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Some Medical Plants Use as Food Additives to Stimulate Antioxidant in Rainbow trout (*Oncorhynchus mykiss*)

***Tarek A. S. Altief**
Department of Basics of Veterinary Sciences - Omar Al-Mukhtar University – Libya.

Ahmed A. Almabrok
Department of veterinary sciences -Higher Institute of Agricultural Technology - Alghairan - Libya

Gamaia A. M. Ali
Department of animal physiology - Azzytuna University - Libya

Soner Bilen
Department of Basic Sciences - Faculty of Fisheries and Aquaculture -Kastamonu University- Turkey

- Tarekhhakam@gmail.com

Abstract

This study was designed to evaluate certain antioxidants, by feeding 1500 of young rainbow trout (*Oncorhynchus mykiss*) on diets containing different levels (0%, 0.1%, 0.5% or 1%) of an aqueous methanolic extract of *Glycyrrhiza glabra*, *Coriandrum sativum* and *Cassia angustifolia* as a feed additive for seventy-five days. Fish with an initial weight of 22.65 ± 0.07 g were distributed to 30 tanks, 50 fish in each tank, and the experiment was started with three replications. At the end of each month, the activities of liver antioxidant enzymes (catalase CAT, superoxide dismutase SOD, glutathione peroxidase GPx, Glutathione GSH and lipid peroxidase LPO) levels were analyzed. According to the study results, the methanolic extracts of *C. sativum* and *C. angustifolia* significantly increased SOD activity in rainbow trout ($P < 0.05$), but showed no significant change in CAT activity ($P > 0.05$). GSH levels increased for the dose by 1% for all experimental groups ($P < 0.05$). When GPx levels were compared, *C. sativum* and *C. angustifolia* showed an overall increase in lipid peroxidase compared to the control, while lipid peroxidase was generally low in the experimental groups ($P > 0.05$).

Key words: *Glycyrrhiza glabra*, *Coriandrum sativum*, *Cassia angustifolia*, rainbow trout, antioxidant status.

1. Introduction

Aquaculture covers the process governing the production and breeding of fish, mollusc, crustacean and aquatic plant species within natural and artificial water resources towards satisfying human demands. Majority of the aquaculture, which has been the food sector with the highest average growth rate that is over 7.7% through the last decade, is realised in Asia (Gjedrem, 2012). It is reported that fish behaviour, environmental conditions and seasonal changes affect the antioxidant defence systems of fishes. That there were seasonal and species-based differences in the antioxidant enzyme activities of the erythrocytes of carp (*Cyprinus carpio*), tench (*Tinca tinca*) and goldfish (*Carassius auratus*) reported by (Gabryelak, 1983). The antioxidant enzymes' activity varied among the 79 freshwater species living in geographically close lakes published by (Palace, 1993). The interspecific sturgeon hybrid juveniles (Russian sturgeon, *Acipenser gueldenstaedtii*, ship sturgeon, *Acipenser nudiventris*) had higher

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antioxidant defence system activity than that of intergeneric hybrids (European sturgeon, *Huso unicentric* and ship sturgeon) reported by (Lozovskaya, 2002). According to (Fitzgerald, 1992), species living in environments with high density light had displayed higher SOD activity as a protection against ultraviolet light. When exposed to increasingly high saltiness levels, Adriatic sturgeon (*Acipenser naccarii*) displayed high antioxidant enzyme levels in their erythrocytes, and normal levels of lipid peroxidation were observed until the saltiness reached sea levels. These changes in the blood did not reflect on liver or heart as a result of the adaptation to marine conditions (Martı´nez-A´ lvarez, 2002). As temperature-dependent organisms, fishes may be exposed to temperature level fluctuations that may affect growth, reproduction and survival (O'Brien, 2000). Both cooling and warming reduce the oxygen levels in the tissues considerably for they both disrupt the oxygen supply-demand balance within the organism and it depends on the organism's aerobic capacity. Adaptation to changing temperatures requires adjustment of both the density and the functional characteristics of mitochondria and thus as a result, it affects ROS formation and the antioxidant defence system. This situation explains the high SOD activity in the liver and blood of active fish species. In addition to temperature, other water quality parameters also change the amount of oxygen dissolved in water and thus may have impacts on the fishes' antioxidant defence system. The most commonly observed response in fishes against hypoxia is their enzymatic and non-enzymatic antioxidant defences. This process was defined as "preparation to oxidative stress" (Hermes-Lima, 2001). According to (Lushchak, 2001) proved for goldfish and (Cooper, 2002) proved for spot croaker (*Leiostomus xanthurus*) that anoxia caused the antioxidant defence mechanism to be activated.

Inclusion of various food additives such as plant extracts and immunity stimulant substances into fish food may increase the fishes' tolerance towards temperature change, and may increase the productivity under adverse effects caused by stress and stock density that generate suppressive impact on natural immunity (Magnadottir, 2006) and (Magnadottir B, 2011). Medicinal herbs and plants are preferred to be used for fish cultures due to being a more adequate and affordable resource in treatment and combatting diseases without products causing toxicity (Madhuri, 2012). Diseases have an important economic impact in aquaculture sector. The losses due to the deaths caused by the diseases in aquaculture sector have been researched globally under various studies (Shinn, 2015). Inclusion of plant extracts to fish food may affect the fishes' food finding capabilities by stimulating their sense of smell and thus promote them to eat more food than normal (Adams, 2005). Such plants have effects towards assisting health and wellbeing such as antibacterial, antimutagenic, anticarcinogen, antithrombotic and vasodilator effects (Bidlack, W.R., S.T. Omaye, M.S. Meskin, and D.K.W. Topham 2000). Those plants have specific activities in terms of the alkaloids,

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flavonoids, colours, phenolic contents, terpenoids, steroids and essential oils they contain indicated by (Citarasu, 2010). Those plants had many antioxidants and antimicrobial properties due to the presence of such compounds and that they may be used as chemotherapeutic molecules in aquaculture activities (Talpur, 2013). Plant extracts have many advantages ranging from reducing environmental impacts, bio-solubility, less residue and low toxicity in fishes. They are also of low cost to producers and formation of resistance by pathogens is unlikely (Ribeiro, 2016) and (Hashimoto, 2016). Those plants use of such natural plants as food additive towards the purpose of animal production yield and animal food usage is acceptable at large scale (Mohamed, 2003). From what was mentioned above, aim of our study was performed to evaluate the effects of three doses of licoricey root (*Glycyrrhiza glabra*), coriander seed (*Coriandrum sativum*), and cassia (*Cassia acutifolla*) on immune and hematological parameters.

