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Ultrastructural Features and Comparative Characteristics of the Common Carp Liver

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Abstract

Cyprinus carpio L., 1758 - apart from its commercial importance, this fish is also considered an ideal experimental model for studying different branches of biology. The liver of fish serves as a good indicator for identifying the toxicity of components of the biosystem. Ultrastructural data on the structure of the liver of this fish are incomplete. This study is devoted to the study of the ultrastructure of the liver of common carp using light and electron microscopy methods. The liver capsule is composed of the epithelial layer and connective tissue elements. The wall of the sinusoids consists of endothelium, which in turn, connect to each other through intercellular connections. Hepatic stellate cells, also known Ito cells, are observed between sinusoids and hepatocytes. The membranes of hepatocytes are connected to each other by desmosomes. Intracellular canaliculi are present in the cytoplasm of the hepatocyte. They are formed as a result of intussusceptions of the hepatocyte membrane. There are numerous microvilli leading to the lumen of the intracellular canaliculi. The biliary ductule wall consists of a epithelial cells and has a elongated nucleus. Biliary ductules wall widen and open into the bile ducts. They are composed of four layers. A comparative ultrastructural characterization of the carp liver with other fish species and vertebrate animal livers was also carried out. The obtained results can be used in the study of ultrastructural changes occurring in the body of living organisms, including fish, during toxic effects.

Keywords: common carp, liver, ultrastructure, comparative characteristics, TEM.

Introduction

Common carp grown in various parts of the world, including Azerbaijan. The common carp belongs to the cyprinid family (Cyprinidae) and is the largest in the number of species of all fish families. There are approximately 2,900 species of cyprinids worldwide [1]. The annual global production of carp is 3.4 - 4.0 million tons, which accounted for almost 14.0% of global freshwater aquaculture cultivation [2, 3]. Despite its commercial importance, this fish is also considered an experimental animal model for studying different branches of biology [4]. In recent years, common carp studies have received more research attention as a model for toxicity analysis [5]. The similarity between the physiology and organ morphology of *C. carpio* and other vertebrates also makes this fish a good model for identifying potential risks to both animals and humans [6]. The fish liver serves as a good indicator for identifying the toxicity of biosystem components [7]. It should also be noted that light and predominantly electron

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microscopic methods make it possible to visually monitor and describe any pathological processes or changes occurring in the body of all living beings (microorganisms, plants, animals), including fishes [8-14]. There are various sources on the study (using histological and TEM methods) of the ultrastructure of the common carp liver in the control group and changes under the influence of substances with toxic effects [7, 15-25]. The liver of the common carp has both similar and different ultrastructural features to the livers of various vertebrates and other bony fishes [22, 26]. As a result of the literature data analysis, it was found that the structural elements that make up the liver of the common carp have not been studied in detail at the ultrastructural level. Taking into account the above, using light and TEM methods, the goal of studying the liver of *C. carpio* at the ultrastructural level and comparing it with the structure of the liver of other bony fishes and some vertebrates was set.

Material and methods

Experimental animals.

The one-year-old fishes (16 examples, juveniles) was obtained from the fishing farm in the Neftchala region of Azerbaijan. Juveniles were euthanized with 3% tricaine. Liver tissues of the fishes were fixed for further study.

Transmission electron microscopic (TEM) investigations.

The abdominal region of the juveniles was incised, and the livers were carefully extracted. Examples were fixated in solution containing 2% glutaraldehyde, 2% paraformaldehyde and 0.1% picrin acid prepared in phosphate buffer (pH 7.4). Araldit-Epon blocks were prepared based on generally accepted methods in electron microscopy. Semithin (1-2 mkm) and ultrathin (50-70 nm) sections were obtained by the aid of Leica EM UC7 (Leica, Germany) ultramicrotome. Simples were studied using a JEM-1400 (JEOL, Japan) Transmission Electron Microscope at a voltage of 80-120 kV [27, 28]

Morphometric analysis.

The morphometric analysis of the images was performed on electrograms taken from TIF images using the ITEM imaging platform program [29]. Morphometric studies were conducted on structural elements, including the cytoplasmic organelles of the cells that make up the fish liver. Different parameters (Min, Max, mean±SD) was studied.

