



The effect of aqueous extract of johar leaves (*Senna siamea*) injection on survival rate and blood profile of snakehead fish (*Channa striata*)

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ABSTRACT

Aqueous extract of johar leaf contains metabolite compounds such as flavonoids, sponins, tannins, phenols, anthraquinones, anthocyanins, and cardiac glycosides which can be used to substitute commercial antibiotics. The use of aqueous extract of johar leaf has been known to have antibacterial activity in vitro. This study aims to determine the effect of aqueous extract of johar leaves (*Senna siamea*) injection on survival and blood profile of snakehead fish (*Channa striata*) in vivo. This research was a laboratory study using a completely randomized design (CRD) with 5 treatments and 3 replications with a total sample of 75 snakehead fish. The fish in KA, KB, KC and KD groups were injected with aqueous extract of johar leaves with concentrations of 10%, 20%, 40%, and 60% respectively, while in KE group the fish was injected with distilled water (control). The parameters of this study were the survival rate and blood profile of snakehead fish. Blood hematology examination included calculation of erythrocyte count, hemoglobin level, and hematocrit percentage, leukocyte count, and thrombocyte count. The research data obtained were analyzed using one-way analysis of variance and followed by Duncan's Multiple Distance test. Injection of aqueous extract of johar leaf did not affect the survival rate of snakehead fish. However, hemoglobin and platelet levels significant decrease in the group given 60% concentration of aqueous extract of johar leaf. It can be concluded that aqueous extract of johar leaf at a concentration of 10%, 20%, 40% is safe to use in snakehead fish.

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Introduction

Snakehead fish (*Channa striata*) is a member of the Channidae family, which consider as an aggressive carnivorous freshwater fish. Snakehead fish usually prey on a variety of small fish, insects, and frogs (Ansari *et al.*, 2020). This fish is named snakehead fish because of its wide head, large scales on the head, pointed mouth, and cylindrical body, show a snake-like appearance (Akbar, 2020). Snakehead fish is good for accelerating wound healing due to its antinociceptive and anti-inflammatory activity (Chasanah *et al.*, 2015). Snakehead fish contains protein, especially albumin, fat, and several minerals such as zinc, copper, and iron. Snakehead fish meat contains 25.1% protein

and 6.22% albumin (Andrie & Sihombing, 2017).

Snakehead fish is a freshwater commodity that has high economic value (Hidayat *et al.*, 2013). However, there many cases of freshwater fish being attacked by bacterial diseases that can cause huge losses for aquaculture activities like mass death (Rahayu *et al.*, 2019). So far, the ingredients commonly used to treat aquaculture diseases are chemicals or antibiotics (Indriani *et al.*, 2014). The use of antibiotic drugs to control diseases caused by bacteria can cause problems, including affecting and killing non-target organisms, the emergence of pathogens that are resistant to drugs and antibiotics, leaving residues on fish, and affecting the growth and reproduction of fish, and causing

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environmental pollution. One solution to dealing with this problem is the use of medicinal ingredients from natural plants that are environmentally friendly (Aisiah et al., 2011). Some of the advantages of using plants are relatively safer, easy to obtain, inexpensive, and do not cause resistance (Hidayah et al., 2016).

The use of antibacterial from plant extracts is an alternative for safer, more effective, and efficient control (Maisyaroh et al., 2018). An example of a plant used in traditional medicine is johar leaf (*Senna siamea*). Johar leaf extract has biological activities such as antimalarial, antioxidant, antibacterial, and antidiabetic. It has also been confirmed that the methanol and ethanol extracts of johar leaves contain flavonoids, saponins, tannins, anthraquinones, anthocyanins, phenols, and cardiac glycosides (Ningrum et al., 2017). Flavonoid compounds that have been isolated from various plants have been known to have interesting biological activities such as cytotoxic effect on cancer cells, inhibiting the release of histamine, anti-inflammatory, antifungal, and antibacterial (Mulyani et al., 2013).

The side effects of using traditional medicine are relatively small if used properly. Things that must be considered in the use of traditional medicines are the accuracy of the dose, the time of use, the method of use, the use of the appropriate materials and by not abusing the use of traditional medicines (Sarwono, 2005).

Blood profile examination can be used to identify abnormalities in fish caused by disease or environmental conditions. Fish with disease or physiological disorders will experience changes in the hematocrit value, hemoglobin level, number of erythrocytes, leukocytes, and platelets (Lestari et al., 2017).

