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## Histological study of the circulatory system of Sulawesi Medaka fish (*Oryzias celebensis*) for animal model research

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**Abstract:** Sulawesi medaka fish (*Oryzias celebensis*) is one part of medaka fish that are endemic in the region of South Sulawesi, Indonesia. This study aims to observe histological structure of the circulatory system of Sulawesi medaka fish. Morphological study showed that the heart is located in the anterior region of the Sulawesi medaka fish body. Histological study of the circulatory of Sulawesi medaka fish showed that the atrium has thin walls whereas the ventricles have thick walls. The contractions of the ventricle have high pressure and then the blood will be pumped through a bulbus arteriosus that is shaped like an onion. Bulbus arteriosus consists of fibroelastic tissue and some smooth muscles. Bulbus arteriosus, is a unique structure and is to dampen the pressure pulse that generated by the ventricle. According to the circulatory system of zebrafish showed that there were similarity between Sulawesi medaka fish and Zebrafish. In general the structure of the circulatory system specially heart and bulbus arteriosus in Sulawesi medaka fish has similarity with Zebrafish as well as other Teleostei fish. This results suggested that Sulawesi medaka fish can be used as model animals especially in the use of fish as animal models in research of cardiovascular disease.

### 1. Introduction

Medaka fish (*Oryzias* sp) or "rice fish" is a native Asian fish that is used as a non-consumption fish or ornamental fish. Whereas, the medaka fish is one of the most well known animal models and is widely used by researchers in the world for studies in various fields of science, especially biology and medicine as well as Zebrafish that have been developed as animal models. [1] introduced medaka fish as model animals for Parkinson's disease testing caused by environmental pollution and genetic factors. Several types of mutant medaka fish have also been made for screening of diabetes drugs, cancer [26] and other degenerative diseases.

Medaka has several advantages as an experimental animal [27] that is easy spawning, short reproduction cycle (initial 2 month maturation), transparent embryo, short generation time, small genome size and has transgenic construction [2]. Various biological information to the molecular systematics of medaka fish, especially Japanese medaka fish (*Oryzias latipes*) has also been found. The need for medaka fish as an animal of this model also made the researchers who use medaka fish as a test animal



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has made various kinds of fish strain *O. latipes* medaka. But in Indonesia as a center for the distribution of medaka fish species, information on medaka fishes, especially those endemic to Sulawesi Island specially South Sulawesi such as *Oryzias celebensis*, is still very poor and has not been domesticated.

Medaka fish belongs to a group of small fish that are taxonomically classified into the Adrianichthyidae family. The Adrianichthyidae family is an indigenous family in Asia, composed of four genera, namely *Oryzias* with 20 species, *Adrianichthys* with two species, *Horaichthys* with one species and *Xenopoecilus* with three species [3]. Most fish in this family are confined to fresh water but some species are found in brackish water and along the coast. This distribution of fish covers a vast area from India to Japan and south along the Indo - Australian islands on the Wallace line of Timor and Sulawesi. The discovery of new species of medaka fish species is also increasing. In 2009, there were 32 species of *Oryzias* sp in Asia, 14 endemic species in Sulawesi waters, 6 species of endemic *Oryzias* in certain lakes on Sulawesi Island [4].

Most of the Medaka fish distribution area is recorded in Central Sulawesi, South Sulawesi and Southeast Sulawesi. Medaka species in Central Sulawesi spread over Lake Lindu and Lake Poso. The endemic species of Lake Lindu consist of: *Oryzias bombrum* and *O. sarasinorum*. While the endemic species of Lake Poso are: *Adrianichthys kruyti*, *A. oophorus*, *A. poptae*, *A. Roseni*, *O. nebulosus*, *O. nigrimas* and *O. Orthognathus* [5, 6].