1.2 Antioxidant System of Fishes

Due to the formation of reactive oxygen species (ROS) that cause oxidative stress, unsaturated fatty acids of the cell membranes become oxidised and the cell loses its functionality. Protein oxidations and DNA and steroid content deterioration are unavoidable as a result of this. There is a certain defence mechanism within the antioxidant system of the body depending on activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione enzymes (glutathione peroxidase (GPx), glutathione reductase (GR)). In addition to these enzymes functioning for the catabolising of oxidative radicals, some small, non-enzyme molecules such as E, K and C vitamins, alpha carotene, beta carotene, flavonoids, isoflavones, carotenoids, catechin, criptoxantin, quercetin, lycopene, lutein, resveratrol and anthocyanins may be obtained from plants to take part in antioxidative processes.

2. Material and Methodology

The design of the study was based on the feeding programme set up for the purpose of increasing the effectiveness of immuno-stimulants of rainbow trout (*Oncorhynchus mykiss. Walbaum, 1792* (Harry J. Grier, 2007)) through using of various medicinal plants. The fish were fed with control diet for a week for adaptation, then fed with experimental food containing three different plant extracts sprayed into the commercial food for a period of 75 days. On the 15^{th}, 30^{th}, 45^{th}, 60^{th} and 75^{th} days of the research,1500 fish were chosen with average weight of 22.65 ± 0.07 g and used in the study which spanned 75 days. (50 fish) were selected randomly from the juvenile growing cages of the Research Centre and placed in each of tanks with dimensions of 1.5 x 1.5 x 1.5 m. Fish were distributed to 30 tanks, 50 fish per tank, and the experiment was started as three replications. Control group fishes were fed only with commercial fish food. Medicinal plant groups were fed with food dosed at 0.1, 0.5 and 1g.

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The licoricey root (*Glycyrrhiza glabra*), coriander seed (*Coriandrum sativum*), and cassia (*Cassia acutifolla*) medicinal plants used in the study were provided from the herbalists inside Kastamonu province. The plants were dried and powdered, and kept in airtight bottles. After being powdered they were weighed as 50 grams each, and after being mixed with 40% methanol inside brown bottles the mixtures were stored for three days. The mixture was screened at the end of 72 hours, and only the liquid part was evaporated through extraction method in the evaporator until it reached the consistency of honey. The extract that is brought to honey consistency was then dissolved in distilled hot water and applied to the fish food via spraying method. Commercial food was used as study rations. All test foods outside of the control group were prepared by spraying the plants' prepared watered methanolic extracts into the commercial food. The amount of daily food given to the fishes was regulated based on the size of the fish and the temperature of the water used in test tanks. Fishes were given daily food based on 2% and 2.5% of their live weights. Throughout the feeding tests, the fishes were fed by hand, twice each day at 09:00 in the morning and 16:00 in the evening. It was emphasized that the food was consumed by the fish during the feeding process. Fish feeding was stopped two days before sampling for analysis.

2.1. Fish Food and Feeding Programme.

Commercial feed was used as study rations. All test feeds outside of the control group were prepared by spraying the plants' prepared watered methanolic extracts into the commercial feed.

Table (1) Content of commercial food

Proximate Content	Macro Elements	Trace Elements	Vitamins
Raw Protein (44%)	Phosphorus 1.10 %	$FeSo_4$ 80 ppm	A 7.400 UI
Raw Oil (21%)	Calcium 1.30 %	KI 2 ppm	D3 1.000
Ash (9%)	Sodium 0.20 %	Cu So_4 7pmm	
Raw Fibre (3.9%)		Mn So_4 15 pmm	
		Zn So_4 110 pmm	

The amount of daily food given to the fishes was regulated based on the size of the fish and the temperature of the water used in test tanks. Fishes were given daily food based on 2% and 2.5% of their live weights. Throughout the feeding tests, the fishes were fed by hand, twice each day at 09:00 in the morning and 16:00 in the evening. Care was shown that the entire food was taken by the fish during the feeding process. Two days prior to each of the days when measurements and analyses were conducted throughout the testing, the feeding process was halted.

2.2. Antioxidant Enzyme Analyses.

2.2.1. Sampling of Tissue and Preparation for Analyses.

Antioxidant enzyme analyses were performed on fish liver tissues. Where the fish was dissected into the water, cleaned of blood, and dried, after sampling, it was placed

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directly inside Eppendorf tubes and stored in liquid nitrogen. Samples were kept at -80 °C before the analyses were performed. For preparation to analyses, the liver tissues were divided into pieces of 0.1 g, and after adding 1 ml EDTA phosphate buffer they were fragmented at the homogeniser. After being adequately fragmented, the tissues were subjected to centrifuge at 20 000 G for 45 minutes, and the supernatants remaining at the top were utilised in analyses.

2.2.2. Superoxide Dismutase (SOD) Analysis.

Superoxide dismutase (SOD) activity was measured spectrophotochemically by use of Ferrocytochrome C method utilising xanthine / xanthine oxidase for source of superoxide radicals (Sun Y, 1988).

% inhibition= [(A blank – A sample) / A blank] X 100

Activity (U/ml) = [(%inhibition/50) X (1/0.1)]

Specific Activity (U/mg protein) = [(U/ml) / mg protein]

2.2.3. Glutathione (GSH) Level

Estimation of total, and nonprotein sulfhydryl groups in tissue with Elman's reagent (Sedlak, 1968).

Calculated Glutathione (GSH) = (buffer A x 66.6) / (g Sample).

2.2.4. Glutathione Peroxidase Analysis.

GPx catalyses the oxidation of glutathione in the presence of hydrogen peroxide (GSH), glutathione (GSH) oxides. Through GPx activity, GSSH produced in the presence of hydrogen peroxidase in the presence of GSH-Px is reduced into GSH via the aid of glutathione reductase and NADPH, analysis according (Wendel, 1980).

