

The Effect Of Polyphenols In Kappaphycus Alvarezii On Blood Biochemistry And Cortisol Level Of Cyprinus Carpio

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Abstract: Research on the effects of polyphenols exposure from red algae Kappaphycus alvarezii that changes blood biochemistry and cortisol levels in Cyprinus carpio has not been widely carried out. C. carpio were injected with polyphenols at a dose of 5.4 mg/kg, 7.2 mg/kg, 9 mg/kg, and 3 mg/kg of tannins concentration. The polyphenols effects on blood glucose and cortisol were observed 3 days after injection. The blood glucose levels and the cortisol hormone had increased significantly.

Keywords: blood glucose, cortisol level, K. alvarezii, polyphenol

1 INTRODUCTION

Environmental changes are affecting the aquatic organism, even some of which are able to cause stress to them. Several factors that causing stress to fish are the existence of handling and transportation, and natural environmental changes such as temperature fluctuation, low oxygen levels and pollution or chemicals entry into the water [1]. One of the chemicals that often found in water bodies is polyphenol compound. It is a compound which able to cause environmental problems. One of the plants that contains polyphenol is Kappaphycus alvarezii seaweed [3]. It contains various types of polyphenols compounds, such as flavonoids, phenols, hydroquinone, and triterpenoids. K. alvarezii is kind of algae that contains natural polyphenols as its secondary metabolites. [4]. Polyphenol compounds which are found in the Kappaphycus alvarezii seaweed acts as immunostimulant to fish [5]. However, feeding fish with brown seaweed extract can be dangerous if given in high dose. This is because the ability of phlorotannin in the extract seaweed able to create hydrogen bounds with proteins, includes the enzyme in digestive tract [6]. This is also supported by a statement [7] that too many seaweed extracts given would not improve the immune systems, because the body could not respond on it, the antibody would not be formed, and probably formed toxicants.

Also, the effect of exposure polyphenol compound is able to increase serum total cholesterol and fat which accumulated in fish tissue, along with decreasing fish growth [8]. The content of polyphenols such as tannins in seaweed extract can cause liver damage (hepatotoxic) even at high doses can result in tissue damage [9]. C. carpio, which is included in the goldfish family, is one type of fish that is often used to evaluate the presence of pollutants that appear in waters [10]. There is not much information about exposure to polyphenol compounds in that fish. Therefore, in this study, we analysed the effects of exposure to polyphenol compounds contained in K. alvarezii on blood biochemistry and the hormone cortisol.

2 MATERIAL AND METHODS

2.1 Materials

Material used in this research were C. carpio size 12-15 gram, K. alvarezii extract, ethanol, n-hexane, ethyl acetate, tannic, anticoagulant.

2.2 Methods

2.2.1 Isolation and characterization of polyphenol in K. alvarezii

K. alvarezii was obtained from farmers in Poteran Beach, Sumenep, Madura, East Java. Maceration for isolating polyphenol using ethanol as polar eluent [11], the ratio between K. alvarezii powder and ethanol is 100 gram: 300 mL (1:3 b/v). Then purified by thin-layer chromatography continued with column chromatography using n-hexane: ethyl acetate (4:1) [12]. Polyphenol was characterized by UV-Vis spectrophotometer and Fourier Transform Infrared Spectrophotometer (FTIR) compared with standard tannin. Liquid Chromatography Mass Spectrometry (LCMS) was done to identify polyphenol on K. alvarezii.

2.2.2 Polyphenol Injection

C. carpio used in this experiment with size 12-15 gram were obtained from Kepanjen, Malang. The 50% deadly concentration of K. alvarezii was at 96 hours. The dose of polyphenol injection from K. alvarezii is 5,4 mg/kg, 7,2 mg/kg and 9 mg/kg based on the result of LC50. Control negative (without treatment) and positive control (tannic 3 mg/kg) [13] was applied. The observations were investigated for 3 days after polyphenol injection.

2.2.3 Blood Sample Collection

Blood samples were taken 3 days (72 hours) after injection. Before taking the blood sample, the fish head was covered by a wet cloth to avoid stress on fish [14]. Blood sample was taken near caudal peduncle. Then entered to a vaculab with anticoagulant inside. The serum was separated and stored for cortisol analysis.

2.2.4 Blood biochemistry and cortisol analysis

Glucose analysis was measured using Glucose Meter. The blood samples were put on glucose strip the entered to glucose meter and the result were appeared on the glucose

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meter screen. The cortisol analysis was performed using ELISA method [15].

2.3 Data Analysis

This research was conducted by using experimental method with Completely Random Design. The result was analysed by Analysis of Variance (ANOVA). The statistical analyses were performed using Microsoft Excel 2013.

