

INFLUENCE OF C₆₀-FULLERENE AQUEOUS COLLOID SOLUTION ON LIVER AND PANCREAS MORPHOLOGICAL STATE AND BLOOD AMINOTRANSFERASES OF RATS WITH EXPERIENCED ACUTE CHOLANGITIS

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Aim of the work was to investigate the suspended C₆₀-fulleren effect on liver and pancreas state under intraperitoneal and intragastrical administration on rat experimental cholangitis model. Acute cholangitis was simulated by a single ingestion of α -naphthyl isothiocyanate — ANIT. C₆₀-fullerene aqueous colloid solution (C₆₀FAS, 0.15 mg/ml) was administered to animals at a volume containing C₆₀-fullerene at a dose of 0.5 mg/kg body weight in 24 and 48 h after ANIT administration. After 72 h of the experiment, the animals were euthanized. Blood serum ALT and AST activities were measured, the liver and pancreas states were analyzed by light-microscopy level. It was found that intragastrical and intraperitoneal administration of C₆₀FAS contributes to the correction of negative effects in the liver and pancreas caused by the induction of acute cholangitis. This was proved by the normalization of ALT activity, reduction of pancreatic parenchymal edema and liver fibrosis, and increased blood flow in these organs. Application of C₆₀FAS could improve the state of the liver and pancreas under acute cholangitis in rats.

Key words: C₆₀-fullerenes, acute cholangitis.

Primary sclerosing cholangitis (PSC) is a chronic cholestatic disease characterized by intrahepatic and extrahepatic bile ducts inflammation, obliteration and fibrosis [1], resulting in biliary cirrhosis, portal hypertension and hepatic failure. The etiology of PSC is unknown. Genetic predisposition (in particular hla b8, dr3, drw52) [2] and environmental factors, including bacterial and viral infections, are considered to be risk factors. Most often this pathology is associated with autoimmune diseases (primary biliary cirrhosis, rheumatoid arthritis, autoimmune hepatitis, systemic sclerosis, systemic lupus erythematosus, cystic fibrosis) and inflammatory bowel disease (70–80% of cases).

PSC-prognosis is extremely unfavorable due to following complications: portal

hypertension accompanied by bleeding and functional renal insufficiency (hepatorenal syndrome), spontaneous bacterial peritonitis, chronic cholestasis accompanied by weakness, itching, steatorrhea, deficiency of fat-soluble vitamins (A, D, E and K) and osteoporosis. Specific complications of PSC include bacterial cholangitis (15–35%), cholelithiasis (25–56%), chronic pancreatitis (20–23%) and cholangiocarcinoma (6–18%). The survival rate of patients in the last case does not exceed 12 months [1].

PSC-therapy is symptomatic and includes medications for treatment itching and jaundice (choleretics, sorbents), antibiotics (for the treatment of infections), diet and vitamin supplements, immunosuppressants and anti-inflammatory drugs. The only effective method

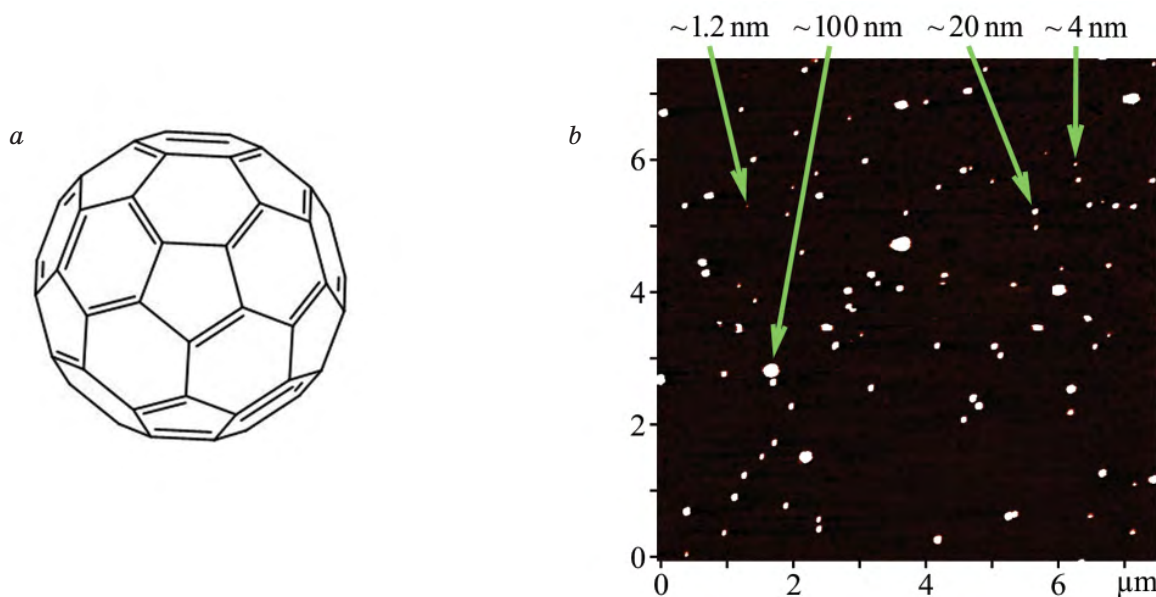


Fig. 1. C_{60} fullerene (a), AFM image (tapping mode) of C_{60} fullerene particles on the mica surface: arrows indicates the height of individual nanoparticles (b)

for PSC treating is liver transplantation, but PSC relapse after transplantation occurs in 15–20% of patients, and the 5-year survival rate is only 66–72% [3].

