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MITIGATIVE EFFECT OF TWO HERBAL EXTRACTS (*ZINGIBER OFFICINALE AND GLYCYRRHIZA GLABRA)* ON THE PRODUCTIVE PERFORMANCE, PHYSIOLOGICAL PARAMETERS AND HISTOPATHOLOGICAL EXAMINATION OF *OREOCHROMIS NILOTICUS*

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ABSTRACT

This study was conducted to evaluate the mitigative effect of *Glycyrrhiza glabra* and *Zingiber Officinale* on the productive performance, physiological and histopathological picture of *Oreochromis Niloticus.* 1800 fingerlings were randomly distributed into four experimental groups; three replicates each, the stocking rate was 150 fingerling /replicate, average initial weight were 17.5 ±0.109 g and the average initial length 10.13± 0.37 cm/fish. Group one fed on the basal diet, group two fed on basal feed fortified by ginger extract 5ml/kg, group three taken basal feed plus liquorice 4ml/kg, and the fourth group was received basal feed treated ginger and liquorice 2.5ml/kg +2ml/kg respectively. After four month data were collected; Productive performance includes body weight, body length, feed intake, feed conversion ratio, mortality rate. Physiological and blood biochemical parameters includes RBCs count, WBCs count, Hb concentration, PCV %, differential leukocytic count, serum proteins (g /dl), serum albumin (g /dl), serum globulins, albumin/globulin ratio and creatinine (mg/dl) finally tissue specimen from, gills, intestine and liver of fish were collected for histopathological examination. The obtained results revealed a high significance difference between the treatments. Group four recorded higher body weight 237.21± 5.6 g followed by group three 211.03±3.2 g, and the lowest body weight was in control group 190.77 ± 3.88 g. Lower feed conversion ratio recorded in group four 1.82 ± 0.03 g, Highest level of hemoglobin conc., white blood cell count, and total leukocytic count recorded in group four followed by group

two. Base up on results obtained we concluded that using one Ginger and or liquorice herbal extract has positive effect on performance, blood biochemical and histological picture in the *Oreochromis niloticus*.

Keywords:

Oreochromis Niloticus, *Glycyrrhiza glabra* and *Zingiber Officinale*, herbal extract, Ginger, Liquorice

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INTRODUCTION

The fish farm and its water quality is considered the main limitation factor for heath condition in both cultured and wild fishes. Pollution of the aquatic environment by inorganic and organic chemicals is a major factors posing serious threat to the survival of aquatic organisms including fish **(Samir and Ibrahim, 2008)**. Heavy metals are considered the most important constituents of pollution from the aquatic environment because of their toxicity and accumulation in fish **(Khansari *et al*., 2005)**.

The exposure to environmental stressors such as pollution, and contaminants can also predispose fish to infectious diseases because the immune system is sensitive target to environmental pollutants. Pollutants have a high potential to induce oxidative stress in aquatic organisms through production of free radicals and reactive oxygen species (ROS) and induce an imbalance between intracellular ROS levels and antioxidant protection, and can subsequently cause oxidative stress in organisms. **(Rice, 2001; Toni *et al.,* 2011).**

Herbal extracts have been reported to favor various activities like antistress, growth promotion, and appetite stimulation, enhancement of tonicity and immunostimulation, maturation of culture species, aphrodisiac and anti-pathogen properties in fish and shrimp aquaculture due to active principles such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils. **(Chakraborty and Hancz 2011).**

Therefore, this study aimed to evaluate the effect of two herbal extracts (Ginger and Liquorice) on the productive performance of tilapia as well as immunological physiological parameters and histopathological examination.

MATERIAL AND METHODS

Fish Location and Management:

This study has been conducted in a private fish farm located in EL- Fayuim Governorate, from April 2016 to September 2016. 1800 Nile tilapia fish (*Oreochromis Niloticus*) monosex, all male fingerlings with average 18 g weight. Fingerling was randomly divided into12 fish hapas (4 X 5 X1.5 m) in earthen pond with surface area 4046.8 m^2. Fish feed was commercial fish diet of 25 % protein and 3235 Kcal energy (Table 1) the Feeding regimen was, hand feeding, twice daily (6 days per week). The feeding frequency was twice; one portion given at 9am and the other portion given at 2 pm. The feed amount was calculated approximately every 21 days' intervals according to the fish body weight, according to **Lucas and Southgate, (2003).**Water exchange and fish sampling: approximately 10- 15% of pond water drained off and replenished by entry of new water in the fish pond. Fish sample collect random representative sample from each replicate in each group every 30 day and record growth performance for each treatment group. Random fish samples were taken at the end of experiment for physiological and histopathological evaluation.