A_{340}/min. = [A_{340} (time 2) - A_{340} (time 1)] / [time 2 (min) - time 1]

GPx activities = [(A_{340} / min) / 0.00373] x [0.19 / 0.02] x sample dilu. on=nmol/min/ ml

Result / (mg protein) = (U/mg protein).

2.2.5. Lipid Peroxidase (LPO) Analysis.

Lipid peroxidation product malonildialdehyde (MDA) and thiobarbituric acid (TBA) 90 enters into reaction at coron and forms a pink colour. The pink colour composition defined as MDA level is based on the spectrophotometric measurement at 532 nm (C. JO, 1998).

MAD = (Sample OD) / (Standard OD) × standard constant

2.2.6. Catalase (CAT) Analysis.

Catalase activity was assayed following the method of (Luck, 1974). Phosphate buffer pH=7.5, H2O2 buffer and methanol were used for catalase analysis, and the readouts were realised at 240 nm absorbance value

K= {[2,3 × log (OD/OD2] / t (sec.)} K / mg protein = K / [(mg / ml protein) × 1000]

CAT= (µM of sample / 20 min) x sample dilu. = nmol /min / ml

Result / (mg protein) = (U/mg protein)

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2.3. Statistical Analyses

The mean and standard deviation (+Std) values of all data obtained in the test were calculated by the assistance of Microsoft Office Excel program. Variance Analysis (ANOVA) was conducted by implementing SPSS Statistics Program (SPSS 23.0) on these data.

3. Results

3.1. Superoxide Dismutase Activity (SOD)

SOD activity values obtained at the end of the overall 75-day study period, through the samplings taken on the 15th, 45th and 75th days are presented in (Graph **(1)**).

Groups with high SOD activity compared to the control group on the 15th day of the study were observed to be 0.5% C, 1% C, 0.1% S, 0.5% S and 1% S ($P < 0.05$), and a significant increase occurred in all test groups excluding SM compared to the control group on the 45th day. Groups for which SOD activity compared to the control group was recognised to be significant at the end of testing were observed to be 1% L, 0.1%C, 0.5% C, 1% C, 0.1% S, and 0.5% S ($P < 0.05$) (Graph **(1)**).

Based on 15th day data, the SOD activities of the 0.1% L, 0.5% L, 1% L; 0.1% C, 0.5% C, 1% C; 0.1% S, 0.5% S and 1% S groups compared to the control group were observed to be 6.79±0.02, 5.77±0.01, 6.81±0.01, 6.30±0.03, 2.63±0.03, 7.37±0.03, 7.70±0.03, 7.94±0.03, 7.45±0.03 and 11.79±0.03 nmol/min/ml. It is observed that coriander (excluding 0.1%) and cassia increased the SOD values compared to the control group on the 15th day (Graph **(1)**).

Based on 45th day data, the SOD activities of the 0.1% L, 0.5% L, 1% L; 0.1% C, 0.5% C, 1% C; 0.1% S, 0.5% S and 1% S groups compared to the control group were observed to be 5.28±0.00, 8.81±0.03, 7.20±0.03, 10.51±0.02, 9.27±0.01, 7.40±0.01, 6.84±0.04. SOD value increases were observed compared to the control group in all test groups excluding 1% S on the 45th day (Graph **(1)**).

Based on 75th day data, the SOD activities of the 0.1% L, 0.5% L, 1% L; 0.1% C, 0.5% C, 1% C; 0.1% S, 0.5% S and 1% S groups compared to the control group were observed to be 6.56±0.18, 5.78±0.01, 6.51±0.00, 7.51±0.03, 7.90±0.03, 8.80±0.02, 8.36±0.02, 7.17±0.01, 8.31±0.01 and 5.26±0.02. Again, on the 75th day it was observed that SOD values increased compared to the control group in all test groups (excluding 0.1% L, 0.5% L and 1% S) (Graph **(1)**).

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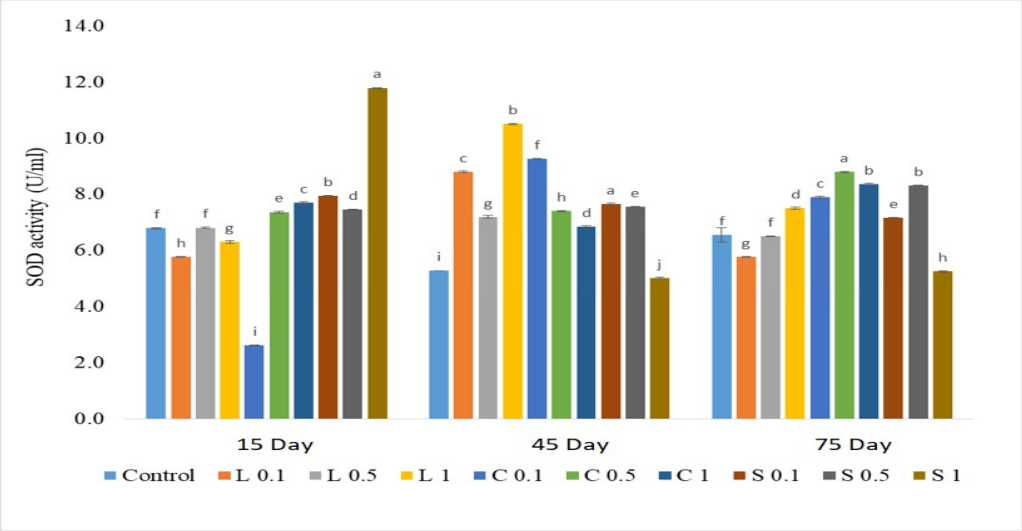

Graph (1) The changes that occurred in the superoxide dismutase activities of the rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (U/ml). (Average values and standard deviations of all data are given, and the small letters at the top represent the difference between groups on the same sampling day).

3.2. Catalase Activity (CAT)

Through the liver samples taken from the fishes on the 15th, 45th and 75th days, changes that occurred in the CAT activities of all groups were examined. The data thus obtained are presented in (Graph **(2)**).

Based on 15th day data, the CAT activities of the groups compared to the control group were observed to be 1.28 ± 0.00, 1.48 ± 0.00, 1.17±0.01, 1.28±0.01, 1.31±0.04, 1.36±0.07, 1.53±0.24, 1.44±0.01, 1.65±0.02 and 2.93±0.04 nmol/min/ml. Overall CAT activity increase was observed compared to the control group in all test groups excluding 0.5% S and 1% S, while this increase was observed to be not statistically significant on the 15th day ($P < 0.05$).