Results

General structure of a common carp liver under a light microscope shown in figure 1A. The image shown hepatocytes (H) and sinusoids (S) of the fish liver. In semi-thin sections (1 μm) of common carp liver, it was observed that vessels and hepatocytes are irregularly arranged. In common carp, the liver is not divided into hepatic lobules. Portal triads were not observed in the studied fish. Figures 1B and 1C show a general view of a polygonal (mainly hexagonal shape) hepatocyte (12.93-17.15 μm (15.25±0.33 μm)) in an electrogram obtained through an electron microscope. Both images show a large euchromatin-rich nucleus (N, diameter 3.37-5.52 μm (4.28±0.20 μm)) and an electron-dense nucleolus (Nu, diameter 1.65-2.14 μm (1.84±0.04 μm)) in the center of the hepatocyte (Fig. 1B and 1C). Large fat droplets (Fd, 0.95-5.26 μm (2.37±0.31 μm)), intracellular canaliculi (Ic), glycogen (Gl) shown in the cytoplasm. In addition, in the larger magnifications of the electron microscope, in the cytoplasm of the hepatocyte, mitochondria (Mt, diameter 0.77-1.18 μm (0.96±0.04 μm)), lysosomes (Ly), Golgi complex, phagolysosomes, a large number of granular endoplasmic reticulum (Er) around the nucleus, and the presence of a nuclear pore between the two membranes of the nucleus can be observed (Fig. 1D). The membranes of hepatocytes are connected to each other by desmosomes (Ds) (Fig. 1E). The parenchyma of the fish is externally covered with simple squamous epithelium (SE) (Fig. 1F). An elongated nucleus (N) of the epithelium, and a large number of vesicles, mitochondria, endoplasmic reticulum and other cytoplasmic structures are observed in its cytoplasm. Between the epithelial layer and hepatocytes, connective tissue elements - collagen bundles (C) are observed (Fig. 1F). Thus, the liver capsule is composed of the epithelial layer and connective tissue elements. Blood vessels are located between the hepatocytes of the common carp liver. The smallest vessels are sinusoids, which in turn join the central vein. In fish, including common carp, central and portal veins do not differ from each other morphologically. Figures 2A and 2B show the sinusoids (S, diameter 2.59-6.48 μm (4.69±0.32 μm)), a blood vessel located between hepatocytes. Erythrocytes (E, diameter 1.40-2.60 μm (1.81±0.08 μm)) are shown in the lumen of the sinusoids. In the center of the erythrocyte cytoplasm is a large, round, euchromatin and heterochromatin-rich nucleus (0.70-1.33 μm (1.06±0.06 μm)). The sinusoid wall consists of a endothelium (En) (Fig. 1E and 2A). Endothelial cells, in turn, connect to each other through intercellular connections. A large nucleus (1.30-2.62 μm (2.01±0.16 μm)) and cytoplasmic organelles - mitochondria, endoplasmic reticulum, ribosome, etc. are observed in the cytoplasm of the endothelium. The subendothelial space between the endothelium and hepatocytes is called the space of Disse (Di) (Fig. 1E and 2A). In addition to the above, hepatic stellate cells, also known as Ito cells or perisinusoidal cells (Hs), are also observed between sinusoids and hepatocytes (Fig. 2B). An irregularly shaped nucleus (N) and fat droplets (Fd) were found in the cytoplasm (Fig. 2B).

The liver, being the largest gland in the internal body of the carp fish, performs functions such as bile synthesis, glycogen storage, and lipid metabolism. Bile synthesis takes place in liver cells, that is, hepatocytes. In the cytoplasm of the hepatocyte, intracellular canaliculi (Ic, 1.20-1.97 µm (1.62±0.10 µm)) are present around the nucleus (Fig. 1B, 2C, 2D). They are formed as a result of intussusception of the hepatocyte membrane. There are numerous microvilli (diameter 0.09-0.11 µm (0.10±0.003 µm)) leading to the lumen of the intracellular canaliculi. Intracellular canaliculi membranes form tight connections with hepatocytes through desmosomes (Ds) (Fig. 2D). Intracellular canaliculi open into biliary ductules. The ductules wall consists of a epithelial cells and has a large and elongated nucleus (N, length 5.61-8.07 µm (6.93±0.28 µm), width 0.70-1.97 µm (1.45±0.14 µm)) (Fig. 2E). We not fined basal lamina in the biliary ductules wall. They open into the bile ducts, diameter 21.52-23.92 µm (22.65±0.24 µm), and wall length 10.18-11.60 µm (11.00±0.18 µm)) (Fig. 2F). It was revealed that the bile ducts consist of several layers. Thus, in the obtained electrograms, it was observed that the wall of the duct is composed of epithelial layer (Ep), basal lamina (BL), connective tissue (Ct), and peritubular cells (PC) from the outside to the inside (Fig. 2F). The nucleus of epithelial cells is large, and the cytoplasm is rich in organelles. These cells are connected to each other through tight junctions. There are microvilli in the part of the epithelium that opens into the lumen (L).

