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Fullerenes: Oral Toxicity and Biological Effects †

Vladimir A. Shipelin [1,2,*]

[1] Federal Research Centre of Nutrition, Biotechnology and Food Safety, Moscow 109240, Russia
[2] Academic Department of Innovational Materials and Technologies Chemistry, Plekhanov Russian University of Economics, Moscow 115054, Russia
* Correspondence: v.shipelin@yandex.com; Tel.: +7-495-698-53-68
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Abstract: The introduction into the circulation of products containing fullerenes is hindered by the ambiguity of the properties exhibited, consisting of the presence of signs of nanotoxicity for biological systems in vitro and in vivo. This study aimed to evaluate the oral toxicity of the fullerene C60 and its water-soluble derivative $C_{60}(OH)_{24}$ at their daily doses from 0.1 to 10 mg/kg body weight (bw) in the long-term experiments on Wistar rats. According to data, C_{60} and $C_{60}(OH)_{24}$ exhibited a general toxic effects in animals: the NOAEL for C_{60} was at least 1 mg/kg bw/day, and for $C_{60}(OH)_{24}$—at least 0.1 mg/kg bw. The results obtained indicate the presence of risks associated with the effects of fullerenes on the human body during the oral route of intake and indicate the need for their regulation in consumer products (including food products) and environmental objects.

Keywords: fullerene; C_{60}; $C_{60}(OH)_{24}$; rats; oral toxicity; NOAEL; intestine barrier; antioxidant effect; selenium

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1. Introduction

Among the various types of nanoindustry products, fullerenes, which represent a new allotropic form of carbon, occupy a special place. The fields of practical application of fullerenes are constantly expanding and include chemical synthesis and catalysis, electronics, optics, printing, paint and varnish industry, pharmacology, production of perfumes and cosmetics, biosensors, packaging materials, plant protection products, etc [1–3]. The search among fullerene derivatives for new biologically active compounds with antioxidant, hepatoprotective, radioprotective and other types of protective effects on the human body led to the development of water-soluble forms of fullerenes, one of the representatives of which is fullerenol $C_{60}(OH)_{24}$ [4,5]. The result of the expansion of the production of fullerenes and products containing them is the transformation of fullerenes into significant environmental contaminants and an increase in the risks of human exposure to fullerenes in various ways of their receipt (skin, oral, inhalation) at the stages of their production, use and disposal of the resulting waste [6]. The issue of ecotoxicity of fullerenes and the possibility of their transfer along trophic chains in the biosphere is relevant today. Unfortunately, all these concerns have not yet been accompanied anywhere in the world by any attempts to regulate fullerenes; in particular, their hygienic rationing in food products and environmental objects is completely absent.

The greatest significance and relevance in the light of possible scenarios of fullerenes' impact on the human body is their toxicological and hygienic assessment in the natural routes of entry into the body, that is, primarily through the gastrointestinal tract, as well as during inhalation and epicutaneous exposure.

The purpose of this work was to assess the possible effects of the most important representatives of the fullerene family—unmodified fullerene C_{60} and its water-soluble

derivative fullerenol $C_{60}(OH)_{24}$, on a wide range of indicators characterizing the state of the body of laboratory animals when administered through the gastrointestinal tract.

2. Materials and Methods

Unmodified fullerene C_{60} (purity of at least 99.8% according to its own HPLC analysis) and its water-soluble derivative fullerenol $C_{60}(OH)_{24}$ (purity of about 98% according to the manufacturer), produced by LLC Fullerene Center, Nizhny Novgorod (Russia), were used as objects of toxicological and hygienic assessment. The study of C60 particle sizes in the dispersion was carried out by the spectroacoustic method, the average particle size of fullerenol $C_{60}(OH)_{24}$—by dynamic laser light scattering. Size distribution in prepared solutions shown in Figure 1.