Based on 45th day data, the CAT activities of the 0.1% L, 0.5% L, 1% L; 0.1% C, 0.5% C, 1% C, 0.1% S, 0.5% S and 1% S groups compared to the control group were observed to be 1.16 ± 0.03, 1.27 ± 0.01, 1.47 ± 0.01, 1.43 ± 0.00, 1.38 ± 0.02, 1.17 ± 0.00, 0.98 ± 0.00, 1.17 ± 0.03, 1.50 ± 0.02 and 1.26 ± 0.02 nmol / min/ml. CAT values of all licoricey groups were increased significantly compared to the control coup on the 45th day, in addition to which 0.1% K, 0.5% SM and 1% SM groups displayed increases for their CAT values (Graph **(2)**).

Based on 75th day data, CAT value increases were only observed in 0.1% L and 1% S groups, respectively as 1.34±0.06 and 1.31±0.01 nmol/min/ml ($P < 0.05$), and no statistically significant difference was observed among the other test groups ($P < 0.05$) (Graph **(2)**.

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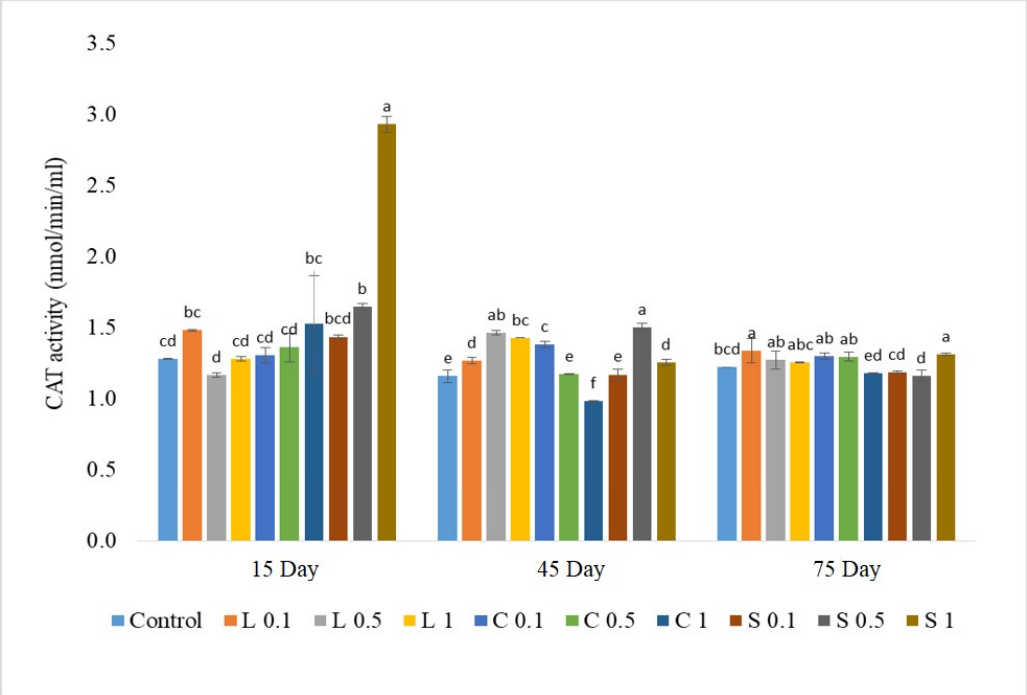

(Graph (2)). The changes that occurred in the catalase activities of the liver tissues of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (nmo/min/ml).

3.3. Glutathione Antioxidant Activity (GSH)

On the 15th day, 0.1% L, 0.5% L, 1% L and 0.1% C, 0.5% C, 1% C groups displayed a significant increase in terms of GSH values compared to the control group (347.77±0.01 nmol/min/ml), respectively as 1135.44±0.03, 682.43±0.03, 697.39±0.07 and 634.81±0.03, 696.72±0.02, 624.83±0.05 nmol/min/ml ($P<0.05$). GSH values of 0.5% S and 1% S groups were observed to be lower than that of the control group (Graph (3)).

On the 45th day of the study, the control group GSH activity (426.42±0.02 nmol/min/ml) was observed to have the lowest value compared to the other groups, while the highest GSH activity values were observed in 0.5% C group (743.94±0.02 nmol/min/ml) and 1% S group (802.78±0.03 nmol/min/ml) ($P < 0.05$) (Graph (3)).

On the 75th day, 0.1% C and 1% C groups displayed significant increase compared to their values on the 15th and 45th days with the values calculated respectively as 1146.58±0.03 and 999.28±0.05 nmol/min/ml. GSH values displayed increases in all groups excluding 0.5% L, 0.5% C, and 0.1% S on the 75th day ($P < 0.05$) (Graph (3)).

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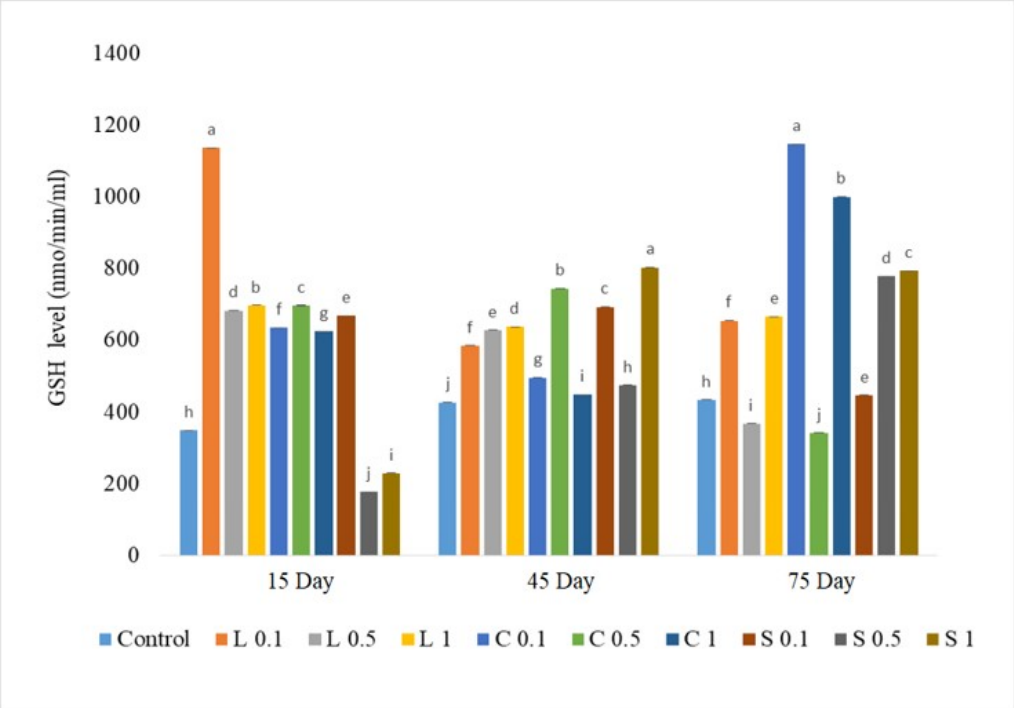

