

## Research Article

# Effects of dietary supplementation of Kakuti, *Ziziphora clinopodioides*, extract on enzymatic and antioxidant parameters of the plasma of common carp, *Cyprinus carpio*

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## Abstract

Kakuti, *Ziziphora clinopodioides*, is a medicinal herb found in Iran, but its potential benefits have not been studied on fish. Thus, the aim of the present study was to investigate the effects of Kakuti aqueous extract on the plasma antioxidant and enzymatic parameters in common carp, *Cyprinus carpio*. The aqueous extract was prepared by boiling Kakuti leaves for 2h. Four diets containing %0 (Ctrl), %0.5 (0.5E), %1 (1E), and %2 (2E) of Kakuti extract were fed to fish within a 60-day period. For each diet, three tanks, each with 15 fish with initial weight of  $46.2 \pm 0.16$ g, were assigned. Then, blood samples were taken from all treatments to assess plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). The results showed that %1 Kakuti extract significantly decreased plasma AST and ALP, but increased CAT activities. There was no significant effect of dietary treatments on plasma ALT activity. 0.5 and %1 Kakuti extract decreased plasma glucose levels significantly, but increased plasma SOD activity. 1 and %2 Kakuti extract decreased plasma MDA levels significantly. According to the results, Kakuti extract is capable of augmenting antioxidant strength, that in turn, may be the reason for boosted tissue health in common carp. Dietary %1 Kakuti extract is recommended for inclusion in common carp diet.

**Keywords:** *Ziziphora clinopodioides*, Common carp, Antioxidant, Immune system, Blood factors, Plasma enzymatic

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## Introduction

Antioxidant system protects living organisms against adverse effects of free radicals and reactive molecules; thus, plays an important role in the organism health and well-being (Halliwell and Gutteridge, 2015). Superoxide dismutase (SOD) and catalase (CAT) are two important antioxidant enzymes that defend an organism against oxidative stress (Fang *et al.*, 2002). If the antioxidant system fails to neutralize reactive molecules, they attack biological materials that damage organisms' tissues (Pérez-Jiménez *et al.*, 2009). Among biological molecules, unsaturated fatty acids are very susceptible to oxidative stress. Under oxidative stress, fatty acids are converted to aldehydes that induce bad flavor (Morales *et al.*, 2004). Measurement of malondialdehyde (MDA) is known as an indicator of lipid peroxidation and oxidative stress (Ullah *et al.*, 2018). Fish have antioxidant system equivalent to higher vertebrates. Antioxidant system is very important in fish health, as they typically have higher percentages of unsaturated fatty acids, compared to terrestrial animals (Fazelan *et al.*, 2020).

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are indicators of tissue health, as they are cytosolic enzymes that are found at different concentrations in various tissues (Haschek *et al.*, 2009). ALT is found at high concentrations in fish liver and elevation of its levels in blood

indicates hepatocyte damage. ALP is found at high concentration in erythrocytes and hemolysis causes blood ALP elevation. AST is not tissue-specific and blood AST elevation means fish tissues are damaged (Taheri Mirghaied *et al.*, 2017). Moreover, AST participates in glucose production from amino acids, thus is known as a stress marker. Measurement of blood AST along with a stress marker such as blood glycemic state may help to diagnose stress in fish (Tejpal *et al.*, 2009).

Herbal additives have recently gained great attentions in aquaculture because of several benefits such as growth promotion, radical scavenging and health boosting in fish (Dawood *et al.*, 2018). They are eco-friendly compared to chemical disinfectants and antibiotics; therefore, have been extensively studied in different fish species (Abdel-Tawwab, 2016). Various herbal products, such as essential oils, extracts and intact plant materials have been successfully incorporated in fish feeds to augment growth and health performances (Chakraborty *et al.*, 2013). Kakuti, *Ziziphora clinopodioides*, is a plant found in Turkey and Iran, which is rich in thymol and carvacrol (Sonboli *et al.*, 2010). These compounds are known for high antioxidant capacity and several studies have shown that application of these compounds in fish diets led to higher antioxidant strength (Ahmadifar *et al.*, 2011; Giannenas *et al.*, 2012). There is no study conducted about using Kakuti extract in fish, despite the

aforementioned positive potentials. Thus, it was hypothesized that inclusion of Kakuti extract in fish diet augments antioxidant system and tissue health. Therefore, the present study aimed to assess the effects of dietary supplementation of Kakuti extract on antioxidant and tissue health indicators of common carp, *Cyprinus carpio*.

