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Effect of the Exposure of Aloe Vera Polyphenol Fraction on Changes in Blood Glucose of Koi Fish (*Cyprinus carpio*) as a Stress Response

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ABSTRACT

Aloe vera polyphenols can cause stress in fish, which results in changes in the blood such as increased blood glucose in fish. The purpose of this study was to analyze the blood glucose parameters of koi fish after being exposed to the polyphenol fraction of A. vera. The method used, previously observed that LC 50% resulted in 150 mg/kg treatment causing 50% death so the dose of A. vera fraction injected into koi fish muscles by the treatment: Control (-) without treatment, Control (+) given tannin/synthetic polyphenols compound 3 mg/kg Biomass Weight of koi fish, treatment A=75 mg/kg BW, B= 100mg/kg BW, C= 125 mg/kg BW. After being injected, starting 72 hours the koi fish blood plasma was taken to see the effect of stress on koi fish by looking at blood glucose parameters. The results of blood glucose parameters were getting higher (from 83,33 mg/dl to 132,33 mg/dl) when exposed to polyphenol fractions. Clinical symptoms after being injected with the polyphenolic fraction of A. vera caused the fish to swim abnormally, with red spots, pale, and hemorrhages.

1. INTRODUCTION

Ministry of Maritime Affairs and Fisheries (KKP) develops the potential of national ornamental fish which is expected to improve the quality of local koi that can compete with imported koi in both domestic and international markets [1] However, this aquaculture still facing various obstacles to obtaining quality seeds. Constraints that often arise among others the viral and bacterial disease attacks and toxic environmental factors [2, 3]. Naturally, the natural processes such as the decomposition of plant material more or less contribute to the accumulation of phenols in the aquatic environment [4, 5]. Buikema et al. [6, 7] added 96% of phenol residues came from industrial, pharmaceutical, and other wastes, while 4% came from the natural decomposition of plants such as aloe vera mostly taken from the flesh of the leaves in the form of gel or latex [8, 9]. Aloe vera is one of the plants that contain polyphenols and their compounds are water pollutants that enter the fish's body affecting metabolism [10, 11], survival, growth and at optimal doses has a function as food, cosmetics [8, 12], as protection against genetic damage or cancer induced by carcinogens [13-15]. Phenolics from Aloe vera as immune and antibacterial against Aeromonas salmonicida bacteria [16]. At high doses it disrupts the endocrine system, this compound has great potential to disrupt the immune system and increase the susceptibility of fish to secondary infections [10, 17, 18]. These compounds are found in Aloe vera and fish body tissues [19]. And is chronic toxicity and immunotoxic [20, 21].

Ibrahem [22] reported in his research in vivo with LD 50 injection of C. gariepinus fish showing abnormal signs such as necrosis of the skin, gills, brain, liver, and kidneys. Added by [23] that the histopathology of these organs can be an indicator of fish stress due to toxicity. Recently, research on the effect of phenolic or polyphenolic compounds in vivo on fish blood parameters has been available, but no information mentions residues and the effect of polyphenolic compounds of Aloe vera, its toxicity to goldfish and phenolic compounds can cause changes in the biochemistry of fish blood, rarely done. Therefore, it is necessary to research the toxicity of phenol on the blood glucose parameters of koi goldfish (*Cyprinus Carpio*).

Toxic polyphenols can cause stress which results in changes in fish blood and chemical tissues such as blood glucose [24]. Stress can cause an increase in glucocortinoids which increases blood glucose to overcome sufficient energy needs. If the blood glucose condition is not normal, the life of the fish will be disrupted and can even cause death. Measurement of blood glucose levels can be used to diagnose fish that are experiencing stress. Recently there has been researched on the effect of phenolic or polyphenolic compounds in vivo on fish blood parameters, but there is no information mentioning residues and effects of polyphenolic compounds Aloe vera (Aloe vera) its toxicity to carp and phenolic compounds can cause changes in fish blood glucose seldom done. Therefore, it is necessary to conduct research on the toxicity of phenol to the blood glucose parameters of carp (*Cyprinus carpio*).

2. METHODS

2.1 The making of aloe vera (aloe vera) powder

The aloe vera leaf midrib that will be used is sorted to obtain a uniform midrib, which is seen from the uniform color and size and no part of the midrib is damaged. The aloe vera leaf midrib is washed in running water to remove the dirt that sticks and the surface of the midrib is rubbed using a clean cloth. Next, the clean midrib is cut into small pieces. Furthermore, the results of the pieces are weighed and put into a blender and added with maltodextrin. The pan containing aloe vera leaf gel is dried until the gel turns into a powder.