Until now, information about the effect of aqueous extract of johar leaf injection on survival and blood profile of snakehead fish is not yet available. Therefore, this study was conducted to determine the effect of aqueous extract of johar leaf injection (*Senna siamea*) on the survival rate and blood profile of snakehead fish (*Channa striata*), so that it can be applied by the community as an effort to control disease in snakehead fish infected with bacteria.

Materials and Methods

This study was designed using a completely randomized design (CRD) with 5 treatments and 3 replications. In groups A, B, C and D the fish were injected with aqueous extract of johar leaves with concentrations of 10, 20, 40 and 60%, respectively,

while in group E the fish were injected with distilled water (control group).

Experimental fish

A total of 75 snakehead fish were kept in tarpaulin tanks ($3 \times 1.5 \times 0.8$ m) for 14 days before being injected with aqueous extract of johar leaves for acclimatization and avoiding stress. During care, the fish were given commercial pellets with a feeding rate of 5% body weight/day. After going through the acclimatization process, the fish were sorted for similar sizes with a weight of ± 100 g and then put into 15 experimental aquariums ($60 \times 30 \times 30$ cm) containing 20 L of water with a total of 5 fish/aquarium. To maintain water quality, a 40-60% water change process was carried out every 2-3 days (depending on water conditions). Siphoning was carried out every day which aimed to clean the remains of feed and fish feces at the bottom of the aquarium. During the treatment, the fish were given commercial feed at a rate of 5% of body weight/day.

Preparation of johar leaves extract

The johar leaves were washed with clean water and air-dried at room temperature to dry. The leaves were then mashed with a blender and stored at room temperature in a closed place. Johar leaves extraction was carried out according to the method of Aggarwal et al. (2015), by dissolving 60 g of johar leaves powder with 300 ml of sterile distilled water. The mixture of johar leaves extract with distilled water was kept at 65°C for 8 hours. The mixture was filtered using Whatman paper and the liquid was evaporated using a vacuum rotary evaporator at a temperature of 70°C. The resulting extract was stored in sterile bottles.

The required extract was 3 ml for each concentration. For this purpose, 0.3 g, 0.6 g, 1.2 g, and 1.8 g extract were dissolved in 3 ml distilled water to obtain concentration of 10%, 20%, 40%, and 60%, respectively. The johar leaf extract was injected intramuscularly into snakehead fish (*Channa striata*). On the 7th day after injection of johar leaves extract, the survival rate and blood profile of snakehead fish were analyzed.

Survival rate

The survival rate of snakehead fish (*Channa striata*) after 7 days of injection, was calculated using the survival formula according to Effendie (1997):

$$SR = \frac{N_t}{N_o} \times 100\%$$

Note: SR = Survival Rate (%); N_t = Final number of fish (head); N_o = Initial number of fish (head).

Blood profile

Blood sampling was carried out twice, before injection (day 0) and on day 7 after injection of johar leaf extract. Blood samples were taken from 3 snakehead fish per treatment group. Blood was drawn using a 1 ml syringe. The snakehead fish were first anesthetized using a fish stabilizer (Arowana™) which was dripped into clean water with 1 ml/1 liter of clean water. The fish were put into water that has been homogenized with fish stabilizer.

The fish were injected using a 1 mL syringe in the linea lateralis behind the anal fin. The needle with a 45° angle was inserted into the muscle until it reaches the spine (Insivitawati *et al.*, 2015). Then the syringe was withdrawn slowly until 1 ml of blood entered the syringe. After that, the blood was put into a green heparinized tube containing heparin anticoagulant. Blood examination included the calculation of erythrocytes, leukocytes, hemoglobin, hematocrit value, and platelet using a hematology analyzer.

Data analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) and continued with the Duncan Multiple Range test.

Table 1. Survival rate of snakehead fish (*Channa striata*) for 7 days after injected with aqueous extract of johar leave in each treatment (Mean $\pm$ SD).

Treatment	Survival rate of snakehead fish (%)
K A	66.67 $\pm$ 11.54
K B	66.67 $\pm$ 11.54
K C	73.33 $\pm$ 23.09
K D	86.67 $\pm$ 23.09
K E	80.00 $\pm$ 0.00

KA: 10% concentration, KB: 20% concentration, KC: 40% concentration, KD: 60% concentration, and KE: Distilled water (Control).

Results

Survival rate

Survival rate is the ratio between the number of animals that live at the end of the study and the number of animals at the beginning of the study expressed in percent (Effendi, 2003). The survival rate of snakehead fish (*Channa striata*) after injected with aqueous extract of johar leave is presented in Table 1.

Blood profile

Blood is one part of the fish that can be used as an indicator to determine the health level of the fish. A normal condition of fish blood is also needed to determine the health status of fish and help diagnose diseases in fish (Insivitawati *et al.*, 2015).