Discussion

There are literary data on the structure of the liver of some fish, studied using light and electron microscopic methods [22, 30-34]. Similar to mammals, the liver of bony fish holds a pivotal role in maintaining metabolic homeostasis within the body. It actively participates in the breakdown of carbohydrates, vitamins, and fats, highlighting its crucial function in the metabolic processes. In addition, it performs many other functions by participating in the synthesis of bile necessary for lipid storage and lipid breakdown in the intestine [35]. Fish livers lack the division into distinct liver lobules, a notable difference from mammalian livers. Furthermore, unlike mammals, fish livers do not exhibit portal triads, and Kupffer cells are not detectable in the ventral part of the sinusoids [22, 26]. Among the fish whose livers have been studied, specific information is limited to *M. merluccius*, revealing a liver structure comprising three lobes and the presence of Kupffer cells in the ventral part of the blood vessels [33]. Additionally, literature data on the presence of Kupffer cells in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), Nile tilapia (*Oreochromis niloticus* L., 1758) and African sharptooth catfish (*Clarias gariepinus* Burchell, 1822) were found [36, 37]. In our studies, we did not identify lobules in the liver; in addition, portal triads and Kupffer cells were not identified in the common carp liver. Earlier studies showed that, the phagocytic Kupffer cells are missing in normal fish livers, hereas in common carp and eels stimulated by prolonged repeated intravenous administrations of dyes for vital staining there appear, in the hepatic sinusoid, Kupffer cells which have probably originated from histiocytes in various tissues and organs including intrahepatic connective tissue [38]. Analysis of the literature data revealed distinct morphological and topographical differences in the livers and their structural elements among the studied fish species. The liver of common carp is externally lined by squamous epithelium. This similarity has also been found in other bony fishes, for example in Oligosarcus jenynsii Günther, 1864 [39]. And according to other authors, the liver of the common carp is covered on the outside with two layers [15]. Our research also confirmed these data. Hepatocytes of common carp are polygonal, and usually hexagonal forms. A large nucleus was identified in the center of the common carp hepatocyte, which is also found in other previously studied fish [21, 40]. Despite the irregular arrangement of hepatocytes, sinusoids are located on their basal parts. According to our data and data from other authors [15], in the common carp's liver, a space of Disse exists between the vessels and hepatocytes. Within this space, hepatic satellite cells (Hs) characterized by a large nucleus and cytoplasmic fat granules are notably observed. Those structures were also described in fish - *D. rerio* [32]. Our electron microscopic studies revealed the presence of intracellular canaliculi (Ic) in the cytoplasm of common carp hepatocytes. These canaliculi are formed through the invagination of hepatocyte membranes around the nucleus, and microvilli (MV) are distinctly observed within their lumens. The bile, synthesized by hepatocytes, flows through the bile capillaries. These capillaries extend from the apical part of the hepatocyte to the biliary ductule, positioned between two hepatocytes, and characterized by a single-layered epithelial wall with elongated nuclei. It, in turn, expands and opens into the bile ducts. Having such a structure of the bile system in the liver is identical in *D. rerio* and many other bony fishes (*O. latipes, Carassius auratus* L., 1758) [31, 32]. But in some fish (*S. salar*), the intracellular canaliculi are not located inside the hepatocyte, but between hepatocytes [30].