Graph (3) The changes that occurred in the glutathione activities of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (nmo/min/ml).

3.4. Glutathione Peroxidase (GPx)

Changes that occurred in the glutathione peroxidase activities of all groups and determined through the liver samples taken from the fishes on the 15th, 45th and 75th days are presented in (Graph **(4)**).

On the 15th day, the glutathione peroxidase activities of all test groups excluding 1% C (1972.32±2.81) displayed statistically significant increases compared to the control group (1979.20±0.16 nmol/min/ml) ($P < 0.05$), and 0.1% L and 1% S groups were observed to display high GPx increases (3684.60±1.77 and 4935.55±0.88 nmol/min/ml, respectively) ($P < 0.05$) (Graph **(4)**).

When the results of the 45th day of the study were analysed. It was observed that the GPx values of all test groups occurred to be lower than that of the control group (2718.41±0.06 nmol/min/ml). However, it was also observed that particularly the 0.1% S and 0.5% S groups among the test groups had higher Gpx activity values, respectively 2683.76±0.36 and 2460.23±3.23 nmol/min/ml, than that of the other test groups (Graph **(4)**).

Based on the 75th day GPx activity values, it is observed that all coriander and cassia groups (highest value belonging to 0.5% L) displayed increases compared to the control

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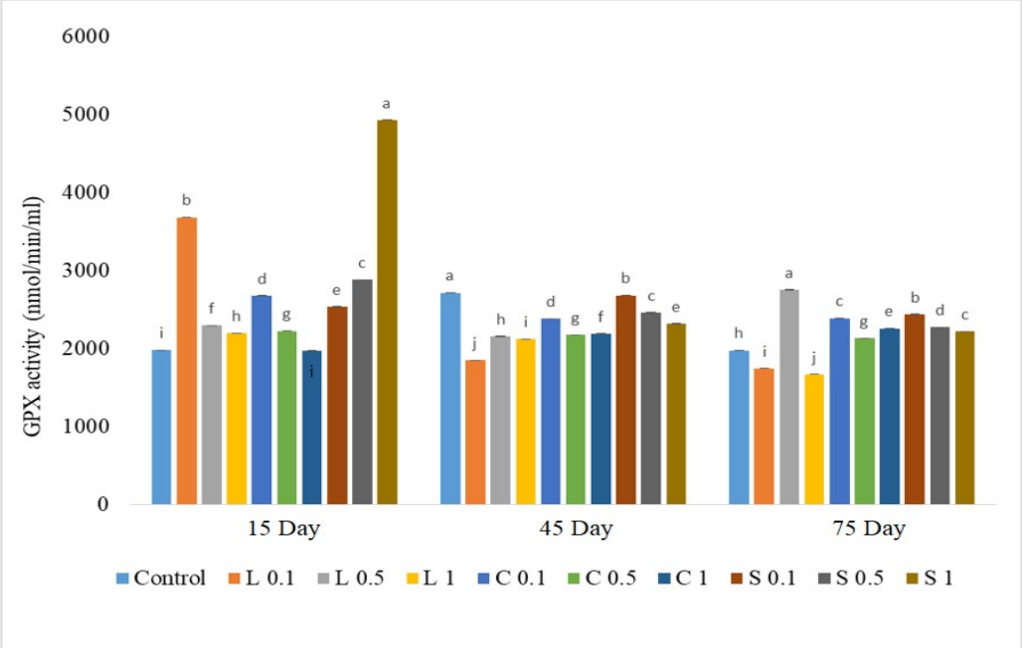
group. 0.1% L and 1% L groups were determined to have lower values than that of the control group; 1747.55±2.08 and 1671.42±1.13 nmol/min/ml, respectively (Graph **(4)**).



(Graph (4)) The changes that occurred in the glutathione peroxidase (GPx) activities of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (nmo/min/ml).

3.5. Lipid Peroxidation (LPO)

15th day LPO values displayed overall increase, with the 0.1%, 0.5%, 1% L, 0.1% C, 0.5% S and 1% S groups having the values 6.56±0.02, 4.98±0.01, 4.25±0.01, 4.85±0.01, 5.14±0.06 and 7.88±0.01 U/ml respectively, compared to the control group (Graph **(5)**). 0.5%, 1% C and 0.1% S groups displayed LPO values lower than that of the control group, with the values 2.75±0.02, 3.59±0.03 and 3.57±0.01 U/ml respectively ($P < 0.05$).

Based on the 45th day LPO values, it was observed that 0.5% L and 0.5% C groups presented similar results with the control group ($P < 0.05$). While 0.1% and 1% L, 0.1% C and 0.5% S groups were observed to have LPO values significantly higher than that of the control group, with the values 4.69±0.03, 4.38±0.03, 4.07±0.02 and 4.91±0.05 U/ml respectively ($P < 0.05$) (Graph **(5)**).

On the 75th day, all S groups, particularly, 0.5% and 1% S groups, displayed lower LPO results compared to that of the control group, with the values 3.48±0.03 and 3.06±0.03 U/ml, respectively ($P < 0.05$) (Graph **(5)**).