Gonnissen et al. [25] stated that flour processing requires filler as a feeder to accelerate drying, preventing heat damage, coating flavor components, increasing total solids, and increasing volume. The filler used in flour processing in this study is maltodextrin. According Sansone et al. [26], maltodextrin is an unsweetened sugar and is in the form of white flour with water-soluble properties, has a low price and the ability to protect the capsule from oxidation, increase yield, ease of re-dissolving and relatively low viscosity.

2.2 Aloe vera fraction

Using the column chromatography method to separate the active compound of the extract into several fractions [27]. The column chromatography method uses a stationary phase and a mobile phase where the stationary phase is silica gel G60 and the mobile phase is an eluent resulting from thin layer chromatography which is a solvent ratio between chloroform: methanol (12: 2). Then various colors will appear which are fractions of aloe vera extract and accommodated in vials according to the colors that can be seen from silica gel G60.

After obtaining several different colors from the fractions resulting from column chromatography screening, the fractions were evaporated using nitrogen and the crust was taken and then conducted UV-Vis testing.

To identify the polyphenol group of aloe vera, was carried out using a UV-Vis spectrophotometer. In the first stage, a sample of 3 ml of column chromatography results was inserted into the cuvette. In the next stage, the spectrophotometer was scanned with a wavelength from 200 nm to 550 nm and calibrated using acetone. Total polyphenols (µg/gr) were calculated by the equation E1%1cm.

This analysis using UV-Vis spectrophotometer serves to indicate the presence or absence of conjugated double bonds and is also able to determine the type of nucleus contained in secondary metabolite compounds [28].

2.3 Lethal concentration (LC 50) test

The LC50 initial test aims to obtain a dose of phenolic compounds that cause 50% mortality within 96 hours and to determine the lower and upper limits of the use of phenolic compounds using the Range Finding Test (RFT) method. In acute exposure, organisms come into contact with chemicals which can be in the form of a single exposure or multiple exposures that occur in the short term, generally within hours to days [29], which can be rapidly absorbed and have an influence /effect [17]. At the beginning of the LC50 test, the doses of polyphenols for injection namely 0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg.

The results obtained were fish mortality up to 50% occurred at a dose of 150 mg/kg for polyphenol treatment 96 hours, so it was assumed that a dose of 125 mg/kg for aloe vera polyphenols was still safe to use for toxicity testing. According to Roche and Boge [3] the determination of the LC50 dose is the result of a polyphenol injection dose with a lethality of 50% less than 96 hours. Meanwhile, for synthetic phenolic compounds in the form of tannins LC50, doses of 0 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, and 5 mg/kg were given as positive controls. At a dose of 4 mg/kg, 50% mortality occurred. So the dose used for synthetic phenolic compounds is 3 mg/kg.

2.4 Research design

The research design used in this study was a completely randomized design (CRD). The treatments in this study were the dose differences with different ranges, positive control, and negative control which were repeated 3 times. The treatment given is as follows:

Treatment A=The given of A. vera extract polyphenol (Dose 75 mg/kg BW)

Treatment B=The given of A. vera extract polyphenol (Dose 100 mg/kg BW)

Treatment C=The given of A. vera extract polyphenol (Dose 125 mg/kg BW)

Control (-)=Without the given of phenolic compounds

Kontrol (+)=The given of synthetic phenolic compounds at a dose of 3 mg/kg.

2.5 Treatment of the given of A. vera fraction

In the next step, 50% of the koi fish from the population were selected based on a uniform size of 15-20 grams and acclimatized for 7 days in a rearing container. On the 8th day, koi fish were injected with polyphenolic compounds of A. vera according to treatment A with a dose of 75 mg/kg BW, treatment B=100 mg/kg BW and treatment C=125 mg/kg BW, while in the control treatment (+) koi fish were injected with synthetic phenolic compounds 3 mg/kg Body Weight. Negative control without treatment. After 72 hours of exposure then conducted observation on the blood for biochemical parameters of koi fish blood. Clinical symptoms of fish were observed up to 96 hours.