PSC, as any inflammatory disease, is accompanied with oxidative stress, increased levels of lipid peroxidation products and antioxidants deficiency. Therefore, the use of the latter is promising to correct at least the disease symptoms, remaining, however, beyond the attention of researchers. Numerous studies have shown the anti-inflammatory effects of natural antioxidants (vitamins, minor amino acids, polyunsaturated fatty acids, plant extracts) *in vitro*, but effectiveness of those in *in vivo* systems is highly questionable. On the other hand, artificial compounds, in particular fullerene, have clearly defined properties and are involved in a number of cellular processes [4–9], which determines their selective action and a more pronounced therapeutic potential. C_{60} -fullerene can effectively scavenge free radicals and thus act as antioxidants [10, 11], revealing anti-inflammatory properties [12, 13]. They are non-toxic in *in vitro* and *in vivo* systems acting in physiological concentrations [6, 8, 14] and are capable to be accumulated in the liver [15]. The last makes them very attractive for direct impact on this organ. Thus, C_{60} -fullerene may be considered as potential therapeutics for effective prevention and treatment of liver diseases associated with oxidative stress.

Given the foregoing, the aim of the study was to investigate the morphofunctional state of the liver and pancreas in rats received suspended C_{60} -fullerene under α -naphthyl isothiocyanate-induced acute cholangitis condition.

Materials and Methods

The study was conducted on 32 white outbred male rats with an average body mass 198 ± 10 g, which were kept under standard vivarium conditions. All experiments were conducted in compliance with bioethics principles, legislative norms and provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), General Ethical Principles for Experiments on Animals, adopted by the First National Bioethics Congress (Kyiv, 2001), and approved by an institutional review committee (protocol № 2 dated April 25, 2017, ESC “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv).

Acute cholangitis was simulated by a single ingestion of α -naphthyl isothiocyanate (ANIT, Sigma, USA) at a dose of 100 mg/kg dissolved in sunflower oil (total volume of 0.1 ml). ANIT is a hepatotoxin which depending on dose and duration of administration causes liver damage, such as intrahepatic cholestasis, acute cholestatic hepatitis, sclerosing cholangitis, biliary fibrosis and cirrhosis [16]. The mechanism of action of

this toxicant is associated with a specific lesion of the epithelial cells of intralobular bile ducts. Cholangiocytes' injury causes bile ducts obstruction with detritus or cells excessive growth with subsequent sclerotic degeneration resulting in bile flow stop and periportal inflammation [16]. The systemic result is blood serum and urine bilirubin increase (mainly due to direct bilirubin) and serum aminotransferases and alkaline phosphatase activity raise, which corresponds to the biochemical manifestations of acute and chronic sclerosing cholangitis in humans.

A highly stable pristine C₆₀-fullerene aqueous solution (C₆₀FAS) with purity of more than 99.95% has been prepared and characterized according to [17, 18]. Briefly, this method is based on transferring C₆₀-fullerene from organic solution into the aqueous phase by ultrasonic treatment. The maximal concentration of C₆₀-fullerene in water was 0.15 mg/ml. The morphological state of C₆₀-fullerene in aqueous solution was monitored using atomic force microscopy and measurement of small-angle neutron scattering (Fig. 1) [19]. Concentrated C₆₀-FAS contained both single C₆₀ molecules and their nanoparticles (aggregates) with sizes of 1.2–100 nm which is in agreement with our previous results [17, 20].

Animals received C₆₀FAS intraperitoneally or intragastrically in a dose equal to 0.5 mg / kg body weight C₆₀-fullerene in 24 and 48 h after ANIT administration. All manipulations with the animals in comparison groups were conducted similarly to the animals of the experimental ones, including the appropriate solutions administration. There were 4 experimental groups ($n = 8$): 1 — control; 2 — acute cholangitis; 3 — cholangitis and C₆₀FAS intraperitoneal administration; 4 — cholangitis and C₆₀FAS intragastrical administration. Since the effect of C₆₀FAS on healthy animals has been studied and described in our previous studies [9, 21], we did not include the description of groups of healthy animals receiving C₆₀FAS in the current manuscript.

In 72 h after the start of the experiment the animals were killed by inhalation of CO₂ and subsequent cervical dislocation. The blood for biochemical analysis was collected immediately after the sacrifice from the femoral vein, left for 20 min to form a clot and then centrifuged 8 min at 1000 g. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in blood serum using standard

reagent kits (Filisit diagnosis, Ukraine) and expressed in μmol sodium pyruvate per ml of blood serum per h.