3 RESULT AND DISCUSSION

3.1 Blood Glucose

The result of this research showed that blood glucose content in the fish has increased after being exposed with polyphenol in which contains inside *K. alvarezii*. Moreover, the highest value of blood glucose was obtained by positive control treatment (synthetic tannins) in the amount of 305.33 g/dl. In addition to that, the result of treatment C (9mg/kg) was 206.67 g/dl, the result of treatment B (7.2 mg/kg) was 131.33 g/dl, and the result of treatment A was 101.67 g/dl. Meanwhile, negative control treatment (without any treatment given) showed the lowest value in the amount of 70.67 g/dl.

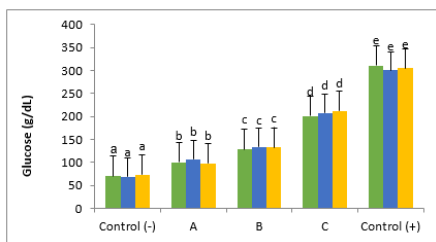


Figure 1. The Blood Glucose of *C. carpio* after polyphenol *K. alvarezii* injection

In general, the normal glucose content in the fish is around 40-90 g/dl [16]. The higher amount of polyphenol content exposed leads to higher glucose content to the fish. Moreover, the increasing blood glucose content in *C. carpio* after *K. alvarezii* polyphenol injection indicates that the fish undergo stress. Fish that experience stress will give a stress response, namely an increase in cortisol secretion and an increase in blood sugar levels [17]. The level of glucose in the blood is greatly influenced by stressful conditions. Fish will experience hyperglycaemia or increased glucose levels as an indicator of stress, because glucose is a very important component in meeting high energy needs when fish are under stress. This is because stress will divert normal metabolic processes into energy which will be used for the activation of the physiological system. [18]. If polyphenol compound is exposed in an appropriate dose, it will act as an antioxidant which suppresses the formation of free radicals. On the contrary, if the polyphenol exposure is too high, it will trigger the formation of free radicals. High exposure to polyphenol compounds will produce free radicals and cause cytotoxicity [19]. The hyperglycaemia conditions is leading to further oxidative stress, because it is able to produce more free radicals in which reducing the work of antioxidants in the body [20].

3.2 Cortisol Level

The result of this research showed that cortisol content increased after exposed to polyphenol form *K. alvarezii*. Furthermore, the highest result of cortisol content was found in

the positive control treatment (synthetic tannins) in the amount of 451.17 nmol/L. In addition to that, the result of treatment C (9mg/kg) was 403.27 nmol/L, the result of treatment B (7.2 mg/kg) was 282.60 nmol/L, and the result of treatment A (5.4 mg/kg) was 181.63 nmol/L. Meanwhile, negative control treatment (without any treatment given) showed the lowest level which was 97.83nmol/L.

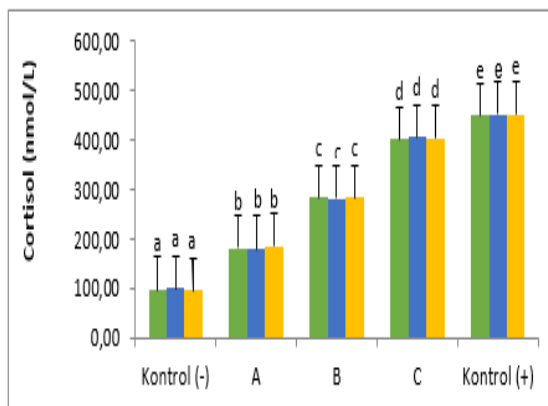


Figure 2. The Cortisol Level of *C. carpio* after polyphenol *K. alvarezii* injection

A normal cortisol content in fish is around 82.75-110 nmol/L [21]. The polyphenol injection from *K. alvarezii* seaweed can cause stress to the fish. It was increasing the cortisol hormone in the fish as a response to primary stress. In normal condition, ACTH hormone (adrenocorticotrophic hormone) from pituitary will be suppressed when glucocorticoids increased. On the contrary, under stressful conditions, the hypothalamus will stimulate the secretion of CRH (corticotropin releasing hormone) which will eventually release ACTH from the pituitary. When there is an increase in ACTH, there will be activation of the adrenal cortex which secretes cortisol. Cortisol levels will continue to increase due to inhibition of the negative feedback mechanism, resulting in continuous secretion of CRH and ACTH [22]. The cortisol hormone regulates the breakdown of glycogen in anaerobic metabolic processes which will produce energy to be used by fish [23]. When there is a breakdown of glycogen, the production of lactic acid is going to increase, also there will be an increase in CO₂ because it enters the bloodstream system [24].

4 CONCLUSION

The result of this research showed that polyphenol injection from *K. alvarezii* in several dose was affecting the blood biochemical content and cortisol content inside *C. carpio*. The increase in blood glucose and cortisol indicates that *C. carpio* was undergoing stress after polyphenol injections.

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