2.6 Blood glucose parameter

The experimental animals were koi fish after injection of A. vera polyphenol then blood samples were taken, before conducting the blood sampling the fish have fasted for ± 24 hours. According [30], to avoid blood clots during collection and storage, the injection syringe and blood tube were given 0.1 ml of EDTA 2.7% anti-coagulant. Blood sampling was carried out at the base of the tail (between the caudal fin and the lateral line) using a 1 ml syringe with the tip of the needle pointing at the bottom of the spine with a slope below 45°C. The blood that has been taken is put in a blood tube and stored in the refrigerator. Blood plasma taken in the form of liquid from blood stored in the refrigerator then observed the blood glucose parameter.

3. RESULTS AND DISCUSSION

3.1 Testing of the aloe vera fraction by UV-Vis

The results of the polyphenol content fraction with compound characterization using an ultraviolet spectrophotometer gave results in the form of spectra and absorbance data, as well as the maximum wavelength of the compound, isolates compared to standard polyphenol solutions using tannins (Figure 1 and Table 1).

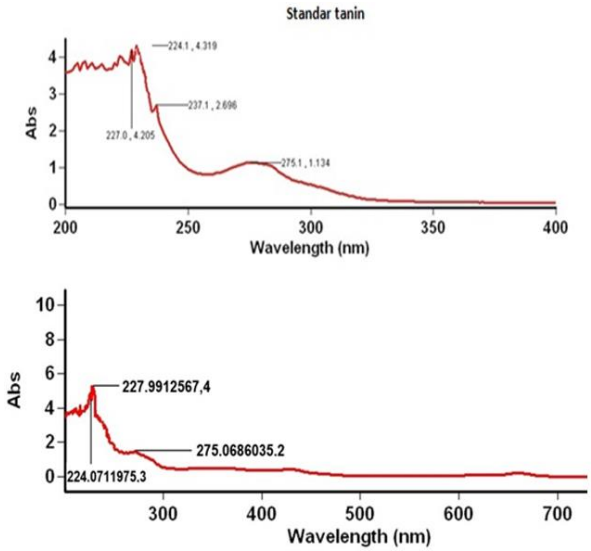

Figure 1. Spectra UV-Vis of compound isolate fraction 8 of aloe vera and standard polyphenols (tannins)

Table 1. The maximum wavelength of ultraviolet spectroscopy results from Aloe Vera and standard polyphenols (tannins)

Sample	Wavelength λmaks (nm)	Absorbance Amaks
Aloe vera	λ1=224,99	A1=1.561
	λ2=227,07	A2=0.824
	λ3=275,82	A3=0.473
Standard polyphenols (Tannin)	λ1=224,03	A1= 1.134
	λ2=227,05	A2=2.694
	λ3=275,05	A3=1.377

Qualitatively, the absorption of fraction showed a strong peak at a wavelength around 227,0 nm and a weak peak at a wavelength near 275,0 nm (Table 1).

According to Ahmad et al. [31], isolates with a spectrum of 210-280 nm came from the flavonoid group. According to Wilapangga and Sari [32] measured in the wavelength range of 200-400 nm and colored compounds were measured in the wavelength range of 200-700 nm including the phenolic compounds. From the data above, it can be concluded that the initial assumption of the UV-Vis spectrophotometer testing of the 8th fraction dominant came from phenolic compounds obtained from Aloe vera extract.

3.2 Blood glucose

Based on observations during the study regarding the effect of aloe vera polyphenol fraction on changes in blood glucose levels, different results were obtained in each treatment. The average blood glucose level (mg/dl) of Koi fish can be seen in Figure 2.

Glucose levels increased when given toxic polyphenols. According to Brandão et al. [33, 34] the presence of polyphenols injected into the koi fish muscle responded by fish as a stressor which was characterized by an increase in blood glucose. Within certain limits, every organism has resistance or tolerance level to environmental changes. According to Barton et al. [4], when experiencing stress, fish will experience primary and secondary responses, an increase in blood glucose is a secondary response of fish experiencing stress, after a primary response occurs, namely an increase in the number of stress hormones such as cortisol and catecholamines from internal cells. According to Supriyono et al. [35]. In a state of stress, there is an increase in glucocorticoids which increases blood glucose levels to cope with the high energy needs at times of stress.