The fragments of liver and pancreas were harvested immediately after the sacrifice and fixed in Bouin mixture for 14 days for histological assay. Then, they were embedded into paraffin, sliced into 5 μm sections, stained with hematoxylin and eosin [22] and examined under the light microscope (Olympus BX-41, Olympus Europe GmbH, Japan). Liver centrilobular and periportal zones, exocrine and endocrine part of the pancreas were assessed separately.

Statistical processing of data was carried out using one-way analysis of variance (ANOVA) with the Tukey post hoc test [23]. The difference between compared groups was considered significant at $P \leq 0.05$.

Results and Discussion

The yellowness of the peritoneum and mucous membranes resulting from the accumulation of bilirubin in plasma blood and tissues, edema and liver granularity suggesting the micronodular fibrosis were observed at the autopsies of all animals from the cholangitis group (group 2). On liver micropreparations (Fig. 2, B) one can see fibrotically altered portal tracts with surrounding cellular inflammatory infiltrate, sites with violation of the parenchymal limiting plate and portal-portal linking septa, suggesting the acute cholangitis. Atrophic and degenerative changes in the bile duct epithelium and some bile ducts replaced by fibrous cords (scars) were observed. Lymphoid follicles and fibrotic loci were detected in parenchyma. Also, thrombosis of some blood vessels including central veins, sinusoids dilation and a marked increase in the number of leukocytes in vessels were detected. Blood serum ALT and AST were higher than those in control group (by 81% and 75% respectively) (Fig. 3) indicating cytolysis of hepatocytes and cholestasis, which may be the result of bile duct obstruction.

The pancreas of rats in this group revealed significant structural changes (Fig. 4, B): large areas of secretory acini destruction; dystrophic changes of acinar tissue; cellular and intercellular edema, which sometimes caused the destruction of both individual pancreatic cells and whole acini; cytoplasm zonation loss in pancreatic cells from areas with destroyed structure. Observed changes could indicate a violation of

pancreatic exocrine parenchyma functional state and the development of acute pancreatitis. The gland microcirculatory also underwent changes: there was a stasis and sometimes expressed thrombosis of small blood vessels, sinusoid hemocapillaries dilation and sometimes microthrombosis. The pancreatic endocrine apparatus wasn't changed significantly, pancreatic endocrinocytes did not differ from control ones.

In rats received C₆₀FAS (groups 3 and 4) the jaundice of mucous membranes and peritoneum was less pronounced compared to non-treated animals (group 2), but liver edema and micronodular fibrosis persisted (Figs. 2, C, D). The area of the liver fibrotically altered parenchyma was smaller compared to non-treated animals, portal-portal linking septa weren't detected. However, necrotic loci and loci of hypereosinophilic cells were observed in fibrotically altered sites. Also blood vessels overflow was markedly higher compared to control, sometimes even blood stasis occurred. Occasionally zones with dark inclusions were detected, which might be caused by C₆₀ fullerene accumulation. It should be noted, that rats received C₆₀FAS intragastrically (group 4) demonstrated significantly less expressed signs of periportal inflammation in comparison with those

received C₆₀FAS intraperitoneally (group 3). Manifestations of necrosis in fibrotically altered sites in this group also were less common and less frequent compared to group 3. ALT activity was restored to control values in rats received C₆₀FAS by both ways, although AST activity remained unchanged (Fig. 3), which might indicate partial persistence of cholestasis. Thus, C₆₀FAS inhibited the symptoms of acute cholangitis but did not prevent hepatocyte cytolysis completely.

Blood stasis in small vessels, wall thickening and edema of middle blood vessels were revealed in the pancreas of animals received C₆₀FAS (Figs. 4, C, D). The vast majority of exocrine pancreatic cells had a normal structure, although there were occasional sites with signs of acinus dystrophy and cells with cytoplasmic vacuolation. The cytoplasm of most cells was clearly delineated into basophilic and acidophilic zones, the nuclei had a rounded form, indicating the normal functional activity of the cells. The endocrine part of the pancreas did not undergo significant changes. However, significantly greater interacinar edema was detected in animals received C₆₀FAS intraperitoneally compared to those received C₆₀FAS intragastrically. Summarizing, C₆₀FAS contributed to mitigate of pancreatic injury due to ANIT-induced acute cholangitis.