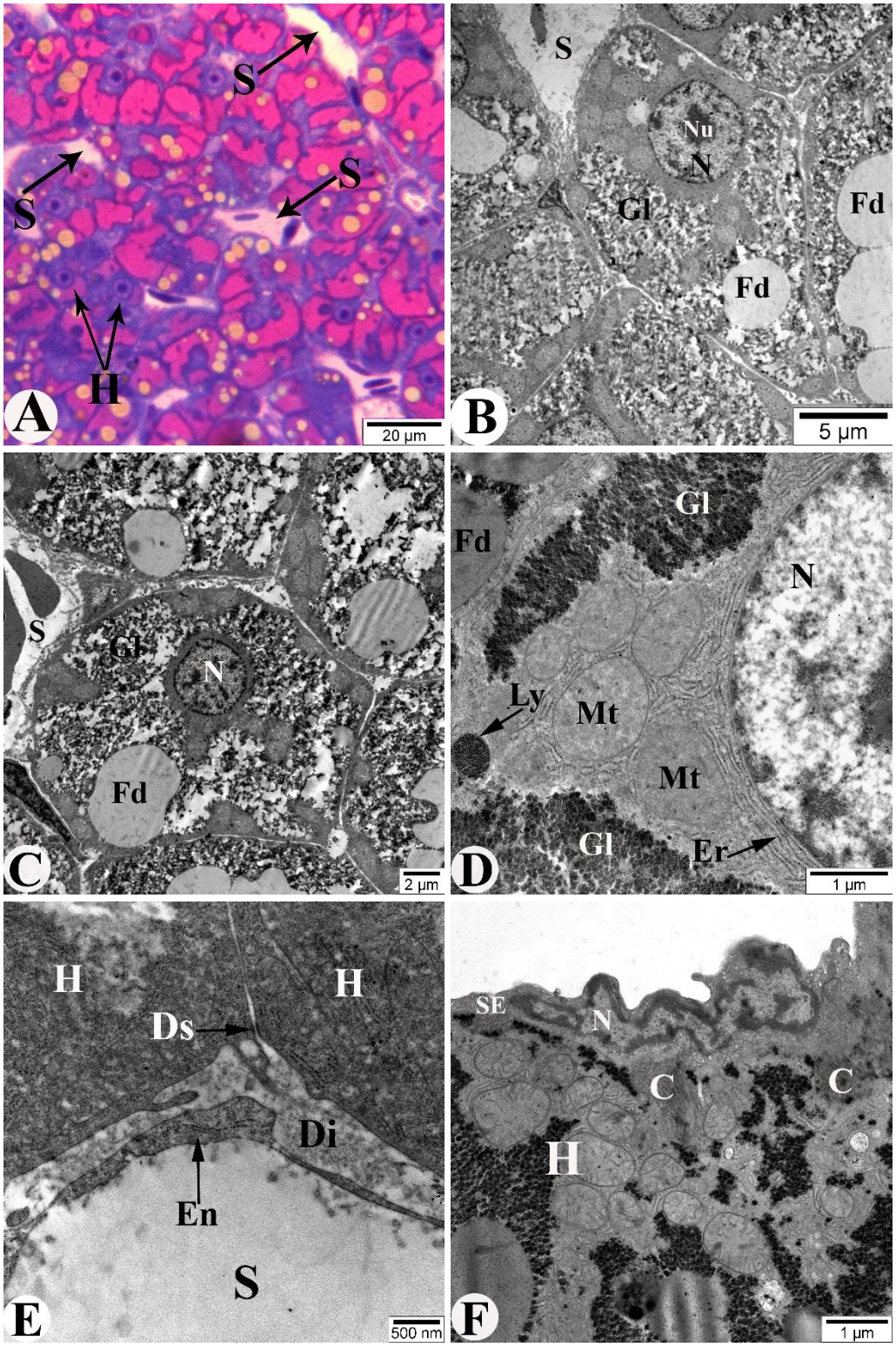

Fig. 1. Light and electron microscopic characteristics of the normal structure of the liver of common carp. A – a general view of the liver, B, C – an electron microscopic view of a hepatocyte, D – structural elements of the cytoplasm of a hepatocyte, E – desmosomes connecting hepatocytes with each other, F – simple squamous epithelium and connective tissue elements covering the liver from the outside. Designations: H – hepatocyte, S – sinusoid, Fd – fat droplets, Gl – glycogen, N – nucleus, Nu – nucleolus, Ly – lysosome, Mt – mitochondria, Er – endoplasmic reticulum, Ds – desmosome, Di – subendothelial space of Disse, En – endothelial cell, SE – simple squamous epithelium, C – callogen.