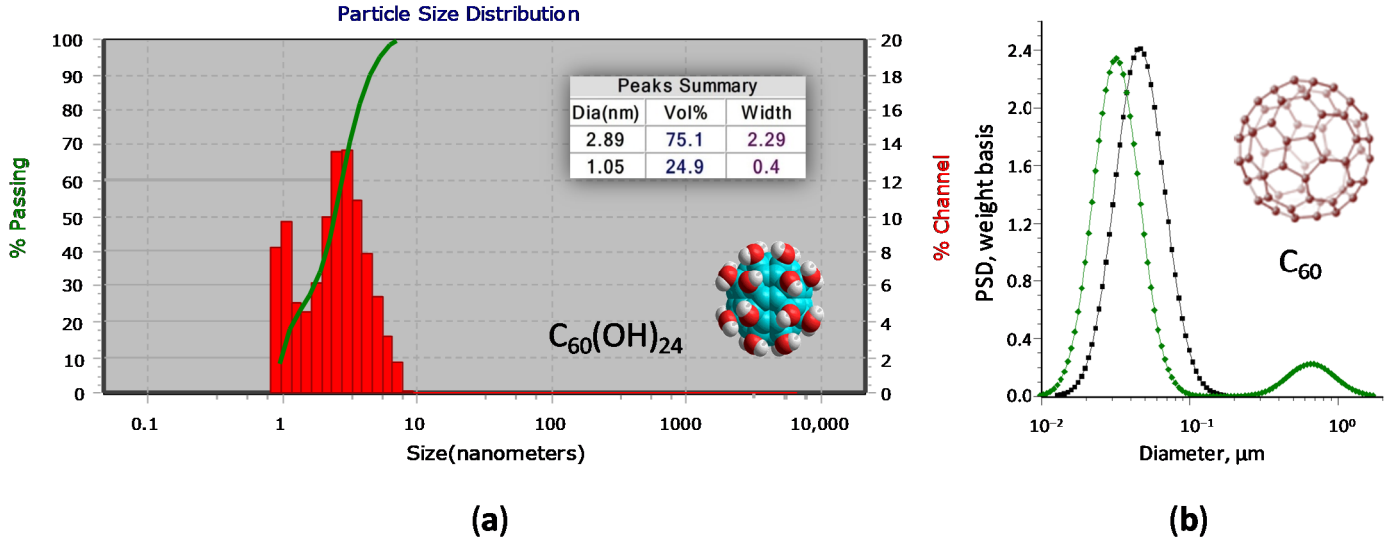


Figure 1. Fullerenes size distribution. (**a**) DLS of $C_{60}(OH)_{24}$ water solution (75.1% represented by particles with average diameter of ~2.89 nm and 24.9% represented by particles with diameter of ~1.05 nm. (**b**) Spectroacoustic study of water colloidal dispersion of fullerene C_{60} in 2% starch solution (75% represented by ~30 nm particles and 25% by ~600 nm).

In the first subacute toxicological experiment with C_{60} lasting 28 days, the study was carried out on 60 rats with an initial body weight (bw) of 111 ± 2 g (M ± s.e.m.). The animals were divided into 4 groups of 15 rats each. Group 1 (control) received distilled water. Group 2 (vehicle) animals received a vehicle (2% water solution of starch). Group 3 rats were received C_{60} dispersion in vehicle at a dose of 1 mg/kg bw, and group 4 rats were administered at a dose of 10 mg/kg bw.

In the second subacute toxicological experiment with fullerene C_{60} lasting 92 days, the study was carried out on 75 rats with an initial bw of 100 ± 5 g (M ± s.e.m.). The animals were divided into 5 groups of 15 rats each. Animals of group 1 (control) received distilled water. Animals of the 2nd experimental group received a fullerene vehicle solution. Rats of 3rd, 4th, and 5th experimental groups received C_{60} in the form of dispersion in the carrier at a dose of 0.1, 1.0, and 10.0 mg/kg bw, respectively.

In the third subacute toxicological experiment with fullerenol $C_{60}(OH)_{24}$ lasting 28 days, the study was carried out on 60 rats with an initial weight of 122 ± 2 g (M ± s.e.m.). The animals were divided into 4 groups of 15 rats each: the 1st group of animals (control) received deionized water; groups from the 2nd to the 4th—fullerenol $C_{60}(OH)_{24}$, dissolved in deionized water, at doses of 0.1, 1, and 10 mg/ kg bw, respectively.

All solutions were administered intragastrically through a gavage. The design of the experiment and the studied indicators are shown in Figure 2.