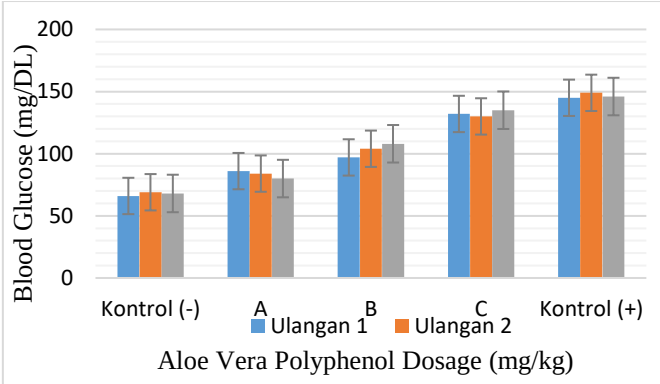

Figure 2. Relationship of the dosage of aloe vera polyphenols and Koi fish glucose (mg/Dl)

Energy needs from glucose to deal with stress can be met if the glucose in the blood can immediately enter the cells. Glucose that has entered the cell will be immediately metabolized to meet the body's physiological needs and energy needs, after being met, high glucose intake will stimulate the process of glycogenesis and lypogenesis [36]. The success of glucose supply into cells is determined by the performance of insulin. Meanwhile, during stress, insulin inactivation occurs so that it closes the use of glucose by cells [37, 38].

At times of stress, the receptor organ will receive information that will be conveyed to the brain in the hypothalamus, then chromaffin cells will secrete catecholamine hormones. This hormone will suppress the secretion of the hormone insulin which serves to help supply glucose into cells, causing the level of glucose that enters the blood to increase. This hormone will also activate enzymes involved in the catabolism of liver and muscle glycogen stores. Added by Varadarajan et al. [39] stated that exposure to phenol causes an increase in blood glucose levels due to increased metabolism in the body, blood glucose homeostasis, and hormonal disturbances.

3.3 Clinical symptoms

Based on observations on the koi fish seeds after being injected with polyphenols, then obtained the data about the signs of stressed fish is shown in Table 2. The observational data on clinical symptoms below show that polyphenols are substances that can make fish unstable, can cause damage to internal organs, pale and red spots on the operculum causing

the number of erythrocytes, hematocrit, and hemoglobin to decrease, this toxic polyphenol is a bioactive compound that is cytotoxic. This poison can disrupt or damage cell membranes, which can interfere with the transport of compounds in and out of cells causing rupture of blood vessels resulting in injury and hematopoietic organs that cannot compensate for the loss of erythrocytes due to bleeding [39, 40].

Table 2. Pathological changes to koi fish seeds after injected with aloe vera polyphenol fraction

Time After Infection	Clinical Signs (Symptoms)	Internal Organ Observation
24 HOURS	▪ Some fish show symptoms of abnormal swimming on the surface of the water decreased appetite ▪ The gills and skin of the fish are still normal ▪ Fish swim abnormally on the water's surface or alone at the tub bottom.	▪ No dead fish
46 HOURS	▪ There are some red spots on the operculum with a slightly pale color ▪ Lots of mucus and the color of some parts of the body begins to look pale ▪ Fish swim abnormally on the water surface and alone at the tub bottom.	▪ No dead fish
72 HOURS	▪ On the operculum pale ▪ A lot of mucus and some parts of the body begin to show a slightly pale blackish color.	▪ Fish that die internal organs are still normal, but there is fluid in the abdominal and intestinal cavities
96 HOURS	▪ Fish swim abnormally on the water's surface, with tilted movement ▪ Swelling in the abdomen	▪ Dead fish internal organs destroyed ▪ Hemorrhage in the liver ▪ Mata menonjol

The toxic mechanism of a compound, for example polyphenols, will work by totally damaging the cytoplasmic membrane by precipitating cell proteins, Ah receptors (AhR) that can bind to ligands such as polyphenolic compounds, polycyclic aromatic HKs or dioxins [41]. So that it can cause damage to fish organs.

The higher the polyphenol fraction, the more stressed fish with higher blood glucose values. Clinical symptoms after injection of polyphenolic fraction caused the fish to swim abnormally near the surface of the water and to be alone at the bottom of the tub. The operculum is pale, a lot of mucus and some parts of the body begin to show a slightly pale blackish color.

4. CONCLUSIONS

Based on the results of the study it can be concluded that: the higher the polyphenol fraction the more stressed fish with higher blood glucose values / For treatment B of 103 mg/dl, treatment C of 132.33 mg/dl and positive control sugar content of 146.87 mg/dl which caused stress, but treatment (K^-) was 66.67 mg/dl and treatment A average glucose value was 83.33 mg normal. It is recommended because it is known that doses B (100 mg/kg) and C(125 mg/kg) cause stress, so to continue to use a dose of 75 mg/kg so that fish life is normal/no stress.

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