Medaka fish species in South Sulawesi are centered around the Karst Maros-Pangkep and Malili Lake complexes in East Luwu regency. Medaka fish found around Karst Maros Pangkep is generally dominated by *Oryzias celebensis*. Medaka fish in East Luwu has a unique distribution pattern, because each lake has its own endemic species, although the lakes are located adjacent to each other. The endemic medaka found in Lake Matano generally has black spots and consists of 2 species, namely *O. marmoratus* and *O. matanensis*. Medaka endemic to Lake Towuti is *O. profundicola* prefers deep habitat. While in the small lake of Masapi (Local Indonesian language), found species *O. hadiatyae*. This species is described in 2010. Other types of Medaka namely: *O. eversi* found in Tana Toraja in 2012 [7,8]

From the explorations of the researchers showed that Sulawesi has a high endemism especially against the fish family Adrianichthyidae. The high endemism is not only supported by the zoogeographic limits as islands located on the Weber line and the Wellacea line, but also because this fish group is not including the traded fish so the chances of migrating of these fish are small. These facts underlie the statements of some researchers that medaka fish can reveal the mystery of the evolution of fauna in the waters of Sulawesi [6].

In order to develop Sulawesi medaka fish as animal model this study aims to observe histology of circulatory system Sulawesi medaka fish especially heart. Previous research has been done by observing the histology of respiratory system of Sulawesi medaka fish and other endemic fish [9,10].

The study of histological observation of respiratory system It is expected that this research can be obtained about the histology of Sulawesi medaka fish as the basis of research development related to medaka fish Sulawesi. Besides, the study of histology structure of this fishes were compared with the circulatory system in zebrafish as the basis of animal model development.

## 2. Materials and Methods

About ten adult Sulawesi Medaka fish were used for this study. The fishes were measured and weighed with same average. Sulawesi medaka fish were obtained from Pattunuang river, Karst, Maros district, South Sulawesi. The fishes and then kept and acclimatization in aquarium for temporary before sampling. Fish samples were kept and stored in 10% neutral buffered formalin. The body of Sulawesi medaka fish sizes are small and the observations made by one part of the whole body of the fish. The fishes and then were processed with routine histotechnique for further observation. Samples were dehydrated through graded series alcohol and clearing using xylol before further embedding with paraffin. The tissues were cut using microtome with 4  $\mu$ m thickness, and then samples were stained using Mayer's Hematoxylin Eosin (HE) and other histochemical stainings. Sampel were observed

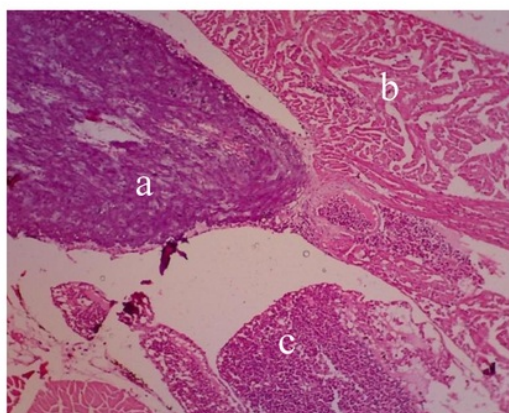
using a microscope which has been connected to the camera microscope (Olympus 22X with advanced Optilab) and analyzed using Image J programme.

### 3. Results and Discussion

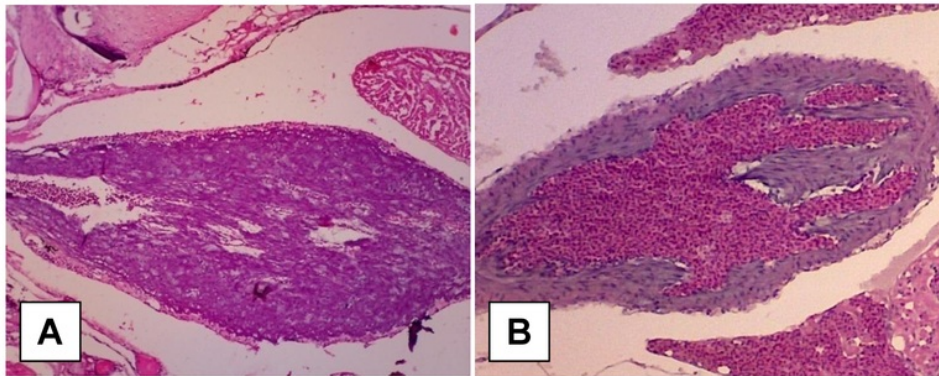
Morphological features of the Sulawesi medaka fish has a long, flat body, terminal mouth, a pair of pectoral fins, a pair of short abdominal fins, a shorter dorsal fin rather than an anal fin located near the caudal fin, has a longer anal fin, thick and jagged on a male fish and has a orange-yellow tail fin on the tail edge of male medaka fish [11]. Morphologic study showed that the heart is located in the anterior region of the Sulawesi medaka fish body. The heart is the pump that generates the driving pressure for the circulation of blood. The fish heart has one atrium and one ventricle. This is in contrast to the mammalian heart that has two separate atrium and two separate ventricles. In the fish heart, two other additional-like chambers can also be found: the sinus venosus and the bulbus arteriosus.