Under stress, there is a decrease in the number of erythrocytes, hematocrit values, and hemoglobin levels, while the number of leukocytes tends to increase in fish (Rachmawati, 2010). The average blood profile of snakehead fish on day 7 after injection of johar leaf aqueous extract is presented in Table 2.

Discussion

Survival rate

The results showed that the survival rate (SR) in the KA, KB, KC, KD, and KE treatment groups were 66.67 $\pm$ 11.54; 66.67 $\pm$ 11.54; 73.33 $\pm$ 23.09; 86.67 $\pm$ 23.09; and 80.00 $\pm$ 0.00%, respectively. Statistically, the result showed that the survival rate (SR) of snakehead fish was not significantly different ($P > 0.05$) among treatment.

The survival rate of snakehead fish observed in this study was below 90 % even in the control group. This is presumably because snakehead fish is a predatory fish that has cannibalistic nature. According to Putra *et al.*, (2015), snakehead fish in the wild are aggressive predator with their main food is natural food. These fish prey on a variety of small fish, insects, and various other aquatic animals including tadpoles and frogs (Listyanto *et al.*, 2009). While during the period of this study, the snakehead fish were given commercial pellet feed. Sasanti & Yulisman (2012) observed that the low survival of snakehead fish was because of the lack of response of snakehead fish in eating pellet feed given at the time of feeding, thus the high mortality in their study could be explained due to the lack of energy obtained from feed and cannibalism, similar to the observation in this study. In addition, the fights that occurred between snakehead fish for food and space also contribute to the low survival rate found in this study.

Blood profile

Erythrocytes

The number of erythrocytes on day 0 was 2.95×10^6 cells/mm³. This value is still within the normal range. The normal value of fish erythrocytes according to Hartika *et al.* (2014) is $0.2 - 3 \times 10^6$ cells/mm³. There was no significant difference in snakehead fish ($P > 0.05$) (Table 2). The erythrocyte values from highest to lowest were found in the KE, KC, KB, KA, and KD treatment groups (3.05 ± 0.12 ; 2.60 ± 0.67 ; 2.56 ± 0.12 ; 2.52 ± 0.26 ; and $2.30 \pm 0.99 \times 10^6$ cells/mm³). The number of erythrocytes in each of these treatments was also within the normal range.

Table 2. The blood profile (Mean±SD) of snakehead fish on the 7th day post injection with aqueous extract of johar leave in each treatment.

Treatments	Erythrocytes (10 ⁶ cells/mm ³)	Hemoglobin (g/dL)	Hematocrit (%)	Leukocytes (10 ³ cells/mm ³)	Platelets (10 ³ cells/mm ³)
K A	2.52 ± 0.26	8.67 ± 0.30 ^{ab}	24.73 ± 2.32	67.50 ± 24.86	8.66 ± 4.04 ^{ab}
K B	2.56 ± 0.12	9.97 ± 0.90 ^{ab}	28.50 ± 6.32	61.35 ± 53.03	18.00 ± 9.53 ^b
K C	2.60 ± 0.67	10.33 ± 2.57 ^{ab}	26.00 ± 7.56	40.37 ± 17.73	18.33 ± 6.50 ^b
K D	2.30 ± 0.99	7.23 ± 2.66 ^a	22.70 ± 10.38	20.90 ± 11.17	5.33 ± 4.16 ^a
K E	3.05 ± 0.12	11.73 ± 0.75 ^b	29.57 ± 1.90	71.31 ± 20.35	17.67 ± 2.94 ^b

^{a,ab,b} Different superscripts in the same column show significant differences (P<0,05)

KA: 10% concentration, KB: 20% concentration, KC: 40% concentration, KD: 60% concentration, and KE: Distilled water (Control).

The results also showed a decrease in the value of erythrocytes on day 7 compared to day 0 in all treatment groups except the distilled water treatment group. The decrease in the number of erythrocytes in fish is thought to be the impact of saponin compounds because saponin compounds can affect the lysis of red blood cells. According to [Fajriyani et al. \(2017\)](#), the low total erythrocyte is suspected because saponin compounds can lyse red blood cells, resulting in a decrease of total erythrocytes in snakehead fish. However, this condition does not adversely affect the snakehead fish. During the trial, all the fish showed normal conditions.

Hemoglobin

The hemoglobin level on day 0 was 10.5 g/dL. According to [Hastuti and Subandiyono \(2011\)](#), normal hemoglobin level in fish ranged from 9-13 g/dL, so hemoglobin levels on day 0 obtained in this study were still in the normal range. On the 7th day post injection, hemoglobin levels in the treatment group injected with johar leaf extract at 60% concentration (7.23 ± 2.66 g/dL) was significantly lower compared to group injected with distilled water (11.73 ± 0.75 g/dL).