Experimental Design:

After 15 days of Adaptation time, the fish (1800 fingerlings) were randomly distributed into four experimental groups each in three replicates (three hapas) representing one of the dietary treatments and stocked in the experimental hapas at a rate of 150 fish /hapa. With an average initial weight were 17.5 ±0.109 gm and the average initial length 10.13± 0.37 cm/fish. Two herbal extract used in our study the first one was Glycyrrhiza glabra (Liquorice) extracted according to **Zargari, (1997).** The second was *Zingiber Officinale* (Ginger) that extracted according to **Masoud, *et al.* (2014).** Both extracts obtained from national research center, Department of Medicinal and Aromatic Plants. Control group was fed on basal commercial diet without any additives, while the basal commercial diet mixed with ginger extract was sprayed on fish feed in the rate of 5 ml for 1 kg feed according to **Rahimi, *et al,* (2015)** and used for group two (Ginger group). In group three (Liquorice group) was fed on basal fish diet mixed with liquorice extract sprayed on fish feed 4 ml for 1 kg feed according to **Amani *et al,* (2015)**. Finally, group four (Mix group) was fed on basal fish diet mixed with (2.5 ml ginger extract and 2 ml liquorice extract) sprayed on 1kg fish feed, all diet in different treatment groups were sprayed 12 hours before feeding.

Table (1): The composition of commercial feed used in the study.

Ingredients	%
Soybean meal (44%Cp)	37.5
Yellow corn	22.5
Fishmeal (Sardine,60%Cp)	6
Rice bran	23
Oil	3
Calcium carbonate	4.7
Salt	0.5
Vitamin mixture	0.3
Antioxidant	0.025
Binder	2.5
Total	100%
Composition	
Crude protein	25%
Crude fat	8.8%
Energy kcal/kg	3235
Crude fiber	5.7%
Ash	4.3%

Measuring Parameters:

Growth Performance parameters.

The initial body weight was recorded at the beginning of the experiment. Random samples of fish were taken every month during the whole experimental period; individual body weight, body weight gain, feed intake, feed conversion ratio and mortality rate were recorded **(Abdel-Halim, 2009).**

Hematological and Immunological Parameters:

Blood samples were collected from the caudal vein. Blood samples were subjected to RBCs count, WBCs count, Hb concentration, PCV % and differential leukocytic count. Blood indices (M.C.V, M.C.H. and M.C.H.C) were calculated from the obtained values of RBCs, PCV and Hb according to **Feldman *et al*. (2000).**

Serum Biochemical Analyses:

Blood samples were collected from the caudal vein and kept without anticoagulant for serum separation for measuring of serum proteins (g /dl) according to **Henry (1974);** serum albumin (g /dl); serum globulins albumin/globulin (A/G) ratio according to **Dumas and Biggs (1972).** Determination of Urea (mg/dl) according to **Patton and Crouch (1977)** was carried out also measuring creatinine (mg/dl) according to **Henry (1974).**

Histopathological Picture:

Tissue specimen from, intestine and Liver of fish were collected at the end of the experiment and fixed in 10 % neutral buffered formalin for 48 h. Then it was processed by paraffin embedding technique. Tissue sections 5 µm thick were prepared using microtome then it were stained with Hematoxylin and Eosin stain for microscopic examination **(Suvarna *et al*. 2012**).

Heavy metal in pond water and fish flesh:

Water samples were obtained, from the pond water for heavy metal determination specially cadmium, lead and copper according to **American Public Health Association (APHA). (1992).** At the end of experiment; fish samples taken from each tested groups to check the residual heavy metals in water and fish flesh in Central Lab of Residue Analysis of Pesticides and Heavy Metals in Food (QCAP Lab), Ministry of Agriculture Egypt. At laboratory, the fish samples were washed with deionized water and wrapped separately in acid washed polyethylene bag and stored frozen at-20°C until analysis was carried out according to **Finerty *et al.*, (1990).**

Statistical Analysis:

The data pooled through the experiment were statistically analyzed by General Linear Model procedures (GLM) described in SAS User's Guide, 2004. The differences among treatments means were subjected to significance by Duncan's Multiple Range-test **(Duncan, 1955**). A probability of 0.05 was utilized to account for the statistical difference between the means.

RESULTS

Table (2): The Final growth performance in fattening monosex *Oreochromis Niloticus* in different treatment groups.