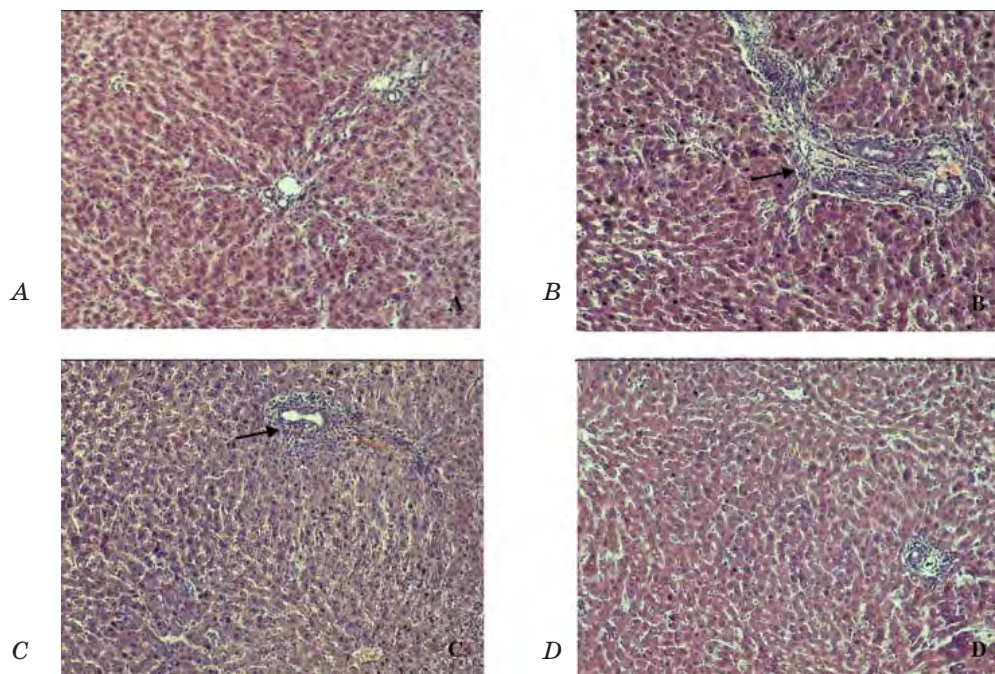


Fig. 2. Microphotographs of liver of rats experienced acute cholangitis and treated with C₆₀FAS: control (A), acute cholangitis (B), cholangitis+C₆₀FAS intraperitoneal (C) and intragastrical (D) administration. Hematoxylin-eosin staining, ×100 magnification; the arrows indicate fibrotically altered portal hepatic tracts with surrounding diffuse cell inflammatory infiltrate

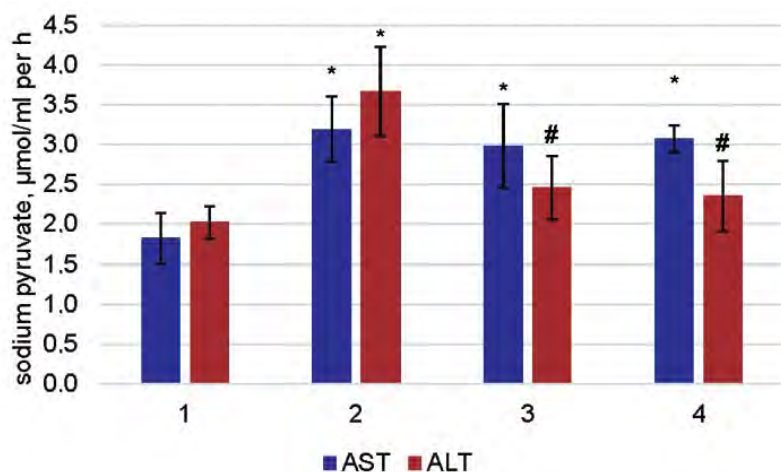


Fig. 3. Blood serum ALT and AST activities of rats experienced acute cholangitis and treated with C₆₀FAS: control (1); acute cholangitis (2); cholangitis+C₆₀FAS intraperitoneal (3) and intragastrical (4) administration;

**P ≤ 0.05 compared to control, #P ≤ 0.05 compared to acute cholangitis group*

One of the mechanisms involved in the pathogenesis of PSC is an abnormal immune response, which leads to the activation of auto-reactive T and B lymphocytes and the subsequent production of numerous inflammatory mediators [24]. As a result, the liver undergoes significant destructive impacts of, in particular, excessive amounts of bile acids (having detergent properties), toxins, inflammatory mediators, autoimmune complexes. This event is the cause of oxidative stress development [25]. Thus, patients with PSC demonstrate significantly increased lipid and protein peroxidation, cholesterol auto-oxidation and downregulation of antioxidant defense system [26–28]. In addition, ANIT-induced liver damage in rats (a valid model of human sclerosing cholangitis) is accompanied by oxidative stress development and is widely used to study hepatoprotective efficacy of different compounds including ones possessing antioxidant properties [29–31].

Biliary pathology is the most common cause of acute pancreatitis and exacerbation of chronic one. The main mechanism of biliary pancreatitis is bile influx into the pancreatic duct (due to the pressure difference in common bile duct and pancreatic duct under the biliary hypertension) with subsequent interaction of bile with pancreatic enzymes and bacteria. Hence, the release of bound bile acids and the activation of pancreatic enzymes immediately in the pancreatic duct system occur. As a result, its protective barrier is damaged and the parenchyma of the gland is affected. Based on clinical and pathological studies the association of PSC with pancreatic disease

was proven [32], and the joint autoimmune component of both diseases was noted [33].