The hemoglobin level on the 7th day decreased from day 0 in each treatment except in the distilled water treatment group. This hemoglobin value was related to the value of erythrocytes. [Ridwal et al. \(2020\)](#) said that the lower the number of erythrocytes, the lower the hemoglobin content in the blood. According to [Fajriyani et al. \(2017\)](#), the damage in erythrocytes both in membranes and in hemoglobin result in decreased hemoglobin levels. However, this condition did not adversely affect the fish during the study. The fish showed normal conditions as seen from the lack of a decrease in feed consumption as compared to feed consumption before injection of johar leaf extract. In general, sick fish will show symptoms such as decreased appetite ([Hardi, 2016](#)).

The low hemoglobin value in the KD treatment group was assumed to be due to the high content of saponins in the johar leaf extract with a concentration of 60%. The high level of saponins causes more erythrocyte cells to burst and ultimately decreases the amount of hemoglobin ([Fajriyani et al., 2017](#)).

Hematocrit

The percentage of hematocrit on day 0 was 30.05%. This percentage of the hematocrit is still within the normal range. The hematocrit percentage of healthy fish ranges from 15-40% ([Ridwan et al., 2020](#)). On the 7th day, the percentage of snakehead fish hematocrit was not significantly different among the treatment groups (P>0.05) ([Table 2](#)).

The percentage of hematocrit from day 0 to day 7 decreased in all treatment groups. This is presumably because total erythrocyte count is very closely related to the hematocrit value. According to [Hastuti & Subandiyono \(2011\)](#), hematocrit is a description of the percentage of red blood cells in the blood. [Campbell \(2015\)](#) revealed that hematocrit levels vary depending on nutritional factors, age, sex, body size, and spawning period. Measurement of hematocrit can also be used as an indicator due to several factors such as environmental factors, handling when taking blood or being exposed to pathogenic infections ([Hardi et al., 2011](#)).

Leukocytes

Leukocytes are components of blood cells that act as the defense system of the fish body ([Robert, 2012](#)). The number of leukocytes is influenced by several factors, i.e. fish species, age, nutrition, and stress ([Lestari et al., 2017](#)). In this study, leukocyte count on day 0 was 51.75 × 10³ cells/mm³ within the normal value for freshwater fish which ranged from 20 - 150 × 10³ cells/mm³ ([Hartika et al., 2014](#)). The number of leukocytes on the 7th day of blood collection did not show a significant difference between treatment groups (P>0.05) ([Table 3](#)). However, the number of leukocytes tended to decrease in fish injected with

johar leaves extract with the lowest number being in the KD group (60 % concentration). Sundaryono (2011) stated that the administration of dewa leaves (*Gynura segetum*) extract containing flavonoid compounds can reduce the number of leukocytes in mice (*Mus musculus*). Furthermore, Lisdiana et al. (2017) reported that the flavonoids contained in rambutan peel extract were shown to be able to reduce the total leukocyte count in white rats. This is because the flavonoids contained in rambutan peel extract can inhibit the process of lipid peroxidation at the initiation stage, so that free radicals cannot develop into new free radicals. Therefore, it can be concluded that the higher the dose of an extract used, the lower the number of leukocytes.

Platelets

The platelet count on day 0 was $11,000 \times 10^3$ cells/mm³. The number of platelets on day 7 in the KD treatment group was very low (5.33 ± 4.16) and significantly different from other treatment groups except for the KA treatment group. The low levels of platelets in the KD treatment group are presumably due to the higher amount of flavonoid compounds in the johar leaf extract compared to the other treatment groups. Kartikasari et al. (2019) reported that flavonoids are compounds that can produce antiplatelet effects by inhibiting the metabolism of arachidonic acid by cyclooxygenase.

Overall, the results of this study showed that the injection of aqueous extract of johar leaf did not affect the survival of snakehead fish. However, hemoglobin and platelet levels experienced a significant decrease in the group given johar leaf extract with a concentration of 60%. Therefore, it can be concluded that johar leaf extract at a concentration of 10%, 20% concentration, 40% concentration is safe for use on snakehead fish.

Conclusion

Based on the results of the study, it can be concluded that the injection of aqueous extract of johar leaves (*Senna siamea*) to snakehead fish (*Channa striata*) with a concentration of 10, 20, 40 % do not have a negative effect on survival and blood profile of snakehead fish.

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