Gp. Parameters	Group one	Group two	Group three	Group 4
Initial weight (g)	18.16 ± 0.32 a	15.53 ± 0.39 b	18.22 ± 0.61 a	18.22 ± 0.22 a
Body weight increase (g)	172.62 ± 3.55 c	170.9 ± 6.31 c	192.8 ± 3.76 b	218.98 ± 5.9 a
Final Body weight	190.77 ± 3.88 c	186.45 ± 5.9 c	211.03 ± 3.29 b	237.21 ± 5.68 a
Initial length(cm)	10.1 ± 0.37 a	10.1 ± 0.37 a	10 ± 0.8 a	10.33 ± 0.44 a
Final Length (cm)	18.66 ± 0.33 a	18.33 ± 0.33 a	19 a	19.16 ± 0.44 a
Length increase (cm)	8.56 ± 0.47 a	8.23 ± 0.53 a	9 ± 0.81 a	8.83 ± 0.44 a
Final feed intake (g)	357.02 ± 8.19 b	349.38 ± 6.11 b	392.44 ± 4.68 a	399.51 ± 4.18 a
FCR	2.06 ± 0.007 a	2.04 ± 0.04 a	2.03 ±0.02 a	1.82 ± 0.03 b

Result expressed as Mean ± Stander error

a, b, c: Different Letter within the raw means significantly differ at p≤ 0.05 between the groups.

Table (3): The blood parameters in fattening monosex *Oreochromis Niloticus*.

Gp. Parameters	Group one	Group two	Group three	Group four
PCV %	25.40 ± 0.61 b	30.50 ± 0.84 a	30.50 ± 0.76 a	31.16 ± 0.76 a
HB Conc. g/dL	4.170 ± 0.11 b	4.880 ± 0.17 a	4.800 ± 0.15 a	5.240 ± 0.21 a
RBCs ($X10^6$)/ µl	1.730 ± 0.06 a	2.560 ± 0.60 a	1.860 ± 0.01 a	2.150 ± 0.06 a
MCV (Femtoliters)	147.6 ± 5.60 a	128.6 ± 2.48 a	163.7 ± 4.18 a	146.3 ± 8.40 a
MCH (pictograms)	24.28 ± 1.11 a	21.16 ± 3.21 a	25.79 ± 0.93 a	24.67 ± 1.80 a
MCHC %	16.46 ± 0.41 a	14.14 ± 1.77 a	15.76 ± 0.48 a	16.82 ± 0.45 a
WBCS (25 square)	92.20 ± 5.28 b	107.4 ± 20.9 ab	122.7 ± 8.49 ab	136.5 ± 9.90 a
TLC ($X10^3$)/ µl	92.20 ± 5.28 b	107.4 ± 20.9 ab	122.7 ± 8.49 ab	136.5 ± 9.90 a
N/ ($X10^3$) / µl	42.23 ± 5.61 b	49.63 ± 9.70 ab	66.19 ± 4.54 a	68.38 ± 8.20 a
L ($X10^3$)/ µl	49.66 ± 2.61 a	57.45 ± 11.1 a	53.98 ± 5.90 a	66.79 ± 5.90 a
MØ ($X10^3$)/ µl	00.30 ± 0.24 a	01.60 ± 0.8 a	2.480 ± 0.97 a	1.310 ± 0.8 a

Result expressed as Mean ± Stander error.

