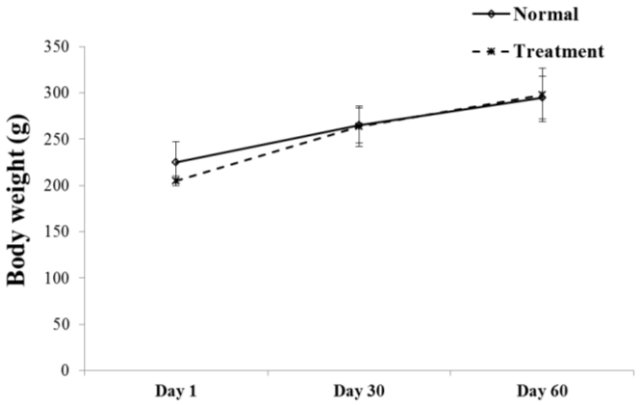

Figure-2. The representative changes of body weight (g) during the study in normal and treatment groups. There were no significant differences in the values of body weight of two groups during the test at the different times. All values are presented as mean±SD (n=6).

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

Figure-3 illustrates the catalase activity of brain at termination of the experiment. The mean value of catalase activity in the brain tissues of untreated rats was 0.12±0.03 U/mg protein. Fullerene treatment significantly increased the catalase activity in the brain tissues of treated rats (0.34±0.07 U/mg protein) compared to untreated animals ($p<0.05$).

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

As shown in Figure-4, the mean value of catalase activity in the liver tissues of untreated rats was 2.07±1.43 U/mg

protein at termination of the experiment. Treatment with fullerene nanoparticles significantly increased the value of catalase activity in the liver tissues of treated rats (6.14±0.76 U/mg protein) compared to untreated animals ($p<0.05$).

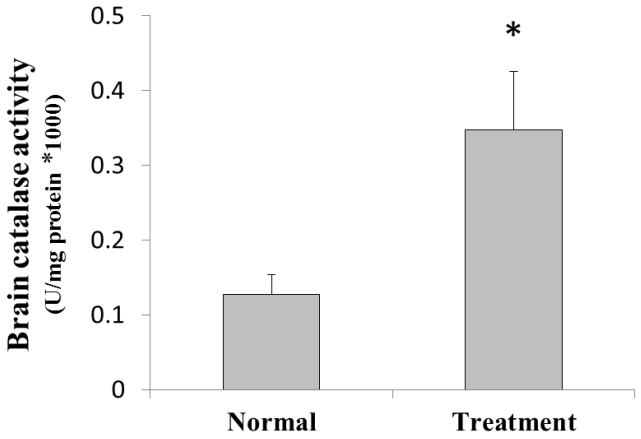

Figure-3. The effects of fullerene nanoparticles on catalase activity of brain (U/mg protein × 1000) in treated rats (treatment group) at termination of the study. All values are presented as mean±SD (n=6). *($p<0.05$) as significant difference compared with normal group

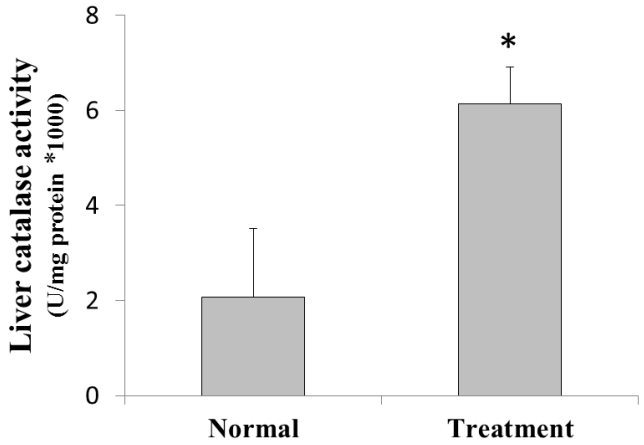

Figure-4. The effects of fullerene nanoparticles on catalase activity of liver (U/mg protein × 1000) in treated normal rats (treatment group) at termination of the study. All values are presented as mean±SD (n=6). * ($p<0.05$) as significant difference compared to normal group