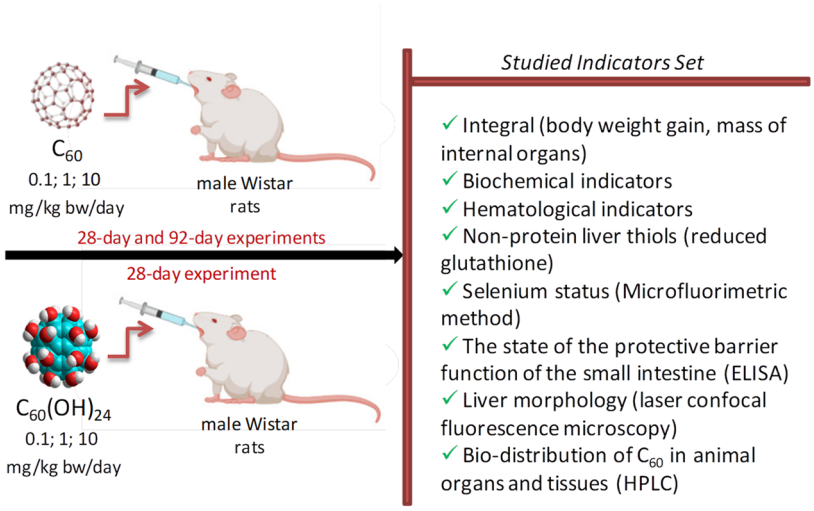
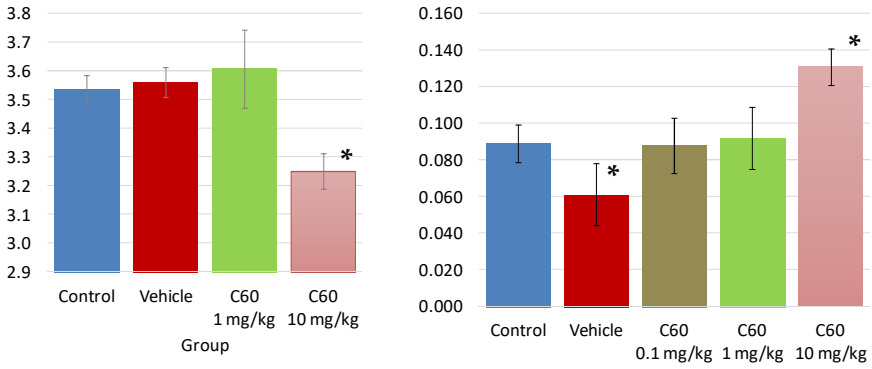


Figure 2. Study design and indicators studied.

3. Results

As a result of intragastric administration of nanoscale dispersion of C_{60} to rats for 28 and 92 days at doses from 0.1 to 10 mg/kg bw daily, changes in endpoints indicating the presence a general toxic effect of this compound on the animal body, including a dose-dependent decrease in the relative weight of the liver (Figure 3a), an increase in the permeability of the small intestine barrier for protein macromolecules up to 100% (Figure 3b), an increase in the number of $CD106^+$ granular cells in the liver parenchyma (Figure 4). Based on the analysis of the data obtained, the maximum inactive dose of fullerene C60 with subacute oral administration is in the range of 1–10 mg/kg bw/day.



(**a**) (**b**)

Figure 3. Integral indicators of rats. (**a**) Relative weight of the rat liver at the end of the 28-day experiment, % of body, M ± S.E.M. (**b**) Absorption of the antigenic ovalbumin protein into the blood of animals, % of the administered dose at the end of the 92-day experiment, M ± S.E.M. *—the difference with control groups is significant, $p < 0.05$.

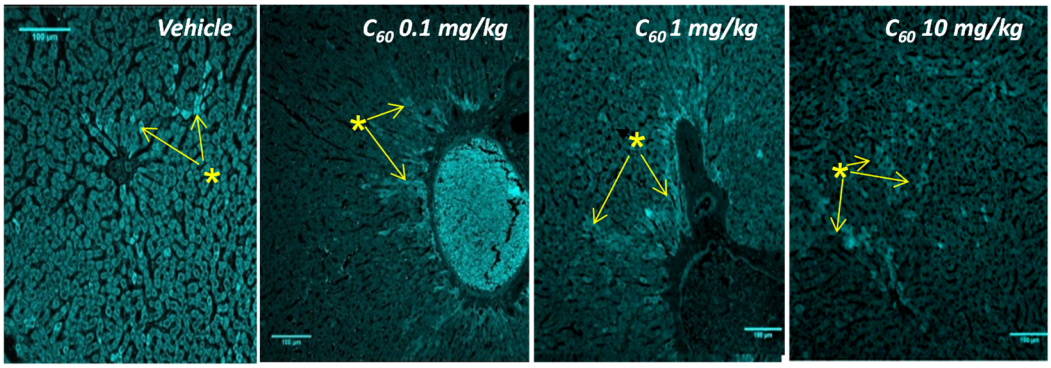
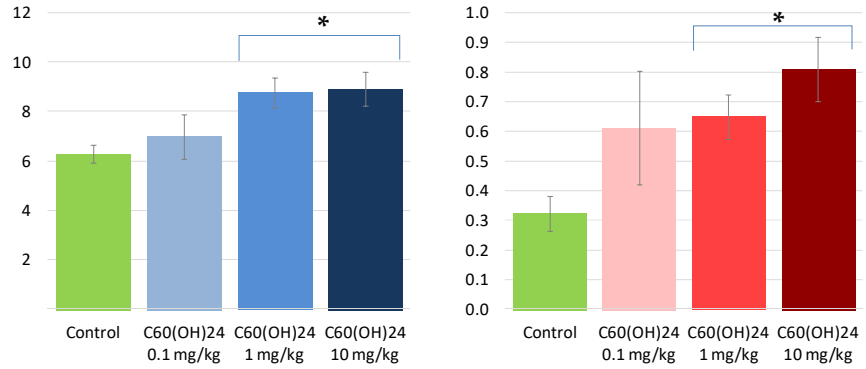