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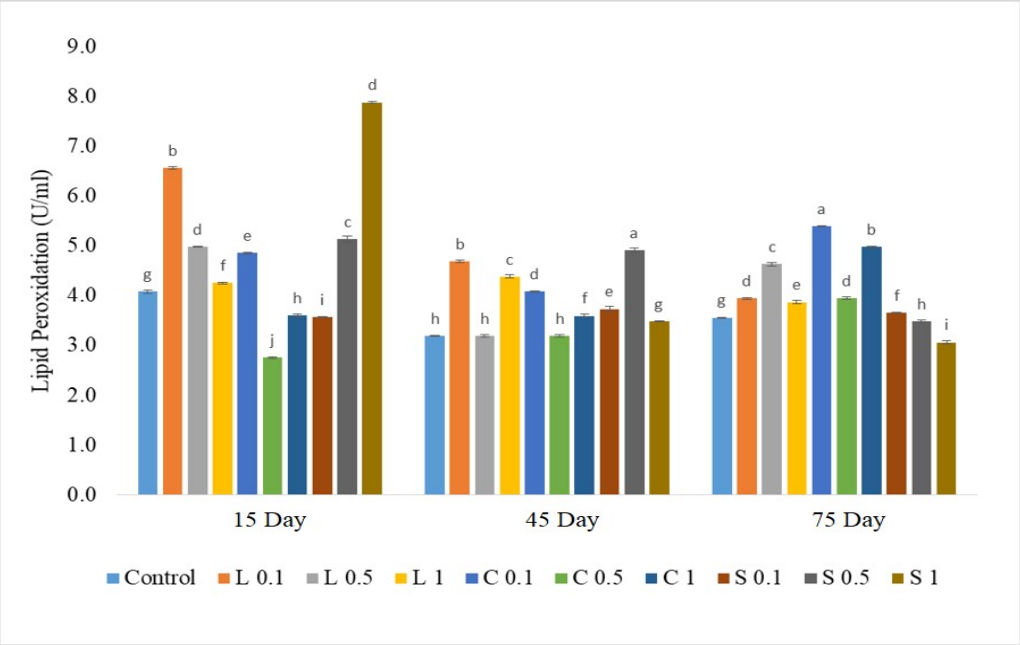

(Graph (5)). The changes that occurred in the lipid peroxidation (LPO) activities of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (U/ml).

4. Discussion

Through this study, licoricey root (*Glycyrrhiza glabra*), coriander (*Coriandrum sativum*) and cassia (*Cassia angustifolia*) added to the food of rainbow trout were determined to have provided positive changes in the food intake, antioxidant enzymes, of the rainbow trout compared to the control group.

Antioxidants Enzyme System

The changes that occurred in the antioxidant systems, SOD, CAT, GSH, GPx activities, and lipid peroxidation (LPO) of the rainbow trout fed with differing ratios of licoricey root, coriander and cassia watered methanolic extract for a period of 75 days were determined through samplings conducted on the 15th, 45th and 75th days. Catabolism of reactive oxygen types is of considerable importance for the continuation of vital activities. Catabolism of reactive oxygen types is important in regards to the vital functions of the cell, and establishment of this balance in a delicate manner is important towards elimination of pathogens. Increases in SOD activity may rise in line with an increase in the superoxide radicals within the cell (Chang, 2006).

At the end of the total study period of 75 days, it was determined that particularly (*C. sativum* and *C. angustifolia*) methanolic extracts caused significant increases in the SOD activities of rainbow trout ($P < 0.05$), while not causing any significant change in CAT activity ($P < 0.05$). (Keleştemur, 2013) determined that rainbow trout fed with food with added Vitamin A and Vitamin E experienced significant increases in their

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SOD activities. (Amr A. A., 2015), fed tilapia fish (*Oreochromis niloticus*) with food containing (*Spirulina platensis*) at 0%, 0.5%, 1% and 1.5% ratios for a period of 75 days, and observed increases in the CAT activities regarding the groups of fish fed with *S. platensis*. It was also reported that implementations of sage and thyme likewise increased SOD activity in trout juveniles (Adem Yavuz Sönmez, 2015). (Gabriel, 2015) failed to determine any change in the SOD parameters of tilapia juveniles tested with different doses of aloe vera in regards to liver SOD activities. (El-Badawi., 2015) reported that they have observed significant increases in the SOD values from *O. niloticus* fishes tested with lupine plant.

Glutathione (GSH) is an important antioxidant and is contained within all cells at milimol concentrations (Lu, 1999). As an endogenous tripeptide, GSH prevents ROS and peroxide components from harming the cell. GSH also takes up role as a substrate for GPx and GST (Pompella, 2003). When the GSH values obtained in this study were examined, it was found out that 1% concentration MK, K and SM groups increased GSH values significantly compared to the control group ($P < 0.05$).

GPx enzyme catalyses NADPH and GSSG formation, enabling the functioning of glutathione reductase enzyme. GPx activity in K and SM groups generally has higher values compared to the control group ($P < 0.05$), and this increase in comparison to the control group gets highlighted especially on the 75th day.

According to the study results, it may be mentioned that GPx activity is positively affected through long-term use of coriander and cassia methanolic extract. (Adem Yavuz Sönmez, 2015) observed increases in GPx activities of rainbow trout juveniles tested with thyme and sage plants. In their study concerning feeding of tilapia juveniles with aloe vera powder supplements with 0.5%, 1%, 2% and 4% concentrations for a period of 2 months. (Gabriel, 2015) observed significant increases in liver CAT activities of the groups of tilapia juveniles fed with 4% supplement, and in GPx activities of the groups of tilapia juveniles fed with 0.5% and 1% supplements ($P < 0.05$). (Metwally, 2009) found out GPx activity decreases in their tilapia feeding study implemented with garlic-content food.

Malondialdehyde (MDA) is the final product of the lipid peroxidation caused by free oxygen radicals, and plays active role in the determination of oxidative stress caused by lipid peroxidation. In this study, lipid peroxidation values were observed to be lower compared to the control group especially according to the 75th day observation of 0.5% and 1% S groups ($P < 0.05$). (Adem Yavuz Sönmez, 2015) defined decreases of MDA levels of the rainbow trout they fed with food containing sage plant. Increases of MDA levels of the Nile tilapia they fed with food containing garlic and cumin (Manal, 2016).