Histological study of the circulatory of Sulawesi medaka fish showed that the atrium has thin walls whereas the ventricles have thick walls (Fig. 1). The contractions of the ventricle have high pressure and then the blood will be pumped through a bulbus arteriosus that is shaped like an onion. Bulbus arteriosus consists of fibroelastic tissue and some smooth muscle. Bulbus arteriosus, is a unique structure and is to dampen the pressure pulse that generated by the ventricle. The bulbus arteriosus is believed to be a vascular adaptation, which to accommodate the distance between bulbus arteriosus and gills and maintained blood perfusion [12, 13]. From the heart, the ventral aorta distributes blood through the afferent branchial artery.

The blood from the body, which is low in oxygen enters the atrium via the sinus venosus, which contains the pacemaker cells that initiate the contractions. The blood is pumped into the ventricle by the atrium, which is a thin-walled muscular chamber. Then the blood is pumped out into the bulbus arteriosus by the ventricle: a thickwalled chamber with lots of cardiac muscle. The ventricle is responsible for the generation of the blood pressure. The last chamber, the bulbus arteriosus, is a unique structure and one of the functions is to dampen the pressure pulse generated by the ventricle. The next organ after the bulbus arteriosus are the gills, and they are thin walled and may be damaged if the pulse pressure (or absolute pressure) becomes to high. The bulbus arteriosus contains elastic components but not many muscle fibres [14].



**Figure 1.** Heart section of Sulawesi medaka fish. Consists of atrium (c), ventricle (b) and bulbus arteriosus (a). 4x10 magnification. HE staining



**Figure. 2.** The difference between the bulbus arteriosus Sulawesi medaka fish (A) and the Zebrafish (B). The bulbus arteriosus wall of Sulawesi medaka fish was thicker than Zebrafish. Magnification: 10x10. HE staining.

According to the circulatory system of zebrafish showed that there were similarity between Sulawesi medaka fish and Zebrafish except in Sulawesi medaka fish the wall of bulbus arteriosus was thicker than Zebrafish (Fig. 2). In general the structure of the circulatory system specially heart and bulbus arteriosus in Sulawesi medaka fish has similarity with Zebrafish as well as other Teleostei fish. Sulawesi medaka fish as well as Zebrafish were easily maintained and relatively economical in comparison to mammalian species such as mice, rats and others [15]. Zebrafish and Sulawesi medaka fish will not be able to fully substitute the mouse or other mammal in animal models, however it will reduce the number of experiments using mammals. Although the Zebrafish novel disease model for cardiac hypertrophy and other heart failure, suggested that Sulawesi medaka fish can also be used as cardiac disease model especially in the use of fish as animal models in research of cardiovascular disease.

#### 4. Conclusion

The structure of the circulatory system specially heart and bulbus arteriosus in Sulawesi medaka fish has similarity with Zebrafish although some different in structure of circulatory system also similar with other Teleostei fish. This results suggested that Sulawesi medaka fish can be used as model animals especially in the use of fish as animal models in cardiovascular research such as cardiovascular development and cardiovascular disease in human and animal

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