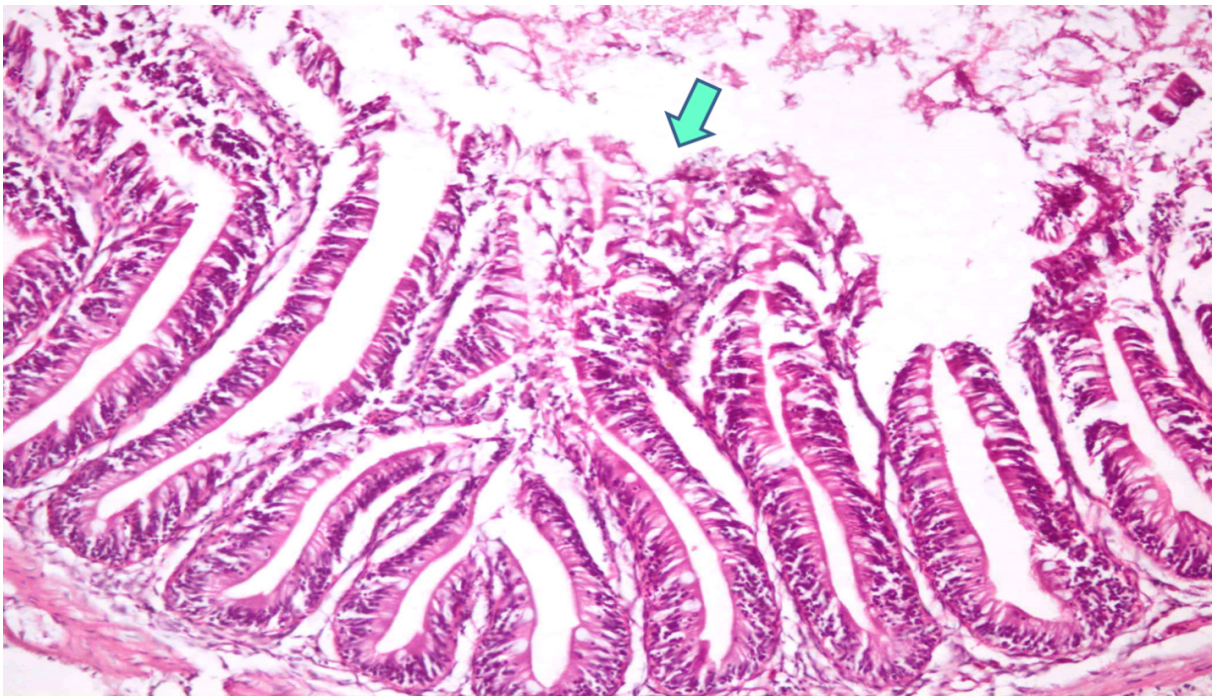
a, b, c : Different Letter within the raw means significantly differ at p≤ 0.05 between the groups.

Table (4): The blood biochemical parameters in fattening monosex O*reochromis Niloticus*.

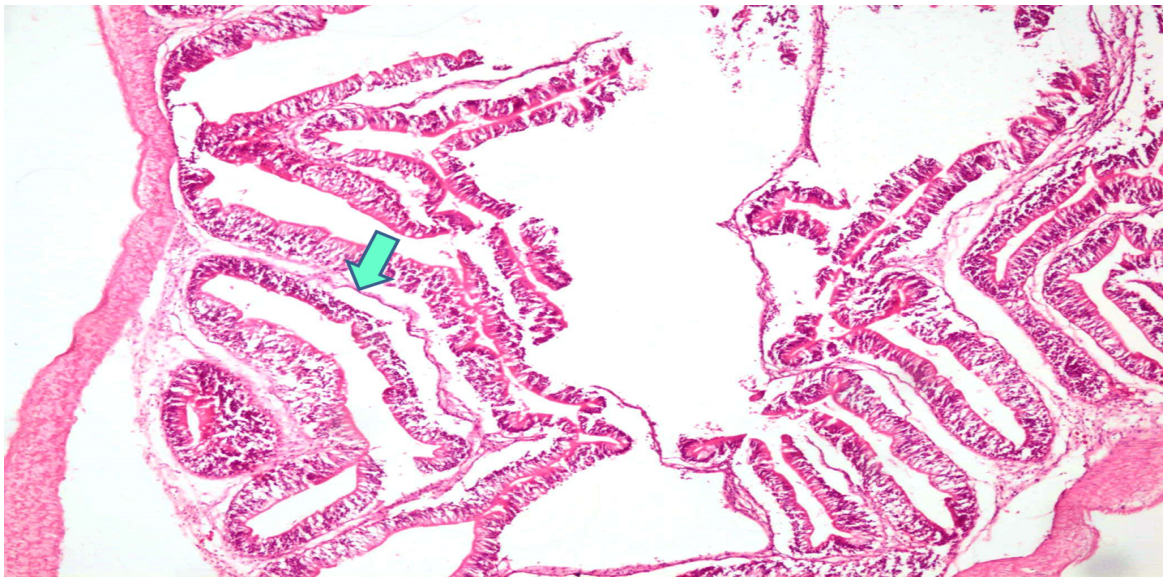
Gp. Parameters	Group one	Group two	Group three	Group four
Total Prot. (g/dl)	**4.95 ± 0.05 a**	**5.50 ± 0.05 a**	**5.95 ± 0.60 a**	**6.90 ± 0.60 a**
Albumen (g/dl)	**2.75 ± 0.25 c**	**3.90 ± 0.00 ab**	**3.30 ± 0.30 cb**	**4.00± 0.00 a**
Globulin (g/dl)	**2.50 ± 0.10 a**	**2.60 ± 0.15a**	**2.55 ± 0.35 a**	**2.90 ± 0.30a**
A/G ratio	**1.18 ± 0.08 a**	**1.30 ± 0.09 a**	**1.40 ± 0.10 a**	**1.40 ± 0.10 a**
Creatinine (mg/dl)	**0.67 ± 0.12 a**	**0.40 ± 0.12 a**	**0.40 ± 0.12 a**	**0.40 ± 0.12 a**
Urea (mg/dl)	**12.1 ± 0.63 a**	**6.70 ±0.00 c**	**8.05 ± 1.95 b**	**6.00± 0.00 c**
Blood Urea Nitro.	**5.68 ± 0.28 a**	**3.10 ± 0.00 b**	**3.75 ± 0.90 b**	**2.80± 0.00 b**