Figure 4. Comparison of confocal images of the liver stained with AT to CD106 (92-day experiment). The position of $CD106^+$ cells morphologically similar to Kupfer macrophages was noted *. Magnification ×400.

In a 28-day experiment with fullerenol C60(OH)24, its daily oral intake at a dose of 0.1 mg/kg bw or more caused a number of significant changes in the indicators of the rat body, in particular, a significant increase in the mass of the adrenal glands up to 15%, changes in the leukocyte formula of the blood, which were manifested in a significant increase in the number of monocytes and the relative content of immature granulocytes (Figure 5). These data may indicate that the negative effect of fullerenol $C_{60}(OH)_{24}$ on animals is manifested starting from a dose of 1 mg/kg bw. This suggests that the NOAEL of fullerenol in a monthly experiment is not less than 0.1 mg/kg and not more than 1.0 mg/kg of body weight.



(**a**) (**b**)

Figure 5. Hematological indices of the rats (92-day experiment). (**a**) The average content of monocytes (M ± S.E.M). (**b**) The average content of immature granulocytes (M ± S.E.M). *—the difference with control group is significant, $p < 0.05$.

It is worth noting an unexpected biological effect—the selenium content in the blood and liver of rats treated with fullerene C_{60} for 92 days indicated an increase in the accumulation of this trace element in the liver of rats treated with fullerene C_{60} at doses of 1 and 10 mg/kg bw, when compared with the second control group receiving a carrier solution. The dependence of the selenium content on the dose of fullerene C_{60} was also observed in the blood of rats, at least for the two largest doses of fullerene C_{60}. It should be noted that there is no reason to interpret the revealed effect as unfavorable, taking into account the role of selenium compounds as indirect antioxidants. The effect characterizing a pronounced dose-dependent increase in the selenium content in the brain (Figure 6) of animals treated with fullerene C_{60}, in its magnitude and orientation, also cannot be unambiguously considered as a sign of an adverse effect on the body. This result speaks in favor of the works published in the literature [7], in which the presence of labeled

fullerenes in the brain was established and their penetration through the blood-brain barrier was suggested.

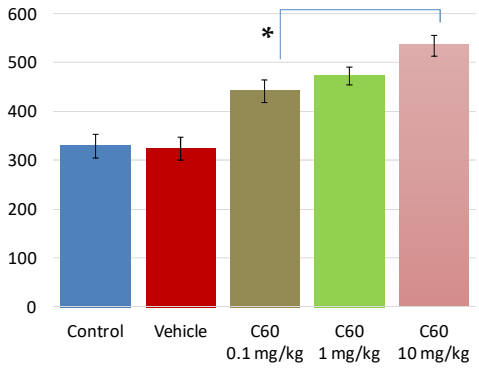


Figure 6. The average (M ± SD) selenium content in the brain of rats treated with C_{60} for 92 days. *—differences between experimental groups and control groups are significant, $p < 0.05$.

4. Conclusions

The results of the toxicological assessment of fullerene C_{60} and fullerenol $C_{60}(OH)_{24}$ indicate the presence of risks associated with the impact of these compounds on the human body when ingested and indicate the need for hygienic regulation of these compounds in consumer products (including food products) and the environment.

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Institutional Review Board Statement: The design of the experiment was approved by the Ethics Committee of the Federal Research Centre of Nutrition, Biotechnology and Food Safety.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request due to restrictions e.g., privacy or ethical.

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Conflicts of Interest: The author declares no conflict of interest.

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