5. Conclusion

Many studies are being carried to study the effectiveness of herbal supplementation in fish feed to manage fish diseases and produce healthy fish. Medicinal herbs have

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bioactive ingredients, for example, polyphenols, flavonoids, tocopherols, essential oils and medicinal herbs have antioxidant properties and can be used as an anti-stress treatment in aquaculture. This approach can reduce costs and side effects of synthetic or chemical products. Although there are many antioxidant compounds in most medicinal plants, few are considered food additives in the diet of fish. The outcomes of the studies suggest the use of herbs and herbal products feed supplements for healthy fishes in culture. Conclusively, the herbal feed supplements promote growth, minimizes stress, improves antioxidant and prevents various infections in fishes that will help to produce healthy fishes for human consumption. Our knowledge of their application to the health and production of aquatic animals appears to be still limited and more studies are needed on this topic.

References

Abdel-Khalek A. A., Kadry M., Hamed A., & Marie, M. A. (2015). Marie Ecotoxicological impacts of zinc metal incomparison to its nanoparticles in Nile tilapia;Oreochromis niloticus. *The Journal of Basic & Applied Zoology.*, 72,115 - 125.

Adams, C. (2005). Nutrition-based health. *Feed international.*, 2, 25–28.

Adem, Y. S., & Bilen, S. (2015). Effects of Dietary Supplementation of Herbal Oils Containing 1,8-cineole, Carvacrol or Pulegone on Growth Performance, Survival, Fatty Acid Composition, and Liver and Kidney Histology of Rainbow Trout (Oncorhynchus mykiss) Fingerlings. *Turkish Journal of Fisheries and Aquatic Sciences.*, 15: 813-819.

Chang, G. R., Mao F. C., Yang C., & Chan F. (2006). Hematological profiles of Formosan black bear (Ursus thibetanus formosanus). *Zoological Studies*, 45(1), 93-97.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. . *Aquaculture International.*, 18, 403-414.

Cooper, R. U., Clough, L. M., Farwell, M. A., & West, T. L. (2002). Hypoxia-induced metabolic and antioxidant enzymatic activities in the estuarine fish Leiostomus xanthurus. *J. Exp. Mar. Biol. Ecol.*, 279, 1–20.

El-Badawi, A. A. (2015). Studies on Tissue Residual Level of NeemAzal T/S in Stressed African Catfish" Clarias gariepinus" with Reference to the Protective Role of Lupine. *World Journal of Fish and Marine Sciences.*, 7 (2): 114-122.

El-Barbary, M. I. (2016). Detoxification and antioxidant effects of garlic and curcumin in *Oreochromis niloticus* injected with aflatoxin B1 with reference to gene expression of glutathione peroxidase (GPx) by RT-PCR. *Fish physiology and biochemistry, 42*, 617-629.

Fitzgerald, J. (1992). Comparative analysis of superoxide dismutase activities in a range of temperate and tropical teleost fish. *Comp. Biochem. Physiol.* , 101B, 111–114.

Gabriel, N. N., Qiang, J., Ma, X. Y., He, J., Xu, P., & Liu, K. (2015). Dietary Aloe vera improves plasma lipid profile, antioxidant, and hepatoprotective enzyme activities in GIFT-tilapia (Oreochromis niloticus) after Streptococcus iniae challenge. *Fish physiology and biochemistry*, *41*(5), 1321–1332.

Gabryelak, T., Piatkowska, M., Leyko, W., & Pérès, G. (1983). Seasonal variations in the activities of peroxide metabolism enzymes in erythrocytes of freshwater fish species. *Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology*, *75*(2), 383–385.

Gjedrem, T., Robinson, N. & Rye, M. (2012). The importance of selective breeding in aquaculture to meet future demands for animal protein: Areview. *AQUACULTURE*, 350 pp. 117-129

Grier, H. J., Uribe, M. C., & Parenti, L. R. (2007). Germinal epithelium, folliculogenesis, and postovulatory follicles in ovaries of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (Teleostei, protacanthopterygii, salmoniformes). *Journal of morphology*, *268*(4), 293–310.

Hashimoto, G. S. D., Neto, F. M., Ruiz, M. L., Acchile, M., Chagas, E. C., Chaves, F. C. M., & Martins, M. L. (2016). Essential oils of Lippia sidoides and Mentha piperita against monogenean parasites and their influence on the hematology of Nile tilapia,Aquaculture,Volume 450, 182-186.

Hermes, M., Storey, J. M., & Storey, K. B. (2001). Antioxidant defenses and animal adaptation to oxygen availability during environmental stress.Cell and Molecular Responses to Stress. Amsterdam, pp. 263–287.

JO, C., & AHN, D. U., (1998). Fluorometric Analysis of 2-Thiobarbituric Acid Reactive Substances in Turkey. *Poultry Science*, 77:475–480.

Keleştemur, G. T., & Özdemir, Y. (2013). Effects of dietary viamin A and E on growth performance and antioxidant status in blood of juvenile rainbow trout (*Oncorhynchus mykiss*, W. 1792)exposed to flow rate stress. *Journal of Animal and Plant Scienc.*, 23(3), 821-827.

Lozovskaya, M. a., Lozovskii, A. R., (2002). Free-radical lipid oxidation in intergeneric and interspecific sturgeon hybrids at early stages of ontogen. *Bull. Exp. Biol. Med.* , 134, 333–334.

Lu, S. C. (1999). Regulation of hepatic glutathione synthesis: current concepts and controversies. *The FASEB Journal.*, 13(10), 1169-1183.

Luck, H. (1974). *Methods in Enzymatic Analysis 2.* New York, p 885.: (Ed Bergmeyer) Academic Press.

Lushchak, V. I., Lushchak, L. P., Mota, A. A., & Hermes-Lima, M. (2001). Oxidative stress and antioxidant defenses in goldfish Carassius auratus during anoxia and reoxygenation. *American journal of physiology. Regulatory, integrative and comparative physiology*, *280*(1), R100–R107.

Madhuri, S., Mandloi, A.K., Govind, P., & Sahni, Y.P. (2012). Antimicrobial activity of some medicinal plants against fish pathogens. *International Research Journal of Pharmacy.*, 3, 28-30.

Magnadottir, B., Audunsdottir, S. S., Bragason, B. T., Gisladottir, B., Jonsson, Z. O., & Gudmundsdottir, S. (2011). The acute phase response of Atlantic cod (Gadus morhua):

humoral and cellular response. *Fish & shellfish immunology*, *30*(4-5), 1124–1130. https://doi.org/10.1016/j.fsi.2011.02.010Magnadottir, B. (2006). Innate immunity of fish (overview). *Fish Shellfish Immunol.*, 20:137–151.