Result expressed as Mean ± Stander error

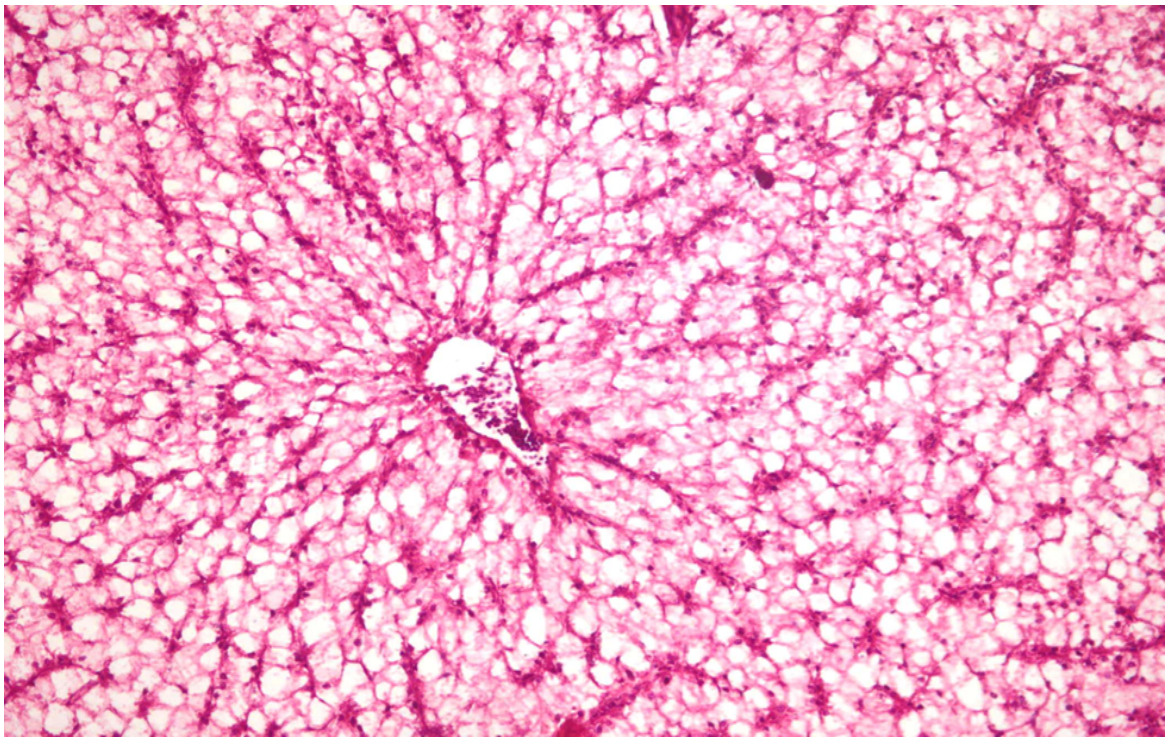
a, b, c : Different Letter within the raw means significantly differ at p≤ 0.05 between the groups