C₆₀-fullerene is a molecular form of carbon having a spheroidal structure. Due to the presence of π -conjugated double bonds between hexa- and pentagonal structures on the surface, C₆₀-fullerene is capable to scavenge reactive oxygen species (ROS) effectively [4, 11, 14]. Its unique properties include strong electron acceptor activity and high polarization and hydrophobic ability, which enables them to effectively bind free radicals not only in the extracellular space, but also penetrate into the cell [5, 10, 34]. Hence, C₆₀-fullerene are capable not only to scavenge free radicals directly [10], but also to be involved in the regulation of intracellular signaling pathways associated with ROS overproduction [4, 11]. An important feature of C₆₀-fullerene is its relatively low toxicity [11], non-immunogenicity [4] and the ability to be accumulated in liver [6, 15], which makes this compound attractive for selective impact on liver and treatment of this organ's diseases associated with oxidative stress. In addition, the ability of C₆₀-fullerene to suppress colonic inflammation under systemic and topical application and to correct its systemic effects [9], to prevent the development of toxic hepatitis [12] and to realize antitumor properties [10] were demonstrated in many studies.

One of the leading roles in the development of autoimmune inflammation belongs to oxidative stress. The last is accompanied by a violation of the pro- and antioxidant balance and overproduction of ROS and reactive

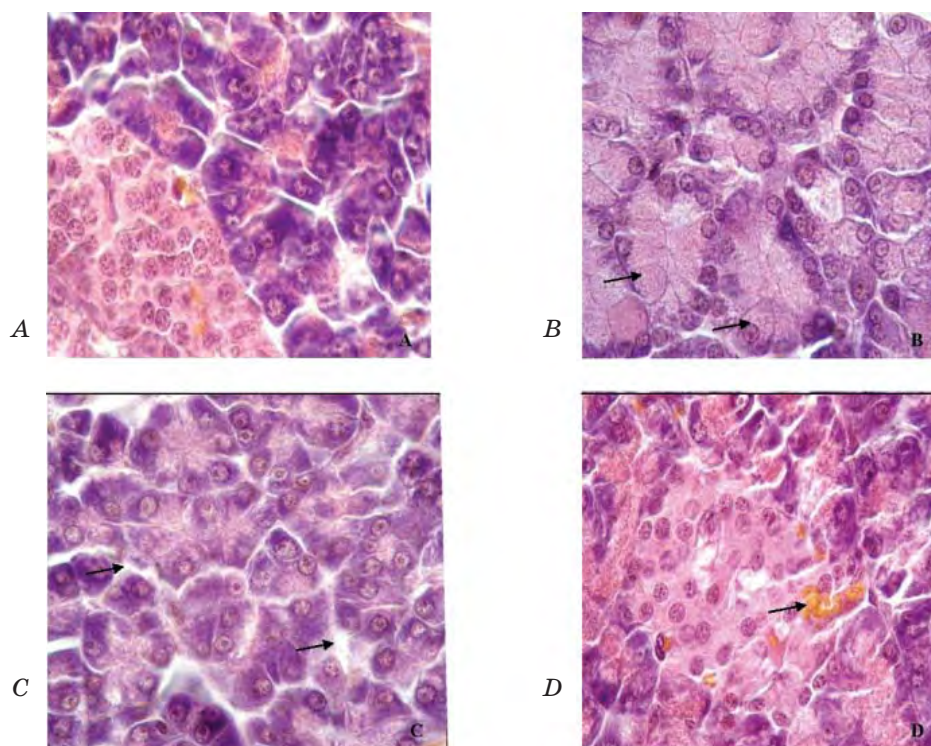


Fig. 4. Microphotographs of pancreas of rats experienced acute cholangitis and treated with C_{60} FAS: control (A); acute cholangitis (B); cholangitis + C_{60} FAS intraperitoneal (C) and intragastrical (D) administration. Hematoxylin-eosin staining, $\times 400$ magnification; the arrows indicate acinar cell edema and cytoplasm zonation loss (B), interacinar edema (C), blood vessels overflow (D)

nitrogen species. The consequence is the initiation of cell phagocytosis and apoptosis leading to tissue damage and intracellular autoantigen release, which in turn results in the production of autoantibodies. In addition, the oxidative modification of macromolecules, in particular DNA, in apoptotic cells leads to the formation of new epitopes, which causes the production of a wide range of polyspecific autoantibodies and the escalation of the autoimmune response process [34, 35].

As mentioned above, most researchers suggest the autoimmune cause of PSC [36, 37], therefore it is logical and reasonable to assume the efficacy of antioxidant use for correction at least the disease symptoms. Therefore, we suppose that C_{60} FAS suppressed the symptoms of acute cholangitis and pancreatitis precisely because of its antioxidant properties. Indeed, the only drug approved for the treatment of PSC—ursodeoxycholic acid—possesses antioxidant properties and normalizes the content of reduced glutathione in liver tissue and blood serum by activating of γ -glutamylcysteine synthase [38], which in turn can cause a positive systemic effect on the organism and particularly on the pancreas. In addition, in our previous studies we demonstrated the ability of C_{60} -fullerene

to suppress lipid and protein peroxidation and to upregulate the antioxidant enzymes in liver under an inflammatory process in the organism [9].