## Materials and methods

### *Preparation of Kakuti extract*

In this study, aqueous extract of Kakuti was used for dietary supplementation. Kakuti was purchased from a local shop and pulverized by a mixer. Then, the pulverized materials were mixed with water at a proportion of 1:20 and boiled for 2h. The extract was separated through a cotton filter and kept in refrigerator until use (Hoseini and Yousefi, 2019).

### *Experimental design*

A basal diet (Table 1) and three supplemented diets were prepared by mixing feed ingredients. Then, water was added to the mixture to create dough. The dough was pelleted by passing through a mesh and the pellets were dried against a fan blow. The Kakuti extract was added at 0, 0.5, 1, and %2 to the basal diet. Accordingly, there were four dietary treatments including Ctrl (fed with basal diet), 0.5E (fed with basal diet+0.5% extract), 1E (fed with basal diet+%1 extract), and 2E (fed with basal diet+%2 extract).

**Table 1: Ingredients and composition of the basal diet.**

Ingredients	Amount (%)
Corn meal	5
Barely meal	5
Wheat meal	22.84
Soybean meal	53.39
Wheat gluten	3
Fish meal	3
Soybean oil	7.27
Vitamin/Mineral premix	0.5
<b>Chemical composition</b>	<b>Amount (%)</b>
Moisture	10
Crude protein	32.7
Crude lipid	7.4
Crude ash	4.2

Common carp juveniles were stocked in 12 tanks (200l volume) at a density of 15 fish with initial weight of  $46.2 \pm 0.16$ g per tank. Continuous aeration and water flow rate of 1 l/min was established for each tank. After a 7-day feeding with the basal diets as acclimation period, the tanks were divided into four triplicated groups that fed either %0 (Ctrl), %0.5 E, %1 E, and %2 E diets for further 60 days. Feeding rate was %3 of biomass per day and the tanks' biomasses were measured biweekly for feed amount adjustment. At the end of the feeding trial, blood samples were taken from all treatment for further analyses.

### *Blood sampling and analyses*

For blood collection, the fish were first anesthetized by eugenol (100 mg/L); blood samples were then taken by caudal puncture using heparinized syringes (Yousefi *et al.*, 2018). Plasma was separated after centrifugation (7000

rpm; 7 min) and kept at  $-70^{\circ}\text{C}$  until analyses.

Glucose levels of plasma were measured by glucose-oxidase method, using a commercial kit provided by Pars Azmun Co. (Tehran, Iran). ALT, AST, and ALP activities of plasma were measured kinetically using a spectrophotometer and Pars Azmun Co. (Tehran, Iran) kits (Yousefi *et al.*, 2018).

CAT activity of plasma was measured according to Goth (1991) based on decomposition rate of hydrogen peroxide. SOD activity of plasma was measured based on conversion of superoxide anion to hydrogen peroxide using a commercial kit (Zellbio, Veltinerweg, Germany). MDA level of plasma was measured based on reaction with thiobarbituric acid at  $95^{\circ}\text{C}$  using a commercial kit (Zellbio, Veltinerweg, Germany, Yousefi *et al.*, 2018).