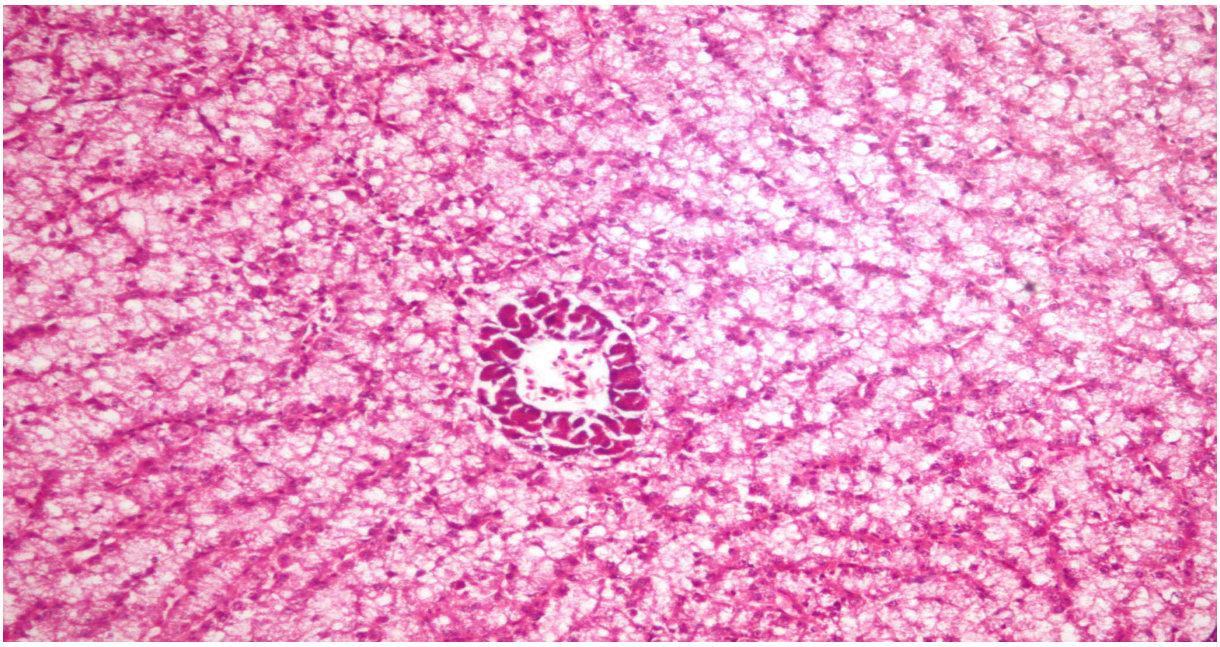
Plat (1): Histopathological examination of intestine in *O. Niloticus* of control group showing intestinal villi with sloughed tips **(H & E X 200).**



Plat (2): Histopathological examination of intestine in O. *Niloticus* of group fed on liquorice showing long intestinal villi **(H & E X 100).**



Plat (3): Histopathological examination of Liver of O. *Niloticus* in control group showing normal vacuolation of hepatocytes **(H & E X 200).**



Plat (4): Histopathological examination of Liver of O. *Niloticus* in group fed on ginger showing moderate vacuolation of hepatocytes **(H & E X 100).**

Table (5): Heavy metals concentration as part per million (ppm) in water source, Pond water and fish.

Heavy metal	Pond water (ppm	Fish flesh (ppm)				Permissible limits (ppm)
		C	G	L	G/L	
Lead (Ld)	0.03	1.5	0.84	0.8	0.38	0.1-0.5
Cadmium (Cd)	0.024	0.3	0.2	0.18	0.07	0.05-0.1
Copper (Cu)	0.034	0.65	0	0.46	0.07	1.00

DISCUSSION

Growth performance parameters of monosex *Oreochromis Niloticus* which fed diet supplemented with ginger and liquorice during four months are shown in (Table 2). The present results showed that fish consumed liquorice and mixture of liquorice and ginger showed significant improvement in appetite than the control and ginger group. Increased feed

intake resulted from improved appetite because of sensory stimulation resulting from the presence of liquorice in the diets **(Amani *et al.*2015**). Further support derived from earlier fish study for **Abd El-Hakim (2008)** where he fed brood stock tilapia fish on liquorice or ginger included diets, there were improved survival rate and led to better feed utilization. Promoted growth in fish fed liquorice and ginger may be due to improving nutrients digestibility and growth-stimulant effect. Improvement in the growth performance has been observed in *Litopenaeus vannamei* **(Chen *et al.,* 2010),** *Siniperca Chuatsi* **(Chen *et al.,* 2000**), and *Apostichopus japonicus* **(Chen *et al.,* 2010)** fed with glycyrrhizin (liquorice roots) confirming the previous findings. In general, phytogenic bioactive compounds increase growth performance through activation of digestive enzymes (amylase and protease) **(Jang *et al.,* 2003)**. Significant increasing in final body weight observed in ginger-liquorice supplemented fishes in the current study indicated increased body weight gain in the mixture group (ginger and liquorice). Also, the diet supplemented with liquorice alone, had significant increasing in final body weight gain than the ginger group. Hematological parameters are used to provide information about the health and physiological status of fish, feeding conditions and water quality in which they live.