It should be noted that intragastrical administration of C_{60} -fullerene in a dose of 10 mg/kg body weight causes an increase of CYP 2B1 enzyme activity in liver, reduces serum blood uric acid, increases serum blood urea and enhances the small intestinal wall permeability for macromolecules [39]. This can testify the occurrence of some local and systemic toxic effects of the chemical in case of its ingestion. This fact might be an explanation of more expressed positive effect of C_{60} FAS intraperitoneal administration on liver and pancreas compared to intragastrical alone. In addition, incomplete absorption of C_{60} -fullerene in the gastrointestinal tract might occur, and, correspondingly, a lower dose of the compound enters into the systemic circulation. This assumption may be confirmed by the data of low absorption of C_{60} -fullerene from the gastrointestinal tract [36] and by information of the dose-dependent manner of C_{60} -fullerene hepatoprotective activity when realized due to their antioxidant properties [14].

Consequently, C₆₀FAS when administered intragastrally or intraperitoneally could correct the negative effects of ANIT-induced acute cholangitis on liver and pancreas. The prove is the normalization of blood serum ALT and diminishing of pancreatic parenchyma's edema and liver fibrosis.

REFERENCES

1. Shyrokova Ye. N. Primary sclerosing cholangitis: etiology, diagnosis, prognosis and treatment. *Clinical perspectives in gastroenterology, hepatology*. 2003, V. 1, P. 2–8.
2. Gidwaney N. G., Pawa S., Das K. M. Pathogenesis and clinical spectrum of primary sclerosing cholangitis. *World J. Gastroenterol*. 2017, 23 (14), 2459–2469.
3. Skrypnyk I. M. Primary sclerosing cholangitis is: a momentary glance at the problem. "News of medicine and pharmacy" *Gastroenterology*. 2008, V. 264, P. 3.
4. Prylutska S., Grynyuk I., Matyshevska O., Prylutsky Yu., Evstigneev M., Scharff P., Ritter U. C₆₀ fullerene as synergistic agent in tumor-inhibitory doxorubicin treatment. *Drugs R&D*. 2014, 14 (4), 333–340.
5. Prylutsky Y., Bychko A., Sokolova V., Prylutska S., Evstigneev M., Rybalchenko V., Epple M., Scharff P. Interaction of C₆₀ fullerene complexed to doxorubicin with model bilipid membranes and its uptake by HeLa cells. *Mater. Sci. Eng. C Mater. Biol. Appl.* 2016, V. 59, P. 398–403.
6. Hendrickson O. D., Morozova O. V., Zherdev A. V., Yaropolov A. I., Klochkov S. G., Bachurin S. O., Dzantiev B. B. Study of distribution and biological effects of fullerene C₆₀ after single and multiple intragastric administrations to rats. *Fuller Nanotub. Carbon Nanostruct.* 2015, 23 (7), 658–668.
7. Didenko G., Prylutska S., Kichmarenko Y., Potebnya G., Prylutsky Yu., Slobodyanik N., Ritter U., Scharff P. Evaluation of the antitumor immune response to C₆₀ fullerene. *Mat.-wissu Werkstofftech.* 2013, 44 (2–3), 124–128.
8. Johnston H. J., Hutchison G. R., Christensen F. M., Aschberger K., Stone V. The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity. *Toxicol. Sci.* 2010, V. 114, P. 162–182.
9. Byelinska I. V., Kuznietsova H. M., Dziubenko N. V., Lynchak O. V., Rybalchenko T. V., Prylutsky Yu. I., Kyzyma O. A., Ivankov O., Rybalchenko V. K., Ritter U. Effect of C₆₀ fullerene on the intensity of colon damage and hematological signs of ulcerative colitis in rats. *Mat. Sci. Engin.* 2018, V. 93, P. 505–517.
10. Ferreira C. A., Ni D., Rosenkrans Z. T., Cai W. Scavenging of reactive oxygen and nitrogen species with nanomaterials. *Nano Res.* 2018, <https://doi.org/10.1007/s12274-018-2092-y>
11. Prylutska S. V., Grynyuk I. I., Matyshevska O. P., Prylutsky Yu. I., Ritter U., Scharff P. Anti-oxidant properties of C₆₀ fullerene in vitro, fullerene, nanotubes. *Carbon Nanostruct.* 2008, 16 (5–6), 698–705.
12. Halenova T. I., Vareniuk I. M., Roslova N. M. Hepatoprotective effect of orally applied water-soluble pristine C₆₀ fullerene against CCl₄-induced acute liver injury in rats. *RSC Adv.* 2016, 6 (102), 100046–100055.
13. Kuznietsova G. M., Dziubenko N. V., Chereschuk I. O., Rybalchenko T. V. Influence of water soluble C₆₀ fullerene on the development of acute colitis in rats. *Studia biologica.* 2017, 11 (1), 41–50.
14. Gharbi N., Pressac M., Hadchouel M., Szwarc H., Wilson S. R., Moussa F. C₆₀ fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett.* 2005, V. 5, P. 2578–2585.
15. Sumner S. C. J., Snyder R. W., Wingard C. Distribution and biomarkers of carbon-14-labeled fullerene C₆₀ ([¹⁴C(U)]C₆₀) in female rats and mice for up to 30 days after intravenous exposure. *J. Appl. Toxicol.* 2015, V. 35, P. 1452–1464.
16. Fickert P., Pollheimer M. J., Beuers U., Lackner C., Hirschfield G., Housset C., Keitel V., Schramm C. Characterization of animal models for primary sclerosing cholangitis (PSC). *J. Hepatol.* 2014, V. 60, P. 1290–1303.
17. Ritter U., Prylutsky Yu. I., Evstigneev M. P., Davidenko N. A., Cherepanov V. V., Senenko A. I., Marchenko O. A., Naumovets A. G. Structural features of highly stable reproducible C₆₀ fullerene aqueous colloid solution probed by various techniques. *Fullerene, Nanotubes, Carbon Nanostruct.* 2015, 23 (6). P. 530–534. <https://doi.org/10.1080/1536383X.2013.870900>