Discussion

It has been demonstrated that fullerene C_{60} nanoparticles show a powerful antioxidant activity in the biological environments.[5,8,15] Since accumulation of oxygen free radicals and development of tissue oxidative stress plays a crucial role in pathophysiology of damage during tissue degenerative diseases as well as aging,[1,2] the current study examined the effects of fullerene C_{60} nanoparticles on the antioxidant capacity of brain and liver through assessment of catalase activity at normal condition in rats. The results of present study indicated that fullerene C_{60} administration during the test increased catalase activity in brain of the treated normal rats. Also, the catalase activity of liver increased in fullerene-treated normal rats during the study. Hence, the results of present study revealed that fullerene C_{60} nanoparticles could potentiate the antioxidant capacity of brain and liver and also enhance the capability of resistance of the mentioned tissues against oxidative stress.

According to the previous findings, fullerene C_{60} nanoparticles behave as a free radical sponge, and therefore these nanomaterials are able to eliminate the various oxygen and nitrogen free radicals in the biological environments.[5,8,14] Since ROS is involved in the pathogenesis of tissue degeneration during various disorders as well as aging process,[1,2] fullerene might be helpful for reduction of these tissue damages. Based on our results, administration of fullerene C_{60} nanoparticles markedly increased the catalase activity in the brains of treated rats. Catalase is an enzyme responsible for detoxification of H_2O_2 formed by the action of superoxide dismutase.[20] This finding has been confirmed by another study that showed that fullerene C_{60} could act as catalase mimetic an *in vitro* study.[10] Hence, it is concluded that fullerene enhance the antioxidant capacity of normal brain against ROS accumulation through enhancement of catalase activity. Because the power of the antioxidant defense system of brain is feeble compared to other tissues,[21] potentiation of the brain antioxidant capacity might be helpful for prevention of brain damage against ROS in several neurodegenerative disease as well as aging phenomena. Therefore, application of these nanomaterials might prevent neurodegeneration and delay the aging process in brain through enhancement of the brain antioxidant capacity.

According to the previous studies, oxidative stress induced by various endogenous and exogenous compounds may play a crucial role in the incidence of

hepatotoxicity.[22-24] Hence, potentiation of the liver antioxidant capacity might be helpful for prevention of liver damage and hepatotoxicity induced by ROS accumulation. The results of present study indicated that administration of fullerene C_{60} nanoparticles noticeably increased the catalase activity in the livers of treated normal rats. In agreement with our results, fullerene C_{60} could act as the superoxide dismutase and catalase mimetics in an *in vitro* experiment.[8,25] Catalase enzyme in accompany with superoxide dismutase protects the hepatic cells against damage caused by free radicals such as hydroperoxides and lipoperoxides.[26] Hydrogen peroxide (H_2O_2), as a toxic and main oxygen free radical, is generated endogenously or produced by action of superoxide dismutase on superoxide anions.[20] Catalase inhibits cellular oxidative damage through neutralizing these hydroperoxides by metabolizing there to water and oxygen.[20] In the lack of catalase activity, the hydrogen peroxide can be converted to hydroxyl radicals through Fenton reactions and induces an oxidative damage in numerous macromolecules such as lipids (peroxidation), nucleic acids and proteins (oxidation).[1] Also, fullerene C_{60} regulates hydrogen peroxide-dependent signal transduction pathways by influencing the cellular levels of catalase activity, because hydrogen peroxide can act as a physiological signal transduction molecule.[27] Therefore, administration of these nanomaterials might exhibit hepatoprotective effects against ROS-induced toxicity in various pathophysiological states or in the conditions of ROS accumulation at liver through enhancement of liver antioxidant capacity.

Conclusions

It is concluded that fullerene C_{60} nanoparticles might exhibit the neuroprotective and hepatoprotective effects against ROS-induced brain and liver toxicity. These protective effects of fullerene C_{60} might be due to potentiation of the antioxidant defense systems through enhancement of catalase activity. Hence, it is suggested that administration of fullerene C_{60} nanomaterials can be useful for prevention of brain and liver tissue damage in the conditions of ROS accumulation.

Acknowledgment

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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

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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