In the present study hematological parameters are illustrated in (Table3),the Hb content was significantly increased ($p \leq 0.05$) in experimental groups compared to control group. Group fed on mixture of ginger and liquorice showed increasing in Hb concentration than the liquorice group alone or ginger group all over the experimental period (4 months). Significant increases in RBC, Hb and RBC indices were observed in the groups supplemented with mixture of liquorice and ginger compared to the control group ($P \leq 0.05$).

On the immunity side, WBC and percentage share of lymphocytes, monocytes, neutrophil and eosinophil cells, which form the first step of the body defense, and basic elements of the non-specific immune system, increased significantly in fish supplied with mixture of liquorice and ginger or liquorice alone ($P \leq 05$). (Table 4), shown a significant decrease in the concentration of creatinine, urea, and blood urea nitrogen in fish groups supplemented with Ginger and Liquorice or both **(Kawamura and Kokko, 1976; Green *et al.,* 1981)** they observed that reduction of blood urea nitrogen in animals receiving ginger extract related to a mechanism of reabsorption of urea in the nephrons. The obtained results are agree with, **Fukai *et al.,* 1998**, who showed a significant decrease in the concentration of urea, uric acid

and creatinine after oral administration of liquorice extract. It was found that there were differences in histopathological pictures for intestine and liver of fish within different groups. From (plates 1, 3) for control group, the intestinal villi showed sloughed tips and the liver had vacuolation in hepatocytes. while in the group fed on liquorice, intestine showed long intestinal villi (plate 2) and the liver exhibited normal vacuolation of hepatocytes (plates 4) **i**n group fed on ginger and liquorice, So we can concluded that, prolonged exposure to water pollutants even in very low concentrations have been reported to induce morphological, and histological alterations in the fish tissues **(Kaoud and El-Dahshan 2010).**

Finally, it was observed that herbal extract supplement able to decrease heavy metal accumulation in the fish body (Table 5), cadmium, Lead and copper were higher in the pond water than the permissible limit, due to contamination the water used agricultural drainage water in which heavy metals and pesticides are the main pollutants according to **(Kalay and Canli, 2000).** Our result, come in accordance with **Gehan and Ayman (2010)** who observed that ginger expressed an antagonistic action on cadmium toxicity. and he result agree with **Egwurugwu *et al.* (2007)** who concluded that Ginger and liquorice through the antioxidant activities, first improved the blood balance with resulting improved the liver functions followed by improving, the kidney functions. Based on the results described above, it is obvious, that herbal extract of antioxidant activity (liqurice and ginger); either separate or in combination with each other in fish feed are effective immunostimulant, growth promotor and has a mitigative effect against heavy metal pollution in earthen ponds of Nile tilapia (*Oreochromis Niloticus*).

REFERENCES

Abd El-Hakim, A. E. (2008): Effect of type of diet on productive and reproductive performance of Nile tilapia reared on commercial scale. Ph. D. Thesis, fac. Of agric., fayoum univ.

Abdel-Halim,H.A.(2009): Studies on intensive culture of Nile Tilapia (*Oreochromis Niloticus*) in tanks. Dissertation, Al-Azhar University.

A.P.H.A. "American Public Health Association" (1992): Standard methods for the Examination of water and waste water. American public Health Association, American water work Association and Water Environmental Federation, 18th Edition, Washington, DC. Pp.11-19.

Amani M.D. El-Mesallamya, Hany I. El-Marakbya, Ahmed M.A.Soulemanc, and Fatma S. Abd El-Nabyb. (2015): Evaluation of phenolic extract of liquorice roots in diets of Nile tilapia (*Oreochromis Niloticus*). Egyptian Pharmaceutical Journal, 14:117-122.

Chakraborty SB and Hancz C. (2011): Application of phytochemicals as immunostimulant, ant pathogenic and antistress agents in finfish culture. Reviews in Aquaculture 3: 103-119.

Chen X, Mai K, Zhang W, Wang X, Ai Q, and Xu W. (2000): Effects of dietary glycyrrhizin on growth and nonspecific immunity of white shrimp, *Litopenaeus vannamei*. J World Aquaculture Soc; 41:665 - 674.

Chen J, Xie P, Zhang DW, KE ZX, and Yang H. (2010): studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (Hypophthalmichthys molitrix) stocked in Lake Taihu with dense toxic Microcystis blooms. Aquaculture, 261:1026-38.

Dumas, B.T. and Biggs, H.G. (1972): Standard Methods of clinical chemistry. Vol. 7, Academic press, New York, pp.175.

Duncan, D., B., (1955): Multiple range and multiple F tests. Biometrics. 11: 1 - 42.