18. Prylutskiy Yu. I., Yashchuk V. M., Kushnir K. M., Golub A. A., Kudrenko V. A., Prylutska S. V. Biophysical studies of fullerene-based composite for bionanotechnology. *Mater Sci. Engin.* 2003, 23 (1–2), 109–111.
19. Kuklin A. I., Islamov A. Kh., Gordeliy V. I. Two-detector system for small-angle neutron scattering in instrument. *Neutron News.* 2005, V. 16, P. 16–18.
20. Avdeev M. V., Khokhryakov A. A., Tropin T. V., Andrievsky G. V., Klochkov V. K., Derevyanchenko L. I., Rosta L., Garamus V. M., Priezhev V. B., Korobov M. V., Aksenov V. L. Structural features of molecular-colloidal solutions of C₆₀ fullerene in water by small-angle neutron scattering. *Langmuir.* 2004, V. 20, P. 4363–4368.
21. Lynchak O. V., Prylutskiy Yu. I., Rybalchenko V. K., Kyzyma O. A., Soloviov D., Kostjukov V. V., Evstigneev M. P., Ritter U., Scharff P. Comparative analysis of the antineoplastic activity of C₆₀ fullerene with 5-fluorouracil and pyrrole derivative *in vivo*. *Nanoscale Res. Lett.* 2017, 12 (8), 1–6.
22. Kiernan J. Histological and histochemical methods: theory and practice. 4th ed. *New York: Cold Spring Harbor Laboratory Press.* 2008, 608 p.
23. Sergienko V. I., Bondareva I. B. Mathematical statistics in clinical studies. *Moscow: GeoterMedicine.* 2006. 304 p.
24. Bell L. N. Serum metabolic signatures of primary biliary cirrhosis and primary sclerosing cholangitis. *Liver Int.* 2015, V. 35, P. 263–274.
25. Grattagliano I., Calamita G., Cocco T., Wang D. Q-H., Portincasa P. Pathogenic role of oxidative and nitrosative stress in primary biliary cirrhosis. *WJG.* 2014, 20 (19), 5746–5759.
26. Sorrentino P. Oxidative stress and steatosis are cofactors of liver injury in primary biliary cirrhosis. *J. Gastroenterol.* 2010, V. 45, P. 1053–1062.
27. Shearn C. T., Orlicky D. J., Petersen D. R. Dysregulation of antioxidant responses in patients diagnosed with concomitant Primary Sclerosing Cholangitis. *Exp. Mol. Pathol.* 2018, 104 (1), 1–8.
28. Petersen D. R., Orlicky D. J., Roede J. R., Shearn C. T. Aberrant expression of redox regulatory proteins in patients with concomitant primary Sclerosing cholangitis. *Exp. Mol. Pathol.* 2018, 105 (1), 32–36.
29. Ohta Y., Kongo-Nishimura M., Hayashi T., Kitagawa A., Matsura T., Yamada K. Saikokeishito extract exerts a therapeutic effect on alpha-naphthylisothiocyanate-induced liver injury in rats through attenuation of enhanced neutrophil infiltration and oxidative stress in the liver tissue. *J. Clin. Biochem. Nutr.* 2007, 40 (1), 31–41.
30. Zhao Y., Zhou G., Wang J., Jia L., Zhang P., Li R., Shan L., Liu B., Song X., Liu S., Xiao X. Paeniflorin protects against ANIT-induced cholestasis by ameliorating oxidative stress in rats. *Food Chem. Toxicol.* 2013, V. 58, P. 242–248.
31. Li Y., Yu H., Xu Z., Shi S., Wang D., Shi X., Wang Y., Zeng B., Deng H., Deng X., Zhong X. Melatonin ameliorates ANIT-induced cholestasis by activating Nrf2 through a PI3K/Akt-dependent pathway in rats. *Mol. Med. Rep.* 2018. <https://doi.org/10.3892/mmr.2018.9746>
32. Jani N., Buxbaum J. Autoimmune pancreatitis and cholangitis. *World J. Gastrointest. Pharmacol. Ther.* 2015, 6 (4), 199–206.
33. Kawa S., Hamano H., Umemura T., Kiyosawa K., Uehara T. Sclerosing cholangitis associated with autoimmune pancreatitis. *Hepatol. Res.* 2007, 37 (3), S487–495.
34. Eswaran S. V. Water soluble nanocarbon materials: a panacea for all? *Curr. Sci.* 2018, 114 (9), 1846–1850.
35. Ahsan H., Ali A., Ali R. Oxygen free radicals and systemic autoimmunity. *Clin. Exp. Immunol.* 2003, 131 (3), 398–404.
36. Takahashi M., Kato H., Doi Y., Hagiwara A., Hirata-Koizumi M., Ono A., Kubota R., Nishimura T., Hirose A. Sub-acute oral toxicity study with fullerene C₆₀ in rats. *J. Toxicol. Sci.* 2002, 37 (2), 353–361.
37. Zen Y. The pathology of IgG 4-related disease in the bile duct and pancreas. *Semin. Liver. Dis.* 2016, 36 (3), 242–256.
38. Pemberton P. W., Aboutwerat A., Smith A., Warnes T. W. Ursodeoxycholic acid in primary biliary cirrhosis improves glutathione status but fails to reduce lipid peroxidation. *Redox. Rep.* 2006, V. 11, P. 117–123.
39. Shipelin V. A., Gmoshinski I. V., Tutel'yan V. A. Study of the fullerene C₆₀- stability in biological substrates using in vitro model system. *Nanotechnologies in Russia.* 2013, 8 (11–12), 810–815.