### Statistical analyses

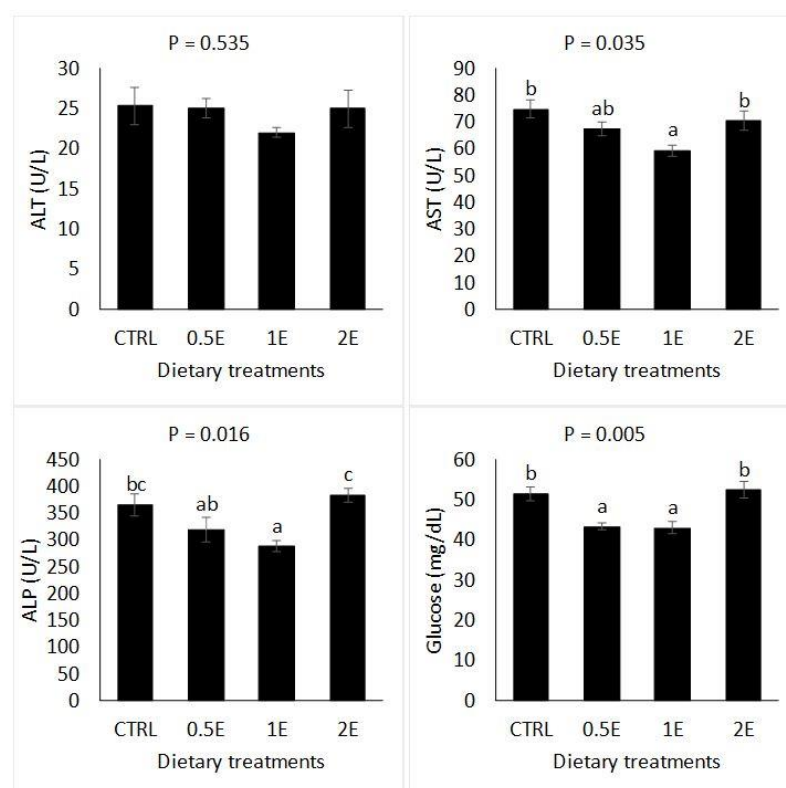
Normal distribution of the data and variance homogeneity were confirmed by Shapiro-Wilk and Levene tests. The data were then subjected to One Way ANOVA to find any effects of the treatments on the variables. Significant differences among the treatments were checked by Duncan's test at  $p < 0.05$ . Data were analyzed using SPSS v.22 and presented as mean  $\pm$  SE.

### Results

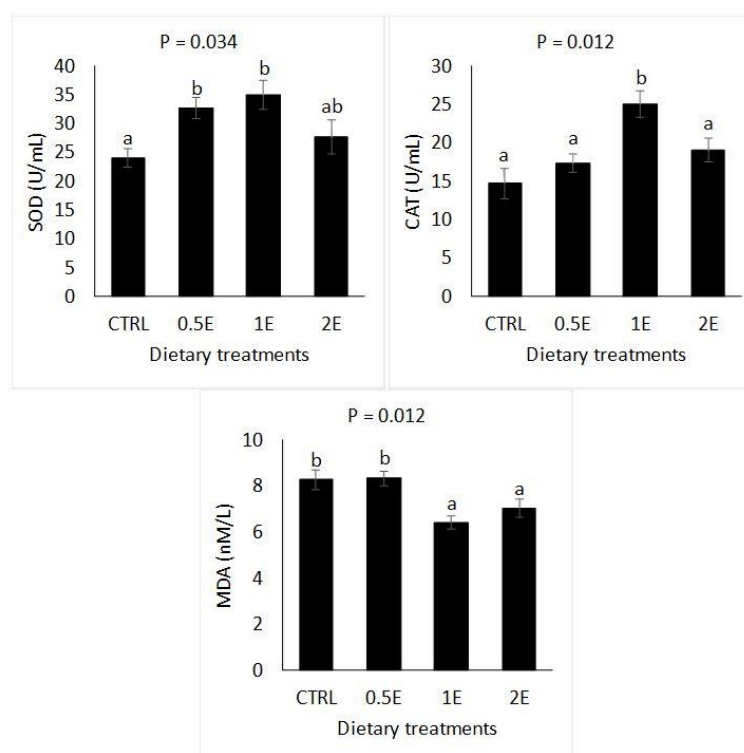
There was no significant difference ( $p=0.535$ ) in ALT activities of plasma among the treatments after 60 days rearing. Dietary treatments affected AST activities of plasma significantly ( $p=0.035$ ); the lowest AST activity of plasma was observed in 1E treatment, which was significantly lower than Ctrl and 2E treatments (Fig.1).

Dietary Kakuti extract affected ALP activity of plasma significantly ( $p=0.016$ ). The lowest ALP activity of plasma was observed in 1E treatment, which was significantly lower than Ctrl and 2E treatments. Moreover, the ALP activity of plasma of 0.5E treatment was significantly lower than that of 2E treatment. Glucose levels of plasma exhibited significant differences ( $p=0.005$ ) among the treatments, which the lowest values were observed in 0.5E and 1E treatments and highest values were related to Ctrl and 2E treatments (Fig. 1).

SOD activities of plasma of 0.5E and 1E treatments were statistically similar, both were significantly higher than that of Ctrl treatment ( $p=0.034$ ). There was no significant difference in CAT activities of plasma among Ctrl, 0.5E and 2E treatments; all were significantly lower than that of 1E treatment ( $p=0.012$ ). MDA levels of plasma of Ctrl and 0.5E treatments were statistically similar and significantly higher than those of 1E and 2E treatment ( $p=0.012$ ). 1E and 2E treatments had similar MDA levels of plasma (Fig. 2).