Egwurugwu H J N, Ufearo C S, Abanobi O C, Nwokocha C. R, Duruibe J, Adeleye G S, Ebun I, lomo A O and Onwufuji O. (2007): Effect of ginger (Zingiber Officinale) on cadmium toxicity. African J Biotechnol, 6 (18): 2078-2082.

Feldman, B. F.; Zinkl, J. G. and Jain, N. C. (2000): Schalm's veterinary hematology 5th ed., Lippincott Williams and Wilkins, New York

Finerty, M. W., Madden, J. D., Feagley, S. E., and Grodner, R. M. [(1990): Effect of environs and seasonality on metal residues in tissues of wild and pond-raised crayfish in Southern Louisiana. Archives of Environmental Contamination and Toxicology. 19, 94 -100.

Fukai T., Baosheng C, Maruno K., Migakawa Y, Konoshi M. (1998): An isopernylated flavonone from Glycyrrhiza glabra and re-assay of liquorice phenols. Phytochemistry, 49: 2005-2013.

Gehan, A.E. El-Emery and Ayman Y. Amin (2010): Cadmium-ginger two-way antagonistic relationship. Arab J. Biotech, Vol. 13, No. (1). 115-124.

Green, R., R.J. Moriarty and G. Giebisch (1981): Ionic requirements of proximal tubular fluid reabsorption: Flow dependence of fluid transport. Kidney Int., 20: 580 -587.

Henry T.J. (1974): Clinical Chemistry Principles and Techniques. 2nd. Harper and Row Publishers; New York.

Jang M.H., Lucas M.C. and Joo G.J. (2003): The fish fauna of mountain streams in South Korean national parks and its significance to conservation of regional freshwater fish biodiversity. Biol. Conserv., 114, 115 -126.

Kalay M and Canli M. (2000): Elimination of essential (Cu, Zn) and nonessential (Cd, Pb) metals from tissue of a freshwater fish Tilapia Zillii following an uptake protocol.Tukr. J. Zool. 24: 429 - 436.

Kaoud, H.A. and El-Dahshan, A.R. (2010): Bioaccumulation and Histopathological Alterations of the Heavy Metals in *Oreochromis Niloticus* Fish. Nature and Science, 8, 147-156.

Kawamura, S. and Kokko, J. (1976): Urea secretion by the straight segment of the proximal tubule. J. Clin. Invest. 58: 604 - 612.

Khansari FE, Ghazi-Khansari M, and Abdollahi M. (2005): Heavy metals content of canned tuna fish. Food Chem., 93: 293 - 296.

Lucas,J.S.,and Southgate,P.C.(2003): Aquaculture,farming aquatic animals and plants, by Blackwell Publishing.

Masoud Sabouri Ghannad, Avid Mohammadi, Sohayla Safiallahy, Javad Faradmal, Mona Azizi, Zohreh Ahmad V. (2014): The Effect of Aqueous Extract of *Glycyrrhiza glabra* on Herpes Simplex Virus. Jundishapur J Microbiol.; 7 (7):11616.

Patton C, Crouch S. (1977): Determination of serum urea. Anal Chem; 49: 464 - 469.

Rahimi Yadkoori, Nasrin; Zanguee, Nasim Mousa VI, Seyed Mohammad; and Zakeri, Mohammad (2015): Effects of Ginger (Zingiber officinale) extract on Digestive Enzymes and Liver Activity of *Mesopotamichthys Sharpeyi* Fingerlings. Journal of the Persian Gulf (Marine Science). Vol. 6. No. 19: 1-10

Rice CD. (2001): Fish immunotoxicology. In: Schleck D, Benson WH (eds) Target Organ Toxicity in Marine and Freshwater Teleosts, Vol. 2. Taylor and Francis, London.

Samir M. Saeed and Ibrahim M. Shaker (2008): Assessment of Heavy Metals Pollution in Water and sediments and their Effect on *Oreochromis Niloticus* in The Northern Delta Lakes, Egypt. 8th International Symposium on Tilapia in Aquaculture 2008 475 - 490.

Suvarna, K. S.; Layton, C. and Bancroft, J. D. (2012): Theory and Practice of Histological Techniques. 7th ed., London, Churchill Livingstone.

Toni, C., D. Ferreira, L. C. Kreutz, V. L. Loro and Barcellos L. J. G. (2011): Assessment of oxidative stress and metabolic changes in common carp (*Cyprinus Carpio*) acutely exposed to different concentrations of the fungicide tebuconazole. Chemosphere, 83: 579-584.

Zargari, A., (1997): Medicinal Herbs,6th edition University of Tehran Publication, 620 P. (In Persian).