**ВПЛИВ ВОДНОГО КОЛОЇДНОГО
РОЗЧИНУ C₆₀-ФУЛЕРЕНУ НА
МОРФОЛОГІЧНИЙ СТАН ПЕЧІНКИ,
ПІДШЛУНКОВОЇ ЗАЛОЗИ
ТА АМІНОТРАНСФЕРАЗНУ
АКТИВНІСТЬ СИРОВАТКИ КРОВІ ЩУРІВ
ЗА ГОСТРОГО ХОЛАНГІТУ**

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Метою роботи було дослідити вплив C₆₀-фуллерену на морфологічний стан печінки та підшлункової залози за інтраперитонеального та інтрагастрального введення на моделі експериментального холангіту щурів. Гострий холангіт відтворювали одноразовим інтрагастральним введенням α -нафтил-ізоціанату — ANIT. Водний колоїдний розчин C₆₀-фуллерену (C₆₀FAS, 0,15 мг/мл) вводили тваринам в об'ємі, еквівалентному кількості C₆₀-фуллерену на 0,5 мг/кг маси тіла, через 24 та 48 год після введення ANIT. Через 72 год після початку дослідів тварин піддавали евтаназії. У крові тварин вимірювали рівень АЛТ та АСТ. Печінку і підшлункову залозу аналізували на світлооптичному рівні. Встановлено, що інтрагастральне та інтраперитонеальне введення C₆₀FAS сприяє корекції негативних ефектів у печінці та підшлунковій залозі, спричинених індукцією гострого холангіту. Свідченням цього є нормалізація рівня активності АЛТ, зменшення набряку паренхіми підшлункової залози і фіброзу печінки, а також посилення кровонаповнення цих органів. Застосування C₆₀FAS позитивно впливає на морфологічний стан печінки і підшлункової залози у щурів з індукованим гострим холангітом.

Ключові слова: C₆₀-фуллерен, гострий холангіт.

**ВЛИЯНИЕ ВОДНОГО КОЛЛОИДНОГО
РАСТВОРА C₆₀-ФУЛЛЕРЕНА
НА MORFOLOGICHESKOE СОСТОЯНИЕ
ПЕЧЕНИ, ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ
И АМИНОТРАНСФЕРАЗНУЮ
АКТИВНОСТЬ СЫВОРОТКИ КРОВИ
КРЫС ПРИ ОСТРОМ ХОЛАНГИТЕ**

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Целью работы было исследовать влияние C₆₀-фуллерена на морфологическое состояние печени и поджелудочной железы при интраперитонеальном и интрагастральном введении на модели экспериментального холангита крыс. Острый холангит воспроизводили однократным интрагастральным введением α -нафтил-изоцианата — ANIT. Водный коллоидный раствор C₆₀-фуллерена (C₆₀FAS, 0,15 мг/мл) вводили животным в объеме, эквивалентном количеству C₆₀-фуллерена на 0,5 мг/кг массы тела, через 24 и 48 ч после введения ANIT. Через 72 ч после начала опыта животных подвергали эвтаназии путем цервикальной дислокации после анестезии. В крови животных измеряли уровень АЛТ и АСТ. Печень и поджелудочную железу анализировали на светоптическом уровне. Установлено, что интрагастральное и интраперитонеальное введение C₆₀FAS способствует коррекции негативных эффектов в печени и поджелудочной железе, вызванных индукцией острого холангита. Свидетельством этого является нормализация уровня активности АЛТ, уменьшение отека паренхимы поджелудочной железы и фиброза печени, а также усиление кровенаполнения этих органов. Применение C₆₀FAS положительно влияет на морфологическое состояние печени и поджелудочной железы у крыс с индуцированным острым холангитом.

Ключевые слова: C₆₀-фуллерен, острый холангит.