**Figure 1: Effects of dietary Kakuti extract on ALT, AST, ALP of plasma, and glucose levels in common carp after 60 days rearing. Different letters above the bars indicate significant differences among the treatments, error bars show standard deviation.**



**Figure 2: Effects of dietary Kakuti extract on SOD, CAT, and MDA levels of plasma in common carp after 60 days rearing. Different letters above the bars indicate significant differences among the treatments, error bars show standard deviation.**

## Discussion

ALT of plasma is known as specific indicator of fish hepatic health, because hepatocytes are rich in this enzyme and damage to these cells leads to ALT leakage into circulation (Haschek *et al.*, 2009). Studies on different fish species have demonstrated that dietary herbal product administration may decrease ALT activity of plasma or have no effects on the enzyme's activity. For example, dietary *Polygonum minus* extract administration significantly decreased ALT activity of plasma in rainbow trout, *Oncorhynchus mykiss* (Adel *et al.*, 2020). However, dietary Oregano, *Origanum vulgare*, extract had no significant effect on ALT activity of common carp (Abdel-Latif *et al.*, 2020). Thus, it seems that effects of herbal product on ALT of plasma and liver health are context-depended. Moreover, a herbal product may better show its hepatoprotective effects under stimulated conditions. For example, Taheri Mirghaed *et al.* (2020a) showed that dietary Sweet wormwood, *Artemisia annua*, leaf extract had no effect on ALT of plasma of common carp under normal conditions, but mitigated ALT elevation after the fish were challenged by ambient ammonia (a hepatotoxic condition).

ALP concentration in erythrocyte is several folds higher than that in other tissues, thus, plasma levels of the enzyme is known as indicator of hemolysis (Taheri Mirghaed *et al.*, 2017). The present results are in line with findings reported on dietary menthol administration which

decreased ALP activity of plasma significantly along with increase in blood erythrocyte number in rainbow trout (Hoseini *et al.*, 2020). AST is found in various tissues and the enzyme level in plasma is an indicator of tissues health (Haschek *et al.*, 2009). The present results are in line with Li *et al.* (2019) findings that showed dietary flavonoids from wild onion, *Allium mongolicum*, significantly decreased AST on plasma in snakehead fish, *Channa argus*. Likewise, dietary rosemary leaf powder significantly decreased AST activity of plasma in Nile tilapia, *Oreochromis niloticus* (Naiel *et al.*, 2020).

One of the benefits of herbal supplements is their anti-stress effects (Chakraborty and Hancz, 2011) and plasma glucose is known as an indicator of physiological stress (Barton, 2002). The present results suggest that Kakuti extract at 0.5 and 1% has anti-stress effects on common carp. Similarly, dietary rosemary administration to common carp (Yousefi *et al.*, 2019) and green tea extract administration to black rockfish, *Sebastes schlegeli* (Hwang *et al.*, 2013), and decreased plasma glucose levels significantly.

One of the well-known benefits of herbal additives is their antioxidant effects (Jeney *et al.*, 2015). Kakuti extract significantly stimulated enzymatic antioxidant system and mitigated oxidative stress. The present results are in line with several previous studies on other herbal products such as *Zataria multiflora* (Taheri Mirghaed *et al.*, 2020b), *Eichhornia crassipes*

(Rufchaei *et al.*, 2020), *Quercus castaneifolia* (Paray *et al.*, 2020), and *Melissa officinalis* (Bilen *et al.*, 2020) extracts stimulated antioxidant system in different fish species. Such an antioxidant effect of Kakuti extract might be due to the presence of antioxidant compounds such as thymol and carvacrol (Ahmadifar *et al.*, 2011; Giannenas *et al.*, 2012; Quiroga *et al.*, 2015). Moreover, the antioxidant effects of Kakuti extract might cause boosted health of different tissues, as 1E fish treatment exhibited stimulated antioxidant system and improved tissue health indicators (ALP and AST of plasma). Such a linkage between the antioxidant parameters and tissue health have been previously reported when fish were fed *Curcuma longa* (Rajabierabadi *et al.*, 2020) and *A. annua* (Taheri Mirghaed *et al.*, 2020a) extract.

In conclusion, dietary supplementation with %1 Kakuti extract is recommended for common carp dietary inclusion, as it stimulates antioxidant system and protects different tissues against damages.

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