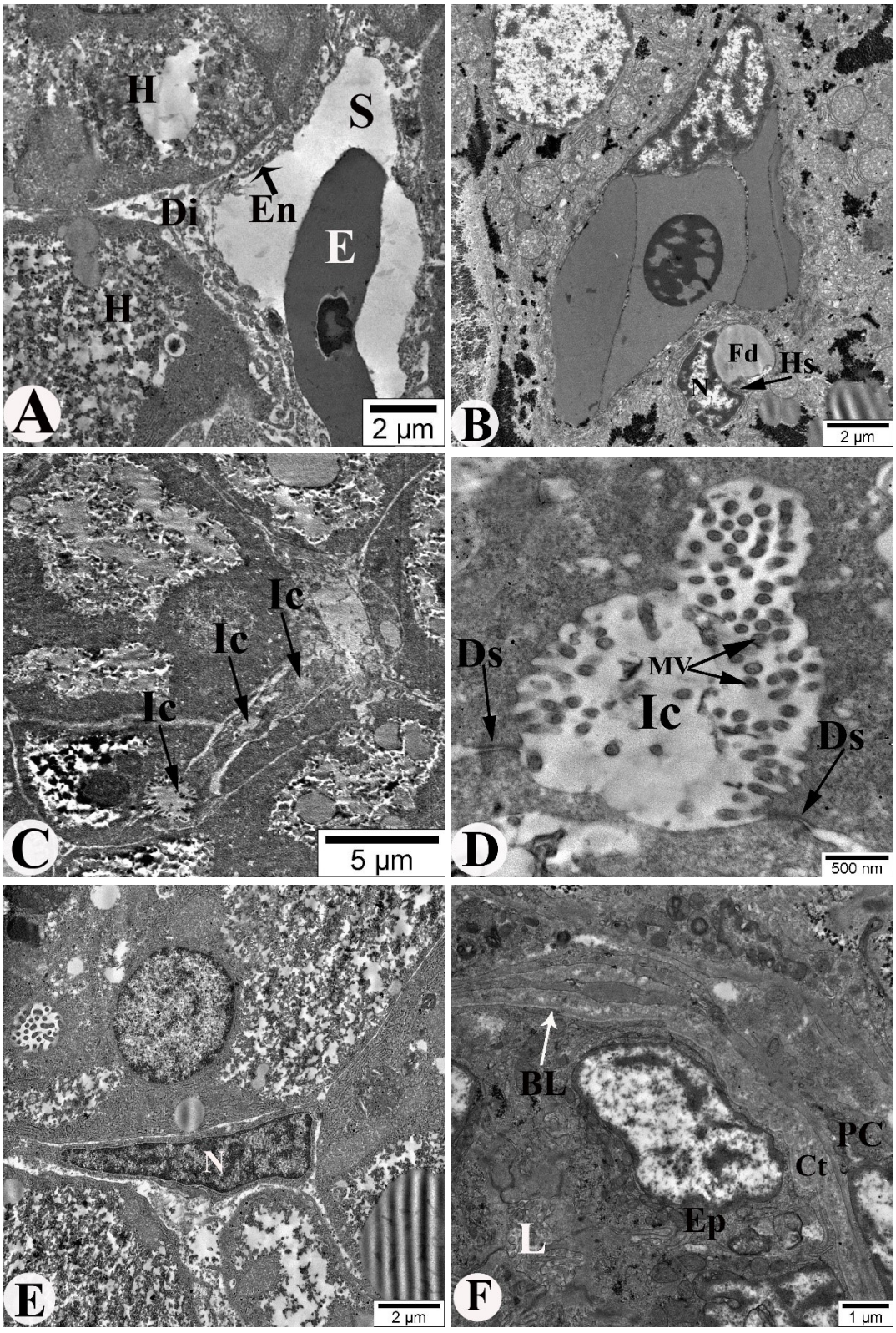

Fig. 2. Ultrastructural characteristics of the liver of common carp in norm. A – sinusoid structure, B – hepatic stellate cell, C – intracellular canaliculi in hepatocyte, D – intracellular canaliculi with desmosomes, E – nucleus of the epithelial cell of the biliary ductule wall, F – structure of the bile duct. Designations: H – hepatocyte, S – sinusoid, Fd – fat droplets, Ic – intracellular canaliculi, N – nucleus, L – lumen, Ds – desmosome, Di – subendothelial space of Disse, En – endothelial cell, Ep – epithelial cell, Ct – connective tissue, PC – peritubular cell, BL – basal lamina, MV – microvilla, Hs – hepatic stellate cell, E – erythrocyte.

Conclusion

Using light and TEM methods, the liver of the common carp and its structural elements have been studied in detail at the ultrastructural level. Ultrastructural characteristics of hepatocytes, sinusoids, intracellular canaliculi, biliary ductules, and bile ducts that make up the parenchyma of the liver are given. The obtained results were compared with the structure of the liver of different bony fishes and vertebrates. The data obtained from this work can be used to study the structure of the liver of other fish species, as well as to conduct toxicological monitoring of biological objects, including vertebrates.

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Conflict of interest: The authors claims that there are no competing interests.

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