Martínez-Alvarez, R. M., Hidalgo, M. C., Domezain, A., Morales, A. E., García-Gallego, M., & Sanz, A. (2002). Physiological changes of sturgeon Acipenser naccarii caused by increasing environmental salinity. *The Journal of experimental biology*, *205*(Pt 23), 3699–3706.

Metwally, M. (2009). Effects of garlic (Allium sativum) on some antioxidant activities in Tilapia Nilotica (Oreochromis niloticus). *World Journal of Fish and Marine Sciences.*, l:56-64.

Mohamed, A. E. (2003). Influence of some medicinal plants supplementation: 1- On digestibility, nutritive value, rumen fermentation and some blood biochemical parameters in sheep. *Egyptian Journal of Nutrition and Feeds.*, 6 (2): 139-150.

O'Brien, C. M., Fox, C. J., Planque, B., & Casey, J. (2000). Climate variability and North Sea cod. *Nature*, *404*(6774), 142.

Palace, V. P., & Klaverkamp, J. F. (1993). Variation of hepatic enzymes in three species of freshwater fish from precambrian shield lakes and the effect of cadmium exposure. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, *104*(1), 147-154.

Pompella, A., Visvikis, A., Paolicchi, A., De Tata, V., & Casini, A. F. (2003). The changing faces of glutathione, a cellular protagonist. *Biochemical pharmacology*, *66*(8), 1499–1503.

Ribeiro, HYPERLINK "https://www.scienceopen.com/search" \l "author/3db0d235-39aa-4fc1-b02d-642c93b7c378" S. C. , Castelo, HYPERLINK "https://www.scienceopen.com/search" \l "author/0a3c69ed-c1a5-4551-ac57-3600145ef6bd" A. S. , Silva, HYPERLINK "https://www.scienceopen.com/search" \l "author/881eae1b-26b0-40a6-b993-b3e9e93ebb85" B. M. P., Cunha, HYPERLINK "https://www.scienceopen.com/search" \l "author/8f17dc8a-6271-46f0-8d90-0f03ec32f542" A, d., HYPERLINK "https://www.scienceopen.com/search" \l "author/2d3d05f7-8216-4081-b803-2b56991fff2a" Proiett Junior, A. A. , & Oba-Yoshioka HYPERLINK "https://www.scienceopen.com/search" \l "author/318ae81e-2356-42b4-a93d-d91c153e8deb" E. T. (2016). Hematological responses of tambaqui Colossoma macropomum (Serrassalmidae) fed with diets supplemented with essential oil from Mentha piperita (Lamiaceae) and challenged with Aeromonas hydrophila. . *Acta Amazon.* , 46, 99–106.

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical biochemistry*, *25*(1), 192–205.

Shinn, A., Pratoomyot, J., Bron, J., Paladini, G., Brooker, E., & Brooker, A. (2015). Economic effects of aquatic parasites on global finfish production. *Global Aquaculture Advocate*, pp. 58–61.

Sun, Y., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical chemistry*, *34*(3), 497–500.
Talpur, A. D., Bolong, A. A., & Ikhwanuddin, M. (2013). Nutritional effects on ginger (Zingiber officinal Roscoe) on immune response of Asian sea bass (Lates calcarifer) and disease resistance against Vibrio harveyi. *Aquaculture*, 400-401, 46–52.
Wendel, A. (1980). *Enzymatic Basis of Detoxication.* NY: Academic Press Volume 1, p. 333.

بعض النباتات الطبية و استعمالها كإضافات غذائية لتنشيط مضادات الأكسدة في سمك السلمون Rainbow trout (Oncorhynchus mykiss)

***طارق عبد السلام سالم إلطيف**
قسم العلوم الأساسية البيطرية/ جامعة عمر المختار/ البيضاء/ ليبيا

أحمد الهادي سعيد المبروك
قسم العلوم البيطرية/ المعهد العالي للتقنية الزراعية/ الغيران/ ليبيا

جمعية علي محمد علي
قسم علم وظائف الحيوان/ كلية العلوم/ جامعة الزيتونة/ ليبيا

سونر بيلان
قسم العلوم الأساسية/ كلية الزراعات المائية/ جامعة كاستامونو/ تركيا

- Tarekhhakam@gmail.com

المخلص

صممت هذه الدراسة لتقييم بعض مضادات الأكسدة لـ 1500من سمك السلمون اليافع Rainbow trout Oncorhynchus (mykiss) الذي تم تغذيته على علف يحتوي مستويات مختلفة (0%، 0.1%، 0.5%، 1%) من المستخلص الميثانولي المائي لكل من نباتات(Glycyrrhiza glabra و Coriandrum sativum و Cassia angustifolia) كإضافة غذائية لمدة 75 يوم. كان وزن الأسماك الأولي عند بداية التجربة 22.65 ±0.07 جرام و التي تم توزيعها على 30 حوض يحتوي كل منها على 50 سمكة و بدأت التجربة بثلاث مكررات. في نهاية كل شهر تم تحديد نشاط الإزيم المضاد الأكسدة بالكبد (catalase CAT و superoxide dismutase SOD و glutathione peroxidase GPx و Glutathione GSH و lipid peroxidation LPO). وفقا لنتائج الدراسة المستخلص المائي الميثانولي لـ C. sativum و C. angustifolia كانت هناك زيادة معنوية لنشاط إنزيم SOD لسمك الـ rainbow trout ($P < 0.05$) ، ولكن لم تظهر أي تغييرات معنوية في نشاط CAT ($P > 0.05$). زيادة جرعة GSH لـ 1%لكل مجموعات التجارب ($P < 0.05$). عند مقارنة مستويات GPx، الـ C. sativum و C. angustifolia أظهرت زيادة بصفة عامة في إنزيم الـ lipid peroxidase بالمقارنة بمجموعة التحكم، بينما إنزيم lipid peroxidase كانت مستوياته منخفضة بمجموعات التجربة ($P > 0.05$).

الكلمات المفتاحية: عرق السوس ـ الكسبرة ـ القرفة ـ سمك السلمون ـ مضادات الاكسدة.